POSTERS

Biologia Celular- Cell Biology

BC01 - Implication of microdomains and plasma membrane-derived parasitophorous vacuole in *Trypanosoma cruzi* cell invasion

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A novel PI 3 kinase-dependent mechanism for cell invasion and vacuole maturation by tissue culture trypmastigotes was recently proposed (Woolsey, 2003). The implication of this pathway in extracellular amastigotes (EA) and metacyclic trypomastigotes (MT) invasion has not yet been defined. Here we examined the distribution of lipid products of class I PI 3-kinase PIP_3/$PI(3,4)P_2$ in Vero cells expressing the pleckstrin homology *PH* domain of AKT fused to GFP during the entry of EA, and MT forms of parasite strains belonging to the two major groups: *T. cruzi* I (G), and *T. cruzi* II (CL). Our results indicate that MT can also associate with host cell plasma membrane (PM) as a first step in an alternate mode of entry, prior to interaction with lysosomes. In addition, Vero cells transfected with PLC-PH-GFP confirmed intimate association of invading and recently internalized MT with the PM marker. Surprisingly, no association was observed with invading EA. We are currently investigating if the transfection procedure influences EA invasion. In addition we examined endosomal-lysosomal sequential labeling with EEA1 and LAMP-1 of the parsitophorous vacuoles formed during parasite entry. Our results reveal that EA and MT can also enter host cells via endosomal pathway, and after 60 min parasitophorous vacuole positive for EEA1 and LAMP-1 were observed. In view of the parasites' ability to invade cells through a plasma membrane bound vacuole, the involvement of host cell cholesterol was investigated. The participation of microdomains enriched in cholesterol and sphingolipids in *T. cruzi* cell invasion had not yet been reported. Preliminary results demonstrate that host cell cholesterol depletion caused a significant reduction of both MT and EA invasion, indicating for the first time, that lipid rafts are also involved in *T. cruzi* internalization. We are now investigating which invasion pathway is/are impaired with cholesterol depletion and possible components involved in the raft mediated entry.

BC02 - Phospholipase A2 antagonist inhibits fluid phase endocytosis in epimastigotes of *Trypanosoma cruzi.*

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The endocytic pathway in *Trypanosoma cruzi* presents a singular architecture. The morphological aspect and the nature of the distinct compartments differ from that previously described for mammalian cells. Despite the morphological and biochemical evidences of different compartments, almost nothing is known about the control of vesicle traffic in trypanosomatids. In the present study we have explored the involvement of iPLA2 in membrane trafficking, in particular along the endocytic pathway of epimastigote forms of *T. cruzi*. Epimastigote forms of the Y stain of *T. cruzi* were incubated in LIT medium for 30 min at 28^o C in the absence or the presence of 7.5μM Bromoenol lactone (BEL) an irreversible antagonist of iPLA2. Samples were pulse-labelled for 4h in presence of Alexa Fluor 488- BSA (10μg/ml) and analysed by flow cytometry. For electron microscopy analysis parasites were incubated with BSA-gold complex (10nm) for 4h followed by incubation in complex-free medium for 20h. Parasites were the treated with BEL, as described above, and then submitted to a second pulse-chase in the presence of a BSA-gold complex of 6nm in diameter. Cell were fixed, dehydrated, embedded and observed at the transmission electron microscope. BEL reduced significantly, 60-65%, the amount of BSA internalized by epimastigotes forms. At the electron microscope, we observed that BEL inhibited the fusion of pre-labelled reservosomes and induced profound modifications on the ultrastructure of Golgi complex.

BC03 - A 21kDa protein from *T. cruzi* is involved in mammalian cell invasion by extracellular amastigotes

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The molecular basis of mammalian cell invasion by *T. cruzi*amastigotes is poorly understood. In order to search for proteins from the parasite that could be involved in the invasion process, we performed DNA comparative microarray analyses of extracellular amastigotes from two isolates that showed distinct infectivity, G isolate that was highly infective toward host cells and CL isolate that showed lower infection rates. Prediction softwares available on the internet were used to screen the 205 genes with greater expression in G isolate and the 111 more expressed in CL. We looked for proteins that could be on the parasite membrane or else be secreted. The sequence of a 21 kDa hypothetical protein, XM 812182, showed in G isolate an increased expression of 2.2 times over CL isolate and the prediction programs showed that it had 78 % of probability to be located on the membrane or to be secreted and showed a signal peptide. We cloned this protein

on pET28 vector and expressed in *E. coli*. The recombinant protein, His-p21 was found to bind to HeLa cells in a dose-dependent manner. Inhibition invasion assays showed that His-p21 inhibited invasion of extracellular amastigotes from G isolate also in a dose dependent manner but this effect was not observed on extracellular amastigotes from 588 isolate. Immunofluorescence showed an intracellular diffuse distribution of the protein. Western blotting revealed the expression of this protein on epimastigotes, metacyclic trypomastigotes from G and CL isolates and on amastigotes from G and 588 isolates but not on CL. We are current verifying if the protein is secreted, glucosylated and also if the signaling events mobilized by this protein during cell invasion involves the activation of Oligopeptidase B. Financial support: FAPESP, CAPES, CNPq.

BC04 - CELL INVASION BY *Trypanosoma cruzi* EXTRACELLULAR AMASTIGOTES: ROLE OF CRUZIPAIN AND BRADIKININ CELL RECEPTORS

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Chagas disease occurs in 18 countries of South America, Central America and Mexico, 16-18 million of people are infected and 120 million live in risk areas. *Trypanosoma cruzi* naturally acquired infections are initiated by metacyclic trypomastigotes (MT) transmitted by haematophagus triatomine bugs. After uptake, MT transform into amastigotes, replicate, transform back to trypomastigote that are released into the bloodstream. In an alternative subcycle, intracellular amastigotes released by prematurely lysed cells or extracellular amastigotes (EA), originated from bloodstream trypomastigotes, may also invade new cells. Several invasion pathways are proposed to explain parasite invasion. Cruzipain, a parasite cysteine proteinase has been implicated in invasion. This enzyme can release bioactive kinins from kininogens. Kinins may increase the tissue culture trypomastigote (TCT) invasion in cells with constitutive levels or overexpressing bradikinin 2 receptor (B_2R). Moreover, this proteinase can also increase the TCT uptake in a bradikinin receptor independent pathway. G and CL strain parasites belong to different *T. cruzi* phylogenetic groups and have different patterns of invasion in cell cultures: CL strain MT invade in higher rates than G parasites, while extracellular G strain amastigotes are more infective than CL. The aim of this study is to test the influence of cruzipain in extracellular amastigote uptake in Chinese hamster ovary (CHO) cells transfected with B_1R, B_2R or B_1R/B_2R in comparison to CHO mock cells. Captopril, an angiotensin converting enzyme inhibitor was used to increase the bradikinin stability. There were no differences in CL strain uptake by transfected cells and CHO mock cells, while for G strain, significant increases occurred in parasite uptake in B_2R or B_1R/B_2R cells, but not in B_1R in comparison with CHO mock cells, and only when cells were treated with captopril. These preliminary results disclose the importance of B_2R and, probably, of cruzipain in EA invasion. Financial support: CNPq/CAPES/FAPESP.

BC05 - Trypanosoma dionisii: morphological aspects and stablishment of expression and distribution of epitopes defined by monoclonal antibodies anti- T. cruzi

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Trypanosoma dionisii is a bat trypanosomatid phylogenetically related to *Trypanosoma cruzi* and of our work. During its life cycle *Trypanosoma dionisii* alternates through different developmental forms alternating between vertebrate and invertebrate hosts: epimastigotes and metacyclic trypomastigotes in the invertebrate host, and bloodstream trypomastigotes and amastigotes in the mammalian host. The aim of this study is detail morphological aspects of each developmental stage and using available anti-*T. cruzi* monoclonal antibodies (mabs), establish the pattern expression of the epitopes defined by these reagents. Confocal fluorescence microscopy imaging studies show strong positive reactions with some anti- *T. cruzi* G strain Mabs (4B5, 4D9, 3B9) and weaker reactions with other Mabs (3B2, 4B9). These reactions occur for both extracellular and intracellular forms. The strongest reaction was observed with mab 4D9 that reacts with a high weight molecular protein on the flagellum and cellular body contact. Mab 4B5 gives a punctuate labeling of a protein next the kinetoplast and also on the opposite pole. Mab 3B9 reacts with a carbohydrate epitope labeled only few parasites in multiply infected cells. Mabs 3B2 and 4B9 that react with protein and carbohydrate epitopes respectively, labeled the parasite surface in some amastigotes nests. By contrast, when we used anti- *T. cruzi* CL strain Mabs, the reactions were almost all negative, with the exception of Mab 2G5 that stain the cellular membrane of some parasites. This results confirm previous studies that *T. dionisii* and *T. cruzi* G strain share several immunochemical characteristics. By transmission electron microscopy observations were made on distinct characteristics of the flagellum, kinetoplast and nucleus of the two closely related species. Financial support: FAPESP, CNPq& CAPES.

BC06 - Polymorphism in Trypanosoma cruzi: parasite:host cell interaction

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Studies concerning Trypanosoma cruzi polymorphism made since last century to the present days suggest a different role for slender and broad trypomastigotes on the parasite-host cell interaction. Differences on the cell surface antigenic composition of slender and broad trypomastigotes were observed and such differences reflected the behavior of the two trypomastigote forms on the host-cell invasion process. In the present study we observed the relative importance of slender and broad forms of the Y strain and CL Brener and Dm28c clones on the host cell infection. Tissue culture trypomastigotes (TCT) have been used. LLC-MK2 cells were plated on 13 mm round coverslips and cultivated in 24-well plates for 24 h at 37 C in a 5 % CO2 atmosphere. TCTs were added to host cells (ratio of 50:1) and the interaction proceeded for 30 min at 4 C. After that cells were fixed with 2.5 % glutaraldehyde and prepared to be observed at the scanning electron microscope. Preliminar results obtained with CL Brener trypomastigotes showed that after the period of incubation at 4 C only 3.7 % of cells showed adhered parasites on their surfaces. We also observed that there was only one parasite per cell and that they were mostly adhered to the nuclear or perinuclear region of the host-cell. In the case of CL-Brener clone 65 % of the adhered forms were broad ones and the parasites were adhered by the posterior or the central region of their bodies. The analyses of the other samples are in course.

BC07 - First record of *Trypanosoma chattoni* and of new hosts for *Trypanosoma rotatorium* and *T. chattoni* in Brazil

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This study is the first record of *Trypanosoma chattoni* Mathis & Leger, 1911 in Brazil and the report of *Leptodactylus chaquensis* Cei, 1950 and *Leptodactylus fuscus* Schneider, 1799 (Anura, Leptodactylidae) as new hosts for *Trypanosoma rotatorium* and *T. chattoni* respectively. The anurans were captured during the month of May 2002 in the state of Mato Grosso, Brazil. Blood samples were obtained by cardiac puncture, and blood smears were examined for the presence of hemoparasites. The *Trypanosoma rotatorium* observed in this study is a short-bodied trypomastigote with an elongated nucleus located in the posterior end of the body. A rounded kinetoplast is located near the nucleus in the posterior end of the body. The cytoplasm is granular, the undulating membrane is conspicuous and no flagella were observed. *Trypanosoma chattoni* is a round-bodied monomorphic parasite with a spherical nucleus located in middle of the body and centrally positioned with adjacent kinetoplast. The cytoplasm is granular, the flagella are short and no undulating membrane was visualized.
Financial support: FAPEMIG

BC08 - EFECTS OF ESPECIFIC TREATMENT ON PARASITOLOGICAL PARAMETERES IN BALB/c MICE INFECTED WITH *Trypanosoma cruzi* DUAL-CLONAL INFECTIONS

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Herein the goal was to verify comparatively the effect of Benznidazole treatment on biological parameters between dual-clonal infections treated (T) and not-treated (NT). Groups of 12 BALB/c mice were infected with two clonal stocks from genotypes 20, 19 (*T.cruzi* I) and 39 and 32 (*T.cruzi* II), chosen based on their sensibility to Benznidazole and virulence verified in monoclonal infections. Twenty-four combinations were studied. Groups of six animals were treated with Benznidazole, 100mg/kg/20days and comparatively evaluated with controls. The parameters percentage of positive hemoculture (%HE+), mortality (%MOR), patent period (PP), maximum of parasitemia (MP), day of maximum of parasitemia (DMP) and area under the curve of parasitemia (PAR) were analyzed. Percentages of positive hemoculture were similar in animals infected with more virulent mixture 19x20 in T and NT groups. On the other hand, mice infected with mixtures 39x32 (less virulent) showed lower (%HE+) in 75% of them. In mixtures 20x39, 19x39, 19x32, 100% +HE were verified in NT groups while in T groups approximately 50% and 100% were observed for (20x39 and 19x32) and (19x39) mixtures. In T group the MOR reduced to 0% in 6/6 mixtures while in NT groups the MORT ranges from 12.5 to 40%. Benznidazole-treatment induced reduction of the level of parameters related to parasitemia independent of the genotype combination in the majority of the mixtures. Besides, in mixtures 20x19 (Gambacl1+Cuicacl1, Gambacl1+P209cl1) and 19x39 (Gambacl1+Bug2148cl1) a parasitemia reactivation was observed after treatment reaching lower levels than the NT group, persistant until 120th day of infection and later DMP. In mixtures 39x32 it was not observed differences. These mixtures displayed inhibitory effects when compared with their monoclonal infections. Data demonstrated that treatment present an important suppressor effect on parasitemia in mice infected with all genotypes combinations as previously observed in monoclonal infections. **Supported by FAPEMIG, CNPq and UFOP.**

BC09 - Evaluation of PCR positivity in tissue of mice with dual-clonal *T. cruzi* infection from major genotypes.

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In this study was evaluated the parasite kDNA presence in tissues of BALB/c mice infected with *T. cruzi* dual-clonal infections. Two polar clonal stocks of each *T. cruzi* major genotypes 19 and 20 (*T.cruzi* I), hybrid 39 and 32 (*T cruzi* II) were chosen based on virulence. Twelve mixtures were studied. After patent parasitemia animals were necropsied in acute (AP) and chronic phases (CP). Parasites kDNA was amplified by PCR from 30 organs/tissues. The percentage of positive PCR (%PCR+) was similar in AP (56-90%) and CP (40-96%) for the majority of combinations, except for P209cl1+IVVcl4 (83vs.26%), Gambacl1+IVVcl4 (80vs.24%) and Gambacl1+Bug2148cl1 (70vs.40%). The less virulent mixtures (LVM) presented during AP a %PCR+ similar than MVM for combinations 20+39 (86vs.96%), 20+32 (93vs.83%) and 19+39 (66vs.70%); higher for 19+20 (80vs.56%) and lower for 39+32 (20vs.80%) and 19+32 (53vs.80%). In relation to tissue distribution in AP, parasites kDNA was observed in most of organs/tissues in mixtures 20+19, 19+39, 20+32 19+32, in MVM of combination for 39+32 and in both phases for 20+39. The LVM of 39+32 were positive only in AP in heart, muscles, lymphnode, spleen and gland while in CP the positivity was observed in spleen, liver and trachea. By the way, for the MVM of this combination PCR+ was verified on the heart, muscle, bladder, kidney, esophagus, duodenum. During CP, mixtures 20+19 (MVM and LVM) presented PCR+ in heart, muscles and lymphatic organs and in TGI only of LVM. In both (MVM and LVM) of 19+39 mixtures PCR+ were observed in heart, muscles and TGI; in heart and muscles of 19+32 and in TGI for LVM of this last combination. Although preliminar, data suggested that dual-clonal infections (39x32 X 20x39) presented the lower and higher PCR positivity and kDNA tissue tropism, respectively.**Supported by CNPq, Fapemig and UFOP**

BC10 - ACTIN CYTOSKELETON-DEPENDENT AND -INDEPENDENT HOST CELL INVASION BY *TRYPANOSOMA CRUZI* IS MEDIATED BY PARASITE SURFACE MOLECULES GP35/50 AND GP82

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This study was aimed at further elucidating the mechanisms of host cell invasion by *T. cruzi* metacyclic forms, and also at investigating the basis of a discrepancy in the literature concerning the involvement of actin cytoskeleton. The disassembly of host cell actin cytoskeleton as facilitator of *T. cruzi* invasion has been reported by some authors, while others claim that it rather inhibits the parasite internalization. Experiments were performed with metacyclic forms of *T. cruzi* strains G and CL, which enter target cells by engaging, respectively, the Ca^{2+} signal-inducing surface molecules gp35/50 and gp82. When HeLa cells were treated with F-actin disrupting drug cytochalasin D or latrunculin B, the invasion of G but not of CL strain was inhibited. In contrast to cells penetrated by CL strain, which exhibited a disrupted actin cytoskeleton architecture, no such alteration was seen in HeLa cells invaded by G strain, which was associated with target cell actin. Co-infection with enteroinvasive *Escherichia coli* (EIEC), which recruits host cell actin for internalization, drastically reduced HeLa cell entry of CL but not of G strain. The purified mucin-like gp35/50 molecules promoted an increase of EIEC uptake by HeLa cells, as opposed to gp82, in its recombinant form, which induces F-actin disruption and inhibits EIEC invasion. These data, plus the finding that drugs that interfere with mammalian cell signaling differentially affect the internalization of G and CL strains, indicate that the host cell invasion mediated by gp35/50 is associated with signaling events that favor actin recruitment, in contrast to gp82-dependent invasion that triggers the signaling pathways leading to F-actin disassembly. Work supported by FAPESP and CNPq

BC11 - From bacterium to organelle: co-ordination of division between the endosymbiont and the host trypanosomatid

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In trypanosomatids cell division requires a co-ordinated replication of different structures: the nucleus, the kinetoplast and the flagellum. Previous studies have shown that cell division proceeds in the sense flagellum-kinetoplast-nucleus. In endosymbiont-containing trypanosomatids this process is more complex, since the symbiont replicates synchronically with the host. In this study we used microscopy and molecular approaches to correlate the timing of endosymbiont divi-

sion with the host cell cycle. Morphological observations revealed that in *Crithidia deanei* the symbiont is the first structure to replicate. The cell cycle in this trypanosomatid lasts 4 hours and curiously the symbiont persists in the dividing form for 2 hours. The endosymbiont is enclosed by two membranes, but lacks a peptidoglycan layer and the septum during the cytokinesis. Molecular analysis showed that the dcw cluster (for **d**ivision and **c**ell **w**all) from *C. deanei* endosymbiont presents the order and identity of genes very conserved when compared to other bacteria, but some genes were lost. FtsZ assembles into a membrane-associated ring in prokaryotic division. This protein is also present in eukaryotes, where it is nuclear-encoded and imported into chloroplasts and mitochondria of primitive microorganisms. In higher eukaryotes, mitochondrion division lacks FtsZ and a detectable dividing ring, but depends on dynamin on the cytosolic face for fission. Intriguingly, a typical Z ring is not observed in the endosymbiont after immunocytochemical assays. In order to understand the absence of Z ring in dividing symbionts, we also performed molecular analysis of minCDE genes, which encode proteins that regulate the precise positioning of the ring. Our preliminary results indicate that Min C is not expressed in the symbiont. Taken together, we conclude that the endosymbiont division is tightly regulated by the host trypanosomatid, recapitulating lessons taken from eukaryotic organelles. Supported by CNPq, FAPERJ, FAPESP.

BC12 - THE CHARACTERIZATION OF NEUROPEPTIDES AND NEUROCHEMICAL MARKERS IN THE ENTERIC NERVOUS SYSTEM OF CHAGASIC PATIENTS WITH MEGACOLON

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Neuronal destruction had been considered the hallmark of pathogenic mechanisms in chagasic megacolon. The characterization of neuropeptides in the enteric nervous system from chagasic patients with megacolon could elucidate some aspects about the development of this syndrome. In the present work we demonstrate the changes of expression in neuropeptides and neurochemical markers presents in the neuronal plexuses from the colon of chagasic patients with megacolon. Colon samples of non-infected control individuals and patients with megacolon were obtained from endemic area of Goiânia. Sections of frozen tissue samples were immunohistochemically labelled for anti-calretinin (IPANs), cChat and substance P (excitatory motor neurons), VIP and NOS (inhibitory motor neurons) and NPY (interneurons). Immunoreactivity was observed by the use of confocal microscope. Our results demonstrated that among all analyzed enteric neurons in chagasic patients with megacolon, the inhibitory motor neurons (VIP and NOS immunoreactive) are preferentially destroyed by the T. cruzi and/or inflammatory process. These results suggest a selective destruction of enteric neurons in the colon of chagasic patients with megacolon, pointing to an important discovery in the mechanism of the Chagas disease pathogenesis.
Support : CAPES.

