POSTERS

Biologia Celular - Cell Biology

BC01 - IN VITRO TRYPANOCIDAL EFFECT OF O-3-HEXOSE DERIVATIVE

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In trypanosomatids D-glucose is a crucial source of energy to metabolism and D-glucose transport has been shown to be the rate-limiting step. The parasites can adapt their specific transporters for the different environment, which are confronted, mid gut, salivary glands, hemolymph and blood. In the present work we evaluated the action of one O-3-hexose derivative 3-O- ((undec-10 en)-1-yl)- D-glucose on *Trypanosoma cruzi* and *Trypanosoma rangeli* growths. *T. cruzi* and *T. rangeli* epimastigotes forms were grown in liver infusion tryptose (LIT) medium supplemented with 15 % fetal calf serum at 28 ^{0}C, in an initial rate of 5 x 10 ^{6} cells / ml in culture flasks. The assays were performed in triplicate by adding different concentrations (0.2, 1 and 5 mM) of 3-O-((undec-10 en)-1-yl) - D-glucose to the culture. Trypanocidal activity of this compound on parasite growth was evaluated by counting the number of live parasites and by morphologic aspect. As control, we have a parasite culture without the agent. Our data showed that treatment of *T. rangeli*epimastigotes with 5mM of 3-O- undec-10 en-1-il - D-glucose led to a 100 % cell death soon after 24 hours of cultivation, while the treatment with 1 mM led to a 99.3 % at the same time. Treatment with 0.2 mM led to a cell death around 37.9 % with viable parasites until the 5th day. For the *T. cruzi* our data showed no inhibition with 0.2 mM and a less effective inhibition with 1mM when compared with *T. rangeli* but with more altered morphology. At 5 mM we obtained a cell death of just 22.4 %. The results suggest that the 3-O- ((undec-10 en)-1-yl) - D-glucose can be a promising trypanocidal agent. Experiments with trypomastigotes forms are under investigation. Supported by: CNPq and Funcitec

BC02 - Dimorphism in Trypanosom cruzi:phenotypic expression of cell surface antigens on slender and broad trypomastigotes

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Several studies have been dedicated to explain the biological reason of the dimorphism in T. cruzi albeit the real purpose of it has not been totally explained yet. Previous studies analysing distinct aspects of the behavior of slender and broad forms pointed important characteristics such as: a) the appearance and persistence in the bloodstream; b) the infectivity to mammals and vectors; c) the prevalence of each form in different strains; d) tissue tropism; e) motility; f) behavior in tissue culture; and g) survival in different host environments. In the present study we investigated the phenotypic expression of different surface antigens by slender and broad parasites. Trypomastigotes forms of the Y strain and CL-Brener and Dm28c clones were used. Briefly, parasites were harvested from tissue culture medium, rinsed in PBS and fixed in formaldehyde. Cells were incubated in the presence of first antibody and the in the presence of the fluorescent secondary antibody diluted in PBS containing 1.5 % BSA for 30 min. Coverslips were mounted on glass slips in a 1:9 solution of PBS/glycerol containing 0.2 N n-propylgalate. All trypomastigotes belonging to the three samples were labeled with anti-SAPA antibody. However, for Y strain and CL-Brener clone, labeling of broad forms was more intense than those observed for slender forms. Dm28c trypomastigotes presented a different pattern of labeling with two distinct populations. Seventy percent of the thin trypomastigotes presented an intensity of labeling, which were as stronger as the observed for Dm28c broad forms. Otherwise, thirty percent of thin forms presented a pattern of labeling similar to that described for thin trypomastigotes from the Y and CL-Brener samples. Labeling using different antibodies as well as a flow cytometric analysis are in course. Supported by: CNPq, FAPERJ, Pronex, CNPq/PIBIC-UFRJ.

BC03 - Effect of suramin on the intracellular differentiation of *Trypanosoma cruzi*

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Suramin is a synthetic, polysulfonate naphthylurea, most often used in the treatment of parasitic infections, but also in cancer treatment. Although the mechanism of action of suramin is not fully known it is believed to inhibit growth-factor-induced mitogenesis besides alters the enzymatic activity of protein-tyrosine kinases and phosphatases. Different effects of suramin have been described in different cell types including changes on cell proliferation and differentiation. The aim of this study is to show the effect of a long lasting suramin treatment on the amastigote-trypomastigote differentiation process. Briefly, LLC-MK2 were cultivated on coverslips in a multi-well plate and 24 h after the infection of with T. cruzi trypomastigotes, cells were incubated in RPMI-1640 medium supplemented with 2% of FCS, containing 500 micromolar of suramin that was maintained in culture medium during the intracellular period of parasite cell cycle. Coverslips were fixed and processed to light microscopy and immunofluorescence after 48, 72, 96 and 120 h of infection. We observed an accentuated increase in the number of intermediate and trypomastigote forms in the cytoplasm of suramin treated cells after 72 hs infection, suggesting an increase in the intracellular differentiation rate. The immunofluorescence analysis of intracellular parasites in control and suramin treated cells showed no difference on the labeling against the antigens Ssp4, that recognized by the 3B2 monoclonal antibody, which is present only on flagellated forms, and SAPA. However, trypomastigote forms ob-

tained from suramin treated cells remained positive for the Ssp4 antigen. Our results suggest that suramin interferes with cell cycle and the phenotype expression of cell surface antigens in *T. cruzi*. Supported by CNPq, FAPERJ, Pronex and PIBIC/UFRJ.

BC04 - Modified Giemsa staining technique: a useful tool for studying the invasion of *T. cruzi* extracellular amastigotes in host cells

Silva, C.V. *(UNIFESP/EPM)*; Mortara, R.A. *(UNIFESP/EPM)*

Invasion assays are used frequently in laboratories that study the biology of intracellular parasites. In our laboratory we use indirect immunofluorescence (IF) as gold standard to determine the invasion index of *Trypanosoma cruzi* amastigotes. However, it is a laborious, time-consuming and expensive method to be routinely used. On the other hand, Giemsa staining technique is a simple and cheap method but the pattern of staining does not allow the observer to precisely distinguish parasites that are undoubtedly inside the host cell. To overcome these problems we have modified a previously described Giemsa staining technique (MGS) (Giaimis et al., 1992) to be used in invasion assays with live parasites. The modified protocol consists, briefly, in pretreating the slides with tannic acid before fixation with methanol and Giemsa staining. We used extracellular amastigotes of G and CL strains for the invasion assays with HeLa cells since it is already known that these parasites invade host cells in different fashion (Mortara et al., 1999). Our results showed that the number of parasites inside host cells was similar for IF and MGS techniques in both strains. After MGS, intracellular amastigotes appeared pink colored, showing a strong labeling of kinetoplasts and nuclei; by contrast, parasites that were only attached to the cells developed a distinct violet hue. This study showed that a simple procedure could be useful in determining the number of extracellular amastigtotes that indeed invaded host cells. This technique could also be applied to other infective forms of *T. cruzi* and intracellular parasites. Financial support: FAPESP, CAPES and CNPq.

BC05 - Distribution of epitopes defined by monoclonal antibodies in *Trypanosoma cruzi* amastigotes derived from an acute phase patient from the recent outbreak in Santa Catarina state, Brazil

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During the recent outbreak of Chagas disease by oral infection in Santa Catarina, a *Trypanosoma cruzi* isolate (SC2005) was obtained from blood sample of a male patient with symptoms of the acute phase. This patient was admitted to hospital in Sao Paulo after consuming sugar-cane juice from the identified point of origin. Sanitary authorities confirmed that the juice was contaminated with *T. cruzi*, probably due to grinding of infected triatomines together with the sugar-cane. Blood samples were cultured in LIT medium and this isolate was characterized as *T. cruzi* II according to Briones et al. (1998). Vero cells infected with SC2005 were examined by confocal microscopy. The fluorescence distribution following Mabs 1D9, 2B7, 3B9and 4B9 labeling showed membrane and intracellular compartments staining. Unlabeled parasites were also observed. Mab 3B2 that preferentially reacted with trypomastigotes in all isolates already studied, showed an anomalous reactivity; cells harboring trypomastigotes showed a strong reaction with the cytoplasm suggestive of an unforeseen antigen shedding. Flow cytometry analysis of intracellular (IA) and extracellular amastigotes (EA) revealed that the epitopes recognized by Mabs 1D9 and 2B7 are more expressed in extracellular amastigotes. Immunoblotting analyses revealed a polymorphic pattern of bands for Mab 1D9 and reactivity with a high molecular weigh protein for Mab 2B7. Invasion assays showed that SC2005 poorly invade host cell and that 588 isolate is the only isolate from chagasic patient that infect HeLa cells in a degree comparable to the G isolate, although EA of all these chagasic patient isolates express the epitope recognized by Mab 1D9 similar to G isolate. We concluded that besides the importance of the epitope defined by Mab 1D9 in the amastigote invasion process there could be other factors that may modulate the invasiveness of extracellular amastigotes from the different isolates. Financial support: FAPESP, CAPES and CNPq.

BC06 - DISRUPTION OF THE KDNA ARRANGEMENT OF TRYPANOSOMATIDS

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The trypanosomatid protozoa present remarkable biological structures such as the kinetoplast, a specialized region of the mitochondrion, which contains the mitochondrial DNA (kDNA). Unlike any other DNA in nature, the kDNA of trypanosomatids is composed by circular molecules, which are topologically interlocked to form a single network. The kDNA replication is complex and requires the participation of type II topoisomerases, enzymes that cut and attach minicircles to the network. A variety of compounds are able to disturb the kDNA arrangement. Drugs that target the bacterial topoisomerase II, as nalidixic acid and ciprofloxacin, inhibit cell growth and differentiation in trypanosomatids. Furthermore, the kDNA arrangement can be disrupted by intercalating agents as acriflavine and ethidium bromide, which bind directly to DNA and can give rise to dyskinetoplastic organisms. In this work, we investigated the effect of different topoisomerase II inhibitors and intercalating agents on trypanosomatid proliferation and ultrastructure. We observed that nalidixic acid, novobiocin, ciprofloxaxin and acriflavine promote distinct effects on kinetoplast, since they present different mechanism of action. For example, the ciprofloxacin

and the nalidixic acid (500μg/ml) did not promote dyskinetoplasty, but induced drastic morphological changes in kDNA, especially in *B. culicis* and in *C. fasciculata*. Such alterations are probably related to inhibition of topoisomerase activity, as catenation and decatenation of kDNA. The acriflavine (50μg/ml) promoted the formation of a compact and round structure, which was seen associated to the basal body. Furthermore, this intercalating drug disassembled the kDNA network, which resulted in dyskinetoplasty. In our next approach we will evaluate if such morphological alteration are related to the inhibition of the mitochondrial metabolism. The use of drugs which affect the kDNA ultrastructure can improve our understanding about the mechanism of kDNA organization and replication, offering new clues about the role of mitochondrion in the energetic metabolism of trypanosomatids.

BC07 - *IN VITRO* NEURONAL DEATH IS MEDIATED BY NITRIC OXIDE PRODUCED BY *T.CRUZI*-INFECTED AND IFN-GAMMA ACTIVATED MACROPHAGES

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Significant loss of neurons is seen in hearts and gastrointestinal tracts committed by Chagas disease. Neuron degenerative events occur in the acute phase. Chronic phase manifestations are a result of acute phase neuronal destruction and consequent denervation of these organs. Neuronal death and peripheric denervation are related to local inflammation which is triggered by *T.cruzi*-infected macrophages and activated lymphocytes. Nitric oxide is an inflammatory cell product that has been implicated in Chagas disease gastrointestinal tract denervation. We used a previously described co-culture model of murine neurons and macrophages (Arantes et al, 2000) to study the role of nitric oxide (NO) produced by *T.cruzi*-infected and IFN-gamma activated macrophages in mediating *in vitro*neuronal lesions. Primary cultures of C57BL/6 (WT) mice sympathetic cervical ganglion neurons were maintaned for 48 hours before co-cultured with peritoneal macrophages from WT and i-NOS KO mice. Co-cultures were infected with Y strain of *T.cruzi* (MOI 10) and received recombinant murine IFN-gamma in a final concentration of 200UI/ml. The supernatants were collected and the NO concentration was measured by the Griess method. The NO inhibitor aminoguanidine was added to co-cultures with WT macrophages in a final concentration of 100μM. *T.cruzi*-infected and IFN-gamma activated co-cultures with WT macrophages showed 51%reduction in neuronal survival and significant increase in NO production related to controls 48 hours post infection. Co-cultures with i-NOS KO macrophage and with WT macrophages and aminoguanidine showed neuronal survival similar to controls and no relevant NO production as expected. The inibition of NO production by i-NOS KO macrophages and aminoguanidine is able to revert the neuronal lesions observed in our model, attributing a direct role for NO in the neuronal death mechanisms in *T.cruzi*-infected and IFN-gamma activated co-cultures. Financial support by WHO, FAPEMIG, CAPES.

BC08 - *Trypanosoma cruzi* DNA FRAGMENTATION INDUCED BY NAPHTHOIMIDAZOLES DERIVED FROM BETA-LAPACHONE

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Plants containing naphthoquinones are commonly employed in folk medicine, and these compounds present a variety of biological activities, most of them associated with oxidative processes. The anti-tumoral effects of beta-lapachone, isolated from Tabebuia (ipes), are related to apoptosis induction and topoisomerases inhibition (Li et al, 1995; Pink et al, 2000). From beta-lapachone, 45 derivatives were synthesized and assayed against Trypanosoma cruzi trypomastigotes. The highest activity was achieved with three derivatives with the aromatic moieties linked to the imidazole ring (N1, N2 and N3) (Pinto et al, 1997; Neves-Pinto et al, 2000; Moura et al, 2001). Our previous studies showed the susceptibility of different forms of the parasite, being mitochondrial swelling, reservosomes disorganization and chromatin condensation the relevant ultrastructural alterations (Menna-Barreto et al, 2003). In the present work, we investigate the effect of three naphthoimidazoles on DNA of epimastigotes and trypomastigotes. After treatment of trypomastigotes with N1, N2 or N3, it was observed intense DNA fragmentation by TUNEL assay, a spread pattern by total DNA electrophoresis, and atypical condensation of nuclear DNA and total disruption of kDNA network by electron microscopy. On the other hand no similar alterations were observed in treated epimastigotes. Our findings showed that N1, N2 and N3 interact with DNA of trypomastigotes, but the exact mechanism is not known yet. Further studies are necessary to understand the molecular and biochemical mechanisms that led to the parasite death.

BC09 - Macrophage Endocytic Pathway during "Trypanosoma cruzi"infection

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Considering that (i) the endocytic pathway plays a role in important cellular functions, and (ii) pathogens are capable to reprogram the endocytic pathway of their own host cells, our goal was investigate the endocytic pathway of macrophages during parasite infection in vitro as well as in vivo. In these studies different endocytic ligands were used, includ-

ing fluid phase (FITC-BSA, FITC-Dextran) and receptor-mediated (FITC-Transferrin) ligands. During in vivo assays, peritoneal macrophages were obtained from Swiss mice infected or not by T. cruzi (Y strain at 7dpi), and during in vitro analysis, peritoneal macrophages collected from healthy mice were infected for 48h by trypomastigotes of T. cruzi (Y strain) in vitro. The cells resulted from both infections were incubated at 37C/30min with the endocytic ligands (50-100 μ/ml), fixed with paraformaldehyde and then, the incorporation index was determined by flow cytometry. After the infection T. cruzi in vivo, the macrophages displayed reduced ligand uptake: the analyses at R1 (region enriched with macrophages) showed a reduction of 37% and 63% in the fluorescent mean number (FM) regarding the incorporation of Dextran and BSA, respectively, as compared to macrophages from uninfected mice. Macrophages from infected mice also displayed less 89% in the FITC- Transferrin internalization as compared to the cells from the uninfected mice. After 48 h of T. cruzi infection in vitro, the phagocytic cells presented 60 % reduction in the FM of dextran uptake as compared to uninfected cells, suggesting that the infection induce an impairment in the endocytic pathway of the macrophage in a similar ways as has been described during the infection of non-phagocytic cells such as cardiomyocytes. Further studies are under way to deep investigate the endocytic pathways of host cells during T. cruzi invasion. Supported by FAPERJ, FIOCRUZ and CNPq

BC10 - MODULATION OF ADHERENS JUNCTIONS IN CARDIOMYOCYTES INFECTED BY *TRYPANOSOMA CRUZI*

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Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, is an important manifestation of cardiomyophathy in Latin America, leading to considerable morbidity and mortality. Therefore, a detailed investigation of the *T. cruzi* - cardiac muscle cells interaction may contribute to the knowledge of biological and molecular events that occur during the infectious process. Given the cardiac conduction abnormalities evidenced in Chagas' diseases, we evaluated the expression of cadherin, a cell-cell adhesion molecule that play critical role in the coupling of cardiomyocytes, and also focal adhesion proteins during *Trypanosoma cruzi*-cardiomyocyte infection in vitro. Indirect Immunofluorescence assay was performed to examine the distribution of adherens junctions during cardiomyogenesis and after infection by *Trypanosoma cruzi* in vitro. Uninfected and *T. cruzi*-infected cardiomyocyte cultures were fixed with acetone (-20°C), washed in PBS and antigenic sites were blockage with BSA 4% in PBS. Thereafter, the cells were incubated overnight at 4°C with an anti-talin (1:20, Sigma) and anti-cadherin antibody (1:400, Sigma). The antigen-antibody complex was revealed by FITC-conjugated secondary antibody. Controls were performed with homologue serum or in the absence of the primary antibody. Laser scanning confocal microscopy revealed an intense labeling of N-cadherin at cardiac cell-cell adhesion sites, while talin was visualized at cell-extracellular matrix adhesion domains. Our results revealed a downregulation of N-cadherin expression and alteration in its distribution in cardiomyocytes infected by *T. cruzi*. Talin distribution was also modulated by *T. cruzi* infection. These data suggest that modulation of adherens junctions proteins may contribute to the alterations in the transmission of cardiac contraction force, leading to cardiac dysfunction in Chagas' disease. Supported by FIOCRUZ, FAPERJ and CNPq

BC11 - Intracellular traffic of different infective forms of *Trypanosoma cruzi* from the two major phylogenetic lineages: role of the intracellular bacterium *Coxiella burnetii*

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Our aim in this work is to study the fate of *Trypanosoma cruzi* extracellular amastigotes (A), metacyclic trypomastigotes (MT) and tissue-culture derived trypomastigotes (TCT) forms after invading Vero cells or Vero cells persistently colonized with the obligate intracellular bacteria *Coxiella burnetii*. This bacterium resides in acidified vacuoles with secondary lysosomal characteristics and our results show it to be a modulator of the intracellular traffic of these forms of *T. cruzi*. We examined endosomal-lysosomal sequential labeling with EEA1 and LAMP1 of the parasitophorous vacuoles (PV) formed during the entry of A, MT and TCT forms of parasite strains belonging to the two major groups: *T. cruzi* I (G), and *T. cruzi* II (CL and Y). Results with Tulahuen (*T. cruzi I*) strain are being processed. Our findings revealed that persistent *C. burnetii* infection did not interfere with the pattern of EEA1 and LAMP1 labeling of A, MT and TCT parasitophorous vacuoles. When we raised vacuolar and cytoplasmic pH with Chloroquine, a there was a distinct pattern in the labeling dynamic of amastigotes PVs. This effect was also different when comparing strains belonging to different *T. cruzi* groups. The alkalynization of Vero cells alone with Bafilomycin A1 also showed to be important in the labeling process only when cells where infected with the amastigote form. Here, no difference was seen between phylogenetic groups. When comparing A, TM and TCT containing vacuoles noticeable differences in the proportion of LAMP1 positive PVs were found. After 90 minutes of infection, TM parasitophorous vacuole showed the higher percentage of LAMP-1 labeling. The fact that TM are retained for longer periods in LAMP1 positive PV, are compatible with the lowest hemolysin and transialidase activities in TM of all strains, when compared to A and TCT. Financial Support: FAPESP, CNPq, and CAPES.

