# *POSTERS*

# Biologia Celular- Cell Biology

## BC01 - *Giardia lamblia* adhesive disk: structural study and proteomics analysis

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*Giardia lamblia* is a flagelated protozoon that inhabits the small intestine of vertebrates. The parasite life cycle is composed of two stages: a vegetative trophozoitic form and a resistant cyst. Trophozoites possess a remarkable cytoskeleton made of stable microtubular structures such as the adhesive disk, four pairs of flagella, median body and funis. They remain attached to the intestinal epithelial cells by means of the adhesive disk, whose structure is organized as a microtubular spiral also containing microrribons and cross-bridges. The protein composition of the adhesive disk has been focused by many studies, mainly by Holberton (1981) who obtained an isolated fraction and observed a 30 kDa protein, which he called giardin. Tubulin has been identified as the major protein in disk extracts, showing localization diversity and post-translational modifications. In the present work, we used a new method for isolating the disk. Adhesive disks have been isolated from *G. lamblia* by mechanical and chemical treatments. The cells were extracted with 1% Triton-X 100 in 0,1M PHEM buffer for 20 minutes, sonicated and centrifuged for 20 min at 15000g. Transmission electron microscopy and negative staining were used to evidence the purity of the fractions. The isolated disks showed the typical spiral structure, showing no sign of procedure damage. Thin sections also confirmed the preservation of the disk structure. The protein profile of the isolated disks was obtained by SDS-PAGE gels, which showed two prominent bands, one with molecular weight of 50 kDa and another with 30 kDa, evidencing the two major disk constituents: tubulin and giardin. Proteomics analysis by mass-spectrometry is being carried out in order to obtain more information about the composition of the disks, since several disk associated structures, such as cross bridges, lateral crest and banded collars have not been biochemically described. Supported by: CNPq,FAPERJ and CAPES.

## BC02 - *Trypanosoma cruzi* and $\beta$-lapachone derived naphthoimidazoles: induction of alternative death styles

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$\beta$-Lapachone, a naphthoquinone obtained from vegetal sources, possesses a variety of biological activities, mainly associated with oxidative processes. Between 45 derivatives of $\beta$-lapachone synthesized and assayed against *T. cruzi*, the highest activity against trypomastigotes was achieved with the naphthoimidazoles **N1**, **N2** and **N3**, presenting aromatic moieties linked to the imidazole ring. In epimastigotes, these compounds blocked the cell cycle, inhibited succinate cytochrome c reductase, metacyclogenesis and induced damage to mitochondrion, Golgi and reservosomes. Treated trypomastigotes demonstrated alterations in the mitochondrion, nucleus, kinetoplast and acidocalcisomes (Menna-Barreto et al., 2005, 2007). In this work, we investigate cell death events in *T. cruzi* induced by these naphthoimidazoles using different techniques. The treatment of trypomastigotes induced strong disruption of kDNA and nuclear DNA, leading to an intense DNA fragmentation. It was also observed an important membrane blebbing, as well as a dose-dependent low % of annexin V+/PI- and high % of annexin V-/PI+ cells. These alterations were not observed in treated epimastigotes, despite the release of cytochrome c to the cytosol in this case. The pre-treatment of both forms with E64, zVAD.fmk, calpain inhibitor I or wortmanin caused a partial reversion of the trypanocidal effect of the naphthoimidazoles, suggesting the involvement of proteases in the induction of cell death. Our results with wortmanin, together with an increase in the fluorescence of treated parasites labeled with monodansyl cadaverine, suggest a possible role of autophagy in the parasite death. It is interesting to observe that induction of an apoptosis-like process occurred only in treated trypomastigotes, while the autophagic pathway was observed in both forms, being more accentuated in epimastigotes. The overall analysis indicates a co-existence of a variety of cell death programs triggered by drug stimuli, and trypanocidal agents could be also an important tool for the study of death mechanisms in trypanosomatids.

## BC03 - Determination of cell viability of merozoite and sporozoite stages of *Eimeria tenella* with different dyes

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*Eimeria tenella* is one of the most important causing agents of chicken coccidiosis. Determination of cell viability is an essential step before performing many biochemical and cell biology experiments. Currently available methods for determining viability of sporozoites and merozoites are based on morphology under phase contrast microscopy, motility, and the ability to invade cultured cells. Most of these methods are indirect, subjective and time-consuming. Vital dyes are substances that differentially stain live and dead cells due to distinct accumulation and/or exclusion properties. Trypan blue has commonly been used as a vital dye in *Toxoplasma gondii*, but *Eimeria* cells are not properly stained with this dye. In order to obtain a fast and direct method to measure cell viability in *E. tenella*, we tested seven other vital dyes. Sporozoite and merozoite stages were incubated for 0, 1,