BC13 - *Trypanosoma cruzi*: CHARACTERIZATION OF CELL DEATH INDUCED BY NAPHTHOIMIDAZOLES DERIVED FROM BETA-LAPACHONE

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Beta-Lapachone, a naphthoquinone obtained from vegetal sources, possess a variety of biological activities, mainly associated with oxidative processes. Naphthoimidazoles N1, N2 and N3 are the most active derivatives of this quinone against *Trypanosoma cruzi*. Their mechanism of action against the parasite involves interference with the mitochondrial membrane potential. In the present work, we investigate cell death events in *T. cruzi* induced by these naphthoimidazoles. Trypomastigotes treated with N1, N2 or N3 displayed an intense DNA fragmentation (TUNEL assay), a spread DNA pattern (electrophoresis technique), an atypical condensation of nuclear DNA, total disruption of kDNA network and plasma membrane blebing (electron microscopy). Also, flow cytometry analysis demonstrated a dose-dependent increase in 7-AAD labeling, together with a low % of annexin V+/PI- and high % of annexin V-/PI+ cells. Neither of these alterations was observed in treated epimastigotes. Pretreatment of both forms of *T. cruzi* with zVAD.fmk, E64 or calpain inhibitor I caused a partial reversion of the trypanocidal effect of the naphthoimidazoles. DNA fragmentation, increased 7-AAD labeling, high percent of annexin V-/PI+ and bleb formation were observed in treated trypomastigotes. Besides, death induced by the naphthoimidazoles depends, at least in part, on cysteine proteases, such as calpain I. Although some of our experiments indicate an apoptosis-like phenotype, the overall analysis are in accordance with the hypothesis, based on in vitro and in vivo models, of a co-existence of a variety of cell death programs triggered by distinct stimuli. The understanding of alternative death routes operating in *T. cruzi* can lead to new targets for the development of chemotherapeutic agents.

BC14 - P2X$_7$ activity in *Trypanosoma cruzi* infection

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P2X$_7$ is a member of a receptors family whose main agonists are extracellular nucleotides and nucleosides, including extracellular ATPe. This receptor is well known for its ability to open a large pore in plasma membrane upon activation and is normally associated to cell death, either by necrosis or apoptosis. However, the receptor is also involved in cytokine secretion, killing of intracellular parasites etc. Our group has recently published that P2X$_7$ receptor may be involved in thymic atrophy induced by *T. cruzi* infection, showing a positive correlation between thymic involution [death of double positive thymocytes (DP)] and ATP$_{e-}$ induced pore opening in vitro. DP cells from non infected mice are refractory to ATP$_{e-}$ induced permeabilization or death, while DP thymocytes from infected mice are susceptible. Therefore, we decided to evaluate the expression and functional activity of this receptor in normal mice and to investigate the participation of this receptor in thymic atrophy using P2X$_7$ KO mice and other aspects of purinergic receptors. We observed that P2X$_7$ receptor is expressed on the cell membrane of control thymocytes, although it is not able to induce cell permeabilization or Ca^{++} influx by incubation with ATP$_e$. This result suggests that the receptor expressed on normal thymocytes may be in a non functional configuration. Regarding thymic atrophy, we observed that P2X$_7$ *in vivo* does not seem to be a central molecule, once this process is still observed in KO mice. The study of functional pattern of P2X$_7$, P2X$_4$ and P2Y, through different agonists and antagonists indicated a possible interdependence between some receptors that could be better evidenced in the context of infection.

BC15 - Distinct Pathways of Smooth Muscle Invasion by *Trypanosoma cruzi* Dm28 Trypomastigotes: Differential Roles of G Protein-Coupled Receptors, Adenylyl Cyclase and PI3 Kinase Mediated Signaling.

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Host cell invasion by *T. cruzi* trypomastigotes is a highly dynamic process involving coordinated activation of host cell signaling pathways by the parasites. Studies on the interaction between Y strain-derived tissue culture trypomastigotes (TCT) and epithelial cells led to the characterization of a novel invasion mechanism that starts with invagination of the plasma membrane, followed by fusion of the parasitophorous vacuole with lysosomes. These studies showed that parasite entry is a reversible process and that the acquisition of lysosomal markers in the vacuole is essential for parasite retention inside the host cell. We have previously shown that the invasion of human smooth muscle cells by Dm28c TCT is to a large extent dependent on the activity of the parasite cysteine protease, cruzipain. Here, we studied the molecular mechanisms underlying entry and retention of Dm28c using genetically modified TCT that overexpress chagasin (pChag), the parasites′ natural cysteine protease inhibitor. pChag presented reduced cruzipain activity and were significantly less infective than wild type parasites (WT). Treatment of host cells with pertussis toxin or with the adenilyl cyclase inhibitor MDL12 significantly reduced entry of WT parasites, while entry of pChag was increased, suggesting that residual invasion by pChag is mediated by a distinct pathway. Interestingly, while WT entry was unaffected by host cell pre-treatment with wortmannin or with BAPTA-AM, parasite retention was subsequently increased. In contrast to data published with Y strain TCT, cytochalasin D drastically blocked entry of WT and pChag, suggesting that actin polimerization contributes to the initial steps of invasion by Dm28c. We propose that cAMP production and activation of G-protein coupled receptors underlie cruzipain-mediated pathways of smooth muscle cell invasion by Dm28c, without obvious involvement of raises in $[Ca^{+2}]_i$. Furthermore, we suggest that retention of Dm28c parasites within smooth cells is negatively modulated by activation of PI3K.

BC16 - Detoxification of heme in *Trypanosoma cruzi* epimastigotes: the role of ABC transporters

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Trypanosoma cruzi epimastigotes propagates in the triatomine insect. These insects usually ingest about 10mM of heme bound to hemoglobin. Heme is a generator of reactive oxygen species (ROS). Our group has demonstrated that heme uptake shows a faster kinetic than hemoglobin. ATP-binding cassette (ABC) transporters, such P-glycoproteins (Pgp) and multidrug resistance-associated proteins (MRP), correspond to a group of proteins involved in multidrug resistance (MDR). Cyclosporin A (CsA) and verapamil (Vp) are Pgp inhibitors, while indometacin (Indo) inhibits MRP. We investigated the detoxification of heme, and the involvement of ABC transporters in this process. Epimastigotes Dm28c strain, were maintained in BHI plus 10% FCS,28 ^{o}C without addition of heme for 14 days. Afterwards cells were incu-

bated with different concentrations of CsA in the absence or presence of heme, the growth of epimastigotes was evaluated. A decrease in parasites growth was observed when treated with 10μg/mL CsA in the presence of heme. Once PgP is inhibited, heme would be trapped in the parasite membranes yelding ROS so, we assayed it by flow cytometry analysis of CM-H2DCFDA fluorescence. We observe that CsA promoted ROS formation in the presence of heme. Pd - Mesoporphyrin IX (an analogous of heme) fluorescence was used as a label to trace the fate of heme taken up by the parasite in the presence of CsA, Vp and Indo in different concentrations. We followed the uptake of Pd-mesoporphyrin IX by microscopy and observed that in the presence of CsA, Pd-mesoporphyrin IX is taken up more efficiently if compared with the other drugs, suggesting a stronger inhibition of PgP. Our data suggest that *T. cruzi* developed mechanisms to efficiently deal with high concentrations of heme found in its environment, and one of them is an ATP-binding cassette transporter, probably a Pgp. Supported by FAPERJ and CNPQ

BC17 - LIPID UTILIZATION IN LOWER TRIPANOSOMATIDS

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Leptomonas wallacei a flagellated trypanosomatid that naturally infects the phytophagous hemipteran *Oncopeltus fasciatus*.The genus *Phytomonas* contains etiological agents of diseases affecting fruits and plants of great economical importance including tomato, cashew, oil palms, coconut, cassava and coffee. Most of them are transmitted to the plants via the saliva of phytophagous insects. In this work, we are studying the capacity of *Leptomonas wallacei*, *Phytomonas françai* and *Phytomonas serpens* to incorporate and metabolize lipids. ^{3}H-palmitic acid was added to the culture medium and after 24h incubation, cells were subjected to lipid extraction. Labeled lipids were detected by thin-layer chromatography followed by exposition of the plate to a phosphoimager screen or the spots were scraped, lipids were re-extracted and the radioactivity associated to each spot was determinated by scintillation counting. The results showed that ^{3}H-palmitic acid was used to synthesize other lipids such as triacylglycerol, cholesteryl-ester and phospholipids. In order to investigate the fate of these lipids in the parasites we used fluorescent microscopy. The parasites were incubated for 24h in the presence of labeled Bodipy - Fatty acid (Bodipy-FA) and Texas red - phospholipids (Texas red-PL). In *L. wallacei* and *P. serpens* the exogenous Bodipy-FA was incorporated into membranes of vesicular structures, while Texas red-PL were localized around the nucleus. However in *P. françai* both lipids were localized concentrated inside the same vesicles. Altogether these data suggest that *P. françai*, *P. serpens* and *L. wallacei* are able to uptake free lipids from the medium. These molecules are used by the parasite as precursors for de novo synthesis of their own lipids. The fate of these lipids in the parasites and the molecular mechanisms involved in their transfer remains to be investigated.
Supported by FAPERJ, CNPQ and IFS

BC18 - Fluorescent *Trypanosoma cruzi*: a potent new tool for parasite studies

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Stable transfection protocols have been described for a number of protozoan parasites. In *T. cruzi*, stable transfections can be achieved by the integration of the foreign genes in the genome through homologous recombination, or by the episomal maintenance of the transfected plasmid. We have generated a vector derived from pROCKNeo, which allowed the integration of GFP and RFP genes into β-tubulin locus of different strains of the parasite genome. The integration of the GFP and RFP markers in the tubulin locus was confirmed by Southern blot analyses. Two clones expressing GFP and 4 expressing RFP were obtained by serial dilution of transfected populations of the Tulauhén strain in 96 wells plates. For all clones, 80% of the cells were found to express high levels of GFP or RFP as assessed by FACS analyses. To evaluate the stability of the GFP and RFP markers in the transfected populations and clones, parasites were cultivated for 1 and 2 months in the absence or presence of G418 (200 μg/mL and 400 μg/mL) and were analyzed by FACS. The selected clones were able to maintain high levels of expression of the fluorescent protein markers even in the absence of G418, whereas in the transfected population, expression of GFP and RFP markers was significantly reduced when cultured in the absence of G418. To evaluate the infectivity of the transfected population, GFP and RFP expressing parasites were inoculated into VERO cells culture. When compared with the wild type population, no significant difference in the VERO cells infectivity was found. Furthermore, GFP and RFP expressing parasites were able produce significant levels of parasitemia 12 days after being inoculated into GKO mice (interferon-γ knockout mice). We observed fluorescent parasites in the heart and diaphragm of infected animals. Financial support: FAPEMIG, CAPES, CNPq, WHO and the Howard Hughes Medical Institute.

BC19 - Multiplex-PCR for diagnosis and molecular characterization of Trypanosoma cruzi in specimens of Triatoma vitticeps from Itanhomi (MG).

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Triatoma vitticeps (Stal, 1859) presents high levels of infection with *Trypanosoma cruzi*; however it is considered a secondary vector in the human transmission of the parasite. In Itanhomi (Minas Gerais state - Brazil), the invasion of the houses for adult insects of *T. vitticeps* is very frequent and any research about prevalence and/or characterization of *T. cruzi* in this area had been realized. In this work, we evaluated the prevalence of *T. cruzi* infection in *T. vitticeps* specimens from Itanhomi. For detection and classification of *T. cruzi*, we used a PCR-multiplex (Liarte *et al.* 2006, manuscript in preparation) of the intestinal content of 65 dead insects placed in filter paper and stored during four years. Besides this, 36 insects alive were examined (61,1% of infection) and 12 *T. cruzi* strains were isolated. From 65 samples of dead insects 26 (40%) were negative and 39 (60%) were positive for *T. cruzi*. Of these infected insects, 35 samples (89,7%) were identified as *T. cruzi* I group and 4 (10,3%) as *T. cruzi* II. The cryopreserved strains 10 (83.3%) were identified as *T. cruzi* I and 2 (16.7%) as *T. cruzi* II. The high prevalence of *T. cruzi* I in this population (88,2%) is in agreement with literature data which show an association between *T. cruzi* I and the sylvatic cycle of the parasite. However, the presence of *T. cruzi* II (11,8%) suggests the possibility of human or domestic animals infected. Thus, the PCR multiplex allowed the parasite detection and classification of *T. cruzi* group in cases where it was not possible the isolation of strain, preserving the natural diversity of the parasite. In addition, our results showed the occurrence of *T. cruzi* I and II in *T. vitticeps* from sylvatic and domestic cycles. Supported by: WHO, CNPq, FAPEMIG and CPqRR/FIOCRUZ.

BC20 - Acriflavine affects mitochondrial activity and ultrastructure in trypanosomatids

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The mitochondrial DNA in trypanosomatids is enclosed in an unusual structure, the kinetoplast, which is composed by minicircles and maxicircles that are held together by catenation into a single network. The kinetoplast is a potential target for drug treatment, since the kDNA arrangement is susceptible to a great variety of compounds. Previously, we showed that inhibitors of type II topoisomerases promote cell arrest and ultrastructural alteration in the kinetoplast. In this work we analysed the effect of acriflavine, a DNA intercalating drug, in trypanosomatid growth and structure, as well as in mitochondrion metabolism. Results showed that monoxenic protozoa, as *Blastocrithidia culicis* and *Crithidia fasciculata* are more affected by acriflavine than *Trypanosoma cruzi*, which regard to cell proliferation and ultrastructural modification. This drug affected protozoa in a dose-dependent manner and transmission electron microscopy analyses showed kDNA disassembly, which resulted in dyskinetoplastic phenotype. Biochemical approaches demonstrated the acriflavine ability to decrease oxygen consumption, indicating that the energetic metabolism of mitochondrion is affected by this drug. Supported by CNPq and FAPERJ.

BC21 - DAPI staining of nuclear DNA of *Trypanosoma cruzi* isolates

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The genome size of CLBrener has been estimated as 110Mbp (∼120fg). The DNA content of *T. cruzi* isolates was estimated employing DNA fluorochromes as 125 to 280fg. Molecular karyotype data were also used to estimate the genome size of: CLBrener(70Mbp); Esmeraldocl3(78Mbp); SO3cl5(94Mbp) and SilvioX10cl11(47Mbp) (Vargas *et al.*, 2004). This study aimed at verifying whether the fluorescence intensity of DAPI-stained nuclei of *T. cruzi* isolates correlates with the genome size estimated by karyotype data. We measured the nuclei fluorescence intensity in CLBrener epimastigotes exponentially growing and in stationary phase; and in 20mM hydroxyurea synchronized cultures (G1 phase). The cells were formaldehyde/methanol fixed on glass slides and stained with 10nM DAPI. Optical section images through nuclei were acquired in BIORAD confocal microscope. The fluorescence intensity of the sum of the sections of at least 20 nuclei was estimated using Image J (http://rsb.info.nih.gov/ij). Similar intensity values were obtained for the three CLBrener samples. Because in hydroxyurea synchronized cells the nuclei were well-defined and a good separation from the kinetoplast was observed, this condition was adopted further. The mean and sem of the intensity values were: CLBrener, 1.478±0.676; Esmeraldocl3, 3.252±0.723; VL10, 1.047±0.171; SO3cl5, 0.515±0.089; SilvioX10cl11, 0.697±0.170. The data indicate variation up to 6.5-fold in TcII isolates (all but SilvioX10cl11). We noticed variation of the nuclei size among the isolates. Therefore, we determined the nuclei diameter (D) and fluores-

cence/nuclei volume ratio (F/V): CLBrener, D=2.4±0.2; F/V=255; Esmeraldocl3, D=2.2±0.2; F/V=739; SO3cl5, D=1.8±0.2; F/V=225; SilvioX10cl11, D=2±0.2; F/V=234. The data indicate that stainability with DAPI does not correlate with the DNA content estimated by karyotype analysis. DAPI is an intercalating dye that appears to associate with AT clusters in the DNA minor groove. Restriction in DAPI stainability may be related to the coiling of DNA in the chromatin. Support: FAPESP and CNPq

BC22 - Endosymbiosis in trypanosomatids: the protein composition of the bacterium envelope

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Some trypanosomatids, such as *Crithidia deanei*, presents an endosymbiotic bacterium in the cytoplasm. Both partners maintain a mutualistic relationship; the symbiont supplies the host protozoa with some essential nutrients, but depends on ATP production from host glycosomes. The endosymbiont is enclosed by two unit membranes, like Gram-negative bacteria, but its cell wall is degenerated and the septum is not observed during the cytokinesis. The origin of the symbiont envelope is controversial; some authors consider that the outer membrane originated from the host cell during the entry process, while others believe that it presents prokaryotic origin. The presence of porins in the endosymbiont envelope would confirm its Gram-negative ancestral, since molecular analysis classified this bacterium in γ subdivision, close to Bordetella genus. In this study, isolated endosymbionts, obtained by cell fractioning, were subjected to bi-dimensional electrophoresis, in order to characterize its protein membrane composition. The mitochondrion was is used as a comparative evolutionary model, since this organelle presents symbiotic origin. Isolated symbionts and mitochondria were also analyzed by transmission electron microscopy, in order to verify the purity and ultrastructure of these fractions. The electrophoretic assays produced gels with well-defined spots of proteins, which have been identified by mass spectrometry. Supported by: CNPq and FAPERJ.

BC23 - Effects of miltefosine in the phospholipid biosynthesis of *Crithidia deanei*, an endosymbiont-bearing trypanosomatid

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Some protozoa of the Trypanosomatidae family, as *Crithidia deanei*, present an obligatory intracellular bacterium, which maintains a mutualistic relationship with the host. The endosymbiont envelope lacks sterols and the phospholipid composition is characterized by a major quantity of cardiolipin (CL), followed by phosphatidylcholine (PC) and phosphatidylethanolamine (PE). PC is the major phospholipid in eukaryotes, being an essential structural component of cell membranes and playing essential physiological roles. However, in prokaryotes PC is present only in species closely associated with eukaryotes, either in symbiotic or pathogenic interactions. In this study we tested the effects of miltefosine in *C. deanei*, considering that this drug inhibits the phosphocholine-cytidyltransferase (CTP), a key enzyme in the PC biosynthesis. Cell growth was inhibited in a dose dependent manner, with a minimum inhibitory concentration of 10μg/mL after 72 h of incubation with the drug. Preliminary data, obtained by transmission electron microscopy, showed that miltefosine promoted ultrastructural effects in *C. deanei*, like plasma membrane shedding and mitochondrion swelling. It is important to mention that the endosymbiont envelope was also affected by the drug treatment. Phospholipid analyses will be performed using drug concentrations which significantly decrease cell growth, but without inhibiting completely protozoa proliferation. In such conditions, we will be able to test the effects of miltefosine in the metabolic exchanges involving the symbiont and the host trypanosomatid. Supported by: CNPq, FAPERJ.

BC24 - DIFFERENTIAL USE OF GLUCOSE AND AMINO ACIDS BY METACYCLIC FORMS OF *TRYPANOSOMA CRUZI* STRAINS CL AND G AS SOURCE OF ENERGY FOR HOST CELL INVASION

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Attachment of *T. cruzi* to host cells requires parasite energy. When grown in rich medium containing glucose and amino acids, insect stages of trypanosomatids appear preferentially to consume glucose. Using metacyclic trypomastigotes, the *T. cruzi* forms responsible for natural infection of mammals, we investigated the transport of glucose, proline and glutamic acid, as well as their participation as energy source in the process of host cell invasion. Assays performed to measure V_0 at 37^{o}C for 30 sec, showed in CL and G strains the transport of glucose, but not of proline. Measurements for up to 1 h revealed also the transport of proline in both strains. For cell invasion assays, the parasites were pretreated with 1 mM glucose, 0.75mM or 3 mM L-proline, or 1 mM L-glutamic acid, and then incubated for 1 h with HeLa cells in absence of glucose or amino acid. HeLa cell invasion by CL strain was

increased by pretreatment with glucose or 0.75 mM proline, but not with glutamic acid. A similar profile was observed when CL strain parasites were energy depleted by pretreatement with 2-deoxy-glucose for 1 h, before treatment with glucose or amino acid. G strain entry into HeLa cells was not altered by pretreatment with glucose, was reduced by 0.75 mM or 3 mM proline and somewhat increased by glutamic acid. This profile was unaltered by treatment of G strain with 2-deoxy-glucose. The apparent differential use of energy source by CL and G strains during host cell invasion further highlights the differences between CL and G strains, which belong to phylogenetically distant groups. Work supported by FAPESP, CAPES and CNPq.