BC12 - Vero cells doubly infected with *Trypanosoma cruzi* and *Coxiella burnetii*: differential effects on the host cell cytoskeleton upon single and double infection

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The invasion of Vero cells harboring the intracellular bacteria *Coxiella burnetii* by different forms of *Trypanosoma cruzi* is a co-infection model well established in our laboratory. The large bacteria containing vacuole is an important modulator of the intracellular traffic of *T. cruzi* and is used to study the fate of these parasites inside the cells. Since intracellular pathogens are able to modify the host cell cytoskeleton to their benefit, we decided to examine the effect that the bacterial growth on Vero cells cytoskeleton and also, what might be the effect caused by the coinfection of these cells with infective forms of *T. cruzi*. For these studies, single and doubly-infected Vero cells were labeled by immunofluorescence using monoclonal antibodies directed to microtubules (tubulin) and intermediate filaments (vimentin) and phalloidin (to stain actin microfilaments). Cells were fixed at different time periods between 2h and 96h post infection. Preliminary results obtained by confocal microscopy suggest that the host cell cytosleleton undergoes substantial rearrangements to accommodate for the large cytoplasmic bacterial vacuole. The relatively stable distribution of cytoskeletal components appears to be gradually disrupted as T. cruzi amastigotes develop within the *C. burnetii* vacuole. Financial Support: FAPESP, CNPq, and CAPES.

BC13 - Fluorescent Genetically Modified *Trypanosoma cruzi*: a new tool for research in Chagas disease

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Stable transformations systems have been described for the trypanosomatids *Leishmania*, *Trypanosoma brucei* and *Trypanosoma cruzi*. In *T. cruzi*, stable DNA transfections can be achieved by the integration of the foreign gene in the genome, through homologous recombination, or by the episomal maintenance of the transfected plasmid. We generated an integrative expression vector derived from pROCKNeo, which allows the integration of GFP and RFP genes into β -tubulin locus of different strains of the parasite genome by homologous recombination. Clones of transfected parasites were obtained by serial dilution in 96 wells plates. We have obtained two clones of the Tulauhén strain expressing GFP and four expressing RFP; all these clones presented levels of heterologous expression higher than 80 %.To evaluate the stability of the GFP and RFP marker between the transfected population and clones, we performed analysis by fluorescence activated cell sorter. The parasites were cultivated for one or two months in the presence of G-418 (200 μ g/mL and 400 μ g/mL)and in the absence of G-418. We observed that the clones carrying GFP and RFP were able to keep the level of expression of the fluorescent protein even in absence of G-418,while in the population the expression of GFP and RFP was significantly reduced, when cultived in the absence of G-418. We used Southern blot of pulse field karyotype gels to confirm that the GFP and RFP marker integrated in the tubulin locus. The transfected parasites present no significant differences in their infectivity for VERO cells when compared with the wild type populations and the fluorescent can be easily visualized by confocal microscopy. Furthermore, when inoculated into Balb/c mice, GFP and RFP expressing parasites were able produce significant levels of parasitemia andopens new perspectives in the evaluation of tissue tropism by different *T. cruzi* strains. Support:CNPq, FAPEMIG and WHO

BC14 - CHARACTERIZATION OF TYPE I DNA TOPOISOMERASES OF Trypanosoma cruzi.

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DNA Topoisomerases are cellular enzymes that modify the topological state of DNA and participate in metabolic processes such as replication, recombination, transcription and chromosome segregation. We decided to clone and characterize the genes encoding type I topoisomerases in T. cruzi as little is known about the repertoire of these enzymes in this parasite. Our search revealed that the genome of T. cruzi encodes four type I topoisomerases: One topoisomerase from the sub-family IB (topoisomerase I) and three topoisomerases from the sub-family IA. We have cloned the genes TcTOP1L and TcTOP1S that share homology with those encoding the two subunits of the topoisomerase I of T. brucei and L. donovani, suggesting that Tctopo I also displays a heterodimeric structure. This seems to be a characteristic feature of trypanosomatids, since all eukaryotic topoisomerases I characterized so far are monomeric. Immunoelectromicroscopy assay shows that TcTOPO I is present either in the nucleus and in the kinetoplast of the parasite, suggesting that this enzyme might be involved in solving topological problems arisen during the replication and transcription of kDNA. The genome of T. cruzi encodes two genes for topoisomerase III (TcTOP3alpha and TcTOP3beta). The sequence of TcTOPO IIIalpha showed to be highly divergent from that of TOPO IIIbeta. This indicates that T.cruzi TOPOIII might have different DNA substrate specificity and consequently different functions in the cell. Immunolocalization assays showed that both TOPOIII are nuclear enzymes in T. cruzi. We have also characterized a third type I topoisomerase in T. cruzi. Interestingly, this protein (TcTOPOIc) share significant homology with a type IA topoisomerase from cyanobacteria. As far as we know this is the

first report of a type I topoisomerase with a prokaryotic characteristic found in an eukaryote.

BC15 - Biological aspects of the *Trypanosoma cruzi* (Dm28c clone) intermediate form, from epimastigote to trypomastigote, obtained in modified liver infusion triptose (LIT) medium

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The life cycle of *Trypanosoma cruzi*, etiological agent of Chagas disease, comprises three different morphological and functional forms, namely amastigote, epimastigote and trypomastigote (Brener, 1973; De Souza, 1984). Cell surface previous analysis of these protozoan stages have pointed out the existence of differences in carbohydrate, protein, and lipid compositions (Bourguignon et al, 1998; De Souza 1989, 1995). Surface components are assumed to play some role in basic biological characteristics, such as resistance to complement-mediated lysis, and recognition of the host cell. However, in vivo and in vitro metacyclogenesis studies have demonstrated the presence of intermediated forms. These forms are morphologically identified through the position of the kinetoplast relative to the nucleus. Despite morphological identification, little it is known about the biological characteristics of these intermediate forms. In this work, some biological aspects are evaluated (i.e., electrophoretic mobility-EPM-, complement lysis, and infectivity on Vero and macrophage cells) of *T. cruzi* intermediate forms (Dm28c clone) cultivated in modified LIT medium. **Materials and Methods** - All experiments were performed using *T. cruzi* Dm28c clone. Epimastigotes forms were maintained in LIT filtered medium as described by Camargo et al. (1964). Afterwards, instead of filtration, autoclavation was applied (20min) before serum addiction. Trypomastigotes were obtained from LIT medium at day 10 and purified by chromatograph. Macrophages- Normal peritoneal resident macrophages were obtained as described by Meirelles et al. (1986), Cell electrophoresis was measured as described by Bourguignon et al. (1998). **Result and Conclusion** - Parasites ultra structural analysis from this medium showed kinetoplast enlargement located beside the flagellate nucleus. Despite displaying a similar trypomastigote surface charge, the intermediate forms are not resistant to complement mediated lysis as epimastigotes. Moreover, the intermediate forms are unable to infect fibroblastic culture cells but develop basal infections in macrophages.

BC16 - COMPARATIVE CYTOTOXITY STUDY OF EPOXIDES ON VERO CELLS AND ON *Trypanosoma crusi*

Cavalcanti DFB *(UFF)*; Zuma AA *(UFF)*; Bourguignon SC *(UFF)*

Epoxides are derivated from naftoquinones, wich are known by its biological activity, anti-microbicidal, anti-*Trypanosoma cruzi*, and also known for its ability to induce the formation of reactive oxygen species. The Chaga's Disease is a South American's endemic disease caused by an protozoa called *Trypanosoma cruzi*. It affects millions of people and remains without an affective treatment that do not causes collateral effects. Thus, our aim is to do a comparative cytotoxity study of Epoxides on Vero cells and on *Trypanosoma cruzi*. **Methods** - *Vero Cells*- Analysis of the epoxides effect on the Vero cells were done as reported by Margis et al, 1989(Anal Biochem 181:209-211). A stock solution of substance- Epoxi-β-lap, Ester-Sulfo, Epoxi-Sulfo, Epoxi-Norβ-lap, Metoxi, Metoxi-p was prepared in DMSO (1.0%), in concentrations of 2,5; 12,5 and 50 μM. *Parasite-Trypanosoma cruzi* was kept in liver infusion BHI-medium. The toxic effects of the substances used were investigated by counting in an optical microscopy. **Results** - Surprisingly, Epoxi$N\beta$ did not show cytotoxity in concentrations 2,5 and 12,5 μM. In 50μM concentration, the cells remained alive. In this last concentration, almost all the protozoa were killed, mean in 2,5μM more than 50% died and in 12,5 μM near 60% were killed. Therefore, Epoxi-β-lap lysed nearly 15% of Vero cells in 2,5 and 12,5 μM concentrations, but in 50μM, only approximately 10 % of the cells remained alive. The porcentation of protozoa alive was higher in 2,5μM and in 12,5μM than in 50 μM concentration. Epoxi-Sulfo presented 54% and 25% of Vero cells alive in 12,5 e 50μM concentrations, respectively. This substance killed 16% of the protozoa in 12,5 μM, and 54% in 50μM concentration. **Conclusion** - This study demonstrates that Epoxi addition in some substances greatly reduced its cytotoxity activity. Epoxi-β-lap was the substance that showed higher Trypanocidal effect.

BC17 - Trypanosoma cruzi infection leads to distinct modulation of co-stimulatory molecules in monocytes and lymphocytes from patients with the indeterminate or cardiac Chagas disease

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Chronic human Chagas disease ranges from an asymptomatic to a severe cardiac clinical form. Several studies have demonstrated that host's immune response is critical in controlling parasitemia but also in leading to the tissue pathology associated with cardiac disease. Interactions between macrophages and lymphocytes through co-stimulatory molecules and cytokines are essential for the establishment of an efficient immune response. In order to determine the influence of *T. cruzi* infection on the expression of co-stimulatory molecules and cytokines by monocytes and T-cells from indeterminate and cardiac chagasic patients, we analyzed the expression of CD80 and CD86 by CD14+ monocytes, CD28 and CTLA-4 by CD4+ and CD8+ T-cells, and immunoregulatory cy-

tokines (IL-4, IL-10, IFN-gamma and TNF-alpha) by these cells. These analyses were carried out in freshly isolated cells as well as after in vitro infection with the parasite or exposure to parasite antigens. Our results demonstrated that circulating monocytes and lymphocytes from chagasic patients with distinct clinical forms showed important phenotypic and functional differences after *T. cruzi*infection or parasite antigen exposure. This suggests that immune cells from chagasic patients with different clinical forms may direct differently the immune response in the presence of *T. cruzi* or their antigens, influencing the development of pathology. Financial support: CAPES, PRONEX, WHO, HHMI and TMRC/NIH.

BC18 - Phages expressing *Trypanosoma cruzi* Tc85 domains bind to the vasculature of the mammalian host *in vivo*.

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The infective trypomastigote stage of *Trypanosoma cruzi* expresses a set of surface glycoproteins collectively known as Tc85 family. A cloned member of the family, Tc85-11, shows adhesive properties to laminin and cytokeratin-18 (CK18) on host cells [JBC 274:3461, 1999, JBC 276:19382, 2001, BBRC 325, 612, 2004]. Using epithelial $LLC-MK2$ cells, it was shown that the mammalian cell - binding domain is the conserved sequence VTVXNVFLYNRPLN (FLY domain) present in all members of the gp85/trans-sialidase gene superfamily. Fusion bacteriophages expressing the FLY domain (FLY-phage) or a modified FLY sequence (FLY-Ala-phage) were constructed and employed for *in vitro* and *in vivo* binding assays. The binding of FLY-phage to $LLC-MK2$ cells is 2.5 fold and 17 fold higher than control FLY-Ala-phage and Fd-tet, respectively. FLY-phage also binds to recombinant CK18 with high affinity, 8 fold higher when compared to insertless phage Fd-tet, confirming previous results from our laboratory [JBC 276:19382, 2001]. In addition to $LLC-MK2$ cells, FLY-phage was able to bind to other epithelial cells (MDCK and HeLa cells), macrophages (J774 cell line) and cardiomyocytes (HL-1). Specific inhibition of phage binding to $LLC-MK2$ by the cognate synthetic peptide was observed when FLY-phage was incubated with $LLC-MK2$ cells in the presence of increasing concentrations of FLY peptide. FLY-phage had no homing to the vasculature of any specific organ analyzed, binding to heart, skeletal muscle, liver, brain, thymus and spleen. The results suggest that the FLY sequence may be an important domain that provides *T. cruzi* with the means for subsequent extravasation to target specific organs and establishment of successful infection of mammalian host. Financial support: FAPESP, $CNPq$.

BC19 - Isolation by phage display libraries of a peptide that binds to the *Trypanosoma cruzi* Tc85 protein

RR Tonelli *(USP)*; U Urias *(USP)*; RJ Giordano *(mdanderson)*; W Colli *(USP)*; MJM Alves *(USP)*

Our laboratory was the first to describe trypomastigote-specific 85-kDa surface glycoproteins, suggesting their role in host cell invasion by the parasite. These proteins are collectively known as Tc85 family and belong to the gp85/trans-sialidase gene superfamily. Recently the corresponding receptor of a member of this family (Tc85-11) on $LLC-MK2$ cells was characterized as cytokeratin 18 [JBC 276, 3461, 1999]. Phage display libraries were used to isolate minimal receptor sequences that bind to trypomastigote Tc85-45, another member of the family, by affinity panning. Aliquots containing $10\hat{9}$ TU from the peptide library consisting of random cyclic nonapeptides (CX7C) fused to the N-terminus of the minor filamentous fUSE5 phage coat protein III were incubated with Tc85-45- or BSA- coated 96 wells plates (0.65 mg/well) [Science 228, 1315, 1985]. After washing, bound phages were recovered, amplified in *E. coli*, and the selection repeated 4 more times using the same procedures. An increased selectivity of target protein over BSA (6.5 fold enrichment) used as control was observed after successive panning rounds. Sequence analyses of randomly selected clones showed a common sequence in 22% of the Tc84-45 binding clones. Other minor sequences appeared 2-3 times being also present in BSA control sequences. The predominant cyclic motif exhibited sequence identity to prokineticin receptor 2 and olfatory receptor. Notwithstanding, further investigation is necessary to confirm the relevance of such motifs for *T. cruzi*-host interaction. Supported by: FAPESP, $CNPq$.

BC20 - Effect of TGF-beta on expression and distribution of fibronectin, laminin and TGF-beta receptor type II in *Trypanosoma cruzi* infected cardiomyocytes

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Alterations in heart expression of extracellular matrix (ECM) during *Trypanosoma cruzi* infection have been described both in the acute and chronic phase of Chagas disease. Although ECM expression is enhanced in the myocardium of infected mice during experimental Chagas disease, *T. cruzi*-infected cardiomyocytes display a reduced expression of fibronectin (FN) and alterations in laminin (LN) distribution in vitro. TGF-beta, a multifunctional cytokine capable to stimulate ECM expression, plays an important role in Chagas disease pathogenesis. Nevertheless, the regulation of extracellular matrix expression in *T. cruzi*-infected cells remains to be elucidated. To investigate the role of

TGF-beta in the modulation of ECM expression, primary cultures of cardiomyocytes were infected with *T. cruzi*, Y strain, and treated with 5, 10 and 15 ng/ml of TGF-beta. FN, LN and TGF-beta receptor type II (TRII) distribution was analyzed by indirect immunofluorescence and the images were obtained with confocal laser microscopy. Treatment of cardiomyocytes with TGF-beta leads to a general enhancement in the fibronectin matrix. Surprisingly, highly infected cardiomyocytes still displayed a reduced fibronectin expression, even after TGF-beta treatment. Reorganization of laminin matrix in highly infected cells was triggered by TGF-beta treatment (15ng/ml), showing a widespread distribution like control cells. Since several reports describe a downregulation of surface receptors of *T. cruzi* infected cells, the expression of TRII was also investigated. Normal cardiomyocytes presented TRII distributed in a striated pattern, co-localized with costameres. Most of the striation pattern of TRII was undetectable in *T. cruzi*-infected cardiomyocytes. In addition, the intracellular amastigotes displayed strong positive reaction. Uninfected cultures treated with TGF-beta raised the frequency of TRII striations array. However, treatment of *T. cruzi*-infected cultures with TGF-beta did not elicit any significant alteration, still showing low levels of TRII striation pattern.