12, 24 and 48 hours at both, 41$^o$C and 50$^o$C, and cell viability was further determined under light or fluorescence microscopy. Four of the tested dyes did not stain the cells. Methyl violet stained the non-viable cells very weakly, resulting in a poor differentiation between viable (non-stained) and non-viable (stained) cells. MitoTracker Red CMXRos$^R$, a dye that stains mitochondria of live cells, allowed for a good differentiation. However, because of the tubular shape of the eimerian mitochondrion, a good visualization was critically dependent on a proper orientation of the cells on the microscope slide. A combined staining method using acridine orange and ethidium bromide permitted a reliable differentiation between viable and non-viable cells. In this method, acridine orange stains viable cells in green, whereas ethidium bromide stains non-viable cells in orange. We intend now to use acridine orange/ethidium bromide to estimate cell viability of sporozoites and merozoites of *Eimeria tenella* destined for biochemical and cell assays.

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## BC04 - Epimastigogenesis in vitro and in vivo of *Trypanosoma cruzi*: Comparison of strains isolated from triatomine, opossum and man.

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*Trypanosoma cruzi* transforms from trypomastigotes to epimastigotes (epimastigogenesis) in the intestinal tract of the insect vector (*in vivo*) and in axenic culture media (*in vitro*). It is not known if the morphological changes occurring during *in vitro* and *in vivo* epimastigogenesis are similar. This work compares the morphological transformation events that occur during epimastigogenesis of different *T.cruzi* strains isolated from *Rhodnius prolixus* (RpN2), *Didelphis marsupialis* (DmN2) and man (EPm6 clone). Cell culture supernatant trypomastigotes were used to artificially infect 5th instar *R. prolixus* nymphs and to inoculate LIT medium at 27$^o$ C. The morphological changes were monitored at different times (1, 2, 4, 6, 8 and 10 days) by phase contrast microscopy and Giemsa-stained smears. Polypeptide and antigenic changes occurring during epimastigogenesis were analyzed by SDS-PAGE and Western blot analysis using stage-specific antisera. The results were (a) the percentage of epimastigotes and the time necessary to accomplish the differentiation varied according to the strain (b) transient amastigote-like forms were observed and their release time was dependent upon the strain (EPm6 > DmN2 > RpN2), (c) in vitro transformation kinetics was dependent on the oxygen tension (d) the antigenic profile of transition forms (amastigote-like) showed significant differences as compared to trypomastigote and epimastigote profiles, (e) amastigote-like forms were resistant to complement lysis. These results indicate that epimastigogenesis can be mimicked in vitro at low oxygen tension conditions; the sequence of events occurring during T. cruzi epimastigogenesis is similar irrespectively from the origin of the strain.

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## BC05 - Aspects of the salivary gland maturation of the *Rhodnius prolixus* (Heteroptera, Triatominae)

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*Rhodnius prolixus* is a haematophagous insect and potential vector of *Trypanosoma cruzi* and *Trypanosoma rangeli*. The efficiency of blood feeding is in part due to the secretion of saliva. Saliva is secreted by salivary gland located in thorax and abounds in substances that antagonize hemostatic, inflammatory and immunological systems imposed by the host. We analyzed by scanning electron microscopy (SEM) the morphological aspects of first-instar nymphs (one to five days after the emergence). The SEM showed that salivary complex of nymphs is composed by two pairs of lobes with two lobules similarly to the adult glands. However, in the first nymphs, the salivary complex was distinctly smaller (200$\mu$m length) from the adult (1mm length). Also, we observed adherent cells on the gland surfaces. It was interesting to note the presence of a very thin muscular layer characteristic of the $5^{th}$ day salivary glands. As observed during the dissection process under the stereoscope, the salivary gland is translucent, except the $5^{th}$ day salivary glands that have in the main lobule a reddish color (an outstanding characteristic of the adult glands). At the same time, we offered a mouse for groups of $1^{th}$ - $5^{th}$ day nymphs and the most number of blood feeding (82%) was observed at the $5^{th}$ day. In conclusion, the success in the haematophagy of the *R. prolixus* first nymphs is probably related with the maturation process linked with the presence of nitrophorins (red color), enzymes responsible for many of the anti-hemostatic activities, and the presence of the muscular layer around the gland that helps in the ejection of saliva. Further studies are being done in order to better understand the morphological characteristics of the salivary complex of nymphs and to correlate them with the organ physiology. Acknowledgements: The financial support of FAPEMIG is gratefully acknowledged.