BC25 - EFFECTS OF NAPHTOQUINONES UPON *Trypanosoma cruzi* PROTEINASES

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Naphtoquinones from the heartwood of *Bignoniaceae* and *Verbanaceae* trees are known by their significant antimicrobial properties, including against *Trypanosoma cruzi* (Ferreira et al.,2006; Jorqueira et al., 2006). Still today, naphtoquinones have not been included in drug screenings for Chagas disease, because the target of these drugs is not fully known. The aim of this study is to evaluate the inhibitory activity of three naphtoquinones derivatives (β-Lapachone, α-Lapachone and Oxyran 10) on the *in vitro* growth of *T. cruzi* epimastigotes and, also, on their proteinases activities. β-Lapachone, α-Lapachone and Oxyran 10 final solution (50 μM) was prepared using DMSO. E64 and PMSF (50 μM) were used as positive control and DMSO as negative control. The results showed that Oxyrane 10 presented a significant activity against epimastigote forms of *T. cruzi*. Its trypanocidal activity is similar to the one of β-Lapachone, with the advantage of possessing lower cytotoxicity in Vero Cells lines. The enzymatic assay performed with specific chromogenic substrates revealed that β-Lapachone inhibited mainly the cysteine-proteinases activity, while Oxyran 10 inhibited serine-proteinases in epimastigotes protein extracts. Other proteinases activities are further to be assayed, allowing us to define the inhibition profile of *T.cruzi* proteinases by naphtoquinones. This study can lead to the development of new anti-chagasic drugs based on lapachone analogs.

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BC26 - A RECOMBINANT PROTEIN BASED ON *TRYPANOSOMA CRUZI* SURFACE MOLECULE GP82 INDUCES APOPTOTIC CELL DEATH IN MELANOMA CELLS

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Reports on the anticancer properties of *T. cruzi* include the direct inhibitory effect of epimastigotes on *in vitro* cultured human breast cancer cells (Sheklakova et al., 2003, Bull. Exp. Biol. Med. 135: 89-92) and sarcoma-180 (Kallinikova et al., 2001, Izv. Akad. Nauk. Ser. Biol. 3: 299-311). In this study, we investigated the effect on melanoma cells of a recombinant protein based on *T. cruzi* surface glycoprotein gp82, which binds to mammalian cells in a receptor-mediated manner and induces Ca^{2+}-dependent actin cytoskeleton disruption. We treated a melanocyte lineage melan-a and a melan-a-derived tumorigenic Tm5 lineage with 0.4μM of J18, the recombinant protein containing the full-length gp82 sequence for different periods of time. Quantification of cell viability by MTT assay showed that J18 had a significant growth inhibitory effect on Tm5 cells, but not on melan-a. The inhibitory effect of J18 was first seen at 48h, and further increased considerably at 72h post-treatment. The J18-treated Tm5 cells, but not melan-a cells, displayed an altered morphology, with cytoplasm shrinkage and detachment from the substrate. There was also cell cycle alteration, DNA fragmentation and positive labeling for annexin V in J18-treated Tm5 but not in melan-a cells, indicating that J18 induces cell death by apoptosis. *In vivo* experiments were also performed by inoculating subcutaneously C57BL/6 mice with Tm5 cells, and injecting daily, for 10 days, 25mg of J18 in the site of tumor cell inoculation. In control mice inoculated with Tm5 cells, the volume of tumors reached up to 6.000 mm^3 in 23 days whereas in J18-treated mice, the tumor growth was significantly reduced. Our efforts to study the apoptosis pathway triggered by J18 in Tm5 cells are under way. Work supported by FAPESP and CNPq.

BC27 - Phosphoglucomutase (PGM) is located in the glycosomes of *Trypanosoma cruzi* .

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The surface of *T. cruzi* is covered by a dense coat of *O* - glycosylated sialoglycoproteins and glycoinositolphosphoglycans, which are highly galactosylated and thought to play a role in host cell adhesion and invasion by this parasite.Since *T. cruzi* is incapable of metabolizing galactose by the Leloir pathway, the formation of UDP-galactopyranose (UDP-Gal*p*) by the parasite is strictly dependent on the epimerization of UDP-Glu.Phosphoglucomutase (PGM) is the pivotal enzyme that catalyses the reversible interconversion of Glu-6-P into Glu-1-P, a necessary intermediate required for the synthesis of UDP-Gal*p*. In *S. cerevisiae* and in higher eukaryotes, PGM is exclusively found in the cytoplasm.Differently from these organisms, several enzymes that participate in sugar metabolism in trypanosomatids are confined to specialized peroxisome-like organelles called glycosomes. In *T. brucei*, some glycosomal proteins contain peroxisome-targeting signals, which are thought to drive their transport to glycosomes. Recently, in silico predictions of putative glycosomal enzymes showed that pmm-like genes of *T. cruzi* and *L. major* present a PTS1-type peroxisomal targeting signal sequence which is absent from PGM (Opperdoes and Szikora, 2006, Mol. Biochem. Parasitol. 147:193). In order to investigate the sub-cellular localization of PGM in these parasites, we used His-tagged recombinant PGM of *T. cruzi* to produce anti-PGM polyclonal antibodies. Immunofluorescence microscopy of different forms of *T. cruzi* (epimastigotes, trypomastigotes and amastigotes) and of promastigotes of *L. major* showed co-localization of PGM with glyceraldehyde-3-phosphate dehydrogenase, a *T. brucei* glycosome marker. Partition of membrane and soluble fractions of epimastigote extracts using Triton X-114 revealed that PGM was found in the aqueous phase. Sub-cellular fractionation of *T. cruzi* organelles by centrifugation in a sucrose gradient followed by Western blotting revealed that PGM is present in the glycosomal enriched fraction. Taken together, our results suggest that in *T. cruzi*, PGM is a soluble protein and associated with the glycosomal fraction.

BC28 - PHOSPHORYLATION AND DEPHOSPHORYLATION OF *TRYPANOSOMA CRUZI* HISTONE H1 THROUGH THE CELL CYCLE

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Histone H1 of most eukaryotes is phosphorylated during the cell cycle progression and seems to play a role regulating chromatin structure during DNA replication and chromosome condensation. The phosphorylation of histone H1 in mitosis is also though to promote the attraction of factors that are involved in condensation of chromosomes by a mechanism not yet understood. The histone H1 of trypanosomatids lacks the globular domain and is shorter when compared with the histone of other eukaryotes. We have previously shown that *T.cruzi* histone H1 is phosphorylated in a typical cyclin-dependent kinase (da Cunha et al, Mol. Biochem. Parasitol, 2005,140:75). In the present work, we used specific antibodies against phosphorylated (anti-H1P) and non-phosphorylated (anti-H1) *T. cruzi* histone H1 to detect when it is phosphorylated. By immunofluorescence assays, we found that most of cells (98%) that have one nucleus, one kinetoplast and one flagellum corresponding to cells in G1 and S were weakly labeled with anti-H1P, contrasting with an intense labeling (more than 95%) in the cells with two flagella, one kinetoplast, and one nucleus, which are in the G2 phase of the cell cycle. All mitotic cells were strongly labeled by the anti-H1P and the labeling decreased significantly when the mitotic furrow was formed. In contrast, 95% of parasites were strongly labeled with anti-H1 regardless of the cell cycle stage. We conclude that *T. cruzi* histone H1 phosphorylation and dephosphorylation is cell cycle dependent. The phosphorylation begins in the G2 phase, increasing until the onset of mitosis. Then, the histone H1 is progressively dephosphorylated during cytokinesis.Supported by FAPESP

BC29 - Different localization of transmembrane eif2α kinases from *Trypanosoma brucei* and *Trypanosoma cruzi*

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Translational regulation by phosphorylation of eIF2α in eukaryotes is central to stress-induced programs of gene expression, from yeast to mammals. Trypanosomatids have little control of transcription, implying that most of its gene expression regulation involves post-trancriptional mechanisms, including translation initiation regulation. These parasites display differentiation programs elicited by the milieu of their physiologically distinct insect vector and mammalian host, likely representing stress situations. *Trypanosoma brucei* and *Trypanosoma cruzi* express three potential eIF2α kinases (eIF2αK1-K3). eIF2αK2 is a type I transmembrane protein localized in the flagellar pocket of *Trypanosoma brucei*

procyclic (insect form) and bloodstream (mammalian form) parasites. However, in *Trypanosoma cruzi* epimastigotes and mammalian tissue culture trypomastigotes the protein was detected along the whole flagellum by immunofluorescence assays using the antibody against recombinant TbeIF2αK2. In *T. brucei* bloodstream forms, but not in procyclic form, TbeIF2αK2 shows an elevated basal level of phosphorylation, which is further enhanced upon treatment of parasites with slightly acidic pH. Since acidic pH stress leads to the release of VSG (variant surface glycoprotein) from the bloodstream parasites, it is possible that TbeIF2αK2 activity relates with the proper control of expression of this important family of proteins, which cover the parasite surface and are responsible for the evasion of the parasite from the immune system. Studies about the localization and expression of this kinase during the life cycle of *T. cruzi* are underway. Supported by FAPESP

BC30 - Identification of a specific cytoskeleton antigen associated with *T. cruzi* reservosomes

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By immunizing mice with a cytoskeleton fraction of *T. cruzi* epimastigotes, we obtained a monoclonal antibody (mAb 3G4) that recognized compartments located at the posterior region of the parasite, where are found reservosomes. The reactivity of mAb 3G4 is located at the same regions labeled by an anti-cruzipain antibody. As observed for reservosomes, the mAb 3G4 staining disappeared when epimastigotes differentiate to trypomastigotes. The reactivity of mAb 3G4 remained when the parasites were solubilized with detergents in the presence of Mg/EGTA, EDTA, or CaCl2 before fixation, indicating that it recognized structures associated with the cytoskeleton. By another hand in the procyclic forms of *T. brucei* after detergent treatment the antibody stains the flagellum. In *T. brucei* the antibody-recognized bands between 55 and 70 kDa and the epimastigote forms of *T. cruzi* it recognized bands of 60 and 70 kDa. All these results suggest that this monoclonal antibody recognize cytoskeleton components associated with the reservosomes, not identified yet . Support FAPESP and CNPq

BC31 - African trypanosomes traversal of the human blood-brain barrier requires cysteine protease-mediated calcium signaling.

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In human African trypanosomiasis (HAT), African trypanosomes invade the central nervous system causing encephalitis and subsequent death. While the question as to how African trypanosomes enter the human brain is not yet resolved, animal models suggest that transendothelial migration occurs. Using human brain microvascular endothelial cells (BMEC) as our human blood-brain barrier (BBB) model system, we found that bloodstream forms of *Trypanosoma brucei gambiense* are able to cross these human BMEC monolayers at much higher efficiency than *T. b. brucei*. These differences may be due to differences in cathepsin L-like cysteine protease (brucipain) activity. *T. b. gambiense* crossing of human BMECs was abrogated by N-methylpiperazine-urea-Phe-homopheylalanine-vinylsulfone-benzene (*N*-Pip-F-hF-VSPh), an irreversible inhibitor of brucipain. The N-Pip-F-hF-VSPh-treated *T. b. gambiense* failed to elicit Ca^2+ fluxes in BMECs, suggesting that generation of activation signals for the BBB depend on brucipain enzyme activity. Consistent with this, biochemical analysis revealed that *T. b. gambiense* secreted higher levels of brucipain than *T. b. brucei*. Strikingly, *T. b. brucei* crossing of the BBB was drastically enhanced upon incubation with brucipain-rich supernatants derived from *T. b. gambiense*. Of note, *N*-Pip-F-hF-VSPh, but not the cathepsin B inhibitor CA074, cancelled these effects, and the effects correlated with loss of Ca^2+ inducing activity in *T. b. gambiense* conditioned medium. Taken together, our studies implicate brucipain as a critical driver of *T. b. gambiense* trans-endothelial migration of the human BBB *in vitro*.

BC32 - BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Trypanosoma cruzi* IN INDIVIDUAL AND DUAL INFECTED DOGS

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The histotropic clonal model previously described for *T. cruzi* and the occurence of mixed infections already demonstrated in distinct host species indicate a significant influence of the parasite genetic on its biological properties. The purpose of this work is to verify, the behavior of *T. cruzi* populations in dogs after individual and dual infections, employing the principal laboratorial parameters for chronic Chagas disease evaluation. Two dogs were intraperitonealy infected with 2,000 blood trypomastigotes of JG strain or Col 1.7 clone respectively, and two with dual infection (1,000 blood trypomastigotes of each population). During the acute phase of the infection the parasitemia was confirmed by fresh blood examination. Almost 3 years later, the dogs (except one

double infected that has died unexpectedly after the second evaluation) were evaluated at regular interval of two months during two years by hemoculture (HC), xenoculture (XC), conventional serology (CS - ELISA and IHT) and PCR in blood eluate. In twelve examinations the percentual of positivity for the dogs infected with JG strain and Col 1.7 clone were respectively 41.7 and 16.7% (HC), 58.3 and 25% (XC), 100 and 100% (CS) and 66.7 and 83.3% (PCR). In the still alive mixed infected dog, the percentage of positivity were 33.3% (HC); 33.3% (XC), 100 %CS and 50% (PCR). The PCR was always the most sensitive method. When the PCR was negative the same occured with HC and XC. The analysis of six microsatellites loci did not revealed mixed infections in any animal inoculated with both populations and only the peak correspondent to JG was observed. This result was confirmed by the rDNA analysis performed with the same material. Further parasite identifications will be tried in tissues to better evaluate if the elimination of one of the parasite population occurred at all. **Supported by FAPEMIG, UFMG and UFOP.**

BC33 - In vitro effects of suramin on the ultrastructure and infectivity of the trypomastigote form of Trypanosoma cruzi.

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Suramin, a polysulfonated derivative of urea is an efficient drug in the prophylactic treatment of human trypanosomiasis and onchocerciasis. In trypanosomes, suramin inhibited several enzymes, the endocytosis of some molecules and the binding of LDL to specific receptors. Previous studies of our group showed a significant effect of suramin on the adhesion of epimastigotes of *T. cruzi* to macrophages. Here we analyze the effect of suramin on trypomastigote morphology and interaction with host cells. LLC-MK2 cells previously infected with Y strain tissue culture trypomastigotes were incubated in RPMI-1640 medium supplemented with 2 % FCS containing 0.5 mM suramin. After 5-6 days trypomastigotes were recovered from the supernatant. LLC-MK2 cells were plated on 13-mm round coverslips and cultivated for 24 h. Control trypomastigotes and those obtained after the suramin treatment (TDS) were incubated with host cells at a parasite-host cell ratios of 10:1 and 50:1 for 30 min at 4 C and for 3 h at 37 C. The interaction was stopped by removing parasites and the monolayers were fixed and stained or incubated by an additional period of 48 and 72 h. Adhesion and infection rates were evaluated. TDS parasites presented significant changes on their morphology mainly characterized by the presence of bi-nucleated and bi-flagellated trypomastigotes. TDS invasion was drastically reduced. After 3 h, using a 10:1 ratio, we observed a reduction of about 80 % in the number of parasite per 100 cells. If the same experiment was observed after 48 and 72 h post-infection the reduction in the percentage of infected cells were of 93.7 and 99 %, respectively, with some wells presenting no infected cells. When a 50:1 ratio was used the reduction in the percentage of infected cells was of about 90 % after 48 or 72 h post-infection. Supported by: CNPq, FAPERJ, Pronex and FUJB-UFRJ

BC34 - Putative caveolae-mediated endocytosis in epimastigote forms of *Trypanosome cruzi*

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Eukaryotic cells have an elaborate membrane system that allows ingestion of macromolecules by endocytosis. The biogenesis of endocytic vesicles is regulated by specific proteins and co-factors. The lipid raft endocytosis model proposes that cholesterol and sphingolipids at the plasma membrane are clustered into micro-domains (rafts) that float in the lipid bilayer. Raft-associated proteins can provide analytical landmarks for lipid rafts. For instance, caveolin and flotillin are cholesterol-binding proteins that associate with a subset of rafts named caveolae, and are frequently used as raft markers. Epimastigote forms of *Trypanosoma cruzi* appear as an interesting model for endocytosis, as their polarized endocytic apparatus is concentrated at only two specific membrane sites: the flagellar pocket and the cytostome. To study the occurrence of molecules necessary for caveolae-mediated endocytosis in *T. cruzi*, epimastigote lysates were incubated for 30 minutes at 4°C in 1% Triton X-100 and then submitted to sucrose density centrifugation. Lipids of sucrose fractions were extracted and analyzed by TLC and HPTLC. Cholera toxin B subunit and monoclonal anti-flotillin antibody were used to identify flotillin and GM1 gangliosides by dot-blot in the cholesterol-enriched fraction. To visualize flotillin in *T. cruzi*, whole epimastigotes were immunolabeled with an antibody against flotillin and revealed with a secondary Alexa fluor-457 antibody. The cells were examined in a Zeiss CLSM 510-meta confocal laser scanning microscope. Epimastigote forms also were incubated with gold labeled transferrin and analyzed by routine transmission electron microscopy. In this work we were able to identify a lipid raft domain in *T. cruzi* epimastigotes, correlated with GM1 and flotillin positive detection. Transmission electron microscopy and confocal microscopy showed morphological evidences of endocytosis by caveolae. The images suggest that the cytostome is a lipid raft site in *T. cruzi* epimastigotes.

BC35 - Integrative and stable expression of the Green Fluorescent Protein (GFP) by *Trypanosoma rangeli*

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Trypanosoma rangeli is a kinetoplastid parasite that can be found in Latin America infecting mammalian hosts and triatomine species. In this work we have transfected parasites of *T. rangeli* Choachi and SC-58 strain with linear pTEXGF-Pmut plasmid in order to verify the possibility of stable GFP transfection for biological studies. For that, plasmids were double digested (*SstI/KpnI*) and the target fragment rescued by eletroelution. Five to 18μg of the double-strand fragments were used for electroporation of epimastigote forms (10^8/mL) using standard conditions (2.5kV, 25μF, 400W). After electroporation, parasites were cultivated in LIT medium supplemented with 20% of SBF and with increasing amounts of G-418, up to 600μg/ml. After 2 months, fluorescents parasites were observed and assayed in order to confirm the maintenance of biological characteristics. GFP expressing parasites revealed no alterations on their biological behavior on infectivity and growth, showing the same growth curves when compared to parasites transfected with circular plasmids and non-transfected parasites. Furthermore, in vivo studies carried out with 4-5th instar nymphs of *Rhodnius prolixus* also confirmed the maintenance of biological characteristics, being the parasites able to infect and develop within salivary glands. PFGE analysis of the transfected parasites revealed the integration of the GFP fragment in two chromosomal bands of ∼1.10 Mb and ∼1.25 Mb for SC58 strain and in a single band of a ∼1.25 Mb for Choachi strain. Thus, these results confirm the plasmid pTEXGFPmut integration in the *T. rangeli* genome and the stability of the transfection. Supported by CNPq, CPqRR and UFSC.

BC36 - Nature of host cell heparan sulfate binding domain involved in the *Trypanosoma cruzi* heparin binding protein recognition

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Trypanosoma cruzi invasion is mediated by receptor-ligand recognition between the surface of both parasite and target cell. We have previously demonstrated the role of heparan sulfate proteoglycan (HSPG) in the attachment and invasion of *T. cruzi* in cardiomyocytes. In this study, we evaluated the nature of heparan sulfate binding site to the parasite surface protein and characterized the *T. cruzi* heparin binding protein (HBP-Tc) by biochemical approaches. We describe herein results showing that two major hydrophobic proteins with molecular mass of 59 kDa and 65.8 kDa were isolated from *T. cruzi* using heparin affinity chromatography. Hydrophobic 35S-metionine labeled proteins eluted from heparin-sepharose column revealed the presence of these proteins in both infective and non-infective stages of *T. cruzi*. Isotopic quantitation analysis suggests higher level of HBP in trypomastigotes as compared with epimastigotes. Sulfo-NHS-LC-biotin labeling proteins and cellular fractionation methodology associated with SDS-PAGE supported the presence of both proteins in epimastigotes, which was also revealed in the parasite surface membrane fraction by Dot blot assay. Two protein bands could also be detected by isoelectric focusing (pH 6.7 and pH 5.9) while the native electrophoresis revealed a unique protein band. Additionally, we observed that HBP-Tc promotes the agglutination of rabbit erythrocytes Since HSPG mediates *T. cruzi*-cardiomyocyte invasion, we have performed a glicosaminoglycans competition assay to analyze the role of sulfated proteoglycans, including heparin, keratan sulfate and both acetylated and sulfated fragments of heparan sulfate (HS), in the recognition and invasion process of *T. cruzi*. Trypomastigotes, clone Dm28c, were pre-treated with 20μg/ml of GAGs prior to 2h-interaction with cardimyocytes. Treatment of the parasites with heparin and partially acetylated HS fragment demonstrated a significant inhibition of invasion. Studies are in progress to verify the role of the HBP-Tc in the recognition of insect cells. Supported by FAPERJ, CNPq& PAPES IV.

BC37 - *Rhodnius prolixus* saliva modulates host signaling pathways and enhances *Trypanosoma cruzi* infection.