BC21 - Special Lipid Domains found in Reservosomes from *Trypanosoma cruzi*

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Reservosomes are peculiar structures found in *Trypanosoma cruzi* epimastigotes. Involved in endocytosis, they are named late endosomes due to pH (approximately 6), endocytosis kinetics and absence of lysosomal markers, such as LAMP 1 and 2. On the other hand, there is no evidence about the existence of lysobisphosphatidic acid or Rab 7 and 9 so far, which are characteristic in late endosomes from higher eukaryotes. Mendonça and co-workers (Gene, 243: 179, 1999) showed that TcRab 11, a homologue of a typical GTPase of recycling vesicles in mammals, was present in reservosomes. These findings are contradictory and prompted us to isolate and investigate these structures. Our group had shown that whole purified reservosomes present twice more lipids than proteins, cholesterol and ergosterol being their most important neutral lipids (Cunha-e-Silva et al, FEMS Microbiol Lett, 2002, 214: 7). Now we aim to describe the lipid composition of their purified membranes, and analyze the existence of special lipid domains. The purified fraction was sonicated and treated with 1% Triton X-100 for 20 minutes on ice. After mixing with the same volume of 80% sucrose in 25 mM Tris-HCl pH 7.4, 150mM NaCl, 5mM EGTA, it was applied at the bottom of 5 and 30 % step sucrose gradient and spun for 22 hours at 100,000xg. The detergent resistant fraction was collected and extracted in a methanol/chloroform/water system. The chloroform phase was analyzed by thin layer chromatography for neutral lipids resulting in 70.3% cholesteryl ester, 6.9% cholesterol and 22.4% phospholipids. Moreover, the presence of GM1 ganglioside detected with cholera toxin B subunit was an unexpected result. It is a remarkable finding, once there is few data in the literature indicating its presence in an endocytic organelle. Supports: FAPERJ, PRONEX-FAPERJ, CNPq, CAPES, IFS

BC22 - Studies on the sub-cellular localization of phosphoglucomutase (PGM) in *Trypanosoma cruzi.*

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The surface of the *Trypanosoma cruzi* is covered by a dense coat of sialoglycoproteins highly *O*-glycosylated, which are thought to play a key role in host cell adhesion and invasion by this parasite, and there are evidences that the degree of surface sialylation of Gal*p* residues is critical to parasite virulence. Phosphoglucomutase is the enzyme that catalyses the reversible conversion of glucose-6-phosphate into glucose-1-phosphate, a product required for the formation of UDP-galactopyranose (UDP-Gal*p*). The *PGM* gene was cloned into the *Eco*RI/ *Xho*I sites of pET28a (Novagen), which encodes additional N and C terminal X6-His tags for rapid purification, for expression in BL21(D3) *E.coli* cells (Novagen). His-tagged proteins were purified using Ni-NTA agarose (Qiagen) under denaturing conditions. Eluted proteins were injected into rabbits for production of polyclonal antibodies against recombinant PGM. Immunofluorescence experiments revealed punctate vesicular distribution of PGM although the cell body of epimastigotes. Sub-cellular fractionation of *T. cruzi* organelles followed by Western blotting revealed that PGM is present in the glycosome enriched fraction. In others organisms, PGM is an exclusively cytoplasmic enzyme. PGM transport into glycosomes may be mediated by a cryptic import signal or by its association with other glycosomal proteins that contain classical PTS1 or PTS2 signals. Supported by CNPq; FAPERJ.

BC23 - Mucinase, a novel Trypanosoma cruzi molecule possibly implicated in gastric mucosal infection

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A considerable number of microorganisms that colonize and/or invade the mucosa have been adapted to the adverse conditions, using adhesion factors and enzymes with mucinase activity for survival and propagation. Upon oral infection, metacyclic forms of *Trypanosoma cruzi*, the agent of Chagas disease, can invade and replicate in the gastric mu-

cosal epithelium, wich is the unique portal of entry for systemic *T. cruzi* infection. This invasive capacity is associated with the presence of surface glycoprotein 82 kDa, gp82, a metacyclic stage-specific molecule that promotes host cell entry in vitro by triggering bi-directional Calcium mobilization. In this study we examined the possibility that *T. cruzi* metacyclic forms express molecules that can disrupt the mucin layer that covers and protects the stomach mucosa, thus constituting the first natural barrier for the parasite penetration. By performing mucin degradation ELISA assays, we detected mucinase-like activity in the supernatant from metacyclic forms from different T. cruzi isolates. The supernatant was concentrated and applied to an exclusion column. To determine which fractions contained the mucinase activity, they were analysed by mucin degradation assays. The positive fractions were then subjected to SDS-PAGE and the gel was silver stained to identify the sample containing the enzyme devoid of contaminants. A component of 43 kDa with mucinase activity was obtained. It was not a cysteine proteinase for its activity was unaltered by inhibitor E-64. We used the purified enzyme to immunize mice and the resultant antisera were tested in immublots containing metacyclic trypomastigote extractss. Anti-mucinase antibodies reacted with a 43 kDa protein from CL isolate (*T. cruzi* II) and G isolate (*T. cruzi* I). The structural and functional characterization of *T. cruzi* mucinase is under way. Supported by FAPESP and CNPq.

BC24 - Inactive *Trypanosoma cruzi trans* -sialidase blocks endothelial cell apoptosis by Bcl-2 expression

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Initiation of communication between *Trypanosoma cruzi*, the causative agent of Chagas' disease, and mammalian cells requires contact of parasite molecules with cognate host receptors. A lectin expressed on *T. cruzi* surface, the inactive *trans*-sialidase (iTS), binds and triggers contact-dependent activation of NF- κB pathway on endothelial cells. Since there is evidence for an anti-apoptotic effect of the NF- κ B signaling pathway in endothelial cells it was of interest to investigate the effects of iTS on human bone marrow endothelial cells (HBMEC) apoptosis induction by growth factor deprivation. Incubation in absence of serum, for 48 hours, decreased cell viability as assayed by TUNEL. Activation with iTS, however, not only failed to induce apoptosis in these cells, but also rescued the endothelial cells from growth factor deprivation effect. iTS anti-apoptotic effect is due to iTS binding to α 2,3-linked sialic acid containing molecules on endothelial cell surface since pre-incubation of iTS with α2,3-sialyllactose but not α 2,6-sialyllactose, restored cell apoptosis to control level. In addition, endothelial cells incubated with iTS had a dose dependent staining of annexin V. Further, we demonstrate that iTS blocks endothelial cells apoptosis by increasing expression of Bcl-2 to similar levels induced by the IL-8, a known anti-apoptotic factor. Taken together our results demonstrated that exposure to iTS protects endothelial cells from apoptosis induced by growth factor deprivation. Activation or prevention of cell death is a critical factor in the establishment of infections enabling parasite replication and evolution of disease. Suppression of the death pathway may facilitate the proliferation of intracellular pathogens as *T. cruzi*. Supported by: CNPq, FAPERJ, PRONEX.

BC25 - *trans*-sialidase from *Trypanosoma cruzi* promotes alterations on T-cell subsets in the hearts of infected mice

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The inflammatory process is a crucial factor for *T. cruzi* control and the establishment of host/parasite equilibrium. The *trans*-sialidase (TS) is a surface enzyme expressed in protozoan parasites like *T. cruzi* playing a critical role in establishment of infection. Although several hypotheses have been raised to explain the pathogenesis of chagasic myocardiopathy, the main molecular pathway remains unsolved. In this work we employed flow cytometry to examine the expression of adhesion molecules (LFA-1, CD2, CD28, CD32, CD49d and CD43) and activation markers (CD44 and CD69) in T cells subsets isolated from the heart of infected mice. Mice were treated with 30 μg of TS one hour before infection, as well as at day 2 and 3 post-infection, with blood trypomastigotes (Y strain). Control mice were treated with PBS. Hearts were processed and examined at day 15 post-infection. Our results show that TS-treated mice present a significant increase in CD8 and in CD4+ expression on T cells infiltrating the hearts. However, the percentage of CD4+ and CD8+ T cells remained unchanged. Previous results published in our laboratory have shown that TS is able to bind in CD4+ T cells through the sialophorin CD43 leading to T cell co-stimulation. In the present study, we found that the expression of CD43 was increased in both T cell subsets when infected mice received the enzyme. Taken together, these findings demonstrate that TS is a *T. cruzi* virulence factor, responsible for phenotypic and cellular alterations in the hearts of infected hosts. Moreover, CD43 could be actively involved in the homing of T cells to the heart, thus contributing for the pathogenesis of Chagas disease. Supported by: CNPq (PRONEX), FAPERJ and FIOCRUZ.

BC26 - Characterization and Evaluation of *Trypanosoma cruzi* ecto-nucleotidase activity and its relationship with "in vitro' ' infectivity.

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Extracellular nucleotides are been related as regulatory molecules seen to be involved in some different biological process as: cellular proliferation and differentiation, cell adhesion, immune response and purinergic signaling. The goals in this work are evaluated if ecto-nucleotidases mediate the virulence or infectivity process in *T. cruzi*. We evaluated the trypomastigotes capacity and ecto-nucleotidase activity in intact trypomastigotes, epimastigotes or amastigotes from different strains (Y, CL ,Be-62, CL-Brener) maintained or derived from acellular or cellular culture. Infective capacity was evaluated as the percentage of infected cells compared with the number of parasites per infected cell. In the first cell passage the infection capacity was highest in Y strain and lowest in Be-62 and CL-Brener. Ecto-ATPase activity was significantly higher in trypomastigotes forms showing this decreasing profile CL¿Y¿CL-Brener¿Be-62. ADP hydrolysis was similar in all strains but the resulted ATP/ADP ratio was quite different in some strains (2:1; 2:1; 4:1; 3:1 for Y, Be-62, CL and CL-Brener respectively). The AMP hydrolysis was similar to that obtained for ADP in Y and Be-62, undetectable in CL and very slow in CL-Brener. The amastigotes showed clearly preference for ATP in all strains, undetectable (CL) or very slow ADP hydrolysis (Y e Be-62) and only Y strain presented detectable ecto-AMPase activity (ATP/AMP ratio was 2.3). Epimastigotes (Y strain) ecto-ATPase activity was similar to amastigotes and ecto-ADP and AMPase was higher than ATP hydrolysis. One second passage in cellular culture resulted in a decrease in ecto-AMPase activity. These data indicated a possible higher adenosine generation capacity in the first cell passage. Adenosine was demonstrated as an immune modulator molecule related with inhibition of inflammatory responses in hosts. We suggested that the high ecto-ATPase activity plus high adenosine generation presented in Y strain could be related with highest infection capacity presented in this work. Supported by CNPq, UFOP

BC27 - Platelet-activating factor (PAF) triggers cell differentiation in *Trypanosoma cruzi* through PKC and CK2

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Mechanisms underlying the differentiation of *Trypanosoma cruzi* are poorly understood, although it is known that platelet-activating factor (PAF) triggers the differentiation of epimastigotes into trypomastigotes in vitro. PAF is a potent phospholipid mediator of several cellular functions in diverse biological and pathophysiological processes. *T. cruzi*, like other eukaryotes, present cellular functions mediated by signaling pathways involving protein kinases and phosphatases, G proteins and second messengers. Recently we showed that PAF triggers a cascade of signal transduction events, leading to the activation of casein kinase 2 (CK2), through protein kinase C (PKC) in *Herpetomonas muscarum muscarum*. Here we show the involvement of PKC and CK2 in cell differentiation of epimastigotes into trypomastigotes of *T. cruzi*, clone Dm28c in vitro. The parasites were maintained in TAUP medium for periods ranging from 1 to 6 days, in the absence or in the presence of the following drugs: PAF and/or the CK2 inhibitors DRB and TBB, and/or the PKC inhibitor BIS. The percentage of epimastigotes and trypomastigotes was daily determined. In average, PAF enhances the number of trypomastigotes by 40%, while in the presence of TBB and DRB this number was brought back to the control levels or to half the control number, respectively. BIS was also capable of inhibiting PAF effect on cell differentiation. Taking together these data suggest that PAF triggers cell differentiation in *T. cruzi* through PKC and CK2. Also, polyclonal antibodies able to recognize the catalytic subunit of the mammalian CK2 reacted with a protein from the cytoplasmic content of *T. cruzi*, through immunoblotting. The protein showed a molecular mass of 44 kDa, which is compatible with the mammalian protein. Other events related to signaling pathways involved in cell differentiation stimulated by PAF in *T. cruzi* are currently under investigation. Supported by: CNPq, PIBIC-UFRJ/CNPq and FAPERJ.

BC28 - MAMMALIAN CELL INVASION BY PHYLOGENETICALLY DISTANT TRYPANOSOMA CRUZI ISOLATES REQUIRES ACTIVATION OF DISTINCT SIGNALING PATHWAYS IN THE PARASITE AND THE TARGET CELL

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Metacyclic trypomastigotes of phylogenetically distant *T. cruzi* isolates CL and G differ widely in their ability to enter mammalian cells in vitro. Studies to elucidate the mechanisms of host cell invasion by these parasites have identified some components of the signaling pathways leading to Ca2+ mobilization, an event required for internalization. This study aimed at further identifying molecules of the signaling cascade both in the parasite and the host cell. We have found that in metacyclic forms of CL isolate, which are highly infective, the activations of the signaling cascade initiated at the cell surface by gp82 involves protein tyrosine kinase, phosphatidylinositol 3 kinase (PI3K), phospholipase C that generates inositol-1,4,5-triphosphate (InsP3) and diacylglycerol, in addition to protein kinase C, which is activated by diacylglycerol. On the other hand, a distinct signaling route

also leading to Ca2+ mobilization is induced in metacyclic forms of G isolate, which are poorly invasive in vitro, and enter target cells mainly in a manner mediated by mucin-like molecules gp35/50. Among the components possibly implicated in this pathway are: cAMP produced by adenylyl cyclase, cAMP-dependent protein kinase and PI3K. Both gp82 and gp35/50 induce Ca2+ response in the host cell, by activating distinct signal transduction pathways. Also contributing to invasion and intracellular replication of CL isolate, but not of G isolate, is the major *T. cruzi* cysteine proteinase cruzipain. Work supported by FAPESP and CNPq.

BC29 - ENDOCYTOSIS, VESICULAR TRAFFICKING AND OXIDANT ROLE OF HEME (Fe-PROTOPORPHYRIN IX) IN *Trypanosoma cruzi* EPIMASTIGOTES.

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Trypanosoma cruzi, the etiologic agent of Chagas disease, is transmitted through triatomine insect vectors during the blood meal on vertebrate host. These hematophagous insects usually ingest in a single meal about 10 mM heme bound to hemoglobin. We have demonstrated that the addition of hemin in the medium (from 0 to 1 mM) increased the parasite proliferation in a dose dependent manner. Pd-Mesoporphyrin IX (an analogous of heme) intrinsic fluorescence was used as a label to trace the fate of heme taken up by the parasite. We followed the time course of Pd-mesoporphyrin IX and hemoglobin-rhodamine internalization in parasites. Both ligands are taken up by endocytosis and follow the same pathway inside vesicles nearby cytostome, kinetoplast and nucleus, reaching reservosomes at parasite posterior region. Endocytosis kinetics, however, indicates that heme analogous uptake is much faster. Since heme is a powerful generator of reactive oxygen species (ROS), which can damage a variety of biomolecules, we are investigating how these parasites protect themselves against heme by addition of BSO (inhibitor of glutathione synthesis) or DETC (inhibitor of superoxide dismutase) in the medium. We observed drastically increases in the basal level of ROS when heme 100 μM was added in culture, as determined by microscopy and flow cytometry analysis of CMH2DCFDA fluorescence. GSH is an important precursor of trypanothione. So, taken together, our data suggest that *T. cruzi* takes up the heme from insect blood meal and in the course of their evolution history, had to develop adaptations to avoid the deleterious effects of high concentrations of heme found in this environment.These defenses are involved with GSH and SOD activity. Supported by FAPERJ, CNPq, PADCT, HHMI (to PLO) and PRONEX.

BC30 - EXPRESSION OF HIGHLY ACTIVE CRUZIPAIN IN TRYPANOSOMA CRUZI CLONE CL-14 IS POSSIBLY ASSOCIATED WITH DEFICIENT EXPRESSION OF GP82 ON THE SURFACE

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Metacyclic trypomastigotes of *T. cruzi* clone CL-14 entered epithelial HeLa cells in much lower numbers than the parental CL isolate, and failed to produce patent infection in mice upon oral inoculation, in contrast to CL isolate that is highly invasive *in vitro* and *in vivo*. Analysis of clone CL-14 surface profile revealed low levels of gp82, the main surface molecule of CL isolate implicated in host cell entry *in vitro* and invasion of gastric mucosal epithelium *in vivo*. They also differed in the expression of cruzipain, the major *T. cruzi* cysteine proteinase involved in cell invasion and intacellular replication, much higher activity being detected in clone CL-14. We investigated the possibility of a connection between the high cruzipain expression and low gp82 levels on the surface of clone CL-14. To test that possibility, we treated J18, a recombinant gp82, with the supernatant from metacyclic trypomastigotes of clone CL-14, designated as cruzipain*, and analysed the samples by imunoblotting, using anti-gp82 MAb 3F6. Considerable degradation of J18 was observed upon treatment with cruzipain*, the effect being negligible when J18 was incubated with cruzipain* in the presence of cysteine proteinase inhibitors. In another set of experiments, we used cruzipain*, to treat metacyclic forms of CL isolate and then analysed the expression of gp82 by flow cytometry. Reduced levels of gp82 was found in parasites incubated with cruzipain*, indicating that gp82 was cleaved to the point of losing the cell binding site, which is located next to the epitope for MAb 3F6. Such a decrease in gp82 was not detected in parasites incubated with cruzipain* in the presence of cysteine protease inhibitor. Our data suggests that the highly active cruzipain secreted by clone CL-14 may be an important factor contributing for low expression of gp82 on clone CL-14 surface. Work supported by FAPESP

BC31 - Studies of inactive *trans*-sialidase on the first step in immune response to *Trypanosoma cruzi* infection

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Chagas' disease, caused by the protozoa *Trypanosoma cruzi*, is the major cause of cardiomyopathy in endemic areas of Latin America. *T. cruzi* expresses on its surface an inactive member of *trans*-sialidase family that interacts with sialic acid-containing molecules on host cells. Recently, we

demonstrated that iTS binds to α-2,3-linked sialic acid of CD43 (sialophorin) on humans neutrophils surface. Neutrophils are key players of the innate immune system and provide a first line of defense against invading pathogens. It has also been reported that neutrophils execute several specialized functions including chemotaxis, phagocytosis and generation of reactive oxygen metabolites. In this work we aimed to elucidate the biologic effects of iTS interaction with CD43 on neutrophil surface, during the acute phase of Chagas' disease. Using microscopy, flow citometry and biochemical methods we demonstrated that iTS was able to induce chemotaxis, actin cytoskeleton reorganization and delays human neutrophils spontaneous apoptosis in vitro. In addition, neutrophils activated by iTS produced and released IL-8, is citokyne able to increase the half-life of the neutrophil. Moreover, incubation of human neutrophils with iTS triggered the oxidative burst as observed by the oxidation of sodium borohydride-reduced derivative of ethidium bromide. Finally, we also demonstrated that iTS promotes neutrophils activation through ERK-2 nuclear translocation. These data suggest that iTS may orchestrate the early immunity during *T. cruzi* infection by activating humans neutrophils. Supported by: FAPERJ, CNPq (PRONEX)