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Rhodnius prolixus is a vector *Trypanosoma cruzi* the causative agent of Chagas disease. The parasite is transmitted by vector feces deposited on human skin during blood feeding. One of the routes of host cell invasion occurs through the wound produced by insect bite. Parasite thus faces a cell environment within the wound previously stimulated by saliva. In the present work we tested the role of bug saliva on parasite transmission. Firstly, saliva injection on mice skin induced cell recruitment as evaluated by histological sections. Secondly, treatment of either the macrophages or the parasites with saliva induced a two-fold increase on parasite association index to macrophages. Thirdly, treatment of macrophages with saliva triggered the phosphorylation of several proteins on phosphotyrosine. However, minor differences on phosphoserine profile were detected. On the other hand, protein phosphorylation profile of *T. cruzi* trypomastigotes is also affected by saliva treatment. The above set of results suggests that previous exposition to saliva components manipulates the intracellular signaling system of both macrophages and parasites. The ultimate effect of such manipulation is the enhancement of parasite invasion. We next searched for candidate molecules in saliva able to trigger the above mentioned effects. Lysophospholipids are powerful modulators of cell signaling in mammalian cells. We have recently shown that *R. prolixus* saliva store lysophosphatidylcholine (lyso-PC) during their growth. We have also detected lyso-PC on bug feces which might suggest a general mechanism of parasite transmission. In conclusion we have gathered preliminary evidence that salivary lyso-PC is in fact a molecule which modulates signaling pathways and might help *T. cruzi* infection. We are currently mapping such intracellular signaling systems triggered by saliva on macrophages. In conclusion this is the first demonstration of involvement of a triatominae-derived molecule as an enhancing factor of Chagas disease transmission. Supported by CNPq, CAPES, FAPERJ, PADCT, IFS.

BC38 - Sugar binding proteins from the *Trypanosoma cruzi trans*-sialidase family play distinct roles in the pathogenesis of infection

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A dramatic aspect of Chagas disease is the development of a chronic active myocarditis after a prolonged asymptomatic infection. The pathogenesis of cardiac damage associated with infection is multifactorial, including the persistence parasites at the inflammatory site. Studying the parasite molecules that trigger these effects might help understanding the nature of the myocarditis in chronic Chagas disease. Ours studies demonstrated that co-stimulation and trafficking of T cells during *T. cruzi* infection can be regulated by a family of glycosylphosphatidylinositol-anchored sugar binding *trans*-sialidase (TS) proteins. The parasite uses an active TS (aTS) to sialylate its surface glycoproteins and an inactive TS (iTS) acts as a sialic acid-binding lectin. In this work we studied the effect of aTS and iTS on trafficking of T cells during *T. cruzi* infection. Balb/c mice were injected with 30 μg of aTS or iTS one hour before infection and at days 2 and 3 post-infection (pi) with blood trypomastigotes (Y strain). Parasitemias were evaluated at days 6-10 pi, and the hearts were examined at day 15 pi. In agreement with the increase in parasitemia, we observed an increase in the number of amastigote nests in the cardiac tissue from the aTS treated group, when compared with infected and treated iTS groups. On the other hand, analyses by flow cytometry, histopathology and immunohistochemistry showed a reduction in the number of leukocytes in the cardiac tissue from infected and iTS treated mice. In agreement, there was a reduction in creatine kinase (CK) activity in the serum from infected and iTS treated mice. These results indicate that, although aTS and iTS share conserved sugar binding sites, these related molecules might play distinct roles in the pathogenesis of Chagas disease. Supported by: CAPES, CNPq, FAPERJ.

BC39 - FURTHER CHARACTERIZATION OF MUCINASE, A *TRYPANOSOMA CRUZI* MOLECULE POSSIBLY INVOLVED IN GASTRIC MUCOSAL INFECTION

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Oral infection with metacyclic forms of *Trypanosoma cruzi*, the agent of Chagas disease, leads to invasion and replication in the gastric mucosal epithelium, which is a unique portal of entry for systemic infection. We have proposed that, by the action of a mucinase, the parasites would translocate through the gastric mucous layer and reach the underlying target cells. Initial experiments showed indirectly that metacyclic forms of different *T. cruzi* strains do have mucinase activity. The present study provides more direct evidences that metacyclic forms secrete an enzyme that digests gastric mucin. By electrophoresis of SDS-PAGE gel copolymerized with gastric mucin, two bands around 45 kDa with mucinase activity were detected. The enzyme was active in a broad pH range (2-10), the activity being higher at acid pH, implying that *in vivo* mucinase will encounter favorable conditions for its action. In host cell invasion assays in the presence of gastric mucin, the rate of invasion by metacyclic forms was significantly increased when polyclonal anti-mucinase antibodies were present, as compared to the controls in which serum was absent or normal mouse serum was present. By indirect immunofluorescence, using anti-mucinase antibodies, the cellular localization of the enzyme was determined. The expression of mucinase in metacyclic forms was intracellular, with the enzyme apparently concentrated in multiple spots predominantly in the posterior end of the parasite. Mucinase was also detected in epimastigotes, tissue-culture trypomastigotes and extracellular amastigotes. Efforts are being made to determine the structure of mucinase and its function *in vivo*. Supported by FAPESP and CNPq.

BC40 - *Trypanosoma cruzi* disrupts costameric distribution of TGF- β receptor type II in cardiomyocytes

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Transforming growth factor beta (TGF- β) family are multifunctional cytokines involved in many cellular processes, being implicated in Chagas disease progression. TGF-β signaling pathway is fundamental for *T. cruzi* invasion, and intracellular amastigotes uptake host TGF-β to differentiate to trypomastigotes. However, poor attention has been given to host expression and distribution of TGF-β receptors. Since several reports have demonstrated the ability of *T. cruzi* to modulate host cell surface receptors, we were interested to analyze the TGF-β receptor type II (TβRII) expression during *T. cruzi*-cardiomyocyte interaction. Indirect immunofluorescence assay revealed an unexpected striated organization of TβRII in cardiomyocytes, which was enhanced (38%) after TGF-β treatment. Double staining with anti-vinculin and anti-TβRII antibodies showed a co-localization of TβRII with costameres of vinculin by laser scanning confocal microscopy. The association of TβRII with the cytoskeleton was also demonstrated by cytochalasin D treatment, which resulted in a decrease of 45.3% in the frequency of cardiomyocytes presenting TβRII striations. This association of TβRII with the cytoskeleton may enhance activation of TGF-β signaling cascade, since treatment of cardiomyocytes with cytochalasin D inhibited phospho Smad 2 activation after TGF- β treatment. *T. cruzi* infection elicited a remarkable decrease in the frequency of cardiomyocytes presenting TβRII striations, but we were unable to analyze the host cell TβRII expression due to strong positive reaction of intracellular amastigotes. The treatment of *T. cruzi*-infected cultures with TGF-β did not provoke any significant alteration in the frequency of TβRII striations, still showing low levels of TβRII striation pattern, in contrast with the raise observed in control cultures. Together, these results suggest that the co-localization of TβRII with costameres may be important to activation of the TGF-β signaling cascade, and *T. cruzi* derived cytoskeleton disorganization could result in altered or low TGF-β response in cardiomyocytes. Financial support: FIOCRUZ, PAPES IV and CNPq.

BC41 - Expression of FcgRII/III on cardiac T lymphocytes isolated from Trypanosoma cruzi infected mice

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Experimental infection with Trypanosoma cruzi, the causative agent of Chagas disease, induces dramatic changes in immunecompetent cells and molecular immuneregulatory pathways responsible for the coordinated function of the immune system. It has been described Fas-dependent activation induced cell death (AICD) of CD4+ T cells, reduced secretion of IL-2, hypergammaglobulinemy, reduction of effector activity of inflammatory cardiac CD8+ T cells and others. The infection also induces important myocarditis, with inflammatory infiltration mostly composed by activated/memory T lymphocytes CD62Llow/ LFA-1high/VLA-4high. Regarding to Fcγ receptors (FcgR), our group observed previously an expansion of total FcgRII/III spleen

lymphocytes in infected BALB/c mice and recently we published an expansion of CD8+/FcgRII/III+ cells, but never observed CD4+/FcgRII/III+ cells in the acute phase of the infection. We also described in C57Bl/6 mice, a resistant mouse strain, that most cardiac inflammatory CD8+ T cells were expressing FcgRII/III. In this work, we studied cardiac inflammatory cells from susceptible mouse strains and observed a double negative (DN) population of CD3+ lymphocytes not expressing CD4 or CD8 markers, but expressing FcγRII/III (about 45% of leukocytes region in FACS analysis). Besides, less then 10% of CD8+ were FcgRII/III+ cells. The DN population is mostly observed after 15-17 days of infection and we are now co-aggregating TCR to FcgRII/III *in vitro* to evaluate whether this stimulus negatively modulates cytokines secretion and cytotoxicity and may play a role in the evolution to the chronic phase.

BC42 - ACQUISITION OF VECTOR LIPOPROTEIN IN THE PROCYCLIC FORM OF *TRYPANOSOMA BRUCEI*

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African trypanosomes are flagellated protozoan parasites, causing sleeping sickness in man and related diseases in livestock. *Trypanosoma brucei* has a biphasic life cycle alternating between the mammalian host and the insect vector tsetse fly. In the invertebrate host hemolymph lipophorin (Lp) is a major haemolymphatic lipoprotein which carries and distributes many lipid classes among the tissues involved in lipid absorption, storage and utilization. Trypanosomatids have incomplete de novo lipid synthesis. Therefore, they avidly take up lipids from host lipoproteins presumably satisfying their requirements for growth and differentiation. In this work we demonstrate that the parasite was able to uptake lipophorin. To examine the Lp endocytosis by *T. brucei*, cells were incubated in the presence of LP-I^{125}. After different times, cells were collected and the radioactivity was determined by gamma counting. Lp was found associated with the parasites and the uptake increased up to 180 minutes. We incubated the parasite in the presence of Pi32. The lipids were extracted and analyzed by thin-layer chromatography. We observed that phosphatidylcholine was the major phospholipid found. In order to investigate cellular localization of lipophorin we used fluorescent microscopy. The parasites were incubated for 3h in the presence of lipophorin double-labeled in the lipid moiety with Bodipy - Fatty acid (Bodipy-FA) and Texas red - phospholipids (Texas red-PL) or lipophorin double-labeled in the protein moiety with FITC and in the lipid moiety with Texas red-PL. After incubation the fluorescence was analyzed by microscopy. We observe that the parasites were able to incorporate lipophorin and the fluorescence was distributed around the cytoplasm. These data suggest that *T. brucei* utilizes lipophorin as lipid source while inside the invertebrate horst. Whether this is a specific transfer or and endocytic process, remains to be investigated.

BC43 - Iron chelator (Desferrioxamine) leads to lower parasitemia and mortality in mice infected with Trypanosoma cruzi

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We have studied the role of iron in experimental infection of (n=80) mice with **Trypanosoma cruzi** . Previously animals were daily treated with desferrioxamine (DF 5mg/animal/day) during 14 days before the infection with 500 blood forms of Y **T. cruzi** strain by intraperitoneal route. After infection, forty of these animals continued to receive DF for 21 days. In infected animals (40), treated (20) or not (20), parasitemia, prepatent period, patent period, day of maximum of parasitemia and mortality were evaluated. The animals were sacrificed in 14th and 21th days after infection (DAI). Hemoculture, PCR and ELISA were performed in animals that survived to the acute phase. In all groups of mice the level of hemoglobin, iron in serum and liver were evaluated. The results observed in treated animals were: a) negative hemoculture in acute and chronic phases of infection; b) positive PCR in all animals during acute phase and c) 3 animals with negative PCR in the chronic phase, while not-treated mice presented positive hemoculture and PCR in both phases of the infection. ELISA was always positive in treated and not-treated mice. Infected groups presented significant lower levels of iron in the liver when compared to not-infected treated or no-treated groups. In 14th DAI the infected treated group presented significant lower levels of hemoglobin than the infected group. In 21th DAI, sera of infected group presented significant higher levels of iron when compared to control and infected treated groups. These results suggest that the decrease of iron in the host leads to better clinical evolution of **T. cruzi** infection.

BC44 - Evaluation of inflammation in heart and skeletal muscles in dual-clonal *T. cruzi* infections

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Previous studies in our laboratory have investigated biological properties following dual-clonal *Trypanosoma cruzi* infections in BALB/c mice. Eight *T. cruzi* clonal stocks, two of each principal genotype, including genotype 19 and 20 (*T. cruzi* I), hybrid genotype 39 (*T. cruzi*) and 32 (*T. cruzi*II) were combined into 24 different mixtures. Our findings clearly demonstrated that features resultant of dual-clone infections of *T. cruzi* clonal stocks did not display either the characteristics of the corresponding monoclonal infections or the theoretical mixture based on the respective monoclonal infections. So, inhibit, stimulatory and similar biological properties were observed. Herein we studied the influence of these different dual-clonal infections on the histopathological lesions of the heart and skeletal muscles in acute phase compared to the monoclonal infections. Low parasitism was frequently observed in monoclonal and dual-clonal infections. Like parasitological properties, preliminary inflammation results observed in twenty dual-clonal infections have demonstrated changes in relation to expect based in monoclonal infections. In 12, 6 and 2 dual-clonal infections were observed decrease, increase and no changes of inflammation level of heart, respectively. On the other hand, in skeletal muscles were observed decrease (2), increase (11) and similar (7) inflammation level previously observed by our group. However, these changes apparently were not correlated with the parasitemia alterations. Considering the genotype combinations 20+39 and 19+39, a reduction of the inflammatory lesions were observed in the heart. However in the muscles no changes and increase in inflammatory level were observed in 20+39 and an increase to 19+39 mixtures, respectively. These results were contradictory to the verified in monoclonal infections for 19, 20 and 39 genotypes. For other combinations neither correlation were observed. These data support that dual-clonal *T. cruzi* infections have a great impact on the biological properties of the parasite in the host. Supported by FAPEMIG, CNPq and UFOP

BC45 - Histopathological alterations in spinal cord during acute *T. cruzi* infection in dogs

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Trypanosoma cruzi, the causative agent of Chagas disease, affects not only cardiac and intestinal structures but also neurological structures. We have previously reported that the spinal cord lesions induced by *T. cruzi* are characterized by glial nodules (GN) and perivascular infiltrates (PI) with or without parasites. In the present study our aim was to determine whether the immunossupressive therapy can influence the lesions observed in spinal cord. Twenty four animals were divided into six experimental groups: uninfected, infected with blood trypomastigotes (BT), infected with metacyclic trypomastigotes (MT), uninfected/immunossuppressed, infected with BT and immunossupressed (BTims) and infected with MT and immunossupressed (MTims). Sixteen dogs (120 days old) were inoculate with 2000 BT or MT/kg body weight by intraperitoneal route (Berenice-78 *T. cruzi* strain). Twelve dogs were submitted to daily immunossupressive therapy with azathioprine (2mg/Kg). These were observed during the acute phase of the infection and sacrificed at 42 days after infection. The spinal cord were collected in totum and the histopathological evaluations were performed using Hematoxylin-Eosin to observe the generally histopathological alterations, Cresil-Violet to evaluate and quantify GN and PI, and the immunohistochemical technique against *T. cruzi* and macrophages, to visualize the parasitism and macrophages respectively. MT group presented greater number of cells in PI and GN than BT group. On the other hand, the number of cells in GN and PI were greater in BT and MT when compared to BTims and MTims. Parasites were observed in BT, BTims and MTims. Macrophages were greater in MT than BT and in MTims than BTims. Taken together, these data suggests that MT lead a more intense response than BT and that the immunossupression during acute phase of experimental *T. cruzi* infection in dogs do not permitted greater parasitism or lesions in spinal cord. Supported by CNPq, FAPEMIG, UFOP.

BC46 - Inhibitors of Angiotensin-Converting Enzyme modify signaling pathways underlying invasion of endothelial cells by *Trypanosoma cruzi*

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Angiotensin-converting enzyme (ACE) is a Zinc dependent metalloprotease that catalyzes the conversion of angiotensin I to vasoconstrictor angiotensin II while promoting the degra-

dation of the potent vasodilator bradykinin and angiotensin-(1-7). Two distinct forms of ACE are expressed in humans, a somatic form that is particularly abundant on the endothelial surface and a smaller isoenzyme that is found exclusively in testis. Recent studies suggest that ACE can function as a signal transduction molecule, acting independently of the involvement of angiotensin II and bradykinin or any of their receptors. Our group has previously reported that *T.cruzi* (Dm28c) tissue culture trypomastigotes invade endothelial and cardiomyocytes by activating the B2 and B1 kinin receptors (B2R/B1R). In the present work, we addressed the possibility that *T. cruzi* trypomastigotes (Dm28c) may coordinately engage TLR ligands and kinin-releasing cysteine proteases (cruzipain) to invade host cells. *T.cruzi* trypomastigotes were incubated for 3 h with human umbilical vein endothelial cells (HUVECs) or brain microvascular endothelial cells (BMECs) in the presence or absence of monoclonal antibodies against TLR2 or TLR4 in cultures supplemented (or not) with ACE inhibitors. Assessment of GPCR involvement was made through addition of specific antagonists for (i) bradykinin B2 receptors (ii) MAS receptor (Angiotensin-(1-7) receptor). Assays run in the absence of ACE inhibitors indicated that antibodies to TLR2 or TLR4 and GPCR antagonists did not interfere at all with cellular invasion. However, assays performed in medium containing ACE inhibitors revealed that invasion was markedly reduced by (i) antibodies to TLR2, and to less extent, by anti-TLR4 (ii) B2R antagonist or (iii) MAS antagonist. Additional studies are required to determine (1) How ACE inhibitors might drive TLR2, B2R and MAS "cross-talk"during the invasion process (2) if the cooperative engagement of these multiple receptors is required for parasite penetration and/or retention mechanisms.
Supported by: CNPq, FAPERJ

BC47 - Do reservosomes possess lipid bodies?

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Lipid bodies are peculiar structures found in many cell types. They are involved in storage, transport or as a possible deposit for toxic or useless fatty acids during cell cycle. There is a variety of inclusions and their structural conformation depends on the chemical nature of the lipid. Basically, the most common type of lipids present in the bodies are triacylglycerols, cholesteryl esters, polyhydroxyalkanoates and wax esters. The latter is almost restricted to prokaryotes, once that it has only been described in jojoba so far. Moreover, the shape of wax ester inclusions can vary in function of the strain and growth conditions, from spherical to flat or rectangular inclusions. Here we analyzed the presence of this kind of lipid body in reservosomes from *Trypanosoma cruzi* epimastigotes, whose main function is storing and degrading lipids and proteins from endocytic origin. High resolution electron microscopy of ultrathin sections and whole isolated reservosomes as well as of reservosomes inside intact parasites evidentiated many lipid inclusions with spherical shape presenting a membrane unit, or with retangular shape crossing the entire organelle, similar to the ones found in prokaryotes. Freeze fracture replicas demonstrated that rectangular inclusions present a laminar feature, devoid of intramembranous particles. Three-dimensional reconstruction provided the spatial distribution of the lipid bodies inside reservosomes. By observing many organelles we could hypothesize that spherical inclusions give rise to rectangular bodies and their associated membrane until the rarer stacked retangular bodies. Large amounts of ergosterol and esterified cholesterol were detected as reservosome major neutral lipids (Cunha-e-Silva et al, FEMS Microbiol Lett, 2002, 214:7), but analysis of wax ester was not considered. Now gas chromatography analysis of wax ester in purified reservosomes is in course, as a new attempt to clarify the chemical nature of their peculiar lipid inclusions.

BC48 - RNA Pol II nuclear redistribution and changes in transcription levels during *Trypanosoma. cruzi* metacyclogenisis

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Transcription by RNA polymerase II in trypanosomes deviates from the standard eukaryotic paradigm. Genes are transcribed polycistronically and subsequently cleaved into functional mRNAs, requiring trans splicing of a capped 39-nucleotide leader RNA derived from a short transcript, the spliced leader (SL) RNA. The only identified trypanosome RNA polymerase II (RNA Pol II) promoter is that of the SL RNA gene. As described in our lab, *T. cruzi* RNA Pol II are found concentrated in a domain close to the parasite nucleolus and containing the spliced leader genes. The remaining RNA Pol II is diffusely distributed in the nucleoplasm, and the spliced leader-associated RNA Pol II localization is dependent on the cell transcriptional state (Dossin and Schenkman, 2005). In the present work we have established the changes in nucleus and kinetoplast morphology during the metacyclogenesis. We could detect by western blot and imunoflurecence that the amount of a SL transcription factor (p57TC) and of RNA Pol II decrease only at the end of the metacyclogenis process, about 72 h, when the parasites have already differentiated into metacyclic trypomastigotes with the kinetoplast along with the flagellum reaching the anterior pole of the cell. At this same stage we could detect a decrease in transcription levels by nucleus run on experiments. These results suggest that the differentiation process requires transcriptional activity and that a reduction in this activity is a feature only of completely differentiated cells. We are now investigating the distribution of sites of transcription by RNA Pol II by labeling nascent RNA with 5-bromouridine 5'-triphosphate in *vitro* during the morphological changes in the metacyclogenesis process. *supported by FAPESP* .

BC49 - INVASION OF SPHEROIDS BY *TRYPANOSOMA CRUZI*

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We have previously established *in vitro* primary hepatocyte cultures by using conventional procedures (Porrozzi e cols, 1997. Mem Oswaldo Cruz), and observed that in these cultures the cells usually flatten and spread upon attachment, followed by loss of polarity and disruption of the cytoskeleton. The development of new biological materials as compatible scaffolds for tissue repair, tissue engeneering cell growth and the actual advances in the application of three dimensional (3D) cell cultures systems have been used in biochemical research and systematic studies on tumour cell response (Bartholoma e cols, 2005. J Biomol Screen). We have developed spheroids from hepatocytes primary culture, by seeding freshly isolated embryonic cells at 37^0C, at constant speed, in non adherent substrate, within a Rotary Shaker (New Brunswick Scientific Innova 2000). We have used a neutral charge polymer (2-hydroxyethyl metacrylate pHema) that inhibited the adhesion of the cells to the substrate. Hepatocyte spheroids maintained the viability for long periods in culture, and adhered to each other. Cells in this culture system displayed large number of projections and electron dense regions of cell adhesion. In infected hepatocyte cells, *Trypanosoma cruzi* was localized in cytoplasmatic regions near endoplasmatic reticulum profiles, near the cell nuclei.

BC50 - Biological characterization of different samples in Trypanosoma cruzi. Comparative study of the endocytic activity.

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Parasites from genus *Trypanosoma* have a complex life cycle. They present different developmental forms adapted to each stage of the parasite cycle and their survival in distinct environments requires exogenous growth factors. *Trypanosoma cruzi* present three distinct developmental forms: amastigotes and trypomastigotes in the vertebrate host; and epimastigotes in the invertebrate host. Epimastigote forms, which can be easily maintained in vitro, avidly internalize different macromolecules from the extracellular milieu. Since endocytosis can be used as a gateway for drug delivery, aspects such as: a) the interaction of distinct molecules with cell membrane domains; b) the mechanisms by which they are internalized; c) the amount of internalized molecule; d) the sorting and fate of different molecules inside the cell; and e) the process that control the downstream traffic, have to be well characterized in order to be used as a potential chemotheraptic target. In this study we use flow cytometry and fluorescence microscopy to compare the endocytic process of epimastigote forms of *T. cruzi* of the Y strain and CL-Brener and Dm28c clones. In this way, epimastigotes were incubated in the presence of 5μg/ml Lucifer Yellow, Alexa-488-BSA, Alexa-488-IgG or FITC-IgG for 15, 30, 60, 120 and 180 min. Differences in endocytosis rates could be observed between Y strain, CL-Brener and Dm28c clones using the different tracers. Besides that, we observed that the kind of fluorochrome conjugated to BSA or IgG influenced the amount of macromolecules internalized by the parasites. Some cells were also incubated in the presence of 20μg/ml Alexa-488-BSA during the same periods described above and analyzed by fluorescence microscopy. The morphological investigation of endocytic process among the three samples also revealed differences in the percentage of labelled cells and in the number of labelled compartments according to incubation period.

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BC51 - ENUCLEATED CELLS SUPPORT THE INVASION, DIFFERENTIATION AND MULTIPLICATION OF TRYPANOSOMA CRUZI PARASITES

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Intracellular pathogens extensively modulate in vitro gene expression of host cells, with responses that depend on the kind and functional status of the interacting partners. These responses may protect the host cells, favor the survival and multiplication of the pathogens, or may be neutral. In addition, some of these responses are involved in the production of factors which, acting on other cell types, may restrict or enhance the in vivo dissemination and multiplication of the pathogen. Is the host cell nucleus proximately required for intracellular infection and if so, for which cells, pathogens and at what stages of the infection? Cytoplasts were earlier infected with *Toxoplasma gondii, Chlamydia psittaci, C. trachomatis, Rickettsia prowazekii or Shigella flexneri* 5a. In the present study, monolayers of L929 mouse fibroblasts, enucleated by centrifugation in the presence of cytochalasin B (CB), were infected with strains CL, CL-14 or G of *Trypanosoma cruzi*, the agent of Chagas Disease, a zoonosis carried by hemipteran vectors and widely distributed in Central and South America. Infection of cytoplasts was compared to that of nucleated cells present in the same preparations. The percent infection with *T. cruzi* was increased in cyto-

plasts that their nucleated controls. In both nucleated cells and cytoplasts, amastigotes multiplied on the average three times between 24 and 72 h of infection. After 72 h of infection amastigotes may acquire epimastigote-like morphology, suggesting that the nearly complete in vitro intracellular cycle. The increased in the percent infection of cytoplasts with *T. cruzi* contrasts with the reduced infection of cytoplasts with *Shigella flexneri* or their reduced uptake of tannic acid treated erythrocytes (Yamamoto, et al. 2006). Productive *T. cruzi* infection, differentiation and initial multiplication of L929 fibroblasts can thus take place in cells unable to modulate gene transcription, RNA processing, or nucleus dependent signaling cascades. Supported by FAPESP.

BC52 - Endocytic pathway of Trypanosoma rangeli

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In trypanosomatids, the endocytic pathway has been characterized exclusively in *Trypanosoma brucei*, *Leishmania sp* and *Trypanosoma cruzi*. In these parasites the endocytic system is highly polarized and presents the flagellar pocket and cytostome as entry sites. Along the pathway, nutrients are delivered to a network of compartments that vary significantly in their organization and morphology according to stage and species. *Trypanosoma rangeli* is a hemoflagellate parasite apparently harmless to humans but pathogenic for the insect vector. However, they are the second trypanosome species frequently found infecting humans in Latin America. In this work, we are characterizing for the first time the endocytic pathway of *T. rangeli* using fluid-phase and receptor-mediated endocytic tracers. BSA-Alexa 488nm and transferrin-Alexa 546nm were found in a network of tubules spread along the whole parasite body and in large organelles placed in the posterior region. Pulse-chase experiments have shown that these compartments were attainable for both tracers. These structures have an acidic character as shown by the accumulation of lysotracker. Using the lipophilic dye FM-464 we observed endocytic vesicles containing or not BSA or Tf. Immunofluorescence using the antibody anti- *T. cruzi* cysteine protease showed that the enzyme were present in small vesicles and large organelles. Electron microscopy analysis showed BSA-Au and Tf-Au bound to the parasite body and flagellar pocket surface. We also found the tracers in tubules and vesicle profiles spread along the cell body and accumulated inside large organelles localized at the posterior region and surrounded by a unit membrane, containing an electrondense matrix with electronlucent inclusions. Our results indicate that *T. rangeli* endocytic pathway presents particularities that resemble the endocytic tubule of *Leishmania* promastigotes together with reservosome-like compartments, as *T. cruzi* epimastigotes, representing a very interesting model to study endocytosis in eukaryotic cells.

BC53 - Effect of *Trypanosoma cruzi* infection in a cardiac microtissue produced *in vitro* - A new three-dimensional (3D) cell culture system

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Cardiac damages caused by *in vivo* infection with *Trypanosoma cruzi* are still not fully clarified. Therefore, in the last two decades many *in vitro* models have been proposed in order to understand more aspects about parasite: host cell interaction and also how chagasic cardiomyopathy (CC) can modulate cardiac physiology. In the present work we describe the effect of *Trypanosoma cruzi* on cardiac microtissue produced *in vitro* as a three-dimensional (3D) cardiomyocytes culture system, that mirror aspects observed on tissue *in vivo* as for example gene expression, proliferation and response to chemotherapy, better than cultures in monolayer (Abbot, 2003). In this 3D system, cardiac cells presented spontaneous contractility with typical cardiac morphology and the cells produce extracellular matrix compounds and growth factors (Garzoni et al., 2005). By either electron, light and confocal microscopy we observed trypomastigote forms adhered to cellular surface and inside of parasitophorus vacuole. Amastigote forms were able to divide and differentiate to trypomastigotes again and infect new cells on in the interior of the microtissue. Additionally, we observed a 4 to 6-fold area and volume increase on *Trypanosoma cruzi* infected cardiomyocytes and whole microtissue with a concomitant reduction in 50% of cells number as observed counting the number of nucleus stained with DAPI. By immunofluorescence was possible to observe increase on staining for fibronectin, collagen IV and laminin on infected microtissues. During co-culture of cardiac microtissue with *Trypanosoma cruzi* and macrophage we observed in semi-thin sections the presence of cells containing many vacuoles on interior of microtissue suggesting migration of activated macrophages or cytotoxic effect of these cells. The results achieved by the infection of this cardiac microtissue can contribute to understand even more of aspects observed on *in vivo* CC including fibrosis and cytotoxic effect of inflamatory cells during infection with *Trypanosoma cruzi*.

BC54 - STUDIES ON 24HPI CALCIUM IONOPHORE INDUCED EGRESS OF *TOXOPLASMA GONDII* IN LLC-MK2 CELLS

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Calcium ionophore A238187 has been used to activate the egress of *Toxoplasma gondii*, an obligate intracellular parasite that is the etiologic agent of toxoplasmosis, from host cells. This stage of *T. gondii* life cycle is still poorly understood. In this work, rosette disassemble after calcium ionophore treatment at 24 hours post-infection (24hpi) was observed by videomicroscopy. We also used actin and kinase inhibitors to evaluate the importance of these elements during the induced egress. Confluent monolayers of LLC-MK2 were infected with 5-10 MOI of tachyzoites of the RH strain of *T. gondii* obtained from the peritoneal cavity of infected mice. At 24hpi samples were treated with 0.001% A23187 calcium ionophore and processed for video microscopy or scanning elecron microscopy. Kinase inhibitors used were wortmanin (5; 10; 15 and 20nM), staurosporin (0,5; 1; 2 and 5μM) and genistein (50 and 100μM). The actin inhibitor used was cytochalasin D (5 and 10μM). All drugs were added for 20 minutes and right before calcium ionophore treatment. Video sequences were captured with a high resolution CCD camera (Optronics, Goleta CA, US). The sequences were optimized by analogic processing and recorder with a S-VHS VCR. Samples were observed under a Zeiss-Axioplan light microscope using phase contrast system. For field emission scanning electron microscopy the 24hpi ionophore treated cells were fixed in a solution containing 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.2, post fixed for 1h with 1% OsO_4 in 0.1M cacodylate buffer, pH 7.2, plus 0.8% potassium ferrocyanide, dehydrated in ethanol, critical point dried in CO_2, sputtered with gold and observed in Jeol 6340 field emission scanning electron microscope. Of total intracellular parasites, a medium of 75% left the cell under ionophore treatment at 24hpi. This egress, however, was significantly blocked under kinase and actin inhibitors treatment. Supports: CNPq, CAPES, PRONEX/FAPERJ

BC55 - A view over *Toxoplasma gondii* isolated cysts

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Toxoplasma gondii is one major cause of fatal encephalitis in immunocompromised individuals. Despite the key role played by the cyst in the persistence of infection, little is known about its ultrastructure. Few works have shown that the cyst wall is composed by an outer membrane bounding a thick granular structure of carbohydrate nature and an amorphous matrix filling the space among the bradyzoites. To achieve a better comprehension of the biology of this important life stage, we analyzed the structural aspects of cysts isolated from brains of chronically infected mice. The cysts studied in this work varied in size, ranging from approximately 20 μm to 100 μm in diameter. Ultrathin sections showed that the cyst wall was composed of an outer membrane possibly the once parasitophorous vacuole membrane enclosing a thick electron-dense structure of 350 nm formed by a granular material, positively labeled by the lectin DBA. Vesicles of approximately 300 nm containing a membranous material were seen between the outer and the thick inner part of the wall budding from the bradyzoites. The granulosity within these vesicles was very similar to the material present in the cyst wall indicating the delivering of components to the growing cyst. The bradyzoites were surrounded by a dense matrix composed of small vesicles and tubules of 60 nm of diameter. These vesicles are being analyzed by 3D-reconstruction of serial sections in order to verify the similarity with the components of the intravacuolar network found in tachyzoites parasitophorous vacuole. This work was supported by CNPq and Faperj.

BC56 - Progressive stages of intracellular digestion of *Toxoplasma gondii* in presence of hydroxyurea

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During host cell invasion, *Toxoplasma gondii* establishes inside parasitophorous vacuole (PV) where it avoids microbicide process, mainly fusion lysosomes-PV. In this intracellular environment tachyzoites develop and multiply. We have showed that in the presence of hydroxyurea (HU), multiplication of *T. gondii* and other parasites was interrupted and those parasites were eliminated (2). Following intracellular *T.gondii*elimination, PV-acidification and its fusion with lysosomes were observed (2). The present study aims to elucidate the progressive stages of intravacuolar *T.gondii* disruption in the presence of HU. Vero cells were cultivated in Linbro tissue plates that contained a sterile coverslip and maintained at 37^o C and 5% CO2 overnight. Cultures were infected with tachyzoites of *T.gondii*at multiply 5:1 for 8 hours. Hydroxyurea (4mM) was diluted in 199 medium supplemented with 5% SFB and incubated for 1, 2, 3, 4, 5, 8, 12 and 24 hours at 37^{o}C. Cultures were fixed with Bouin solution, and stained with Giemsa and analyzed morphologically analyses by using a Zeiss AXIOPLAN photomicroscope. Infected cells, parasites and PV number were determined. Acidification process was observed using acridine orange (5ug/ml) in 199 medium and observation was realized in laser scanning microscope. Parasites did not divide following HU incubation; however its morphology was preserved up to 4 hours in the presence of HU. After this time, altered

tachyzoites were observed inside some PV. The number of PV containing altered parasite increased during progressive incubation with the drug. PV-acidification was observed after incubation for 5 hours and a progressive elimination of intracellular parasite was observed after this time. Understanding the process elimination of intravacular *T.gondii* is important to clarify some cellular aspects of this interaction.
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BC57 - Inducible nitric oxide synthase degradation pathway in activated macrophages after *T. gondii* infection

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Activated peritoneal macrophages with lipopolysaccharide and Interferon-gamma produce nitric oxide (NO), which is a microbicidal agent. This production is catalyzed by inducible nitric oxide synthase (iNOS), which uses arginine as substrate producing NO and citruline. However, it has been demonstrated that NO production is inhibited by *T. gondii* infection, the agent that causes toxoplasmosis. This inhibition is caused by iNOS disappearance as visualized by immunofluorescence. Two iNOS degradation pathway have been described: the proteasome pathway or the calpaine pathway. This work aims the identification of the iNOS degradation pathway activated after *T. gondii* infection of activated macrophages. For this, J774-A1 macrophages cell line was cultured with DMEM supplemented with FBS 10 percent. The *T. gondii* (RH strain) was maintained in Swiss mice. Macrophages were activated with lipopolysaccharide and Interferon-gamma for 24h, treated with Lactocystine (proteasome inhibitor), or with Calpeptine (calpaine inhibitor) for 2 or 6h, and infected with the parasite. Coverslips were collected after 2 and 6h to assay iNOS expression by immunofluorescence and the supernatant after 6h of interaction was evaluate to determine NO production. iNOS was not visualized in macrophages infected with *T. gondii*. Lactocystine treated macrophages infected with *T. gondii* show iNOS expression. NO production was not inhibited after Lactocystine treatment, confirming the result. Results obtained with Calpeptine were not conclusive. These results, although preliminarily, indicate that *T. gondii* infection activates the proteasome pathway degrading iNOS.
Supported by: FAPERJ, CNPq

BC58 - EXPRESSION OF CYTOSKELETON PROTEINS IN TISSUE CYSTS OF *Toxoplasma gondii.*

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Toxoplasma gondii is an opportunistic pathogen of immunocompromised hosts, been normally assymptomatic with formation of tissue cysts. The composition and biogenesis of cyst wall have not been fully elucidated. This work aims to study the expression of cytoskeleton proteins in tissue cysts of *T. gondii*. C57BL/6 mice were infected with cysts of *T. gondii* ME-49 strain. To analyze the expression of cytoskeleton proteins in cysts were utilized isolated cysts and cryosections of mice brain after 4-8 weeks of infection. The material was fixed and processed for immunofluorescence with antibodies anti-tubulin, MAPs, actin and smooth and skeletal muscle myosins. The anti-tubulin, anti-MAPS and anti-actin antibodies showed intense and homogeneous labeling all over the cyst wall and also in bradyzoites. Confocal microscopy showed that anti-skeletal muscle myosin presented labeling heterogeneous with distribution at focal points all over the cyst wall, in the matrix diffuse and located between the parasites. When compared the label pattern of brain cryosections in relation to isolated tissue cysts, only actin was different. Some cysts in brain cryosections had not label in the cyst wall, while all isolated cyst were positives to actin. Our previous results showed the negativities of the cyst wall, the incorporation and traffic of surface molecules in the interior of cyst of *T. gondii* suggesting that the cyst wall has endocytic activity. The detection of cytoskeleton proteins in the composition of wall cyst and also their presence in the matrix, reinforce the idea of their participation in the dynamic process of the cyst wall. Our model of study can make an important contribution to the knowledge of: (i) biology of tissue cyst; (ii) the processes involved in the nutrients acquisition by cysts and bradyzoites and, (iii) the direct action of drugs on the cyst. Supported by CNPq, PAPES IV/FIOCRUZ& FAPERJ

BC59 - *Toxoplasma gondii* infection blocks the in vitro myogenesis of skeletal muscle cells.

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The apicomplexans, including *Toxoplasma gondii*, are a large and diverse group of parasitic protozoa that essentially lead obligate intracellular lifestyles. We employed mouse pri-

mary culture of skeletal muscle cells (SKMC) as a model for experimental toxoplasmosis studies, which is involved in the chronic phase of the disease, in vivo. Our aim was to analyse the infective capacity of *T. gondii* tachyzoites in fibroblasts, myoblasts/myocytes and myofibres from SKMC during myogenesis and the potential interference of the infection in this process, monitoring the modulation of actin microfilaments with phalloidin-TRICT. Our results showed that after 24 h of interaction, fibroblasts (76%) were more infective than myoblasts (61%) and myotubes (38%), indicating differences in the ability of tachyzoites to invade the cell types presents in the culture. Herein we discuss, among other possibilities, that such infectivity differences could be explained by the fact that myotubes present low expression of adhesion proteins preventing its interaction with the parasite through the secretion of MIC2 from micronemes, responsible by the recognition parasite-host cell. Cultures pre-treated with EDTA presented a low myogenesis percentage, about 5%, with a reduction of 63% when compared with untreated cells. After 24 h of parasite-host cell interaction, in EDTA-treated cultures myotube formation was totally inhibited, while infected myocytes maintained their alignment capacity. These data suggest that the parasites are able to interfere molecularly the host cell, preventing membranes fusion and consequently affecting the myogenesis process. Potential molecules that could be involved in these cellular events are discussed. Supported by CNPq, PAPES IV/FIOCRUZ& FAPERJ.

BC60 - FELINE ENTEROCYTES AS MODEL FOR STUDY OF THE INTERACTION TOXOPLASMA GONDII-HOST CELL, IN VITRO

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Toxoplasma gondii is a protozoan intracellular that parasites vertebrates of warm blood. In the cats and other felines, the sexual reproduction occurs in the intestinal epithelium, being considered as definitive hosts. The complete cycle results in the formation of immature oocystos that are eliminated in feces of the felines. This work aimed to develop an experimental model in vitro for reproduction of the sexual cycle of T. gondii. Primary cultures of feline enterocytes were established by dissociation of cell fragments removed of intestinal epithelium of cats for endoscopic biopsy. The biopsies were washed with decontamination in PBS solution containing penicillin (1000 U/ml), streptomycin (10 mg/ml) and amphotericin B (25 mg/ml) and were dissociated with PBS containing 1 mM EDTA, 1 mM EGTA and 0,5 mM dithiothreitol and antibiotics in the same concentrations. After wash 3x with Ringer, the dissociates cells were suspended in DMEM/Hams - F12 medium plus penicillin (100 U/ml), streptomycin (1 mg/ml), amphotericin B (2,5 g/ml), 10% bovine fetal serum, 1 mM glutamin and 20 ng/ml growth factor EGF. The cells were kept at 37^oC in 5% CO_2. After 48 hours the cells were infected with $1{,}0x10^5$ bradyzoites of T. gondii ME49 strain and fixed for ultrastructural studies after 48, 72 and 96h of interaction. Our preliminary results show the ultrastructural of enterocytes. After 48 hours of interaction of bradyzoites with enterocytes intracellular parasites were observed into parasitophorous vacuole surrounded by mitochondria, endoplasmatic reticulum and lipid droplets. In this time, the morphology of parasites was compatible with tachyzoites forms,by absent of amylopectin granules and rhoptry with low eletrondensity. This study is new and will make possible approaches of the cellular biology of the both cells involved in the infection, aiming to contribute for a better understanding of the enteric cycle of the T. gondii. FAPERJ, PAPES-4, CNPq and IOC-FIOCRUZ.