BC32 - Reactivity of mAb BST-1, direct to glycosilinositolphosphoceramides, with different strains of *Trypanosoma cruzi*

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The LPPG, a glycosylinositolphosphorylceramide (GIPC), is the major glycoconjugate present in epimastigotes of *Trypanosoma cruzi*. This glycoconjugate is higly immunogenic due to the presence of one or two residues of galctofuranose (Gal$f$) linked β 1-3 to terminal or subterminal residues of mannose of the core 4Man(AEP)GlcN-Ins-P. Carreira et al. (1996) classified *T. cruzi* GIPCs into two series, depending on the substitution at the third mannose distal to inositol, as ethanolamine-phosphate or aminoethylphosphonic acid to Serie-1 (GIPCs of G strain), and β-Gal$f$ to Serie-2 (major GIPCs of CL and Y strains). A monoclonal antibody (mAb) against purified GIPCs of epimastigotes (CL) were produced and termed BST-1. By ELISA it was verified that BST-1 recognizes with high affinity GIPCs from strains CL and Y (mainly Serie-2). On the other hand, BST-1 did not react with GIPL-1 of *Leishmania major*, and with the major GIPCs of strain G (Serie-1). These results indicate that BST-1 does not recognize only the Gal$f\beta$1-3Man, but it recognizes a larger epitope present in GIPCs from Serie-2. By indirect immunofluorescence it was observed high fluorescence with epimastigotes of the three strains. While strains CL and Y presented fluorescence all over the parasite, epimastigotes of G strain presented fluorescence localized at epimastigote posterior region and the flagellar pocket. By Western blotting, it was demonstrated that BST-1 recognizes two components of 80 and 115 kDa in epimastigotes of strain G and not visualized in strains Y and CL. These data indicate that epimastigotes of strain G express the BST-1 recognized epitope in a 80-115 kDa glycoproteins. BST-1 also recognizes intracellular amastigotes and culture trypomastigotes. By Western blotting, high molecular weight components (140-180 kDa) reactive with BST-1 were detected in both amastigotes and trypomastigotes of strains CL, Y and G. These glycoproteins are currently under investigation. Supported by FAPESP and CNPq

BC33 - Cell cycle progression of *Trypanosoma cruzi* is related to flagellum growth

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The duplication and separation of organelles producing two identical daughters must be precisely controlled during the cell cycle progression of kinetoplastid-flagellated protozoa. This includes the division of the single mitochondria containing the kinetoplast DNA, and the flagellum, which grows from a cytoplasmic basal body through the flagellar pocket compartment, before emerging from the cell. To understand when these structures are duplicated, we studied the morphological alterations during the cell cycle progression of epimastigote form of Trypanosoma cruzi. We found that the new flagellum appears and grows at G2 phase, only after the complete replication of nuclear and kinetoplast DNA. Differently from the old flagellum, it emerges and remains unattached to the parasite. It keeps growing freely from the cellular body, until the cells pass through mitosis and enter cytokinesis, when the new flagellum reaches its final size. The attachment of the new flagellum to the cell body occurs independently of the completion of cytokinesis, which can be prevented by okadaic acid, a phosphatase inhibitor. The new flagellum emerges from the same flagellar pocket that contains the old flagellum, and during cytokinesis, when a new cell body is formed, the flagellar pocket divides and the cytoplasm extends and attaches to the new flagellum. These results describe for the first time the cell cycle progression in epimastigote forms and suggest that flagellum growth is strictly controlled during T. cruzi cell cycle. Supported by FAPESP/CNPq

BC34 - Expression of trypomastigote trans-sialidase in metacyclic forms of *Trypanosoma cruzi* accelerates parasite exit from the parasitophorous vacuole

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Trypanosoma cruzi invades host mammalian cells by forming a parasitophorous vacuole. After cell invasion the parasite has to escape from this vacuole to start growing inside the

host cell cytoplasm. *T. cruzi* express trans-sialidase (TS) on its surface, which has been previously implicated on the vacuole escape. Here we show that cell derived trypomastigote forms has 5 to 10 times more TS activity than metacyclic trypomastigote forms. As these two parasite forms invade similarly HeLa cells, we conclude that TS is not rate limiting for cell entry. In contrast trypomastigotes exit much faster from the vacuole as compared to metacyclics. To test whether TS expression in trypomastigotes is responsible for this difference, we expressed constitutively an epitope tagged trypomastigote enzyme in metacyclic forms. We obtained two cell lines; one of them (line 17) lacked the signal sequence and expressed TS in the parasite cytoplasm. The other one contained the TS gene preceded by its signal sequence (line 8) and expressed large amounts of TS activity, which was released into the medium as does trypomastigotes. We found that metacyclic forms of these lines adhere and enter equally HeLa cells. However, line 8 escapes as fast as trypomastigotes from the vacuole, and more rapidly than line 17, or untransfected metacyclic forms. Similar results were obtained with CHO cells, but not with sialic acid-deficient CHO cells (Lec-2). In the later there was no difference in the exit from the vacuole. In addition, we found that line 8 differentiates to amastigote forms as fast as trypomastigotes, and earlier than line 17 and wild type metacyclic forms, suggesting that escape from host cell vacuole is necessary to complete the differentation in amastigote forms. These results definitively provide a role of TS in the exit from the cell phagolysosome. Supported by FAPESP / CAPES

BC35 - Trypanosoma cruzi epimastigote endocytosis dependence on cytoskeleton varies with cargo size

Jeovanio-Silva, A.L. *(UFRJ)*; Sant' Anna, C. *(UFRJ)*; De Souza, W. *(UFRJ)*; Cunha-e-Silva, N. *(UFRJ)*

Trypanosoma cruzi epimastigotes are skilled to uptake big particles. They enter through flagellar pocket and/or cytostome and travel along parasite body to finally reach reservosomes. To study the participation of the cytoskeleton in this process, epimastigotes were pre-incubated with cytoskeleton disturbing drugs, at concentrations compatible with cell viability, before 100 or 210nm fluorbeads or transferrin-Alexa incubation. Only tracer-containing parasites were counted in each experiment. 10ug/mL cytochalasin D completely blocked 100 and 210nm beads entry, and allowed transferrin delivery to reservosomes in only 3% epimastigotes. 3.3ug/mL cytochalasin retained at the entry site 56.9% of 210nm and 29% of 100nm bead-containing parasites, but only 1% of transferrin-containing epimastigotes. Jasplakinolide (5uM) also acted at cargo entrance, retaining there 210nm beads in 68.3%, 100nm beads in 72% and transferrin in 55.1% of the cargo-containing parasites. Interestingly, jasplakinolide allowed bead delivery to reservosomes in only 2.9% (210nm) and 8% (100nm) but transferrin managed to reach reservosomes in 28% of the parasites. Microtubule disturbing drugs were less effective in impairing transferrin uptake (23.5% of 50uM oryzalin treated parasites and 15.1% of 20uM taxol treated epimastigotes presented cargo at the entry site) than bead capture: oryzalin blocked the entry of 210nm in 79.6% and of 100 nm beads in 70% of cargo-containing parasites. 20uM taxol retained beads at the entrance in 61.9% (100nm) and 61% (210nm) of the parasites. Again, drug treatment affected more delivery to reservosomes when the ingested particle was bigger: only 3% of taxol- and less than 1% of oryzalin-treated parasites presented 210nm in reservosomes, whereas 54.7% of taxol- and 42.5% of oryzalin-treated parasites contained transferrin in reservosomes. These data strongly indicate that dependence of endocytic pathway on cytoskeleton dynamics is influenced by cargo particle size, possibly having as critical steps cytostome opening diameter variation and vesicle fusion with reservosomes. Supports: CNPq, FAPERJ.

BC36 - Establishment of mouse embryo cardiomyocytes cultured as spheroids and infected with *Trypanosoma cruzi*

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Cells isolated from many tissues self-organize *in vitro* to form multicellular structures under suitable conditions and frequently retain functional characteristics typical of the tissue of origin. For example cells from adult pancreatic islets that re-associate to form clusters with organization and insulin comparable to that found in native islands (Halban et al., 1987). We have established primary cultures of mammalian cells as cardiomyocytes that usually flatten and spread upon attachment. Following the normal myogenic process, 60 to 80% of the cells attached to gelatin coated substrate exhibiting a bipolar aspect characteristics of myoblasts (Meirelles et al., 1984, 1986). We now developed cardiomyocytes primary cultures as spheroids by placing freshly isolated embryo heart cells in a supplement medium with 15% calf serum, 5% chick embryo extract, 1mM glutamine and antibiotics. The cells infected or not with *Trypanosoma cruzi* were maintained in a rotary Shaker New Brunswick Scientific Innova 2000 at a constant speed. We used a neutral charge polymer (2-hydroxyethyl metachrilate pHema) in non adherent substratum. Spheroids are tridimensional structures that are formed when the adhesion to the substrate is blocked and then the cells adhere to the each other, remaining in suspension for long times and also maintaining in vivo-like morphology as shown by electron microscopy. Our ultrastructural approaches revealed that spheroids display electron dense membrane contacts as junctional complexes, membrane projections, and mantaining attached as cell groups and viability for long periods of culture. In infected spheroids the parasites could be found developing its cell cycle, being also observed in the multiplicative form. The preliminary results of the parasite invasion showed cardiomyocyte spheroids invasion being localized near the cell nucleus and endoplasmic

reticulum. Supported by CNPq, PAPES/FIOCRUZ

BC37 - Studies with Carrageenan in Mice During *Trypanosoma cruzi* Interaction

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Carrageenan (CAR) is a family of polysulphated carbohydrates extracted from marine red alga that in a non-fractionated form is known to induce acute inflammation (Winter et al., 1962; Fukuhara and Tsurufuji, 1969), paw oedema (Vinegar et al., 1969; Henriques et al., 1987), injury to blood vessels (Ward and Cochrane, 1965), inhibition of complement functions (Davies 1965), kinin release (Di Rosa and Sorrentino, 1970), potentiation of endotoxicity (Becker and Rudbach, 1978; Lo et al., 1987) and the formation of air pouch in rodents (Sedgwick and Lees, 1986). Purified forms of CAR vary as to type namely CAR-kappa, -lambda and -iota according to total sulphate-content distribution as well as molecular configuration. CAR contributes to the pathophysiology of inflammation. The present study proposes to evaluate the modulatory action of CAR-lambda. It has been documented that this type of CAR induces the synthesis of prostaglandins by macrophages which could influence the pathophysiology of *T. cruzi* infection in mice. Mice treated with CAR, 4h or 24h, before infection with *T. cruzi* presented a reduced parasitaemia as compared with control group (only infected mice). The histopathology analysis showed the presence of parasite nest but a lower inflammatory infiltrate. An enhancement of TNF-alfa level in sera was observed in mice treated with CAR injected 24h before *T. cruzi* in Swiss mice. Further studies are being carried on to compare the action of CAR with LPS in experimental Chagas' disease. Supported by FIOCRUZ, FAPERJ and CNPq.

BC38 - LIPID RAFT FROM *Trypanosoma cruzi*

Vargas, C.S. *(UFRJ)*; Corrêa, J.R. *(Fiocruz)*; Soares, M.J. *(Fiocruz)*; Atella, G.C. *(UFRJ)*

Trypanosoma cruzi is hemoflagellate that employs a wide variety of mammalian hosts and hematophogus insects in its life cycle. Under natural condition this parasite is transmitted to the vertebrate host by triatomine insects. In the vector the parasite reproduces asexually. Lipid rafts are cholesterol-enriched microdomains that are detergent-resistant at low temperatures and are involved in a number of cellular processes such as trafficking and cell signaling. The objective of this work is the purification and characterization of lipid rafts to define their role on *T. cruzi* endocytosis. Sucrose density centrifugation is the standard method for lipid rafts isolation. The parasites were lysed, homogenized and incubated at 4^{o}C in Triton X-100 1%. After 30 minutes the homogenate was subjected to a sucrose gradient. Cholera toxin B subunit (CTB) is a specific ligand for ganglioside GM1 and can be used for the detection of GM1 containing microdomains. In order to investigate the presence of GM1, the fractions were submitted to a dot-blot using colera toxin. We identified a large GM1 content in the corresponding fractions 5 to 8 of the sucrose gradient. Amplex Red Cholesterol Assay Kit was used for quantification of total content of cholesterol in the gradient fractions. The cholesterol content reached to the maximum on the lipid raft fraction corresponding to a 400 μg/mL of total cholesterol. The raft containing fractions were pooled and subjected to lipid extraction and TLC. The spots were analyzed by densitometry. Lipid composition of rafts is: cholesterol-ester (44.61%), Ergosterol (15.90%), Free Fatty Acid (6.05%), Monoacylglicerol (4.83%), phospholipids (12,90%) and triacylglicerol (15.57%). Supported by CNPq, FAPERJ, IFS

BC39 - Analysis of the shedding process in trypomastigote forms of Trypanosoma cruzi

Torres, PLAS *(UFRJ)*; Souto-Padron, T *(UFRJ)*

Cleavage or shedding of functional proteins is an important phenomenon in modulation of cell surface. Previous studies have shown that two kinds of host immnoglobulins can interact with the surface of Trypanosoma cruzi, a non-specific, bind to the parasite surface trough either Fc or Fab fragments, and specific IgG antibodies, capable to induce mobility of surface antigens of live blood forms to form a cap in the anterior and posterior poles of parasite. Shedding of surface antigens of trypomastigote forms of T. cruzi was described as a spontaneous process involving a plasma membrane vesiculation. The aim of present study was the analysis of the shedding process of specific and non-specific IgG. For this, trypomastigote forms from Y strain or CL Brener clone were incubated in the presence of non-related IgG or chagasic serum first at 4C for binding of IgGs and subsequently at 37C for different periods in way to observe the shedding process. Some samples were pre-treated with sodium azide or PMA. The analysis of all experiments was performed in a FACScan. Shedding of specific IgG is more active than non-specific IgG in both strains used, showing about 70 percent decrease on fluorescence intensity in Y strain after 60 minutes at 37C. In the presence of sodium azide, shedding was significantly impaired. PMA stimulated the shedding of non-specific IgG in Y trypomastigotes when compared to specific IgG. The opposite could be detected in CL Brener clone. We concluded that a more intense shedding occurs when trypomastigote forms are in contact with specific IgGs, that available energy is important in this process and that activation of PkC probably is not major pathway in shedding regulation. Studies in the presence of 1,10-phenantroline are in course to observe its possible involvement in shedding regulation.

BC40 - ANALYSIS OF SEQUENCES OF THE SATELLITE DNA IN Trypanosoma cruzi STRAINS BELONGING TO DIFFERENT ZYMODEMES AND BENZNIDAZOLE SUSCEPTIBILITY.

Liarte DB *(CPqRR)*; Murta SMF *(CPqRR)*; Romanha AJ *(CPqRR)*

The existence of *T. cruzi* strains naturally resistant to benznidazole (BZ) and nifurtimox may be an important factor in explaining the low rates of the cure in treated chagasic patients. The total amount of DNA of *T. cruzi* varies from 120 to 330 fg/parasite and approximately 10% are constituted of 195bp sequence repetitive named satellite DNA. Due its abundance and polymorphism, this sequence has been used for Chagas' disease diagnosis and more recently for phylogenetic purposes. In this study we investigate the possibility of using the DNA satellite polymorphism for *T. cruzi* DNA detection and drug resistance identification simultaneously. Polymorphism of the satellite DNAs from 12 *T. cruzi* strains from different zymodemes and BZ susceptibility were determined. All sequences were amplified, cloned, sequenced and analyzed using different programs. The sensibility for detection of the 195bp fragment DNA in PCR was 1fg. Fourty-five contigs, were sequenced at least three times each. The results showed an association of the polymorphism with *T. cruzi* zymodeme but not with drug resistance phenotype. The strains were divided into the two main groups *T. cruzi* I (Zymodeme 1) and *T. cruzi* II (Zymodemes 2 and B). The alignment of the sequences showed a low degree of entropy with the polymorphisms concentrated in 25 to 30 different positions. The polymorphisms found in seven positions allow the distinction of the two *T. cruzi* main groups. In addition, a search in Transfac database allowed the identification of binding sites of transcriptional factors in 195bp sequence. Some sites showed quantitative and/or qualitative differences among strains. We believe that the knowledge and adequate use of the polymorphism in abundant repetitive DNAs will allow the specific and intra-specific diagnosis of *T. cruzi*. In blood of chagasic patients. Supported by CNPq, FAPEMIG and PAPES/FIOCRUZ.