BC61 - *Plasmodium falciparum* die by a caspase-independent process under chloroquine pressure

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It is well known that nitric oxide (NO) and chloroquine inhibit parasite growth, even though the mechanism of parasite death was never clearly elucidated. In fact, in the studies where *in vitro* parasite culture was used to access cell death in the presence of these compounds, apoptosis could only be identified on the basis of DNA fragmentation using electrophoresis gel or TUNEL assays. Considering the non inflammatory profile of apoptosis, that may represent an escape mechanism for parasite, we decided to evaluate if the *in vitro P. falciparum* death induced by NO, chloroquine or staurosporine - a potent apoptotic inductor on mammalian cells - would be marked by apoptotic events. Firstly we analyzed, by thin blood smears microscopic readings, the capacity of S-nitroso-acetyl-penicillamine (SNAP - a NO producer), chloroquine and staurosporine to inhibit the parasite growth as evaluated in a 24h in vitro culture. Non-treated *P. falciparum* control cultures grew up normally, as expected, but blood forms treated with different concentrations of SNAP, chloroquine and staurosporine presented decreased parasitemia. Flow cytometry analysis of these treated parasites after rhodamine 123 staining showed also a decreased viability of parasites together with a loss of mitochondrial membrane potential. Parasite morphology (assessed by transmission electron microscopy) showing a vacuolization-induced process, as well as the failure of the pretreatment of *P. falciparum* with a general caspase inhibitor to prevent parasite death (as verified by rhodamine staining), lead us to conclude that *P. falciparum* death induced by pharmacological and immune stress is not due to an apoptotic process. This may be true even for parasite death induced by well characterized mammalian cell apoptosis inductor such as staurosporine. The implications of these findings in the immune response to the parasite are under

investigation.

BC62 - *Plasmodium yoelii* sporozoites trickle out of the inoculation site.

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Malaria is responsible for 1 to 2 million deaths annually and remains one of the world's greatest public health challenges. Mammalian hosts are infected when an Anopheline mosquito injects sporozoites in the skin. Then, they exit, enter the blood circulation and go to the liver, where they invade hepatocytes. Although much work has been done to elucidate the mechanisms of hepatocyte invasion, little is known about the interactions between host-parasite before sporozoite enters in the bloodstream. It has been assumed that they rapidly exit the skin, making this interaction brief and difficult to study. We now demonstrated that, contrary to what has been previously thought, the majority of sporozoites spend hours at injection site. Using real time PCR to quantify the number of parasites remaining at the injection site as well as the numbers arriving in the liver, we found that the majority of them take 3 hours to leave the injection site, whether they were injected intradermally by needle or by an infected mosquito. Additionally, the kinetics applied when sporozoites were injected into other inoculation sites. We also performed subinoculation experiments to determine how long the blood of a mouse injected with sporozoites is infectious and our data show that they exit the injection site in a slow trickle rather than a rapid burst. Lastly, we found that approximately 20% of the sporozoite inoculum goes to the draining lymph node after intradermal inoculation and it enlarges significantly compared to intravenously inoculation of parasites. Overall, our data show that *Plasmodium yoelii* sporozoites remain at the inoculation site for hours. Both the prolonged infectivity of sporozoites in the skin and their effect on the draining lymph node are likely to have an impact on the nature of immune response generated against this stage of *Plasmodium* parasite. Supported by NIH and CNPq.

BC63 - GIARDICIDAL EFFECTS OF THE SOLASODINE: A NEW THERAPEUTICAL TOOL

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The flagellated protozoan *Giardia lamblia* inhabits the upper small intestine of humans and is widely prevalent in children, in whom it is a significant cause of diarrhea and malnutrition. The incidence of giardiasis worldwide may be as high as 1000 million cases [1]. The drug of choice for giardiasis is the nitroheterocyclic compound metronidazole. Although generally effective, treatment failures have been reported and drug resistance to all available antigiardial drugs has been observed [2]. The carcinogenic potential of metronidazole was also reported [3]. Thus the search for new giardicidal drugs is required. Natural products may comprise valuable drugs for parasitic disease chemotherapy. Here we tested the influence of the solasodine (isolated from Solanum paludosum), in the growth and ultraestructure of Giardia trophozoites in vitro. 2 x 10^4/mL of *Giardia lamblia* trophozoites (WB strain) were cultured in TYI-S-33 medium supplemented with bovine bile and 10% of bovine serum for 96 hours at 37^{o}C. Every 24 hours, samples were collected for counting at Neubauer chamber and to transmission electron microscopy processing. The IC_{50} of the solasodine is around of 5.7 μM. We observed by TEM that this drug induces some cells to the encystation process. Also, trophozoites presented alterations in membranes surrounding the nuclei, cytoplasmic organization and glycogen particle distribution. These data demonstrate that this compound and or its derivatives may comprise a promising drug for treatment giardiasis.
Supported by CNPq, PROCAD/CAPES, FAPESB.
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[3] Bendesky A et al. Mutat Res., 511: 133-44, 2002 Review.

BC64 - Evaluation of the effects of thioxopyrimidines and thienopyridines on Trichomonadidae

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Trichomonas vaginalis and *Tritrichomonas foetus* are the etiologic agents of trichomoniasis, a sexually transmitted dis-

ease (STD) of worldwide importance. In cows, the disease is associated with infertility, vaginitis, endometritis and abortion. It is the most common nonviral STD in humam and it is associated with many perinatal complications, female genitourinary tract infections and increased HIV transmition (LAGA *et al.*, 1993). Clinical diagnosis is difficult, since the symptoms of trichomoniasis resemble others STDs and microscopy detection methods are often misleading. Since metronidazole, the drug of choice for humam infection is frequently faced with refractory cares and may be carcinogenic (BENDESKY *et al.*, 2002), new trichomonicidal drugs are required and natural products of the Brazilian flora comprise important sources of antimicrobial compounds. There we tested effects of thienol[2,3-*b*] pyridine drugs synthesized in Biochemistry and Molecular Modeling Laboratory and Organic Chemistry Department of Fluminense Federal University (LCP 101, LCP 103, LCP 104, LCP 106, LCP 107 and LCP 109) and hydrophobic drugs which comprise thiol or phenyl groups or resonant rings of the Chemistry Institute of Federal University of Bahia (RMR 63, RMR 72, RMR 78, RMR 80 and RMR 81). Compared to the untreated control, 50μM LCP 107 presented 70% inhibition in the growth of *T. foetus* and 53% of *T. vaginalis*. In screening with drugs codified for RMR the RMR 78 showed higher microbicidal activity towards *T. foetus*. Ultrastructure analysis is in progress and may elucidating the molecular mechanisms responsible for trichomonicidal effects. Acknowledgments: FIOCRUZ, CNPq, CAPES and FAPESB supported this work.

BC65 - Cellular death in *Tritrichomonas foetus* after treatments with $AlPcS_4$ and Photodynamic Therapy

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The *Tritrichomonas foetus* is as amitochondrial parasitic protist which causes a major sexually transmitted disease in cattle, bovine trichomoniasis. No effective drugs are currently approved for this disease. Photodynamic therapy is an experimental treatment which shows great potential for the treatment of bacterial, fungi, yeasts, and viruses. However, the citotoxic effect of the PDT in protozoa has been poorly studied. In this study, PDT with photosensitizer aluminum phthalocyanine tetrasulfonated ($AlPcS_4$) was efficient for killed *T. foetus*. We investigated the mode of cell death in *T. foetus* after PDT by transmission electron microscopy. Morphological changes were observed such membrane projections, resembling apoptotic blebs, nucleus fragmentation with peripheral masses of heterochromatin, endoplasmic reticulum proliferation, intense cytoplasmic vacuolization, myelin-like figure, fragmented axostyle-pelta complex and internalized flagella. This is the first report to demonstrate cell death in *T. foetus* after PDT therefore will open up new lines of investigation into developments of new treatments for bovine trichomoniasis.

BC66 - ADHESION OF *TRITRICHOMONAS FOETUS* TO IMMOBILIZED LAMININ-1 IS MEDIATED BY LAMININ BINDING PROTEINS (LBP) AND RESULTS IN MODULATIONS OF THE PARASITE FUNCTION.

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During the invasion of the urogenital tract *Tritrichomonas foetus* interacts with extracellular matrix (ECM) components present in basal membrane and subjacent tissues. Such interaction is important for the modulation of a series of biological processes. ECM may determine parasite preferred niches, as well as serve as roadmaps into hosts. Data available on the interaction between parasitic protozoa and ECM underlie the existence of signal exchanges from the ECM to parasites and from parasites to ECM. Laminin-1(LMN-1) is the major glycoprotein present in basal membrane and many parasites are able to identify and interact with it. Here, we designed experiments looking for a biochemical and biological characterization of the interaction of LMN-1 with the extracellular parasite *T. foetus*. Adhesion assay demonstrated affinity of *T. foetus* adhesion on LMN-1 biofilm. Dot blot and immunoblotting analyses were made with *T. foetus* extracts with and without pre -treatment with trypsin and periodate, in order to identify laminin binding molecules present at *T. foetus* surface. Immunobloting analyses demonstrated the presence of 5 LBPs in parasite extracts (230, 200, 150, 125, 90KDa), which probably work together at LMN-1 recognition by the parasite. This is reinforced by the *T. foetus* ability to recognize multiple domains of LMN-1. Presence of divalent cations (Ca^{+2}, Mn^{+2}, Mg^{+2}) is important to adhesion processes, which suggests participation of some integrin-like protein. In order to understand the importance of the LMN binding to the pathogenic process scanning electron microscopy, gelatin zymography and cytotoxicity tests were made in the presence or absence of LMN-1, demonstrating the modulation of the parasite function. Our results demonstrate that *T. foetus* is able to recognize laminin-1 and that recognition is made by an array of laminin binding proteins. Supported by CAPES, CNPq, FAPERJ, FUJB-UFRJ and MCT-PRONEX.

BC67 - Effect of kinases inhibitors in growth and encystation of Giardia lamblia

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Giardia lamblia is a world spread protozoan parasite of mammals. It is the causative agent of giardiasis, a waterborne disease which symptoms include diarrhea and malabsorption. The trophozoite - binucleated flagellated form - colonizes the

upper small intestine and the encystation process gives rise to the cyst - the infective form. Although crucial to parasite survival outside the host, the formation of the cyst is not yet fully understood and little is known about the signaling pathways involving this process. To better understand how kinases could be involved in the encystation process we used 5 micromolar Wortmanin, 100 micromolar Genistein and 2 micromolar Staurosporine (respectively PI3 kinase, tyrosine kinase and kinase C protein inhibitors) immediately after inducing parasites to encyst. Wortmanin inhibited the completion of encystation by 90 percent, although it has not shown a great effect on the parasite growth. Staurosporine did not affect growth or encystation. Genistein inhibited the parasite growth, and lowered the encystation rate by 42 percent. PI3 kinase seems to be involved in the signaling pathway that leads to the formation of cyst while tyrosine kinase appears to be involved in the parasite multiplication and growth as well. The next step of this work will be the observation of ultrastructural changes in cysts and cells in the encystation process treated with these kinase inhibitors.

BC68 - Structural and functional characterization of acidocalcisomes in *Euglena gracilis*

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An unusual characteristic of protozoan parasites such as *Trypanosoma* and *Toxoplasma* is the presence of acidic calcium-rich organelles named acidocalcisome. Different functions have been attributed to these organelles such as the storage of high energy compounds, calcium and other cations, pH homeostasis and osmoregulation. From the biochemical point of view, some adidocalcisomal components such as the vacuolar proton pyrophosphatase (V-H+-PPase), possess plant-like characteristics. *Euglena gracilis* is a freshwater flagellate that presents plant and animal-like characteristics. When *E. gracilis* is cultivated in absence of light, it reduces its chloroplasts to proplastids, whereas when cells enter in contact with light, the proplastids enlarge, sintetize chlorophyll and reinitiate photosynthesis. For this reason, *Euglena* has been considered a representative of intermediary organisms between plants and protozoa. In this work, the morphological and physiological characteristics of acidocalcisomes in *E. gracilis* are described. Immunofluorescence and immunocytochemistry using monoclonal antibodies raised against the V-H+-PPase in cells cultivated in presence and absence of light showed localization of the antigen in internal vesicles distributed preferentially located in the region near to the contractile vacuole. To verify whether these internal vesicles were acidic, cells cultivated in absence of light were incubated with Lysotracker, a weak base that accumulates in acidic compartments. We observed labeling of different vacuoles distributed all over the cell body as well as in the vesicles located in the region near the contractile vacuole, as observed in the immunolocalization experiments. These results show that *E. gracilis* presents acidic compartments which presumably are acidified by a V-H+-PPase with similar characteristics to the acidocalcisomes. Altogether, the results suggest the presence of acidocalcisomes in *Euglena*. The functional role of these organelles and determination of their elemental composition in *E. gracilis* are currently under investigation in our laboratory. **Finantial support:** CNPq, FAPERJ, NIH and Programa de Nanociência e Nanotecnologia to K.M.

BC69 - Interaction of *Acanthamoeba* spp. to extracellular matrix glycoproteins: binding characterization and role played on cytotoxicity and amoebic invasion.

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Acanthamoeba are free-living amoebae widespread on nature. In special situations, *Acanthamoeba* can cause severe human infections, as keratitis, dermatitis, encephalitis and other disseminated pathologies. The mode by which these amoebae cause cytopathogenesis is not entirely known, but it is documented that amoebae adhere and destroy mammalian cells. After that, it has been postulated that amoebae gain access to components of the extracellular matrix (ECM), invading it. Knowing that ECM binding could direct host tissue invasion, the aim of this work was to characterize association of *Acanthamoeba* on type I collagen, fibronectin and laminin-1, and also determine the nature of this association and the role played by ECM on amoebic cytotoxicity and tissue invasion. To quantify these interactions, we performed binding assays using 3H-thymidine-labelled amoebae or staining procedures. We observed that amoebae are able to attach to ECM molecules with different avidity (binding on $laminin = collagen > fibronectin$) and different species of *Acanthamoeba* exert differential specific binding to ECM glycoproteins, with clear differences on ECM recognition between pathogenic and non-pathogenic species. Morphologically, by scanning electron microscopy, we observed that collagen and laminin-attached amoebae presented spread shape on these substrata. We also determined that binding on fibronectin and laminin was increased when divalent cations were added on the binding assay medium, and EDTA was able to diminish amoebic association on all ECM substrata tested. We also tested the integrin-recognition peptide RGD and a monoclonal antibody against human beta-1 integrin and no binding inhibition was observed in both conditions. Looking at the effect of ECM binding on cytotoxicity, no differences regarding the ability of *Acanthamoeba* to kill Vero

cells were observed. We determined that *Acanthamoeba* invade collagen matrices better than matrigel matrix. Overall, those results show that *Acanthamoeba* is able to differentially recognize and migrate on ECM. Acknowledgements: PDEE-CAPES/MEC, CNPq, FUJB-UFRJ, MCT-PRONEX and NIH.

BC70 - Record and distribution of the peritrich epibiont Rhabdostyla (Ciliophora, Peritrichia, Epistylidae) on limnic oligochaetes (Annelida, Oligochaeta) in an urban stream

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Epibiosis is a facultative association of two organisms: the epibiont and the basibiont. The term "epibiont"includes organisms that are attached to the surface of a living substratum during the sessile phase of their cycle, while the basibiont provides support for the epibiont. Peritrich Protozoa of the genus *Rhabbdostyla*Kent, 1880 are common constitutes of freshwater ecosystems and attached surfaces of invertebrate metazoa such as Crustacea, Oligochaeta, and Insecta. The present work record the occurrence of the epibiont ciliate *Rhabbdostyla*on some species of oligochaetes and analyses the distribution of this ciliate on the surface of the worms. In order to get oligochaetes infested with ciliates collections of the sediment had been carried in May 2006 at five points along the São Pedro stream located in the southwest part of the urban area of Juiz de Fora, MG, Brazil. Points 1 and 2 was located in a rural region that receives a low sewage load, while Points 3, 4 and 5 were located in populated regions receiving high sewage loads. The peritrich epibiont ciliates of the genus *Rhabbdostyla* were found on oligochaetes only in points 3, 4 and 5. The ciliates were record on Tubiffecidae: *Limnodrilus hoffmeisteri*and Naididae: *Dero borellii*, *Dero digitata*, *Dero sawayai*,*Pristina americana* and *Pristina leidyi*. All of them are new registers of the genus *Rhabbdostyla* on these worms. Most *Rhabbdostyla* were generally distributed on the posterior part of individual worms, although the distribution patterns of the ciliates varied between the worms of the species *L. hoffmeisteri*. On the naidids the distinct distribution pattern was evident for the peritrichs and was presumably based on their ability to obtain food and/or oxygen epibiont. The restriction on the posterior part of the worm may expose more adequately the epibionts to optimal water currents that provide oxygen and food.

BC71 - Influence of bacteria in *Entamoeba histolytica* virulence

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The interaction of *Entamoeba histolytica* and bacteria many times result in increasing of virulence of amoebae. Nevertheless, the mechanism that involves the increasing of of virulence is not well known. In order to clarify this subject we have studied one strain of *E. histolytica* that was kept with their original flora (xenic), in axenization process with *Crithidia fasciculata* (monoxenic) and in axenic condition. This strain was genotyped and evaluated to virulence by inoculation into hamsters liver. For genotyping was used 2 polymorphic loci used to detect inter-strain polymorphism. Hamsters were inoculated intra-hepatically with 100,000 trophozoites, respectively of axenic, xenic and monoxenic strain of *E. histolytica*. The animals were necropsied six days after infection; liver fragments were processed and stained with H&E. The lesions were measured in the Carl Zeiss Image Analiser. The hamster inoculated with the axenic and monoxenic strain developed chronic granulomatous inflammation and moderate tissue destruction. The animals inoculated with xenic strain showed almost total destruction of the hepatic tissue, without granulomas formation. Axenic and monoxenic strain showed similar profile while xenic strain showed different one. These results confirm the significant contribution of bacteria to amoebae virulence and suggest that bacteria can modulate genetically the virulence. Supported by FAPEMIG

BC72 - In vitro effect of lectin Gal/Gal/Nac on Entamoeba histolytica and Entamoeba dispar trophozoites

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Amoebiasis pathogenesis is an event that involves different amoebae functions which results in the death of the cell-target. Cell adherence seems to be the first step for amoebae invades a tissue. The lectin Galactose/N-acetyl-Galactosamine (Gal/GalNac) is involved in this process in E. histolytica which promotes the necessary contact for beginning the destruction of the tissue. Some Gal/GalNac epitopes are present in E. dispar. As galactose inhibits the lectin we decide to evaluate its effect on polixenic cultures of E. histolytica and E. dispar, strains EGG and MCR respectively. The strains were incubated with galactose at the concentration of 180 and 200 mM, 37^o C, for 2 and 3 hours. After incubation it was observed that there was an alteration in the viability for both strains and the amoebas were round and immovable. Two percent of them were dead after 2 hours of incubation in both concentration of galactose. After 3 hours

of incubation in 180 mM galactose, 42% of the trophozoites of E. dispar and 3% of the E. histolytica were dead. In addition, the viability of E. dispar trophozoites suffered a drastic reduction when 200 mM galactose were associated with it, and 63% of them were dead against 7% of E. histolytica. In this way, these results suggest that in addition of being involved in the tissue-amoeba adherence Gal/GalNac is able to protect amoeba, mainly E. dispar, probably by exhibiting important function in the amoeba environment relationship.

BC73 - Salivary glands of *Aedes aegypti* infected by the dengue virus (DENV-2)

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There are several barriers for the *Ae. Aegypti* to become a competent vector of the dengue virus. The last one is the salivary gland that will store the virus for the entire life of the vector. In order to infect the gland, the virus needs to cross the basal membrane, infect the secretory cells, escape to the secretory cavity where the saliva is, and finally, reach the secretory duct during the mosquito bite. With the purpose of following the virus invasion, we infected mosquitoes through a glass feeder with blood mixed with the virus (DENV2). Twelve-days after, the mosquitoes were dissected and the salivary glands removed and fixed in 4% paraformaldehyde in PBS at pH 7.2. The entire samples were immuno-labelled for dengue virus visualization as the following procedure: (a) three times washed with PBS; (b) incubated with RPMI medium; (c) incubated with PBS/BSA; and finally, (d) incubated with a anti-dengue polyclonal antibody and washed again in PBS/BSA. Subsequently, the samples were labeled with a secondary anti-mouse antibody (FITC) and Topro-3 (Rodamine), as a nuclear marker. In the end, the samples were mounted in a glass slide with Mowiol in order to avoid the fluorescence fading. The labeled samples were observed in a laser confocal microscope (LCM). The analyses of the entire labeled salivary glands shows that is possible to visualize details of the organ under the LCM, including the location of the dengue virus. Our results showed that the dengue virus completes its life cycle inside the colonized mosquitoes. According our knowledge, this is the first time that dengue virus are observed in the mosquito salivary gland. New experiments are in process for describing details of the virus invasion. Financial Support: Fapemig, Capes, Fiocruz and Pronex

BC74 - Involvement of platelet-activating factor receptor and protein kinase CK2 in lysophosphatidylcholine-induced cellular differentiation of *Herpetomonas samuelpessoai*

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Herpetomonas samuelpessoai is a trypanosomatid parasite of the insect *Zelus leucogramus*. Lysophosphatidylcholine (LPC) is a major bioactive compound of plasmatic lipoproteins like LDL. The presence of LPC in the saliva of *Rhodnius prolixus* and its anti-hemostatic activities have been demonstrated. Platelet-activating factor (PAF) is a phospholipid with potent and diverse physiological and pathophysiological actions. PAF is known as a powerful inducer of cell differentiation in *Herpetomonas muscarum muscarum* and *Trypanosoma cruzi*. The enzyme phospholipase A2 (PLA2) catalyzes the hydrolysis of the 2-ester bond of 3-sn-phosphoglyceride, transforming phosphatidylcholine (PC) into LPC. We have shown that LPC modulates some signaling pathways that lead to cell differentiation of *H. samuelpessoai* and that PLA2 probably rules this process by converting PC into LPC. Intriguingly, some of LPC actions are dependent on PAF receptor. The present study intends to confirm the involvement of protein kinase CK2, as well as to test the possible participation of PAF receptor and G protein in this process. *H. samuelpessoai* parasites were grown for up to 96 hours in Roitman complex medium, in the absence or in the presence of PC, LPC, G protein inhibitor (pertussis toxin), PAF receptor antagonist (WEB 2086), and CK2 inhibitors (DRB and TBB). The percentage of non-differentiated (promastigote) and differentiated forms (paramastigote and opisthomastigote) were daily determined by Giemsa stained preparations. The best results were obtained at 48 hours of growth: control (60% promastigotes, 40% differentiated); LPC (40% promastigotes, 60% differentiated); DRB + LPC (70% promastigotes, 30% differentiated); TBB + LPC (60% promastigotes, 40% differentiated); WEB + LPC (62% promastigotes, 38% differentiated); pertussis toxin + LPC (55% promastigotes, 45% differentiated). These results highly suggest the involvement of PAF receptor, but not G protein, in cell differentiation of *H. samuelpessoai* triggered by LPC. Supported by: CNPq, FAPERJ, CNPq/PIBIC-UFRJ, CAPES.

BC75 - Recognition of laminin-1 by *Leishmania leishmania amazonensis* parasites: the presence of laminin binding protein

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Leishmaniasis is an important disease in widely spread in the world which *Leishmania leishmania amazonensis* is the causative agent of cutaneous form of the disease. The extracellular route of promastigotes and amastigotes infection

forms is essential for the establishment and maintenance of the infection where host ECM will have a key role on pathogenesis. Recent published data demonstrated that Leishmania alters the extracellular matrix in footpad lesions in mice. ECM may determine parasite's preferred niche as well as to serve as roadmaps into hosts. Data available on the interaction between parasitic protozoa and ECM underlie the existence of signal exchanges from the ECM to parasites and from parasites to ECM. Laminin-1(LMN-1) is the major glycoprotein present in basal membrane and many parasites are able to recognize and interact with it. Here, we designed experiments looking for a biochemical approach concerning the interaction of LMN-1 with the each one of extracellular (promastigotes) and intracellular (amastigotes) forms of *L. amazonensis*. Adhesion assays demonstrated that promastigotes and amastigotes forms are able to associate to LMN-1 biofilm. Interestingly, axenic amastigotes seems to be less able to recognize LMN-1 than amastigotes derived form BALB/c footpad lesions. This result suggests the importance of membrane alterations during vacuolar contact of amastigotes with the extracellular matrix adhesion process. In order to identify the presence of *L. amazonensis* laminin binding molecules, dot blot analyses were made with both forms and parasite extracts were treated with and without trypsin and periodate. Such result demonstrated the presence of laminin binding proteins in promastigotes and amastigotes forms. To identify and quantify those laminin binding proteins, immunoprecipitation assay is currently under investigation. Supported by CAPES, CNPq, FAPERJ, FUJB-UFRJ and MCT-PRONEX.

BC76 - The purinergic receptor ($P2X_7R$) plays a role in macrophage phagocytosis and may be involved in the establishment of *L. amazonensis* infection.

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$P2X_7$ receptor ($P2X_7R$) is a purinergic receptor that when exposed to $\geq$ 1mM of ATP, leads to the opening of 900Da pore in the surface of the cell. Its action on the inhibition of intracellular growth of pathogens, such as, *Chlamydia trachomatis* and *Mycobacterium tuberculosis* was previously described. Our group demonstrated that intralesional treatment with ATP control the parasite load in mice infected with *Leishmania amazonensis*-GFP. In this work we used C57Bl/6 $P2X_7$ receptor knockout mice ($P2X_7R$ -/-) and wild-type (WT) to evaluate the role of this receptor at leishmaniasis. To assay its role in phagocytosis, macrophages from $P2X_7R$ -/- and WT mice were allowed to infest FITC-beads for 2h under gentle. The cells were then analyzed by FACS. $P2X_7R$ -/- macrophages displayed lower fluorescence than WT controls (63,2±3,37 and 82,73±3,25 Fluorescence Unit (FU), respectively). To investigate the capacity of internalization of leishmania by these cells, macrophages from WT and $P2X_7R$ -/- mice ($5x10^6$/ml) were plated in 24-well plate and allowed to rest for 48h or 0h at 37^oC and 5% CO_2. Then, they were infected with promastigotes of *L. amazonensis*-GFP (5:1) for 4h. We observed that after the 4 hour of infection, fresh plated macrophage from WT and fresh and 48h plated macrophage from P2X7R -/- mice had fewer internalized parasites 6444±320, 5241±311 ; and 5008±513 Fluorescence Unit (FU), respectively compared to 9427±334 FU observed on WT macrophage infected 48h after plated. To compare the susceptibility to infection by GFP-promastigotes, the cells were rested for 24h and infected as described above. After additional 48h of incubation, the culture fluorescence was assessed by plate fluorimeter, showing that $P2X_7R$ -/- macrophages were less infected (4286,6±356,5 FU) than WT controls (5781,5±377,4 FU). These results suggest that $P2X_7R$ may favor parasite internalization and play a role in the establishment of leishmaniasis. Financial support: CAPES.

BC77 - THE EFFECT OF PHOSPHOLIPASE A2 ISOLATED FROM THE *CROTALUS DURISSUS COLLILINEATUS* VENOM ON MACROPHAGE INFECTED WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS.*

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There are few studies concerning to the importance of phospholipase A2 (PLA2) in infections by *Leishmania* sp. In some protozoal parasites infections, the PLA2 participate of cell invasion, however some reports suggest its protective effect against some protozoal parasites illness. Thus we evaluated the effect of PLA2 and crotapotin (CRP) from *Crotalus durissus collilineatus* venom on macrophage infected with *Leishmania (Leishmania) amazonensis*. The PLA2 and CRP were isolated according Toyama et al (2005). Mouse peritoneal macrophages were platting in a concentration of 10^5 macrophage/well in a 96 wells plate following to infection for promastigote forms in stationary phase (about to 10 promastigotes/macrophage). After 24 hours the infected macrophages were incubated with PLA2 or CRP at 1.0 to 32.0 μg/mL for 24 hours, following this time, ELISA *in situ* (according Piazza et al, 1994) was performed to quantify the parasites within macrophages. The enzimatic activity from promastigote lysates and supernatant were measured to specific substrate (4-nitro-3-octanoyloxy-benzoic acid). The citotoxicity of PLA2 and CRP was carried through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) under the same concentrations used for ELISA *in situ*. Both PLA2 and CRP did not exhibit any cytotoxic effect for macrophages. Infected macrophages treated with PLA2 induced a significant increase of the infection by dose-

dependent manner and CRP did not affect the progress of infection. We also observed a similar effect for epimastigotes of *Trypanosoma cruzi*. Using a specific substrate, we observed that the supernatant of *L.(L.)amazonensis* showed more PLA2 activity than that of *C.d.collilineatus* PLA2. This effect caused by PLA2, was most likely, inducing the mobilization of eicosanoids into prostaglandin E2. Therefore secretory PLA2 is an active enzyme in the mobilization of eicosanoids. The results obtained suggest the involvement of PLA2 as important factor for cutaneous leishmaniasis progress.
Supported by FAPESP

BC78 - AUTOPHAGY INFLUENCE *LEISHMANIA* UPTAKE AND SURVIVAL IN MACROPHAGES

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Recently, the capacity of autophagy to interfere on the intracellular survival of pathogenic microorganisms during infection has been published. Little is known about the influence of this process in *Leishmania* infection. *In vitro*, CBA/J mouse macrophage can destroy *L. major* and are unable to eliminate *L. amazonensis*. Herein we analyzed the influence of autophagy in CBA/J mouse macrophage infection with *L. amazonensis* or *L. major* and demonstrated that post-infection induction of autophagy by amino acid deprivation (physiological induction of autophagy) reduce both *Leishmania* infection of macrophages. Furthermore, previous autophagy was induced to evaluate its influence in phagocytic capacity of macrophages. It was observed a decrease in the percentage of *L. amazonensis*-infection at 15, 30 and 60 minutes when compared to control cells incubated under full nutrient conditions. We also demonstrated that phagocytosis reduction induced by autophagy in murine macrophages is not a specific mechanism, since the phagocytosis of other particles, such as yeast and zymosan, were also decreased. Reduction of phagocytosis is a reversible mechanism since nutrient reposition recovers the phagocytic capacity of these cells. To verify whether the alteration on the phagocytic capacity of macrophages was related to the amino acid deprivation, we analyzed the effect of the pharmacological induced autophagy. Rapamycin, a drug that inhibit Tor kinase activity, was used to induce autophagy under full nutrient conditions. The results obtained in these assays were similar to those on physiological induced autophagy. The data described herein show that physiological or pharmacological induction of autophagy promotes a decrease in the survival and phagocytic capacity of murine macrophages. Moreover, it was demonstrated that Tor kinase may participate in the phagocytic process. These findings suggest a link between the early stages of phagocytosis and the autophagy pathway with Tor as a possible molecule that promotes this communication.

BC79 - Standardization of CNS glia cell cultures to study the microglia-*Leishmania major* interaction

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Introduction: Leishmania are obligatory intracellular parasites that must enter mononuclear phagocytes to complete their cell cycle. Macrophages are the main parasitized cell. In the CNS, microglia has an important role in host defense, with functions similar to macrophages. There are some evidence of parasite and anti- Leishmania antibodies in the nervous system of Leishmania infantum infected dogs. Also, there is one human study that shows parasitism in cerebroespinhal °uid of a 10 years old child. The objective of this work is to establish glia cell cultures from newborn susceptible (Balb/c) and resistant (C57BL/6) mice to study microglia- Leishmania major interactions. Methods: Newborn forebrains were obtained from both mouse strains. The cerebral cortex was enzimatically and mechanically dissociated and isolated cells in a ¯nal concentration of 106 cells/well were cultured on 24 well plates in 10% Fetal Bovine Serum/Minimal Essential Medium. The cultures were maintained at 37o C in humidi¯ed 5% CO2 incubator from 5 to 12 days. The cultures were washed 24 hours post plating and every 48 hours during entire experimental period. In parallel experiments, cultures maintained for 6-7 days were infected with Leishmania major in parasite to cell ratio of 10 to 1. At 0, 2, 6 and 24 hours p.i. the cultures were ¯xed and Giemsa stained. Slides were analised under optic microscope to study the parasite-cell interaction. Images of GFAP and F4/80 immuno°uorescence were captured for documentation. Results and Conclusions: Balb/c and C57BL/6 glia cell cultures have kept their morphological characteristics during entire experimental period, indicating success in culture standardization and maintenance. We identi¯ed two cell types in our cultures. Most cells presented astrocyte morphological features and GFAP immunopositivity. Microglia F4/80 immunopositive cells were also identi¯ed upon astrocytes. Leishmania major infection of cultures showed adhered or internalizated parasites. Further experiments are needed for quantitative analysis.Support:FAPEMIG(CBB-1048/05),CAPES and CNPq.

BC80 - Mast cell evaluation in low dose *Leishmania major*ear infection in Balb/c and C57BL/6 mice

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Introduction and objectives: Cutaneous leishmaniasis can be experimentally induced by *Leishmania major* infection in different mice strains. Balb/c mice develop severe lesions and exhibit progressive and lethal systemic disease, which is correlated to Th2 response. However, C57BL/6 mice show spontaneously healing small lesions and Th1 response. The usual mouse model employes promastigote high dose infection 10^5 -10^7) and inoculation into subcutaneous sites. The natural model of *Leishmania major*infection (Belkaid et al.,2000) combines two features of natural transmission: low dose infection (100-1000 metacyclic promastigotes) and inoculation into ear derm. Mast cells are source of cytokines that may influence host response to *Leishmania*, but their roles during infection remain unknown. A comparative study between susceptible and resistance mouse strains was carried out to elucidate the role of mast cells in the natural model of infection. Methods: Balb/c and C57BL/6 mice were infected i.d. with 1000 *Leishmania major* parasites into the right ear. The mice were euthanized 4,5; 6,5; 10 and 12 weeks p.i.. Infected and control ears were processed and stained by Giemsa. Images were measured for ear area and mast cell number using the KS300 program (Zeiss). Mast cell density was expressed as number of mast cells/mm2. Results: Balb/c or C57BL/6 mice did not show significant difference in mast cell density among different times p.i.. However, infected Balb/c mice showed decreased mast cell density compared to infected C57BL/6. Some of our preliminary data indicated that mast cell density was not modified in C57BL/6 infected animals compared to controls. However, mast cell density in Balb/c infected animals was lower than in controls. Conclusions: Our data show decreased mast cell density in infected BALB/c mice along 12 weeks of infection when compared to C57BL/6 mice. We intend to study ealier time-points and other inflammatory cells. Financial support by FAPEMIG(CBB-1048/05), CAPES and CNPq.

BC81 - CD4 AND CD8 T LYMPHOCYTE PROFILES IN PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS CAUSED BY SPECIES OF SUBGENUS *VIANNIA*.

Lima, A.C.S. *(Faculdade de Medicina da Universidade de São Paulo)*; Gomes, C.M.C. *(Faculdade de Medicina da Universidade de São Paulo)*; Silveira, F.T. *(Instituto Evandro Chagas-Pará)*; Gama, M.E.A. *(Universidade Federal do Maranhão)*; Laurenti, M.D. *(Faculdade de Medicina da Universidade de São Paulo)*; Fernezlian, S.M. *(Faculdade de Medicina da Universidade de São Paulo)*; Tomokane, T.Y. *(Faculdade de Medicina da Universidade de São Paulo)*; Corbett, C.E.P. *(Faculdade de Medicina da Universidade de São Paulo)*

The T lymphocytes (LT) are the main effector cells in the cellular immune response against intracellular pathogens, which have a CD3 receptor on their surface. They are divided into two subpopulations, CD4 T lymphocytes and CD8 T lymphocytes. Depending of the subpopulation of CD4 T lymphocytes and of the *Leishmania* antigen, the host can manifest resistance or development of the infection. The aim of this project was to characterize the phenotypic profile of $CD4^+$ T cells and $CD8^+$ T cells which were involved in immune cellular response of patients American Cutaneous Leishmaniasis (ACL) caused by species of subgenus *Viannia*. Skin biopsies of 22 patients with ACL were submitted to immunohistochemistry using monoclonal antibodies-clone F7.2.38/DAKO, OPD4/DAKO and C8/144B/DAKO, respective for CD3, CD4 and CD8. The immunolabelled cells were counted with the help of image analysis system (Zeiss). Cell densities were calculated and the mean for each cell population across patients was derived. The density of $CD3^+$ T cells was 3653 cells/mm^2, while the density of $CD8^+$ cells was 2561 and $CD4^+$ T cells was 1268, showing that there was a higher proportion of $CD8^+$ T cell relative to $CD4^+$ T cell lymphocytes in patients with lesions caused by the species of *Viannia* group, these finding may be related to the localized form. Supported by: LIM-50/FMUSP, FAPESP

BC82 - STIMULATION OF GROWTH CONCOMITANT WITH DECREASED INFECTIVITY OF *Leishmania amazonensis* BY ATP.

Figliuolo, V.R. *(Universidade Federal do Rio de Janeiro)*; Chaves, S.P. *(Universidade Federal do Rio de Janeiro)*; Torres-Santos, E.C. *(Universidade Federal do Rio de Janeiro)*; Coutinho-Silva, R. *(Universidade Federal do Rio de Janeiro)*; Rossi-Bergmann, B. *(Universidade Federal do Rio de Janeiro)*

P2XR are receptors belonging to the family of purigenic receptors, that are activated by ATP, and largelly expressed in the cells of the immune system. One of their members, the $P2X_7R$, when activated by $\geq$ 1mM ATP characteristically induces the opening of pores in the cell membrane that allow the entrance of $\leq$ 900 Da sized molecules into the cell. $P2X_7R$ seems to play a role in diseases caused by intracellular microorganisms, such as *Mycobacterium tuberculosis* and *Chlamydia trachomatis* . We have been studing its role in leishmaniasis. Our preliminary results indicate

that incubation of *Leishmania amazonensis* promastigotes for 72h at 27°C with increasing concentrations of ATP leads to increased parasite growth. On the other hand, when promastigotes were treated with ATP (1mM) for 10 min at 34°C prior to infection of peritoneal macrophages, a significant decrease in the parasite load was observed when compared to untreated parasites, or to parasites treated with ATP only during infection. Therefore ATP appears to decrease the infectivity of *Leishmania amazonensis* promastigotes , and at the same time promote their growth. Once leishmania promastigotes have surface ecto-ATPases, the contribution of adenosine binding should also be considered.

BC83 - *Leishmania (V.) braziliensis* Strains Isolated From Different Clinical Cases: Phosphatidylserine exposure and interaction with Murine Peritoneal Macrophages.

Farias, L.H.S.F *(Universidade Federal do Pará)*; Rodrigues, A.P.D.R *(Universidade Federal do Pará)*; Saraiva, E.M.S *(Universidade Federal do Rio de Janeiro)*; Seabra, S.H.S *(Universidade Estadual da Zona Oeste)*; daMatta, R.A.D. *(Universidade Norte Fluminense)*; Silveira, F.T.S. *(Instituto Evandro Chagas)*; Silva, E.O.S *(Universidade Federal do Pará)*

The protozoan of *Leishmania* genus is responsible for different forms of leishmaniasis, a disease spread worldwide. In the Amazonian region of Brazil, seven species of leishmania infect man. The clinical profile of these species can vary from a single cutaneous lesion until mucocutaneous lesion. Leishmania is an intracellular protozoan which lives and multiply in mammalian macrophages and display different mechanisms to evade the microbicidal response of host cells. The inhibition of nitric oxide [NO] and superoxides radical production seams to constitute two of the main microbicidal mechanisms of host cell. In this study we analyse the microbicidal response of murine macrophages infected with two different strains of *Leishmania (Viannia) Braziliensis*, M15970 [mucocutaneous] and M17593 [tegumentar]. The results showed that, after 24h of interaction, both strains were not able to inhibit the superoxides radical production. Moreover, we observed that the levels of NO production by macrophages infected for 24h with M15970 strain, without or with stimulation [100 ng of lipopolysacharide and 1μgof interferon gamma], were significantly higher if compared with M17593 strain. Thus, these preliminaries data revealed that strains of the same specie of Leishmania isolated from different clinical cases exhibit distinct responses in some situations and that this characteristic could be related with the survival of the parasite in the host cell, immune response of the host and the persistence of infection. Supported by: PIBIC-UFPA; CNPQ-PADCT; FUNTEC/MS.

BC84 - Microbicidal response of host cell infected with two isolates of *Leishmania (Viannia) braziliensis* from human patients with different clinical manifestations.