BC41 - Trypomastigotes of *Trypanosoma cruzi* induced TGF-beta production from activated macrophages regulating the expression of iNOS by PS exposure

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Trypanosoma cruzi is the agent of Chagas diseases that infects about 16 to 18 million individuals in Latin America with 120 million people being at risk of infection. *T. cruzi* presents basically three different forms during its life cycle: amastigotes, epimastigotes and trypomastigotes. The latter initiates the infection in humans; thus, it has to deal with the innate immune system, especially macrophages. Our group has shown that tripomastigotes, from blood, supernatant of infected Vero cells, and after metacyclogenesis in chemically defined medium (metacyclic), exposed phosphatydilserine (PS) at their surface. The involvement of transforming growth factor-beta 1 (TGF-beta) secreted by infected macrophages has been implicated in many different evasion mechanisms of pathogens. Recently the induction of TGF-beta secretion by infected macrophages was related to the exposure of PS on *Leishmania amazonensis* and *Toxoplasma gondii*, thus, mimicing the anti-inflammatory response caused by apoptotic cells. The exposure of PS on the surface of *Trypanosoma cruzi* and its deactivating effect on macrophages, seen by downregulation of inducible nitric oxide synthase (iNOS), caused by the induction of TGF-beta were analized. For that, activated macrophages (interferon-γ and lipopolysaccharide) cultured for 24 h were infected with blood trypomastigotes in the presence or absence of annexin V for 2 h and expression of iNOS, and phosphorylated Smad 2 (TGF-beta intracellular signaling protein) assayed by immunofluorescence microscopy. *T. cruzi* infection induced nuclear translocation of phosphorylated Smad 2 in macrophages and abolished iNOS expression. However, the interactions in the presence of annexin V (PS blocker) showed opposite results. Thus, *T. cruzi* have the capacity to subvert the normal microbicidal system of activated macrophages by PS exposure leading to a TGF-beta signaling pathway causing disappearance of iNOS. Supported by: FAPERJ, FIOCRUZ, CNPq

BC42 - ULTRASTRUCTURAL AND CONFOCAL LASER SCANNING IMUNOCYTOCHEMISTRY ANALYSIS OF PROLINE RACEMASE EXPRESSION IN TRYPANOSOMA CRUZI

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The protozoan parasite T. cruzi is the causative agent of Chagas' disease, which affects about 16-18 million people. Its amino acid (proline) racemase has been cloned and immunofluorescent approaches suggested its intracellular localization in replicative non-infective forms while membrane-bound and secreted forms of the enzyme were present upon differentiation of the parasite into non-dividing metacyclic forms. Since the secreted form (i) is a potent host B-cell mitogen supporting parasite evasion of specific host immune responses, and (ii) has been linked to the immunosuppression and pathology of the chronic chagasic phase, our aim was analyze, by ultrastructural, confocal laser scanning microscopy and differential interference contrast (DIC), the proline-racemase enzyme expression in both epimastig-

otes and bloodstream trypomastigotes of T. cruzi. DIC microscopy associated to fluorescent analysis showed positive labeling in several intracellular compartments distributed in the cytoplasm of the epimastigotes, which did not showed plasma membrane labeling. The analysis of proline-racemase expression by transmission electron microscopy confirmed its cytoplasmatic localization. The gold particles could be seen within vesicles of different sizes near the flagellar pocket (FP), which is an important site of endocytic/exocytic in kinetoplastids. In fact, some gold particles were noted at extracellular sites, which is suggestive of enzyme secretion through the FP. In addition, a frequent and curious positive labeling was also noted close at the main structure related to endocytosis of epimastigotes, the cytostoma, deserving further analysis. Negative controls performed by the primary antibody omission and by non immune serum did not showed any considerable staining. Fluorescent assays performed with bloodstream forms showed intracellular labeling mostly at the posterior region. Ultrastructural immunolabeling of both bloodstreams as well intracellular amastigotes are now under way to investigate the expression of proline-racemase throughout the parasite development within vertebrate hosts. Supported by Fiocruz PAPES, PDTIS

BC43 - ANALYSIS OF SEQUENCES OF THE SATELLITE DNA IN Trypanosoma cruzi STRAINS BELONGING TO DIFFERENT ZYMODEMES AND BENZNIDAZOLE SUSCEPTIBILITY

Liarte DB *(CPqRR)*; Murta SMF *(CPqRR)*; Romanha AJ *(CPqRR)*

The existence of *T. cruzi* strains naturally resistant to benznidazole (BZ) and nifurtimox may be an important factor in explaining the low rates of the cure in treated chagasic patients. The total amount of DNA of *T. cruzi* varies from 120 to 330 fg/parasite and approximately 10% are constituted of 195bp sequence repetitive named satellite DNA. Due its abundance and polymorphism, this sequence has been used for Chagas' disease diagnosis and more recently for phylogenetic purposes. In this study we investigate the possibility of using the DNA satellite polymorphism for *T. cruzi* DNA detection and drug resistance identification simultaneously. Polymorphism of the satellite DNAs from 12 *T. cruzi* strains from different zymodemes and BZ susceptibility were determined. All sequences were amplified, cloned, sequenced and analyzed using different programs. The sensibility for detection of the 195bp fragment DNA in PCR was 1fg. Fourty-five contigs, were sequenced at least three times each. The results showed an association of the polymorphism with *T. cruzi* zymodeme but not with drug resistance phenotype. The strains were divided into the two main groups *T. cruzi* I (Zymodeme 1) and *T. cruzi* II (Zymodemes 2 and B). The alignment of the sequences showed a low degree of entropy with the polymorphisms concentrated in 25 to 30 different positions. The polymorphisms found in seven positions allow the distinction of the two *T. cruzi* main groups. In addition, a search in Transfac database allowed the identification of binding sites of transcriptional factors in 195bp sequence. Some sites showed quantitative and/or qualitative differences among strains. We believe that the knowledge and adequate use of the polymorphism in abundant repetitive DNAs will allow the specific and intra-specific diagnosis of *T. cruzi* in the bloodstream of chagasic patients. Supported by CNPq, FAPEMIG and PAPES/FIOCRUZ.

BC44 - *Trypanosoma cruzi* PROTEINS ASSOCIATED TO BENZNIDAZOLE RESISTANCE

de Andrade, H.M. *(CPqRR)*; Murta,S.M.F. *(CPqRR)*; Nirdé, P. *(CPqRR)*; do Rêgo, J.V. *(CPqRR)*; de Chapeaurouge, A. *(CPqRR)*; Perales, J.H *(IOC)*; Romanha, A.J. *(CPqRR)*

In order to identify *T. cruzi* proteins associated with benznidazole (BZ) resistance, we compared proteomic profile of epimastigote forms from *T. cruzi* population and clones with *in vivo* selected resistance to BZ (BZR and Clone27R), its susceptible pairs (BZS and Clone9S) and a pair from a population with BZ-*in vitro*-induced resistance, 17LER and its susceptible pair 17WTS. Total proteins from each sample were fractionated on bidimensional polyacrylamide gel electrophoresis. Gels were made in triplicate, stained with colloidal Coomassie, digitalized and analyzed (PDQuest, Bio-Rad). Gel images were first compared intrastrain and then interstrain. Gel master image of a resistant sample was aligned and compared with gel master image of its corresponding susceptible pair. This comparison allowed the identification of the presence or absence of the same spots and the determination of the ratio between the volumes of each spot in gel pairs. Ratio between spots ≥ 2.5 was considered significant. Differentially expressed spots were excised from the gels for protein identification through mass spectrometry (MS). BZ-resistant samples presented more total and orphan spots than their pairs. Out of 139 spots analyzed through MS, 80 (58.4%) presented well defined spectra and 62/80 (77.5%) were identified as 38 different proteins. Out of the 38 identified proteins, 27 (71%) were associated to drug resistance and 7 (15.8%) to drug susceptible phenotype. The proteins from the resistance phenotype were mainly to detoxification, metabolism and response to stress functions. The proteins from the susceptible phenotype were mainly to the structural function. Financial support: PDTIS/FIOCRUZ, CNPq, FAPEMIG

BC45 - Entrance mechanism of trypomastigotes of *Trypanosoma cruzi* in mice peritoneal macrophages cultured with homologous serum is by phagocytosis

Lobato, C.S.S. *(UENF)*; Mota, L.A.M *(UENF)*; Monteiro,V.G. *(UENF)*; Seabra, S. H. *(UFRJ)*; Souza, de W. *(UFRJ)*; DaMatta, R.A. *(UENF)*

Entrance mechanism of trypomastigotes of *Trypanosoma cruzi* in mice peritoneal macrophages cultured with homologous serum is by phagocytosis Lobato ,C.S.S [1], Mota, L.A.M [1], Monteiro,V.G. [1], Seabra, S.H [1]
&2, Souza, W de [2] , DaMatta R.A [1] UENF [1] Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, UENF; [2]. Instituto de Biofísica Carlos Chagas Filho, UFRJ renato@uenf.br It has been demonstrated that mice peritoneal macrophages cultured in the presence of homologous serum (HS) express sialoadhesin (SD), a surface receptor that recognize sialic acid. Our group has demonstrated that SD increases the association of *T. cruzi epimastigoteandtrypomastigote* to macrophages due to its glicocalix rich in sialic acid. It is not clear whether the entrance mechanism of trypomastigotes in macrophages is by active penetration or phagocytosis. In this work this mechanism was determined through the use of cytochalasin D that blocks the phagocitic process of macrophages. Macrophages were cultured with HS and fetal bovine serum for 48h at 37°C, treated with cytochalasin D for 30 minutes, and infected with trypomastigotes for 2h. After interaction, the cells were fixed and counted under an optical microscopy. As expected, parasites associated more to macrophages cultured with HS, confirming that the expression of SD increases this association. Macrophages cultured with HS treated with cytochalasin D presented higher amounts of adhered parasites. Furthermore, it was not possible to observe interiorized parasites. It is possible to conclude that the entrance mechanism of this parasite in these macrophages is by phagocytosis. Supported by: FAPERJ, CNPq, UENF.

BC46 - Molecular identity and heterogeneity of trichomonad parasites in a closed avian population.

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Trichomonas gallinae is the causative agent of avian trichomoniasis or canker, it occurs worldwide and presents a threat to domesticated birds from the turkey to the ostrich and wildlife species as diverse as the penguin and the goshawk. Despite this prevalence, the possible occurrence of genetic variation within this parasite has not been investigated so far. The Mauritian Pink Pigeon (*Columba mayeri*) is the world's rarest pigeon, and, despite concerted effort to increase the population, several parasites appear to be contributing to low productivity and high mortality rates, with trichomoniasis pre-eminent amongst these. The pink pigeon population is actively managed and thus a detailed record of the life-history and ontogeny of all birds has been compiled. In order to evaluate the genetic variation present in *T. gallinae* populations of the Mauritian islands and its possible correlation with epidemiological aspects, twenty four positive samples were collected from the endangered endemic Mauritian Pink Pigeon, *Columba mayeri* and the introduced Madagascar Turtle Dove, Streptopelia picturata. The 5.8S rRNA region and flanking internally transcribed spacer regions (ITS1 and ITS2) were amplified by polymerase chain reaction, using universal trichomonad primers. Sequencing of this region revealed no variation within this section of the genome and 100% homology with a previously sequenced *T. gallinae* isolate obtained from an unrelated pigeon. Random amplified polymorphic DNA (RAPD) analysis of all 24 isolates revealed significant genotypic variation, while comparison of genotype with the life-history of the avian hosts allowed correlation with factors including with host, age, sex, species, co-infection, geography, virulence and date of isolation.

BC47 - Platelet-activating factor (PAF) and cAMP stimulate cellular differentiation in *Herpetomonas muscarum muscarum* in a PKA activity-dependent fashion

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Herpetomonas muscarum muscarum is a house fly flagellate parasite of the family Trypanosomatidae that presents three evolutive forms: promastigote, paramastigote and opisthomastigote. Cyclic AMP (cAMP) is a second messenger that regulates important functions such as cell proliferation, differentiation and host cell invasion by parasites. Protein kinase A (PKA) is a protein kinase dependent on cAMP, whose targets have not been identified in parasites yet. Platelet-activating factor (PAF) is a potent phospholipid mediator of several cellular functions in diverse biological and pathophysiological processes, such as cell differentiation, inflammation and allergy. PAF is known to enhance cell differentiation in *H. m. muscarum* and in *Trypanosoma cruzi*. Recently we showed that PAF triggers a cascade of signal transduction events, leading to the activation of casein kinase 2 (CK2), through protein kinase C (PKC) in *H. m. muscarum*. Here we demonstrate that the enhancement of cellular differentiation promoted by PAF and cAMP in *H. m. muscarum* is mediated by PKA. The parasites were grown for periods ranging from 1 to 3 days, in the absence or in the presence of the following drugs: PAF and/or cAMP and/or the PKA inhibitor H89. The percentage of non-differentiated forms and differentiated forms was daily determined. On the third day of incubation, the following percentages of differentiated forms were observed: PAF (70%), cAMP (75%), H89 (42%), PAF + cAMP (75%), PAF + cAMP + H89 (43%), cAMP + H89 (38%), PAF + H89 (32%), as compared to the

control parasites, which presented 40% differentiated forms. We also describe an enhancement of PKA activity when the cells were treated with PAF, cAMP and PAF plus cAMP. These results are suggestive that PKA is a mediator of signaling pathways triggered by PAF and cAMP that culminate in cellular differentiation of *H. m. muscarum*. Supported by: CNPq, FAPERJ, CNPq/PIBIC-UFRJ.

BC48 - Signaling pathways modulated by lysophosphatidylcholine and phosphatidylcholine in *Herpetomonas samuelpessoai*

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Herpetomonas samuelpessoai presents three evolutive forms: promastigote, paramastigote and opisthomastigote. Previously we have shown that PAF triggers cell differentiation of *H. m. muscarum* and *Trypanosoma cruzi*. Lysophosphatidylcholine (LPC) is a major bioactive compound of plasmatic lipoproteins like LDL. It has also been demonstrated that LPC is present in *Rhodnius prolixus* saliva, acting as an anti-haemostatic molecule. The enzyme phospholipase A2 (PLA2) catalyze specifically the hydrolysis of the 2-ester bond of 3-sn-phosphoglyceride, transforming phosphatidylcholine (PC) into LPC. Here we describe the effects of LPC and PC modulating signaling pathways related to cell differentiation of *Herpetomonas samuelpessoai*. The parasites were treated for 4 hours with the following drugs: PAF, PC, LPC, the PKA inhibitor H 89, the PKC inhibitors BIS I and BIS T, the calmodulin kinase inhibitor KN 93, the myosin light chain protein kinase inhibitor ML-7, the MAPK (ERK) inhibitor PD 98059 and the CK2 inhibitor TBB. Then, the cells were disrupted and the cytoplasmic content of these parasites was assayed for protein phosphorylation using 32P-ATP. The proteins were separated by SDS-PAGE and the gels were exposed to X-ray film. The results showed differences in the phosphoprotein profiles of the parasites that were treated with PAF, LPC, LPC plus H 89, LPC plus PD 98059 and LPC plus TBB. We also analyzed the effects of the PLA2 inhibitors ACA and MJ 33, as well as the CK2 inhibitor TBB on cellular differentiation of *H. samuelpessoai*. The percentage of differentiated forms (paramastigote + opisthomastigote) observed in these experiments were: 42% (control), 81% (LPC), 39.8% (PC+ACA), 38.5% (PC+MJ 33), 63% (LPC+TBB), 66.7% (ACA), 65% (MJ 33) and 59.5% (TBB) Our results suggest that LPC modulates some important signaling pathways that leads to cell differentiation of *H. samuelpessoai* and that PLA2 probably rules this process by converting PC into LPC. CNPq, FAPERJ, CNPq/PIBIC-UFRJ.

BC49 - Infection of VERO cells and human macrophages by *Blastocrithidia culicis* and *Crithidia deanei*. Role of endosymbiont and Nitric Oxide in the infection process.

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Introduction: Monoxenous trypanossomatids were known as protozoa not able to infect vertebrate cells. However, we have recently reported the infection of mouse dermal fibroblasts (MDF) by *Crithidia deanei* and *Herpetomonas roitmani* (trypanossomatids originally with endosymbiont) (Santos,D.O. et al. 2004). Thus, our purpose is to investigate the role of the endosymbiont and Nitric Oxide in the process of the vertebrate cells infection. **Materials and Methods**: *B.culicis* and *C.deanei* treated or not by cloranfenicol were incubated with VERO cells or macrophages derived from human peripheral blood [5:1] in RPMI medium with 5% FCS in a 32^o C with 5% CO_2 for 24 h in a 24 well plates. After incubation, the cells were fixed in methanol, dried and stained with Giemsa, and analyzed by Optical Microscopy. In some experiments VERO cells were activated by PMA (0,5 ug/ml; Sigma) and human macrophages were treated by L-NAME (20 uM; Sigma). **Results**: *C.deanei* and *B.culicis* are able to infect VERO cells. However, no infection was observed when human macrophage was used as host cell. Interestingly, PMA activated - VERO cells did not present infection by these parasites, while L-NAME treated - human macrophages were able to be infected. No infection in both hosts cells was also observed when these same parasites were endosymbiont-free. Our results suggest that the process of the vertebrate cells infection by monoxenous trypanossomatids depends on two factors: (1) the presence of the endosymbiont in the parasite; (2) the ability of the vertebrate cell to produce Nitric Oxide. Financial support: FAPERJ and UFF.

BC50 - Identification and characterization of 2'-O-methyltransferases involved in cap 4 formation of SL RNA in Trypanosoma brucei.

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The biogenesis of Spliced Leader RNA (SL RNA), involved in trans-splicing of all nuclear encoded genes, includes the formation of a unique cap 4 structure at the 5'end, acquisition of a pseudouridine and removal of the 3' poly U tail. In addition to the m7G cap, the cap 4 structure is composed of ribose 2'-O-methylations of the first four nucleotides (AACU) with additional methylations on the bases of the first (N2,6A) and fourth (N3U) nucleotides. Both the cap 4 of kinetoplastids and viral 2'-O-methylation of mRNAs have been linked

to translational efficiency. Thus, we aimed to identify the enzymes responsible for the kinetoplastid-specific cap 4 to elucidate potential drug targets and challenge the role of these modifications in kinetoplastid translation. Utilizing alignments assembled from known 2'-O-methytransferases to search the T. brucei genome and protein structure prediction, we have identified several candidate methlytransferases. Two candidate genes display similarity to the well-characterized 2'-O-methytransferase of Vaccinia Virus, VP39. To investigate the role of each potential MTase in the formation of cap 4, we have utilized the following approach: First, GFP-MTase fusion proteins were engineered into T. brucei cells to determine the sub-cellular localization. We next performed functional analysis using RNAi knockdown and primer extension to assay for cap 4 formation. To date, two methyltransferases have been shown to be involved in cap 4 methylation. Future experimentation will focus on enzymatic studies of these confirmed 2'-O-methyltransferases and analysis of the role of the methylations in both trans-splicing and translation.

BC51 - ORAL INFECTION OF IN VITRO TISSUE CYSTS FROM TOXOPLASMA GONDII IN CD1 MICE

Fux, B *(WashU)*; L David Sibley *(WashU)*

Tachyzoites and bradyzoites are structurally and biologically different stages of *Toxoplasma gondii* responsible for acute and chronic infectious, respectively. While previous studies have shown that bradyzoites genes and antigens can be expressed *in vitro*, the biological potential of these stages to cause oral infection has not been estabilished. We sought to develop conditions that allow *in vitro* development of fully mature bradyzoites as defined by oral infectivity. We monitored the development of bradyzoites of GT-1 (type I), ME49 (type II) and CTG (type III) *in vitro*. Differentiation was induced by modifying the culture conditions to increase the pH (8.1) of the culture medium. Cysts were identified by fluorescent *Dolichos biflorus* lectin and BAG1 staining. The differential resistance of tachyzoites (susceptible) and bradyzoites (resistant) to digestive enzymes was used as a criterion of differentiate between these stages. *In vitro* cysts were also used to orally challenge mice. After 9 days of culture, tissue cysts were observed by light microscopy and their identity was confirmed by electron microscopy. We used these *in vitro* cysts to orally infect CD1 mice with 100, 1000 and 10,000 cysts of GT-1, ME49 and CTG strains. After 30 days only one animal was infected when challenged with 1000 cysts of GT-1 and died after 11 days of infection. We confirmed infection in 3/5 animals with ME49 by western blot innoculate with 1000 cysts, similarly 5/5 mice were found to be infected with 10,000 cysts. In CTG strain 1/5 animals were inoculated with 100 cysts, 2/5 with 1000 cysts and 3/5 with 10,000 cysts were found to be infected. We have developed a viable model for the study of tissue cysts formation in vitro by *T. gondii* and confirmed the ability of these cysts to cause oral infection.

BC52 - Is calcium ionophore stimulated egress of *T. gondii* similar to spontaneous host cell exit?

Caldas L.A. *(IBCCF)*; Magno R.C. *(IBCCF)*; Seabra S.H. *(IBCCF)*; Attias M. *(IBCCF)*; De Souza W *(IBCCF)*

Toxoplasma gondii, the ethiologic agent of toxoplasmosis, is an opportunistic pathogen capable of infecting all nucleated cells in mammals and birds, where it divides by endodiogeny within the parasitophorous vacuole (PV). At the end of several division cycles, the parasites exit the host cell and invade neighboring ones. Invasion and egress of *T. gondii* are both crucial events to parasite survival and a raise in parasite cytoplasmic calcium seems to be decisive in triggering both. So, calcium ionophores have been widely employed to artificially trigger the egress of parasites at various times after host cell entry (Hoff and Carruthers, Trends in Parasitol. 18:6, 2002). We have used calcium ionophore A23187 to trigger egress of *T. gondii* at early (2 hours) and late (24 hours) times after entry. Videomicroscopy recording of LLC-MK2 monolayers infected with *T. gondii* showed that infected cells treated with calcium ionophore promptly release parasites, which enter other cells, but rapidly exit them. Transmission electron microscopy of these monolayers showed the disagregation of vacuolar membrane and intravacuolar network around exiting parasites. Previous studies made by Seabra et al. (2004) demonstrated that parasites released in the peritoneal exudate of infected mice exposed phosphatidyl-serine complexes in its membrane. Under ionophore stimulation, although parasites were seen to leave the host cells in all intervals, observations made by fluorescence microscopy and flow cytometry showed that under ionophore stimulation fewer of the released parasites exposed phosphatidyl-serine on its surface, when compared to parasites from mice peritoneal exudate. In view of these observations, we think that much care is to be taken when calcium ionophore is employed to evaluate the role of calcium in signaling cellular events and that phosphatidyl-serine must have an important role in both natural invasion and egress of *T. gondii*. Supports: CNpq, Capes, Faperj, Pronex.

BC53 - Morphological aspects of *Toxoplasma gondii* inside neutrophils: is that all there is?

Dias KC *(IBCCF)*; De Souza W *(IBCCF)*; Attias M *(IBCCF)*

Are ultrastructural aspects of the intracellular development of *Toxoplasma gondii* definitively described and established? What new information can transmission electron microscopy bring on parasite-host cell interaction? We have recently been surprised by the fact that the so called PVM-organelle association of the parasitophorous vacuole membrane with the host cell mitochondria was not observed in LLCMK2 cells

infected with tachyzoites (Magno et al. Microsc. Microan., 2005). So we decided it would be worthy to give a second look in cells aspirated from the peritoneal cavity of Swiss mice 48 hours after being inoculated with tachyzoites of the RH strain of *T. gondii*. About 4 mL were aspirated in RPMI medium, centrifuged at 1000g for 10 minutes and the resulting pellet was resuspended and fixed in 2.5% glutaraldehyde in 0,1M cacodylate buffer, post fixed in 1% OsO4 in the same buffer plus 0.8% potassium ferrocyanide, dehydrated and embedded in epoxy resin. Random and serial ultrathin sections were observed in a Jeol 1200Ex transmission electron microscope. Serial sections were used to generate 3-D models of infected neutrophils by using the softwares 3DED and SYNU. The exudates contained both free tachyzoites, that had probably recently emerged from host cells, and a variety of cells (neutrophils, macrophages, granulocytes), most of them containing one to several parasitophorous vacuoles in various stages. The same cell could contain vacuoles with different numbers of parasites. Association of mitochondria to the PVM was not observed in these cells but assembly of endoplasmic reticulum around the PVs was a constant, denoting active penetration of the parasite. Some images suggested that glycogen granules could accumulate between the endoplasmic reticulum and the PVM. These preliminary observations suggest that classic electron microscopy still has a contribution to give to the knowledge of *T. gondii* intracellular behavior. supports:CNPq, FAPERJ, PRONEX.

BC54 - OSTEOPONTIN EXPRESSION DURING *Toxoplasma gondii* - MACROPHAGE INTERACTION

Erika Cortez *(UERJ)*; Ana Carolina Stumbo *(UERJ)*; Carlos Alberto Rodrigues *(FIOCRUZ)*; Helene Barbosa *(FIOCRUZ)*; Luis Cristóvão Porto *(UERJ)*; Laís Carvalho *(UERJ)*

Toxoplasma gondii is a widespread opportunistic pathogen capable of infecting a variety of host cells in which it multiplies within a specially modified compartment called parasitophorous vacuole [1]. Osteopontin (OPN) is a secreted adhesive glycophosphoprotein that contains an Arg-Gly-Asp (RGD) integrin-binding domain and is expressed by several cell types. It is involved in a number of physiologic and pathologic events including cell-mediated immunity, inflammation, tissue repair, cell survival and tumor metastasis [2]. In addition, OPN is also reported as an important factor of host resistance against intracellular bacterial infections [3]. In this study we analyzed the expression of OPN during *T. gondii* - macrophage interaction. After 1 and 24h of infection, the cultures were fixed and immunolabeled with a goat osteopontin primary antibody using a biotinilated anti-goat secondary antibody and Cy-3 streptavidin to reveal the reaction. Extracellular tachyzoites of *T. gondii* presented an intense positive labeling for OPN. However, soon after host cell invasion this labeling is diminished. After 24h of infection, the parasites presented again an intense labeling for OPN. These results demonstrated that tachyzoites of *T. gondii* express OPN and that, probably, this protein could be secreted during invasion, participating of the *T. gondii* - macrophages interaction. (Supported by CNPq, FAPERJ and CAPES). References [1] Cortez et al (2005) J Parasitology. In Press [2] Standal et al (2004) Exp Oncol. 26(3):179-84. [3] Staege et al (2001) Immunogenetics. 53(2):105-13.

BC55 - NAD(P)H-OXIDASE PRESENCE IN *Toxoplasma gondii* TACHYZOITES VACUOLE DURING INTERACTION WITH IFN-gamma ACTIVATED HUMAN ENDOTHELIAL CELLS

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Toxoplasma gondii is an obligated intracellular parasite capable of infecting a variety of host cells where it resides in a specially modified compartment called parasitophorous vacuole (PV) that prevents lysosomal fusion [1]. Tachyzoites of *T. gondii* invade and proliferate in human umbilical vein endothelial cells (HUVEC) [2]. However, IFN-gamma activated HUVEC have been shown to inhibit the multiplication of *T. gondii*. The mechanisms of inhibition have been identified in a variety of cellular types, but IFN-gamma activated HUVEC inhibit *T. gondii* multiplication by an unknown mechanism [3]. In this study we analyzed the NAD(P)H-oxidase presence during *T. gondii* - IFN-gamma activated HUVEC interaction. Activated HUVEC were infected with tachyzoites of *T. gondii* for 2 hours. After interaction the cytochemical localization of NAD(P)H-oxidase activity was carried out and the cultures were processed for transmission electron microscopy. Reaction product, indicative of NAD(P)H-oxidase, was observed in 38% of PVs containing *T. gondii* [4]. We also investigated the co-localization of horseradish peroxidase-colloidal gold and NAD(P)H-oxidase activity during *T. gondii* - IFN-gamma activated HUVEC interaction. Activated HUVEC were labeled with horseradish peroxidase-colloidal gold complex and infected with tachyzoites of *T. gondii* for 4 hours. After that, the cytochemical localization of NAD(P)H-oxidase activity was carried out and the cultures were processed for transmission electron microscopy. The results show in the same PV, the co-localization of peroxidase-colloidal gold particles, indicative of phagosome-lysosome fusion, and a reaction product, indicative of NAD(P)H-oxidase activity [4]. We suggest that NAD(P)H-oxidase may participate in a mechanism by which IFN-gamma activated HUVEC inhibit *T. gondii* multiplication, thereby exerting a microbicidal effect. (Supported by CNPq, FAPERJ and CAPES). References [1] Sibley (2004) Science. 304:248-253. [2] Stumbo et al. (2002) Mem Inst Oswaldo Cruz. 97(4):517-22. [3] Woodman et al. (1991) J Immunol. 147:2019-2023. [4] Cortez et al. (2005) J Parasitol. *In Press.*

BC56 - Expression of HMGB1 during *Toxoplasma gondii*- endothelial cell interaction

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High-mobility group box 1 protein (HMGB1), which previously was thought to function only as a nuclear factor, was recently discovered to be a crucial cytokine that can be translocated to the cytoplasm and then released by activated macrophages, that mediates the response to infection, injury and inflammation [1]. The nucleus of the endothelial cell contains large amounts of HMGB1, and it was suggested that the endothelium would be a potential source of extracellular HMGB1 seen in chronic and acute inflammatory responses [2]. The present study investigated how HMGB-1 is expressed in human umbilical vein endothelial cells (HUVEC) infected with tachyzoites *Toxoplasma gondii*. The HUVEC were cultured, activated or not with IFN-gamma for 24h and then infected with the parasites for periods of 2h and 24h.The cultures were fixed, and immunolabeled with goat anti-HMGB1 primary antibody, using biotinilated anti-goat secondary antibody and Cy-3 streptavidin to reveal the reaction. The non-activated and IFN-activated HUVEC presented intense labeling for HMGB1 through the cytoplasm. In some non-infected cells the labeling was observed in filamentous pattern and after 2h of *T. gondii* infection, a large number of cells presented granular labeling scattered throughout the cytoplasm. However, after 24h of interaction, the filamentous pattern was again observed around parasitophorous vacuole containing rosettes. Probably, this positive HMGB1 labeling is present in organelles surrounding the vacuole, which are essentials for the survival of intracellular *T. gondii*. Supported by FAPERJ, CNPq and CAPES. References: [1] Lotze M T& Tracey K J (2005) Nature Reviews/ Immunology 5: 331-343 [2] Mullins G E et al. (2004) Scandinavian Journal of Immunology 60: 566-573

BC57 - Massive liver infection with *Toxoplasma gondii* tachyzoites

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Toxoplasma gondii, the etiological agent of toxoplasmosis, is claimed to be able to infect virtually any nucleated cell of birds and mammals, where it quickly divides by endodiogeny, generating dozens of parasites from each parasitophorous vacuole. Serial intraperitoneal passage of tachyzoites between mice is one of the most used methods of maintenance of the virulent strain RH of this parasite. An inoculum of 106 parasites results in the animal death in about two days. In order to pursue the course of tissue invasion of parasites in the liver of an intraperitoneally infected mouse we injected 105 parasites per mouse that survived 7 days after it. Prior to death, the mouse was anesthetized and its liver was excised for comparison to the liver of a healthy animal of the same age. After extraction, the liver was quickly rinsed in phosphate buffered saline, cut in thin slices (circa 1 mm) and immediately immersed in a fixative containing 2% freshly prepared formaldehyde and 2.5% glutaraldehyde in cacodylate buffer. Observations were made both by light and transmission electron microscopy. The control uninfected animals showed typical morphology and ultrastructure of liver cells, while hepathocytes of infected animals were very damaged. Typical parasitophorous vacuoles were not observed in hepathocytes but dead and decomposing parasites were found as clusters among cell lisates. Intact inflammatory cells, many of them infected with tachyzoites were found infiltrated among liver cells. Electron microscopy reinforced these observations that indicated to us that, although liver cells die, this may not result directly from parasite invasion. Supports: CNPq/PIBIC, Pronex, Faperj, Capes, CNPq

BC58 - Association of lipid bodies of macrophage cultured with homologous serum with *Toxoplasma gondii*: preliminary results.

Mota, L.A.M. *(UENF)*; Lobato, C.S.S *(UENF)*; Seabra, S.H. *(UFRJ)*; DaMatta, R.A. *(UENF)*

It was demonstrated that mouse peritoneal macrophages when cultured with homologous serum present many vesicles that are positive for Nile red staining (fluorescence microscopy) and for imidazol (transmission electron microscopy) indicating their lipid nature. The presence of lipid bodies in macrophages has been related to inflammation, atherosclerosis and neoplasia. Furthermore, little is known concerning lipid bodies association with obligatory intracellular parasites. *Toxoplasma gondii* is an intracellular parasite capable of infecting macrophages. Here we demonstrate that the parasitophorous vacuoles harboring this parasite associated to lipid bodies. For that, macrophages were cultured for 48h at 37_o C in Dulbecco's Modified Eagle's Medium supplemented with 2% homologous serum. Tachyzoites of *T. gondii*, RH strain, were obtained by peritoneal washes of infected mice. Interactions were performed for 2 and 24h in a 10:1 protozoa:macrophage ratio. After that, cells were washed, fixed with 4% formaldehyde in PBS. Cells were washed, incubated for 15 minutes with Nile red in PBS, washed, mounted in N-propyl-gallate with DAPI and observed in a conventional fluorescence microscope. After 2h, it was possible to observe the association of lipid vesicles with parasite. After 24h, lipid bodies surrounded parasitophorous vacuoles with many parasites. These preliminary results indicate that *T. gondii* can also attract lipid bodies to its parasitophorous vacuoles. This is probably related to its development where lipid bodies may provide essential substance for the parasite. Supported by: CNPq, FAPERJ.

BC59 - THE AREA OF HEPATIC NECROSIS INDUCED BY *Entamoeba histolytica* AND *Entamoeba dispar* AND ITS RELATION TO TROFOZOITES NUMBER

Costa, C.A.X. *(UFMG)*; Pires, E.M. *(UFMG)*; Gomes,M.A. *(UFMG)*; Caliari,M.V. *(UFMG)*

Amoebae isolated from clinical cases of non-dysenteric colitis, identified as *E. dispar*, showed virulence similar to that of *E. histolytica* isolated from symptomatic patients. In this context, it was our objective to study the pathogenesis of amebiasis through comparison of the two species. Twenty-five male hamsters were inoculated intra-hepatically with 100,000 trophozoites of *E. dispar*MCR lineage (group 1). Another 25 animals were inoculated with *E. histolytica* EGC lineage (group 2). Six animals were inoculated with their florae. Groups of five animals were necropsied in days 1, 2, 3, 6 and 8 after inoculation. Liver fragments were stained with H&E, and streptoavidin-peroxidase immunohistochemistry was used for detection of trophozoites. Measures of necrosis and trophozoites were obtained through a Carl Zeiss image analyzer. All animals inoculated with E. dispar or E. histolytica developed liquefying necrosis. Intensity of necrosis increased from the 1st to the 8th day in both groups. At the 6th and 8th days necrosis was more intense in the animals inoculated with *E. histolytica*. The number of trophozoites counted decreased along the period in both experimental groups. A correlation between the increase in the necrosis area and trophozoite reduction was observed (Spearman test: *E. histolytica*: r = -0.6984; *E. dispar*: r = -0.4549). Such reduction may have been produced by the toxic products released by macrophages and neutrophils, or even by damaged trophozoites. Our results suggest a higher influence of inflammation on the expansion of necrosis rather than only the lytic action of the viable trophozoites. The efficacy of the cellular and humoral immunities against trophozoites also needs to be investigated.Supported by: FAPEMIG

BC60 - TROPHOZOITES OF *Entamoeba dispar* ARE ABLE TO PRODUCE INTESTINAL AND HEPATIC LESIONS IN DIFFERENT HOSTS.

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Asymptomatic individuals presenting the clinical form of non-dysenteric colitis is very common in Brazil; they generally show negative serology and are carriers of *E. dispar*. Although *E. histolytica* is known as the etiologic agent of amebiasis, the true role of *E. dispar* in this context is not completely clear. In an attempt to evaluate the pathogenic potential of *E. dispar*, different hosts (Swiss mice, Wistar rats and Mesocrisetus auratus) were inoculated with 100,000 *E. dispar* trophozoites in the cecum and liver (three animals/species for the study of each organ separately). Other animals were inoculated with the respective florae. Liver and cecum fragments were collected 48 hs after infection for processing; histological sections were stained with H&E and streptoavidin-peroxidase immunohistochemistry was used for detection of trophozoites. Amebic lesions were detected in all groups of animals inoculated with *E. dispar*. The most significant lesions were observed in the Hamster and mouse livers, and in rat and mouse cecum. These lesions were indistinguishable from those produced by *E. histolytica*. Trophozoites were also detected on the border or in the interior of the necrosis zones in all groups. The animals inoculated with flora did not exhibit lesions or discreet purulent inflammation. From these results we should consider the possibility that *E. dispar* invades tissues causing lesions, even in the absence of dysenteric colitis.