Nascimento, D.B.P.N. *(Universidade Federal do Pará)*; Farias, L.H.S *(Universidade Federal do Pará)*; Rodrigues, A.P.D. *(Universidade Federal do Pará)*; Silveira, F.T. *(Instituto Evandro Chagas)*; Silva, E.O. *(Universidade Federal do Pará)*

The protozoan of *Leishmania* genus is responsible for different forms of leishmaniasis, a disease spread worldwide. In the Amazonian region of Brazil, seven species of leishmania infect man. The clinical profile of these species can vary from a single cutaneous lesion until mucocutaneous lesion. Leishmania is an intracellular protozoan which lives and multiply in mammalian macrophages and display different mechanisms to evade the microbicidal response of host cells. The inhibition of nitric oxide (NO) and superoxides radical production seams to constitute two of the main microbicidal mechanisms of host cell. In this study we analyse the microbicidal response of murine macrophages infected with two different strains of *Leishmania (Viannia) braziliensis*, M15970 (mucocutaneous) and M17593 (tegumentar). The results showed that, after 24h of interaction, both strains were not able to inhibit the superoxides radical production. Moreover, we observed that the levels of NO production by macrophages infected for 24h with M15970 strain, without or with stimulation (100 ng of lipopolysacharide and 1μg/ml of interferon-γ), were significantly higher if compared with M17593 strain. Thus, these preliminaries data revealed that strains of the same specie of Leishmania isolated from different clinical cases exhibit distinct responses in some situations and that this characteristic could be related with the survival of the parasite in the host cell, immune response of the host and the persistence of infection.
Supported by: PIBIC-UFPA; CNPQ-PADCT; FUNTEC/MS.

BC85 - Leishmania major PROMASTIGOTES INHIBIT iNOS AND NITRIC OXIDE PRODUCTION IN MOUSE PERITONEAL NEUTROPHILS

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Introduction and Objectives: C57BL/6 and BALB/c are two mouse strains that differ significantly in their susceptibility to *Leishmania* parasites. In this study, we compare the nitric oxide (NO) and iNOS enzyme production by C57BL/6 and BALB/c peritoneal neutrophils, in the presence or absence of *Leishmania major* promastigotes. Methods: Peritoneal neutrophils of both mouse strains were isolated by Ficoll-Paque gradient 4 h after thioglycolate 3% i.p. injection. Neutrophils purity was about 99%. 1.5 x 10^6 neutrophils were cultured in RPMI/10% FBS supplemented with LPS and IFN-gamma

in the absence or in the presence of *Leishmania major* promastigotes at a parasite:cell ratio ranging from 0.1:1 to 10:1. At different time points (6 to 18 h) of incubation at 37^o C, NO and iNOS production were detected by Griess reaction and by Western blot, respectively. Results: In the presence of 0.1 micrograms LPS and 50 IU IFN-gamma and after 18 h of incubation, it was detected 20.3 ± 10.3 mM of NO in C57BL/6 and 10.2 ± 4.16 mM in BALB/c cells supernatants. C57BL/6 neutrophils produced larger amounts of iNOS than BALB/c cells and the enzyme can be detected as early as 4 h of incubation. *L. major* promastigotes caused no measurable inhibition in the iNOS expression at a 0.1:1 parasite:cell ratio. At the 1:1 ratio, BALB/c neutrophils were more sensitive to *L. major* inhibition than C57BL/6 cells. Neutrophils of both mouse strains showed similar inhibiton (about 80%) at 10:1 promastigote:neutrophil ratio. Conclusion: BALB/c peritoneal neutrophils produce less iNOS and nitric oxide than C57BL/6 neutrophils, in the presence of unspecific stimulus of LPS and IFN-gamma. However, cells of both mouse strains are inhibited in the NO production and iNOS expression by the presence of *L. major* in a 10:1 ratio. Financial support: CNPq, Fapemig

BC86 - DIFFERENTIAL ACTIVATION OF NF-κB BY DIFFERENT *LEISHMANIA* SPECIES SUGGESTS SPECIFIC IMMUNOMODULATORY EVENTS

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NF-κB is an important cellular transcription factor involved in the expression of immunoregulatory genes such as cytokines, adhesion molecules and other mediators. Several pathogens active NF-κB and the study of this transcriptional factor in parasitic infection can unveil some mechanisms that underlie pathogenicity. Recent studies demonstrated that *Leishmania amazonensis* infected C57/B6 mice presented a delayed expression profile of cytokines, chemokines and chemokines receptors when compared with mice infected with *Leishmania major* (a healing model), suggesting that a deficient activation of early immune responses contributes to the pathogenesis in *L. amazonensis* infected mice. Since NF-κB regulates the expression of some of these proteins we aimed in this work to investigate the modulation of NF-κB activation in murine macrophage lineage (Raw 264.7) infected with either *L. amazonensis* or *L. major*. Eletrophoretic Mobility Shift Assays (EMSA) revealed nuclear translocation of NF-κB in extracts from macrophages infected with promastigotes forms of *Leishmania*. *L. major* activates a NF-κB complex in one hour of infection which seems to be substituted by a distinct dimmer in four hours. However, *L. amazonensis* led to activation of a single NF-κB complex in one or four hour of infection. Strikingly, we also demonstrated by EMSA that *L. amazonensis* inhibits LPS or TNF-α induced NF-κB complex. This observation was supported by Western blot assay, which demonstrated the reduction of p65 nuclear levels induced by TNF-α or LPS when the macrophages were infected with *L. amazonensis*. Our data indicate that both, the kinetics and the composition of activated NF-κB dimmers seem to be dependent on *Leishmania* species which can lead to distinct expression of cytokines and chemokines. Moreover, *L. amazonensis* infection inhibits NF-κB activation induced by LPS or TNF-alpha, suggesting a potential down- regulation of NF-κB-dependent genes.

BC87 - INVOLVEMENT OF MACROPHAGE SCAVENGER RECEPTOR MARCO (Macrophage Receptor with a Collagenous Structure) IN *LEISHMANIA* INFECTION

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CBA mice are susceptible to *Leishmania amazonensis* and resistant to *L. major* infection. CBA macrophages control *L. major* and are unable to destroy *L. amazonensis in vitro*. The data suggest that macrophages participate in determination of immune profile. Phagocytosis represents an early and crucial event in triggering host defenses against invading pathogens. Several receptors have been implicated in promastigote binding to macrophages. In DNAmicroarray studies we showed that both parasites induced significant alterations on macrophage gene expression. We found there was an up regulation of genes encoding MARCO receptor in response to *L. major*. This scavenger receptor has not being previously related to *Leishmania* infection. Here we demonstrated that there was higher in vitro expression of MARCO in *L. major*-infected macrophages when compared to *L. amazonensis*-infected cells. In *in vivo* studies, we demonstrated that there was higher expression of MARCO in lymph nodes and spleen from *L. major*- in comparison to *L. amazonensis*-infected CBA mice. In *in vitro* studies, neutralizing antibody anti-MARCO reduced in 28.5% of *L. major* but not *L. amazonensis* infection. Evaluation of MARCO *Leishmania* interaction by confocal analyses using fluorescent antibody revealed a very early colocalization of *Leishmania* and MARCO receptor in macrophages. In summary, these data describe for the first time an involvement of a scavenger receptor in innate response of *Leishmania* infection. In addition, our observations indicate that MARCO contributes only to *L. major* entry inside macrophages, since MARCO neutralization reduces *L. major* but not *L. amazonensis* infection. Since *L. major* infection is controlled by CBA/J

mice and CBA/J mouse macrophages, herein we discuss that the macrophage higher expression of MARCO receptor is involved in parasite destruction. *In vivo* neutralization of MARCO is being performed in order to establish the actual involvement of this receptor in *L. major* infection of CBA mice.

BC88 - Role of the *Leishmania* cysteine proteinase Lpcys2 pro-domain in lysosomal targeting

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Leishmania pifanoi cysteine proteinase 2 (Lpcys2) is an amastigote specific lysosomal hydrolase, and also a virulence factor important in the host-parasite interaction. We have shown previously that the prepro-domain of this proteinase was sufficient to take the reporter protein GFP to the lysosome. In continuation to this project, we developed antibodies against this domain. Using this antibody we showed that the Lpcys2 zymogen has different isoforms, preferentially expressed in the amastigotes as opposed to stationary promastigotes, as seen by immunofluorescence, electron microcopy and western blots. Using antibodies against the pro, catalytic, and C-terminal extension (CTE) domains, the processing of Lpcys2 was analyzed, and using the same antibodies, it was seen that *L. amazonenses* has a cysteine proteinase with high homology to Lpcys2. To study the interaction of the pro-domain with proteins that take part in Lpcys2 lysosomal targeting, ligand blot and cross-linking experiments were carried out, showing the interaction of the pro-domain with promastigote and amastigote proteins. Using immunoprecipitation it was possible to isolate some candidate interacting proteins from amastigotes as: P4-nuclease, glyceraldehide-3-phosphate dehydrogenase, histone H4, F1 subunit α-ATPase, Lpcys2. We are presently investigating the possible role of these proteins in interaction with the pro-domain of Lpcys2. This work was supported by PAPES-Fiocruz and IOC-Fiocruz.

BC89 - Cell cycle Inhibition of *Leishmania amazonensis* promastigotes by new thiosemicarbazones

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Universidade Estadual do Norte Fluminense, Centro Biociências e Biotecnologia, Laboratório de Biologia Celular e Tecidual. Campos-RJ, Brazil. Interruption of cell cycle was preferential target to drugs development against several pathogens. New thiosemicarbazone (TSC) arrested intracellular *Toxoplasma gondii*, *Leishmania amazonensis* and *Trypanosoma cruzi* leading its elimination (1, 2). In the present study we tested these compounds on extracellular promastigotes of *Leishmania amazonensis*. Promastigotes of *L. amazonensis* was maintained in Warrens medium, supplemented with 10% FBS for 5 days, when they were in exponential phase. Thiosemicarbazones compounds (2e, 2h, 2i, 2j, 3f and 3h), firstly diluted in DMSO and so in Warrens medium (1mM), were incubated in promastigotes for 7 days, at 28^{o}C. During this time, parasite number was determined daily in treated or not cultures, by using a Neubauers chamber. To morphological analyses, promastigotes were fixed in 4% Formaldehydes solution, stained with Toluidines blue solution and observed in Zeiss AXIOPLAN photomicroscope. Extracellular promastigotes arrested its cell cycle after 36 hours in presence of TSC. The multiplications interruption caused drastic parasite morphological alterations. In the treated culture, promastigotes showed a rounded shape with vacuolization in the cytoplasm and nuclear fragmentation. In some cases, disrupted promastigotes were still seen. These results suggest that these thiosemicarbazones were able to synchronize multiplications promastigote in vitro, leading parasites morphological alteration and lysis. References: (1) Beiral, H.J.V.& Melo, E.J.T., 2003. Braz J Med Biol Res 36, 1-5. (2) Tenório, R.P., Carvalho, C.S., Pessanha, C.S., Lima, J.G., Faria, A.R., Alves, A.J., Melo, E.J.T., Góes, A.J.S. (2005). Bioorg. And Med. Chem. Letters 15, 2575-2578.

BC90 - A new defined medium for primary growth of Leishmania sp.

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The primary growth of *Leishmania* constitutes an essential condition to reach some purposes as: confirmation of diagnosis, of etiological agents, biochemical analysis, cellular biology, immunological and chemotherapies studies. The aim of the present study was to evaluate the performance of a new formulation of liquid culture medium for primary growth and proliferation of *Leishmania*. I - Cultures media evaluated:a) Biphasic: BAB + BHI with SHU (Sterile Human Urine); b) TGGY broth - 4 different formulations (TGGY; TGGY + Folic Acid; TGGY + Haemin; TGGY + Folic Acid + Haemin) containing: yeast and malt extract (source of vegetable carbohydrate), soy peptones and soy casein (source of vegetable protein), salts, supplemented or not with Haemin and/or Folic Acid (animal source). II - Biological samples:a) splenic aspirates from 29 dogs suspected of Canine Visceral Leishmaniasis; b) control samples of L(L) chagasi, L(L) amazonensis, L(V) braziliensis and T. cruzi . Gentamycin was added in all cultures media. RESULTS: I - Primary growth

was obtained from 15/29 (51,72%) suspected of Canine Visceral Leishmaniasis. II - Of those 15 dogs, 11/15 (73,3%) *Leishmania* was isolated in biphasic medium (BHI + BAB + SHU); 5/15 (33,3%) in TGGY broth; 10/15 (66,7%) in TGGY + Haemin; 8/15 (66,7%) in TGGY + Folic Acid and 12/15 (80%) in TGGY + Folic Acid + Haemin. III - Different formulations of TGGY make possible more isolations 14/15 (93,3%). IV - Control samples *L(L) chagasi, L(L) amazonensis, L(V) braziliensis* and *T. cruzi* present satisfactory growth in biphasic medium and in TGGY broth supplemented with Haemin and Folic Acid. *T cruzi* keeps up only in biphasic medium.

BC91 - AMERICAN TEGUMENTARY LEISHMANIASIS (ATL) INFECTION: MORPHOLOGIC STUDY OF THE INFLAMMATORY CELLS PROFILE.

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The cell profile at the tissue granulome micro-ambient of lesions in patients with ATL from endemic areas of the State of Rio de Janeiro was analyzed by light microscopy, fluorescence (TUNEL), immunohistochemistry and TEM. Our data allowed to demonstrate alterations that varied according to the time of infection. In recent lesions it was possible to observe an expressive number of eosinophils and lymphocytes, as well as some monocytes and macrophages. In older lesions there was an expressive increase in the number of cells, particularly macrophages and plasmocytes, the later presenting well developed RER profiles and some secretory vesicles, thus suggesting an intense production of immunoglobulins. Later lesions (6 months) presented as typical feature a large number of cells undergoing a process of cell death, such as autophagy, necrosis or apoptosis. Analysis by TUNEL demonstrated an increase in the number of apoptotic cells according to the time of infection. Ultrastructural characterization of the lesions showed some cells, such as plasmocytes, in progressive stages of cell death, suggesting initial stages of autophagy, such as: loss of normal RER architecture, with rounding and occasionally swelling of the cisterns, together with an increase in the intermembranar space of the nuclear envelope; loss of the Golgi complex and the presence of a large number of secretory vesicles with rarefied matrix. These lesions presented macrophages forming a disorganized granulome, amastigote residual bodies inside parasitophorous vacuoles of macrophages, and cells with intense vacuolization suggesting death by autophagy. Cell death by apoptosis could also be demonstrated by the visualization of some cells presenting highly condensed chromatin and loss of cytoplasmic volume. The morphological evaluation of active cutaneous lesions of human ATL described in this work can supply new parameters for a better histopathological diagnosis of L. (V.) braziliensis. This work has been supported by CNPq and FIOCRUZ.

BC92 - THE ROLE OF THE PARASITE LIPOPHOSPHOGLYCAN MOLECULES IN THE PROCESS OF LEISHMANIA CHAGASI-MACROPHAGE INTERACTION.

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Visceral leishmaniasis is the most serious form of the disease and fatal if left untreated, the etiological agent being Leishmania chagasi. Lipophosphoglycan (LPG) is the major glycoconjugate present on the surface of Leishmania promastigotes. For example, in L. major the LPG forms a dense glycocalix that covers the entire parasite surface. Expression of this molecule is developmentally-regulated and modified during metacyclogenesis. This phenomenon appears to be a key determinant in the parasite invasion of macrophages and survival in both invertebrate and vertebrate environments. Aim of this study is to observe the interactions between macrophages and L.chagasi. We identify the distribution of LPG during the infection of BALB/c mice peritoneal macrophage with the procyclic or metacyclic forms. Peritoneal macrophages were obtained from 90-day-old BALB/c mice, the L. chagasi strain used is BH 46. The interaction period studied was at 24h, 48h and 72h. The samples were labeled with FITC-phaloidin for observation of the cytoesqueleton and Tropo-3 as nuclear marker. The parasites inside infected macrophages were labeled with anti-LPG. In control experiments, the slides were labeled with Giemsa. In the period between 24 to 72 hours it was possible to determine the kinetic Leishmania - macrophages interaction. Fluorescence labeling images revealed details of the LPG distribution in the cytoplasm, outside the parasitophorous vacuoles. Further studies are processing in order to understand the role of LPG in the interaction and to show how this process occurs.
Support: Fapemig, Fiocruz and CNPq.

BC93 - Histopathological analysis of initial cellular response in experimental lesions caused by *Leishmania (L.) amazonensis* in different mice strains.

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Leishmaniasis is an important public health problem in several countries. In Brazil tegumentar leishmaniasis is the most common form of the disease. The initial moments of the infection are crucial to determine the evolution of the disease. In order to elucidate the inflammatory answer after infection

with *Leishmania (L.) amazonensis*, we have used BALB/c, C57BL/6, C3H/HeJ e TLR-2^{-}/− mice. The mice were inoculated at the external auricular region with promastigotes of *L. (L.) amazonensis*, the lesion were removed after 1, 7 and 15 days post-inoculum, and processed for light and transmission electron microscopy. For the histopathological analysis, the biopsies were fixed with formalin, embedded in paraffin, and sections stained with HE. For the ultrastructural analysis, fragments of the inoculum areas were fixed with glutaraldehyde and osmium tetroxide and embedded in PolyBed 812 resin. Semi-thin and ultra-thin sections were used for the analysis of the inflammatory infiltrate and the parasite load. Our results demonstrate that lesions in BALB/c, C57BL/6 and TLR-2^{-}/− mice infected by *L. (L.) amazonensis* have cellular profile characterized mainly by macrophages, neutrophils and eosinophils. In C3H/HeJ mice the cell infiltrate is made up predominantly by mononuclear cells and by a gradual increase of mastocytes and fibroblasts. Balb/c mice are the most susceptible, presenting an expressive number of extracellular amastigotes. However, the TLR-2 and TLR-4 deficient mice presented a low parasitary load. Use of TLR-2 and TLR-4 deficient mice allowed to demonstrate that the absence of such receptors is determinant to the control of the parasitary load and to the recruitment of inflammatory cells during the two first weeks after infection with *L. (L.) amazonensis*. Thus, we suggest the study of the TLRs pathway as an alternative for the development of new drugs for treatment of infections caused by *L. (L.) amazonensis*. Supported by CNPq/FIOCRUZ.

BC94 - Primary culture of *Rhodnius prolixus'* salivary gland cells

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Rhodnius prolixus is a hematophagous triatomine vector of Chagas disease. The insects release saliva during the role feeding process, which interfere in the haemostasis mechanism of the host and assure the success in feeding. Some of the components of the saliva are anticoagulants, inhibitors of platelet aggregation, vasodilators, anti-histamines and others. Salivary glands are composed by a single layer of epithelial cells wrapped by a thin basal lamina, tracheal system and muscle fibers. The objective of this work was to develop primary cell culture of salivary glands as well to obtain bioactive salivary components in vitro. Adult insects from both sexes were disinfected by consecutive submersions in detergent, hypochlorite and alcohol and dissected in aseptic environment. The glands were homogenized with a pistil, treated with collagenase and incubated at 28^{o}C in humid environment in Schneider′s insect medium. After 5 days, glandular cells were adhered to the plate. Two cellular types were visualized: the first was grouped, adhered to the tracheal system filaments from the gland, in a monolayer of juxtaposed cells. It was possible to verify the presence of secretion granules being liberated by these cells. The second cellular type is lengthened and fusiform. These cells migrated from the tissue fragments of the salivary gland. The results demonstrated that it is possible to cultivate triatomine salivary gland cells and they are able to remain in culture for 30 to 40 days. Tests are in progress aiming the detection of bioactive components secreted by these cells in culture.

BC95 - INTRASPECIFIC FUSION OF LEISHMANIA PARASITOPHOROUS VACUOLES

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Protozoa of the genus *Leishmania* are intracellular parasites which alternate between two different developmental forms: promastigote and amastigote. Within their mononuclear phagocyte host cells, the parasites are lodged in phagosomes called parasitophorous vacuoles (PVs) which rapidly acquire compositional and functional features of phagolysosomes. *L. amazonensis*' PVs grow markedly in size, can shelter large numbers of amastigotes, and fuse with vesicles containing colloids or killed yeast particles. Although fusion between *L. amazonensis* PVs was earlier detected by time lapse cinemicrography, quantitative studies of intra or interspecific fusion between Leishmania PVs have not been reported. We examined the kinetics of homotypic fusion between *L. amazonensis* vacuoles in bone marrow-derived macrophages infected with lesion-derived *L. amazonensis* amastigotes. After 48 hours to allow for development and maturation of "recipient"PVs, macrophages were re-infected with a second batch of amastigotes transfected with GFP or preloaded with carboxyfluorescein diacetate succinimidyl ester so that parasites from the first and second infections could be distinguished. Fluorescence microscopy of the cultures revealed that 2 hours after reinfection 3% of the fluorescent parasites taken up by the macrophages were found within previously established PVs containing non-fluorescent amastigotes. Whereas co-infected vacuoles (containing both labeled and unlabeled parasites) initially comprised 2% of the total number of PVs, their numbers increased with time, reaching about 15% and 30%, respectively, at 24 and 48h post-reinfection. This increase was most likely due to continued PV fusion as analysis revealed a time-dependent decrease in the number of vacuoles containing either unlabeled or labeled parasites and increased numbers of vacuoles containing both. It was also shown that the numbers of labeled amastigotes increased with time. Our studies thus document substantial fusion can take place between incoming and pre-existing PVs that shelter amastigotes of *L. (L.) amazonensis*. Supported by EPM/UNIFESP, FAPESP and CNPq.