BC61 - Growth, encystment and survival of *Acanthamoeba castellanii* co-cultivated with *Escherichia coli*

Moraes, J. *(USP)*; Alfieri, S. C. *(USP)*

Free-living amoebae of the genus *Acanthamoeba* are frequently isolated from soil and water collections. In the environment, the organisms grow and multiply as phagotrophic trophozoites and encyst under unfavorable conditions. *Acanthamoeba* spp. cause human diseases (granulomatous encephalitis and keratitis) and are recognized as natural hosts of several intracellular pathogens. Since *Acanthamoeba* spp. are predators of a variety of microorganisms, trophozoites seem to play a major role in controlling bacterial population in the environment. Here we monitored growth, encystment and survival of *A. castellanii* placed in non-nutrient media (Ca^{2+}-Mg^{2+}-free PBS and modified Neff's saline) following incubation with live and heat- or gentamicin-killed *Escherichia coli* K12. When given to *A. castellanii* trophozoites (30 minutes contact, multiplicity of 100:1), the Gram-negative bacterium was rapidly internalized and 50 % were killed within 50 minutes. These results confirm *E. coli* as a good food source for *Acanthamoeba*. Trophozoites did not grow when placed in non-nutrient media (an expected result), but their typical morphology was fully preserved for 5 days in Neff's saline. This markedly contrasted with the rapid onset of encystment (within one or two days) of organisms placed in PBS. Addition of *E. coli* (at the time zero or daily) increased the population of trophozoites but only within the first 24 hours. In both PBS and Neff's saline, the growth-promoting effect depended on the multiplicity of bacteria offered, and was enhanced when live bacteria were given. Comparatively to trophozoites incubated in medium alone, encystment was delayed in those receiving higher doses of bacteria (1000:1 to 5000:1) in Neff's saline. By day 10, when only cysts could be visualized, it was remarkably noted the higher recovery of cysts from cultures exposed to bacteria. Thus, *E. coli* K12 stimulates growth, delays the encystment and increases survival of *A. castellanii*. Financial Support: FAPESP and CNPq.

BC62 - Post-translational modifications of microtubules indicate that *Giardia lamblia* cytoskeleton remains "ready to use" during its entire life cycle

Loraine Campanati *(IBCCF)*; Letícia Labati-Terra *(IBCCF)*; Joana Bittencourt-Silvestre *(IBCCF)*; Wanderley de Souza *(IBCCF)*

Giardia lamblia is a multiflagellated diplomonad that possesses a simple life cycle. Its vegetative state colonizes the small intestine of mammals and the cyst is found in stools. Once a susceptible host ingests it, excystation begins in the stomach and finishes in the small intestine. The reverse process, encystation, begins as the trophozoites travel down the intestine. Encystation culminates with the formation of a mature cyst. Trophozoites have an exuberant cytoskeleton with stable microtubular structures. The adhesive disk is involved in cell attachment. Four of the eight flagella are responsible for dislocation and the other are involved in movements of the cell body. The median body is thought to serve as microtubules. Our group is interested in finding out how the cystoskeleton of the trophozoites changes in order to originate the cyst cytoskeleton. Our first approach was to use antibodies against different post-translational modifications (PTMs) of microtubules. PTMs are known to change the intrinsic characteristics of a microtubule, making it more or less stable, for example. We used two antibodies known to label differentially newly assembly and more stable ("old') microtubules. The labeling pattern indicate that the external portion of the flagella become less stable as the trophozoite differentiates into a cyst, while the cytoplasmic portion continues to show stability. Labeling of the adhesive disk with both antibodies indicates that there is a renovation of the disk microtubules, although its structure remains stable (divided into small pieces). These preliminary data indicate that some cytoskeletal structures remain "ready to use' in the cytoskeleton of the cyst. The excystation would expend less energy just re-adapting them to form new trophozoites.

BC63 - *Eimeria acervulina* deactivates chicken macrophages by reducing nitric oxide production and NADPH diaphorase activity.

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Eimeria acervulina is one of the species that causes coccidiosis, a major parasitic disease that infects poultry. This parasite is found worldwide and have an elaborated evasion mechanism. We have demonstrated that tachyzoites of Toxoplasma gondii down modulates nitric oxide (NO) production of activated mice and chicken macrophages with lower expression of inducible NO synthase (iNOS) and NADPH diaphorase activity (iNOS histochemical detection), respectively. The aim of the present work was to determine whether sporozoites of E. acervulina might also reduce production of NO after infecting chicken macrophages. The chicken macrophages cell lines HD11, able to produce NO when treated with LPS, was cultivate with DMEM-F12 supplemented with 5 % of chicken serum. Sporozoites of E. acervulina were obtained from oocysts after mechanical rupture, enzimatical treatment and separation on DEAE-cellulose columns. Macrophages were infected, activated with LPS, NO essayed by the Griess reagent and NADPH diaphorase activity evaluated. Macrophages infection caused 30 % inhibition in NO production. Furthermore, NADPH diaphorase activity was lower in infected macrophages. These results indicate that sporozoites of *E. acervulina* can also reduce NO production of chicken macrophages. This demonstrates an evasion mechanism of these parasites explaining its success. Supported by: FAPERJ, CNPq.

BC64 - Fine structure of acidocalcisomes in Eimeria parasites

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Eimeria species are intracellular protozoan parasites that infect the poultry flocks all over the world, causing a major parasitic disease of great economical importance. The current chemotherapy against these parasites faces problems and the search for new biochemical targets has been the subject of intense investigation. In this regard, the mechanisms of ion homeostasis by acidocalcisomes in protozoan parasites have gained a lot of attention in the last years. Acidocalcisomes are acidic calcium-rich organelles found in several microorganisms. They are characterized by their acidic nature, high electron density and cations content and by the presence of ion pumps and exchangers in their membrane. In this work, we show the ultrastructural and chemical properties of acidocalcisomes in Eimeria parasites. Transmission electron microscopy of sporozoites of E. acervulina and E. tenella showed the presence of several electron dense organelles of 100-300 nm in size. X-ray elemental mapping showed that most of the cations, namely Na, Mg, P, K, Ca and Zn were concentrated in their matrix. Vacuolar proton pyrophosphatase immunolocalization showed intense fluorescence in organelles of size and distribution similar to the acidocalcisomes seen by transmission electron microscopy. Altogether, the results show that Eimeria parasites possess electron dense organelles with structural and chemical properties analogue to the acidocalcisomes, suggesting a similar functional role for these organelles in the cell biology of these parasites. Financial support: FAPERJ, Capes, Finep, CNPq, NIH and Deutsche Forschungsgemeinshaft.

BC65 - Apicomplexa parasites: can nuclear encoded genes lacking a bipartite signal sequence be transported to the apicoplast?

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Parasitic protozoa of the phylum Apicomplexa possess a distinct and essential organelle of algal origin termed the apicoplast. As a homologous organelle is not present in the mammalian host cells, the apicoplast has become the target of intense research to look for alternative control strategies against human diseases such as malaria and toxoplasmosis. Analysis of the 35 kb circular apicoplats genome of *Plasmodium falciparum* yielded no insights into its function as most genes were noncoding or genes coding for ribosomal proteins. Thus, an in silico nuclear genome survey was performed to identify genes containing signal peptide sequences immediately followed by a plastid targeting domain: a bipartite signal sequence. Remarkably, 466 coding genes (~10% of the nuclear genome) encode this bipartite signal and thus were predicted to be transported to the apicoplast (Foth et al. 2003. Science 299:705). Of notice, reverse genetics approaches have validated that some of these genes are indeed transported to this organelle. Recently, a different approach has been pursued to predict genes encoding apicoplast proteins. Thus, based on the trasncriptome analysis of the asexual blood stages of *P. falciparum* at 1h resolution (Bozdech et al. 2003. PLoS 1:1), a probabilistic genetic network of the apicoplast (api-PGN) was predicted (Barrera et al. Critical Assessment of Microarray Data Analysis, in press; http://www.camda.duke.edu/camda/camda04/papers/days/friday/portillo/paper). Interestingly, nuclear encoded genes that lack a bipartite signal and that have homologous plastid genes in *Arabidopsis thaliana* were predicted in this api-PGN. To determine if these genes encode proteins that are transported to the apicoplast and validate the api-PGN, we have constructed expression vectors for *P. falciparum* and *Toxoplasma gondii* containing some of such genes in fusion with GFP. Moreover, we are raising polyclonal peptide antibodies against other of such genes to determine their sub-cellular localization. Results on these experiments will be presented.

BC66 - NF-kappaB activation in human and murine macrophages infected with *Leishmania amazonensis*

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NF-kappaB is an important mammalian transcription factor involved in the regulation of host immune antimicrobial responses through activation of immunomodulatory genes. *Leishmania* is an intracellular parasite that targets macrophages and dendritic cells. Human Leishmaniasis presents symptoms ranging from disfiguring cutaneous and muco-cutaneous lesions to visceral disease affecting the haemopoetic organs. *Leishmania amazonensis* is the main agent of diffuse cutaneous leishmaniasis. In the present work we demonstrated, by gel shift analysis, the nuclear translocation of the transcription factor NF-kappaB in murine and human macrophage infected with *L. amazonensis* promastigotes. Supershift assays indicated that the NF-kappaB complex induced by this pathogen in the human model is formed by RelB/p50 subunits. Moreover, cytochalasin treatment did not inhibited NF-kappaB activation induced in murine macrophages. Thus, the observed NF-kappaB activation does not require *Leishmania* uptake and may occur through surface receptor binding. We demonstrated for the first time the NF-kappaB activation in *L. amazonensis* infection and ongoing experiments are in progress to investigate the NF-kappaB dependent gene expression and its activation pathways in the present model.

BC67 - THE LYSOSOMAL TARGETING SIGNAL OF LEISHMANIA IS RECOGNIZED BY YEAST BUT NOT BY MAMMALIAN CELLS.

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The unique mechanisms of targeting exploited by trypanosomatid parasites could serve as chemotherapy targets. Our previous studies showed that in Lpcys2, an abundant lysosomal cysteine proteinase from *L. pifanoi*, the pro domain plays a role in lysosomal targeting. Lysosomal trafficking in mammals involves mannose-6-phosphate receptors, while vacuolar sorting of carboxypeptidase in yeast was shown to be related to the propeptide region. We are presently searching for proteins that interact with the targeting signal of Lpcys2. We failed to identify interactions using the Yeast Two Hybrid System. We interpreted this result as a possible recognition of the signal by yeast cells. To confirm this hypothesis we fused the Lpcys2 targeting domain (either the pre-pro, or pre or pro alone) to GFP (yeast plasmids pGFP-N-FUS34 and pGFP-C-FUS23), transfected these constructs into *Saccharomyces cerevisiae* and examined the transfectants by fluorescence confocal microscopy. Interestingly, all transfectants showed fluorescence in the vacuole, the equivalent to the lysosome of leishmania, indicating the recognition of the leishmania signal by yeast. A control transfected with the plasmid alone had cytoplasmic fluorescence. We have also investigated the putative recognition of this leishmania targeting signal in a mammalian system. The pre-pro region was cloned into a mammalian expression vector containing a GFP cassette in order to give rise to a fusion of the leishmania signal on the N-terminus of GFP. These constructs were transfected into CHO or VERO cells, that were examined by confocal microcopy. Fluorescence was ob-

served around the nucleus, possibly ER or Golgi. When colocalization experiments were performed, it was apparent the location was not lysosomal or in the ER. The use a of a Golgi marker confirmed this was the cellular location of GFP, thus confirming the non-recognition of the leishmania lysosomal targeting signal by mammalian cells. This work was supported by FAPERJ, PAPES-FIOCRUZ, CNPq, FIRCA-NIH, PDTIS,COLCIENCIAS

BC68 - Observations on the polymorphism of morphostatic individuals and during the morphogenesis in *Gastrostyla setifera* (Ciliophora: Spirotrichea: Stichotrichia)

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Among the genus *Gastrostyla* Engelmann, 1862, *G. setifera* (Engelmann, 1862) is a rare limnetic representant, which differs from the other congeners mainly in the arrangement of transverse cirri, distinctly split in two groups. The morphological aspects of interphasic individuals and the events of the divisional morphogenesis, in particular those related to the somatic ciliature and oral primordia development, are used by authors as data for α-taxonomy in spirotrich ciliates. However, the morphological variability at population level sometimes represents an obstacle in the task of recognizing differences that might justify the attribution of subspecific rank to different intraspecific populations or even species splitting. In specimens of *Gastrostyla setifera*, isolated from cultures made with sediments from the Laranjal beach, Pelotas - RS, we discovered a polymorphism that was not yet described for this species, thus a morphologic and morphogenetic study of this population was conducted through the analyses of living specimens and protargol-impregnated slides. Morphostatic cell size varies from 180-93μm x 93-53μm, values that overlap with the smallest ones from the chinese population studied by Shi *et al.* (*J. nat. Hist.*, 37: 1411-1422; 2003). In our population, we noticed that except for 2 specimens in a sample of 15, the second somatic cirral primordium produces two buccal cirri instead of one, which is the predominant condition in the chinese population. Other differences include the transverse cirri number, that contrary to our observations, is rather stable in the chinese population. In addition, some dividers showed a variation of the cirral primordia formation by exibiting an extra primordium in the proter, between the primordia V and VI. The descriptions of *G. setifera* from Leipzig, Germany, by Engelmann *apud* Berger (*Monographie Biol.*, 78: i-xii, 1-1080; 1999), also possesses some differences, mainly concerning the organization and quantity of cirral products of primordia II and III.

BC69 - Comparison of cytokine expression by circulating and lesion cells from patients with cutaneous leishmaniasis

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Human infection with *Leishmania braziliensis* leads to the development of cutaneous leishmaniasis, a major public health problem in Brazil. Previous studies developed by us and others have shown that peripheral blood mononuclear cells (PBMC) from individuals with cutaneous leishmaniasis produce IFN-gamma and TNF-alpha, as well as IL-10, in response to *Leishmania* antigen *in vitro*. The expression of these macrophage-stimulating, as well as anti-inflammatory cytokines, may be associated with the relatively mild nature of the cutaneous disease. Although most studies concerning immunoregulation of human leishmaniasis have been performed using PBMC from patients, an important question is to determine whether the profile seen in the circulating cells reflects the immune response *in situ*. Thus, the aim of this study was to compare, at the individual level, the cytokine expression between PBMC and lesion cells from individuals with cutaneous leishmaniasis. Our results showed a positive correlation between the frequency of IFN-gamma; and TNF-alpha; producing cells in PBMC stimulated with SLA and the frequency of IFN-gamma; and TNF-alpha; producing cells in cutaneous lesions ($p < 0.02$ and 0.04, respectively). Moreover, we observed a positive correlation between the frequencies of SLA stimulated PBMC producing IFN-gamma; and TNF- alpha; and the frequency of CD4+ (p=0.05) and CD68+ (p=0.05) cells in lesions, respectively. No correlation was seen between the expression of IL-10 by PBMC and lesion cells. These findings show a correspondence between inflammatory cytokine expression *in situ* and in circulating cells, and suggest a role for these cytokine in the recruitment of CD4+ and CD68+ cells to lesion sites. *Financial support: CNPq/PADCT, CAPES, PRONEX, WHO and TMRC/NIH*

BC70 - *Leishmania amazonensis*: a new nondefined culture medium for promastigote-amastigote differentiation *in vitro*

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The parazitic protozoans of the *Leishmania* genus are digenetic and have two distinct stages in their life cicle. The motile flagellated promastigote stage lives in the alimentary tract of this sandfly vector, while the nonmotile amastigote stage occurs inside the macrophages of mammalian hosts. Isolated or cloned strains of these parasites can also be grown in certain culture media in vitro as promastigotes. These

media can be divided into three main categories: semisolid, biphasic, and monophasic. In vitro standardized cultivation of the members of the *Leishmania* genus is a useful approach yielding amount of parasites suitable for diagnosis purposes to provide a better knowledge of host-parasite relationships and for the determination of biologic and immunologic characteristics of the parasite. In addition, the ability to axenically cultivate amastigotes could be an important tool since it is the intracellular amastigote that is associated with the vertebratre patology. In the present work, we report the development of an inexpensive, easily prepared, monophasic medium which supported *Leishmania amazonensis* growth, as well as promoted, in specific conditions, the trigger of the cellular differentiation of this *Leishmania* species. The number of *L. amazonensis* promastigotes reached 6,25 x 10^6 cels/ml at the end of the 5th day in our new medium, though in BHI medium the number of organisms reached only 3,25 x 10^6 cels/ml. Transformation of promastigotes to amastigote-like organisms required o period of growth at pH 4.6 28°C prior to transfer to 32°C. The percentage of amastigote-like obtained was superior 90%. Additionally, we also reported the influence of this novel culture medium in biochemical characteristics of *L. amazonensis*, and in the interaction of these parasites with mammalian macrophages. In conclusion, the culture medium prepared was evaluated as being quite inexpensive, simple, and successful compared with other commercially available liquid culture media.

BC71 - Celular location of arginase in promastigotes and amastigotes forms of *Leishmania (L.) amazonensis.*

Maria Fernanda Laranjeira *(IB - USP)*; Tiago Moreno Castilho *(IB - USP)*; Maíra Nassar *(IB - USP)*; Lucile Maria Floeter-Winter *(IB - USP)*

In the mammal host, *Leishmania* survive inside the macrophages by escaping from their microbicidal mechanisms such as production of nitric oxide (NO) and superoxide radicals (Bogdan et al., 1996). The production of NO by inducible nitric oxide synthase (iNOS) requires L-arginine as substrate, the same aminoacid required by arginase to generate ornithine and urea. Arginase may play a dual role in parasite survival, competing with the iNOS reducing the NO generation and/or providing ornithine, a polyamine precursor that assures DNA synthesis and cell proliferation (Alves et al, 2004). Moreover, inhibition of arginase by N^{ω}-hydroxy-L-arginine enhances NO production and increases parasite killing (Iniesta et al., 2001).

The subcellular site of arginase in promastigotes and amastigotes forms can give a hint of its physiological role. Cytological preparations of promastigotes, submitted to imunofluorescence assays using an antiserum specific against arginase, showed that the enzyme is compartmentalized. This observation corroborated with previous work that showed a glycossomal location of the enzyme. To confirm this we constructed a *Leishmania* expression plasmid containing the coding gene for green fluorescence protein (EGFP) followed by a glycossomal carboxi-terminal targeting sequence (PTS-1) and a selection marker. After transfected promastigotes selection, their confocal images presented the same pattern of compartmentalization observed for arginase.

To locate arginase in amastigotes, J774 macrophages infected with *L. (L.) amazonensis* were used in imunofluorescence assays with anti-arginase. The preliminary results showed spread reactivity in the macrophage citoplasm. This observation can be an indicative of exportation of the parasite enzyme to the host cell, or an activation of the host enzyme, although the control macrophage, not infected, did not show the reactivity. The elucidation of this observation will contribute to determine the involvement of parasite arginase in escaping mechanisms.

Supported by FAPESP and CNPq.

BC72 - Hypoxia-mediated selection of macrophages with reduced susceptibility to *Leishmania amazonensis*

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A hypoxic microenvironment has been proposed to exist in wound non-healing tissues. It is likely that small regions of hypoxia also occur in leishmanial lesions and may play a role in the outcome of infection. In hypoxia, some cells undergo injuries and death. However, subpopulations of cell are resistant to the stress and survive. In this study, we selected death-resistant murine macrophages (J774 cell line) by five cycles of 48 h of hypoxia (< 1% O2) and evaluated susceptibility to *Leishmania amazonensis* infection, heat shock protein (HSP) expression, cytokine and reactive nitrogen intermediates (RNI) production. When *L. amazonensis* infection index (percentage of infected cells X amastigotes per cell) was examined, selected macrophages (Jsel) showed a significant reduction in the infection index (decreased of 60% compared to J774 macrophages) in both normoxic and hypoxic conditions. The HSP70 expression was analyzed in cells exposed to normoxic and hypoxic conditions. Under normoxic condition Jsel macrophages reduced the HSP70 expression (about 40% of J774 macrophages). In contrast, under hypoxic condition, Jsel macrophages showed an increased HSP70 expression (about 30% of J774 macrophages). We also examined TNF-alpha and nitrite production in macrophages activated with interferon-gamma and lipopolysaccharides. Jsel macrophages showed a 2-fold lower level of TNF-alpha secretion than did J774 macrophages and produced 33% less nitrite than did J774 macrophages. These results suggest that populations of macrophages may adapt to potentially pathological hypoxic conditions altering their activities such as killing of *L. amazonensis*, HSP expression, and TNF-alpha and RNI production. Supported by FAPESP, FAEP and CNPq

BC73 - Enucleated L929 Mouse Fibroblasts Support Invasion and Multiplication of *Leishmania (L.) amazonensis.*

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Intracellular pathogens extensively modulate the 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 that, acting on other cell types, can 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? Enucleated cells were earlier infected with *Toxoplasma gondii, Chlamydia psittaci, C. trachomatis, Rickettsia prowazekii* or *Trypanossoma cruzi*. In the present study, monolayers of L929 mouse fibroblasts, enucleated by centrifugation in the presence of cytochalasin B, were infected with amastigotes of *Leishmania (L.) amazonensis*, an agent of cutaneous leishmaniasis. Infection of cytoplasts was compared to that of nucleated cells present in the same preparations. Although the percent infection with Leishmania was similar for cytoplasts and nucleated controls, the average number of amastigotes was higher in the former than in the latter and, in both instances amastigotes multiplied once between 24 and 56 h of infection, which is limited by the relatively short survival of cytoplasts. The similarity in the percent infection of cytoplasts and nucleated cells with Leishmania contrasts with the reduced infection of cytoplasts with *Shigella flexneri* or their reduced uptake of tannic acid treated erythrocytes. Productive Leishmania infection 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.

BC74 - IN SITU EVALUATION OF THE IMMUNOLOGICAL FEATURES ASSOCIATED WITH THE DEVELOPMENT OF EXPERIMENTAL CUTANEOUS LESIONS PRODUCED IN THE MONKEY Cebus apella BY Leishmania (Viannia) braziliensis

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Clinical and histopathological aspects of the evolution of the infection caused by different species of Leishmania in Cebus apella confirm its susceptibility to Leishmania infection and support previous indications that this primate may be useful as experimental model for studies of cutaneous leishmaniasis. In order to characterize the immunopathological mechanisms involved in the evolution of the histopathological changes, skin biopsies collected from C.apella inoculated intradermally with 3.000.000 promastigotes of L.(V.)braziliensis were studied at 2, 30, 60, 90, 120, 150, 180 and 300 days post-infection (PI). The skin sections were stainned by HE and immunohistochemistry performed using mouse anti-Leishmania, mouse anti-human B cell (CD20), rabbit anti-human lysozyme and rabbit anti-human T cell (CD3) antibodies. The histopathological changes were characterized by focal and mild inflammatory infiltrate in the dermis by polimorphonuclear and mononuclear cells at 2 days PI. The inflammatory process increased with the time of the infection and parasitized macrophages, lymphocytes and plasma cells was detected since 30th day. At the 60th and 90th day of infection, granuloma with giant cells envolving areas of necrosis were also present. From the 120th day of infection collagen tissue together with inflammatory cells and necrosis characterized the lesions. The immunohistochemistry showed discreet presence of parasites in the lesion from the 2nd day till the 180th day of infection. Activation of macrophages characterized by positive reaction for lysozyme was mild at the 2nd day PI, moderate at the 30th day and intense between 60th and 300th day PI. CD3+ cells was present since 30th day PI and it was characterized by moderate intensity. B cells were observed from 30th day, was mild until 120th day and moderate from the 150th day. Our results suggest that the immunopathological mechanisms are related with the sequencial events of the histopathological changes were related to immunopathological mechanisms.

BC75 - CLINICAL, PARASITOLOGICAL AND SOROLOGICAL SELECTION OF DOGS FROM ENDEMIC REGION OF VISCERAL LEISHMANIAIS FOR IMMUNOLOGICAL STUDIES AND ITS CORRELATION WITH THE TRANSMISSIBILITY

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Clinical, parasitological and sorological diagnosis of dog from endemic region of visceral leishmaniais was evaluated to future studies on the immunological features and its correlation to the transmissibility for the vector. Blood and tissue fragments have been collected from dogs sacrificed in the Zoonosis Control Center of Araçatuba city, an endemic region located in the Northwest region of São Paulo State. Animals were classified as symptomatic, oligossymptomatic and assymptomatic according to clinical signs. Fragments from spleen, lymph nodes and skin collected for histopathological studies were also used for parasite search in imprint stained by Giemsa. Sera were used for specific antibodies detection by indirect immunofluorescence. From 45 dogs, 10 had negative diagnosis for leishmaniasis using parasitological and sero-

logical tests and 9 animals had positive serology only, which suggest previous infection with control or cross reaction with others pathogens. Parasitological search was positive in 26 dogs but 6 dogs from these animals showed negative serology. By serological tests we observed 26% of false positive and 23% of false negative results suggesting that the serology was not a good trail for the canine disease. The clinical more evident signs were lymphadenomegaly (30), splenomegaly (27), lost weight (24) hepatomegaly (18), skin lesions (10) and onicogriphosis (4). From 12 symptomatic animals, 6 showed parasites in the skin (50%), from 6 oligossymptomatic, 2 showed parasites in the skin (33%) and form 8 assymptomatic, 2 showed parasites in the skin (25%). These preliminary data suggest that the transmissibility of the parasite to the vector from the domestic reservoir is not high, but has some relation to symptomatically pattern. Immunohistochemistry reaction will be used to better characterizing cellular immune response on the viscera and skin to correlate it with the parasitism and transmissibility to the vector.

BC76 - Kinetics of exposure and distribution of phosphatidylserine on the surface of *Leishmania (L) amazonensis* amastigotes

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Phosphatidylserine (PS) exposure on apoptotic cell surface is important for cell recognition and removal by macrophages, without eliciting an inflammatory response. Previous work from our group showed that amastigote forms of *Leishmania (L) amazonensis* use the same process to facilitate macrophage infection, a mechanism described as Apoptotic Mimicry. PS recognition by macrophages on parasite surface results in an anti-inflammatory phenotype in host cell, facilitating maintenance and expansion of infection. The host can modulate PS exposure on parasite surface. Purified amastigotes from lesions in Balb/c mice (susceptible) expose a higher density of PS molecules when compared to amastigotes purified from C57Bl6 mice (resistant). The present work aims to investigate PS exposure by amastigotes during the course of macrophage infection *in vitro* and the distribution of these molecules on parasite cell surface. Previous results suggest that PS exposure on parasites purified from lesions is non random. Confocal images show that some amastigotes display patches of Annexin-V labeling while in others PS molecules are dispersed along all surface, in a "ring-type"fashion. This result suggests that there is an active control of PS molecules distribution on parasite surface. In order to enable us to study the mechanism involved in the macrophage-dependent PS exposure by intracellular amastigotes, we developed an *in vitro* model consisting on infecting peritoneal exudates macrophages with promastigotes and measuring the kinetics of PS exposure in the purified amastigotes. Preliminary results showed that amastigotes have an increase in PS exposition along the time course of infection, with a maximum at 72h of culture. This exposition is similar in amastigotes purified from macrophages of Balb/c or C57Bl6 mice, suggesting that the differences observed in amastigotes isolated from *in vivo* lesions are dependent on differential macrophage activation in the host, not present in the *in vitro* model.

BC77 - IMMUNOPATHOLOGICAL CHARACTERISATION OF HUMAN CUTANEOUS LEISHMANIASIS LESIONS CAUSED BY LEISHMANIA (VIANNIA) SPP. IN THE AMAZONIAN BRAZIL

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American Cutaneous Leishmaniasis (ACL) is a growing health problem worldwide. The clinical expression of the disease is the result of the interaction between parasite and vertebrate host in various phases of the infection. The species of Leishmania and the host immune response seem to be the most significant determinants. This study aims to characterise the histological alterations and in situ cellular immune response of the lesions of patients with ACL in an endemic area through histopathological and immunohistochemical analysis. Forty-five patients were diagnosed as ACL in the Outpatients of UFMA in Buriticupu-MA, based on clinical, epidemiological, parasitological, immunological and histopathological studies. Histopathological studies were performed on 22 biopsies stained by HE. The cellular immune response was characterised by immunohistochemistry, using 7 cellular markers (anti-human CD3, CD4, CD8, CD56, CD68 , lysozyme and CD20 antibody). An image analysis system was used for counting cell populations. The parasite strains analyzed by PCR followed by RFLP were classified as belonging to the Viannia subgenus. DTH was positive in all patients. Histological skin changes were characterized by an inflammatory infiltrate of the dermis was characterized by presence of mononuclear cells and was mostly intense and diffuse with granulomatous features. Well-defined epithelioid granulomas were observed in 76% of the cases. CD3+ and CD8+ T lymphocytes, activated macrophages, B lymphocytes and natural killer cells were identified in nearly all lesions by immunohistochemistry. Activated macrophages (lysozyme +) were almost twice as commonly encountered as inactive macrophages (CD68+). Low levels of NK cells (CD56+) were found. Our findings show that infection caused by species of the Viannia group led to significant macrophage activation, likely mediated by high levels of CD3+ T lymphocytes and CD8+ T lymphocytes which favour the control of the disease (LCL). Further studies are going to carry out looking for cytokines profile and CD4+ subpopulations.

BC78 - The MARCO scavenger receptor is up regulated in the 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 permissive to *L. amazonensis* infection *in vitro*, indicating that macrophages participate in determination of host immune profile. Events of innate immune response are supposed to be determinants of Leishmania infection outcome. 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. In some of the expressed genes were previously related to Leishmania infection but some of them were not yet associated to Leishmania infection. We found that there was a down regulation in response to *L. amazonensis* of genes encoding for surface receptors such as Fc receptor, already known to be involved in parasite recognition. In contrast, other receptors were up regulated in response to *L. amazonensis* and *L. major* which were not previously related to Leishmania infection such as MARCO and others scavenger receptors. This result is correlated with higher in vitro expression of MARCO scavenger receptor in *L. major*-infected macrophages when compared to *L. amazonensis*-infected cells. Furthermore, *in vivo* studies demonstrated that there was higher expression of MARCO receptor in lymph nodes and spleen from *L. major*- in comparison to *L. amazonensis*-infected CBA mice. In addition, cells over expressing MARCO receptor show lower percentage of *L. major* infection than non-infected cell, suggesting a protective role of MARCO. These data described for the first time an involvement of scavenger receptor in Leishmania infection and work in progress in our laboratory will better define the specific role of MARCO in our model.

BC79 - Evaluation of osteopontin expression during in vitro and in vivo infection by Leishmania amazonensis or Leishmania major.

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CBA/J mice are resistant to *L. major* infection and susceptible to *L. amazonensis*, presenting distinct patterns of tissular and immune responses. There is evidence that the early events post-infection are crucial to the course of the disease. Macrophages play a central role in Leishmania infection, since they are the cells that harbor parasites, present antigens to T lymphocytes and have capacity to secrete cytokines and chemokines. Little is known about the mechanisms involved in macrophage recruitment despite it is important for the maintenance and dissemination of Leishmania infection. Osteopontin is a protein involved in cellular migration and adhesion that has been related to attraction of macrophages to inflammatory sites in response to different pathological stimuli. Since macrophages are the main cell which responds to osteopontin stimulus, the goal of this work was to evaluate osteopontin participation during both *in vitro* and *in vivo* Leishmania infection. Peritoneal inflammatory macrophages and CBA/J mice were infected by *L. amazonensis* or *L. major*. *In vitro*, osteopontin expression was evaluated by RT-PCR analysis. In *in vivo* studies, the expression of osteopontin protein was detected in cells of the inflammatory infiltrates and draining lymph nodes of infected animals. During infection of macrophages it was observed a higher expression of osteopontin mRNA in later times of *L. amazonensis* infection. According to this result, the number of osteopontin expressing cells was higher in draining lymph nodes of *L. amazonensis* infected animals. By linear regression analysis it was observed that the increase in both *in vitro* osteopontin mRNA and *in vivo* lymph node osteopontin expression depends on the time of *L. amazonensis* infection. These data suggest that osteopontin is involved in the susceptibility of CBA/J mice to *L. amazonensis* infection and points out to the need to broad the knowledge about the role of this protein in leishmaniasis.

BC80 - Taxis responses in Leishmania amazonensis promastigotes and their role in Leishmania development in phlebotomine sandflies

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During the final phase of their development in the phlebotomine sandflies, promastigote forms of Leishmania migrate to the anterior regions of the digestive tube, from where they are transmitted to the mammal host. Studies have been published indicating that taxis responses are involved in this stage of Leishmania development. Our aim was to investigate the taxis responses of Leishmania promastigotes by using a method capable to distinguish chemotaxic from osmotaxic responses. The methodology consists on observing how frequently promastigote forms alter their course in different concentrations of a chemical stimulus. In the presence of an attractant, promastigotes have a tendency to swim smoothly in a straight line until adapt to this new condition after 30 to 60 minutes. Adapted promastigotes frequently alter their course by "tumbling"and it is not possible to distinguish then to those not exposed to the attractant. By using this methodology, it was possible to demonstrate chemotaxic responses to a very low concentration (0.001 mM) of sucrose, lactose, manitol and glycine. On the other hand, promastigotes were not capable to respond to the same concentration of guanosine, NaCl or HEPES. All of those substances were capa-

ble to induce osmotaxic responses in higher concentrations such as 1 to 100 mM. The affinity constant (Kd) between sucrose and the membrane receptor(s) involved in chemotaxic responses was measured and reached the surprisingly value of 0,6pM. When promastigote forms were exposed to lower osmotic pressures (in relation to that they were adapted to), the frequency by which they usually alter their course increased, indicating that this kind of modification causes repulsion. The opposite happened when they were exposed to higher osmotic pressures. The results obtained in this study are in accordance with the idea that chemotaxis as well as osmotaxis responses could be involved in Leishmania development. Supported by CNPq

BC81 - INVOLVEMENT OF LIPID RAFTS IN LDL ENDOCYTOSIS BY *Leishmania chagasi*

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Leishmania are the etiological agents of a variety of disease manifestations,. Visceral leishmaniasis caused by *Leishmania leishmania chagasi* is a serious health problem in tropical and subtropical countries. During the life cycle it alternates between gut vector and macrophage as an intracellular amastigote. Lipid rafts are cholesterol-rich, and detergent-resistant microdomains found in plasma membranes of many cells. These domains play important roles in endocytosis, secretion, and adhesion in a variety of cell types. The objective of this work is the purification and characterization of lipid rafts and to study its participation in the LDL endocytosis. Sucrose density centrifugation is the standard method for lipid rafts isolation. Parasites were lysed, homogenated and incubated at 4^oC in Triton X-100 1%. After 30 minutes the homogenate were subjected to a sucrose gradient. Cholera toxin B subunit (CTB) is a specific ligand for ganglioside GM1 and can be used for the detection of GM1 containing microdomains. In order to investigate the presence of GM1, the fractions were submited to a dot-blot using colera toxin. We identified a large GM1 content in the corresponding fractions 5 to 8 from sucrose gradient. To examine the involvement of the lipid microdomains on LDL endocytosis, cells were pretreated with MBCD (Methil-β-cyclodextrin) for 60 min and then incubated in the presence of LDL-I^{125}. After 24h, cells were collected and the radioactivity was determined by gamma counting. It was observed that LDL endocytosis was significantly inhibited in cells treated with MBCD, suggesting that, in *L. chagasi* cells; this process is dependent on the presence of lipid raft. Supported by CNPq, FAPERJ, IFS

BC82 - Characterization of the renal lesion in hamster experimentally infected with *Leishmania (Leishmania) chagasi.*

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Renal involvement is frequent in visceral leishmaniasis (VL) and hamsters (*Mesocricetus auratus*) experimentally infected with *Leishmania (Leishmania) chagasi* develop a disease similar to human and canine, but few is known on renal alterations in this species. Since hamster is a good model to study VL, here we searched the renal lesions at different periods of infection. Hamsters were infected by intraperitoneal route with 2 x 10^7 *L. (L.) chagasi* (MHOM/BR/72/cepa 46) and sacrificed at 7, 15 and 90 days post-infection. For histological analysis kidney was fixed in 10% buffered formalin and embedded in paraffin. Sections of 3-4 μm thickness were stained with haematoxylin-eosin, Schiff periodic acid, Masson' s trichrome and examined under a light microscope. The results revealed more glomerular cells in the group of 15 days compared to the group of 90 days ($p = 0.0160$), but there is no difference to the group of 7 days. In animals of 90 days post-infection it was observed a reduction in the number of glomerular cells and in some cases, thickness and duplication of the basal membrane with obstruction of the glomerular capillary. The tubule interstitial lesions were characterized mainly for inflammatory infiltrate of lymphocytes, plasma cells and macrophges and presence of hyaline casts and tubular dilatation. The inflammation increased from day 7 infection onwards and it was more intense in the cortical region in the group of 7 days ($p = 0.00235$), but in the group of 90 days was more intense in the corticomedullary region ($P = 0.00258$). In hamsters experimentally infected with *Leishmania (Leishmania) chagasi* occur proliferative glomerulonephritis and the lesions are progressive. Supported by UFPI, FAPESP, LIM-38 (HC-FMUSP)
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