### BC06 - Characterization of *Trypanosoma cruzi* p67: a putative reservosome marker

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Reservosomes were characterized as pre-lysosomal compartments of *Trypanosoma cruzi* presenting an internal pH of 6.0, and lacking lysosomal molecular markers commonly found in mammalian cells, such as LAMPs and lgp. Although typical lysosomes seem to be absent from *T. cruzi*, in *T. brucei* the single lysosome is defined by the presence of a highly glicosylated type I membrane protein, which contains a long lumenal domain, a transmembrane portion and a short cytoplasmatic tail, designated as p67. Although there is no sequence similarity between parasite p67 and LAMPs, their high structural similarity and distribution in two glycosylated domains defined by intrachain disulfide linkage led to the proposal that these proteins would perform a similar function, protecting the lysosome membrane from the internal hydrolytic millieu. An EST presenting 48% identity to p67 was identified in *T. cruzi*, posing as a candidate marker for lysosome-like organelles in this parasite. Preliminary proteomic analyses of purified reservosome membranes of *T. cruzi* Dm28c indicated the presence of p67, further suggesting this protein as a potential molecular marker of reservosomes. Based on the p67 gene sequence in the *T. cruzi*genome, we designed oligonucleotides directed to regions spanning the p67 luminal domain. The gene fragments encoding the region located between aminoacid residues 32-323 or the entire luminal domain were cloned into pQE-30 and the fragment corresponding to the region between residues 321-578 was cloned in pET28a(+). The histine-6X tagged fusion proteins were produced in *E. coli* as inclusion bodies and the denatured recombinant proteins were purified from SDS-PAGE by electroelution or in nickel agarose resins. The purified proteins were inoculated in Balb/c mice for the production of anti-p67 antibodies. The distinct antisera reacted strongly with the respective recombinant proteins in Western blot and are currently being used to localize p67 in *T. cruzi* Dm28c using immunofluorescence and immunoelectronmicroscopy.

### BC07 - Tubulin specific monoclonal antibody recognizes *Trypanosoma cruzi* reservosomes

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Reservosomes are membrane bound organelles located at the posterior region of epimastigote form of *Trypanosoma cruzi*. We have produced several monoclonal antibodies against a *T. cruzi* cytoskeleton fraction. One of them (mAb 3G4), reacts in indirect immunofluorescence assays with organelles found in similar positions as reservosomes showing a significant colocalization with cruzipain. Differently from cruzipain, the antigen recognized by mAb 3G4 remained associated with the cells after detergent solubilization prior to fixation. The antibody recognizes by immunoblot a protein of 55 kDa that also remains insoluble after mild detergent lysis in the presence of EGTA and $MgCl_2$. Immunoblot analysis of total and cytoskeleton enriched extracts after 2D-gel electrophoresis revealed that it reacts with different isoforms of tubulin. The presence of cytoskeleton fibers is also associated with the reservosomes which are decorated by mAb 3G4 as seen by immunoelectron microscopy. We concluded that tubulin is associated with reservosomes and that a specific post-translational modification or a unique epitope exposition is present in tubulin in this organelle. Supported by FAPESP, FAPERJ and CNPQ.

### BC08 - Characterization of the putative pre-replication complex component Orc/Cdc6 of *Trypanosoma cruzi*

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The assembly of pre-replication complexes on eukaryotes begins with the binding of an origin recognition complex (ORC1-6) to chromatin. Chromatin-bound ORC recruits Cdc6 and Cdt1. Cdc6 stabilizes the binding of ORC and allows Cdt1 to load the hexameric Mcm complex, whose helicase activity is essential for replication. The assembly of the pre-replication complex occurs in G1. In order to ensure that DNA molecules will be replicated only once in each cell cycle, Orc1 is degraded upon entry into S phase and re-accumulates after mitosis. In yeast, however, all six Orc subunits remain in a complex bond to replication origins throughout the entire cell cycle. Very little is known about the DNA replication in trypanosomes. Differently from other eukaryotes, trypanosomes do not contain sequences in their genome that could codify for ORCs, Cdc6 or Cdt1. Unlike, these parasites contain one open reading frame homologous to Orc1 and Cdc6 similar to Archaea. Here, the Orc/Cdc6 encoding gene from *T. cruzi* was cloned, expressed in E. coli, and the recombinant protein was used to immunize animals. The anti-Orc/Cdc6 serum recognized a band of expected molecular mass in extracts of *T. cruzi*. The Orc/Cdc6 localizes in the nucleus and this nuclear localization is not dependent of the cell cycle stage, since cells in G1/S, G2 and even mitotic cells contain Orc/Cdc6 in the nucleus. We have previously showed that replication sites are located at nuclear periphery of epimastigote cells (Elias et al. 2002). Indeed, Orc/Cdc6 is constrained at nuclear periphery in most replicative cells and is dispersed through the entire nuclear space of the infective/non replicative forms. These data point to a role of

Orc/Cdc6 in the replication of trypanosomes. Supported by FAPESP

### BC09 - Storage pools of proliferating cell nuclear antigen PCNA in the nucleus of *Trypanosoma cruzi*

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The proliferation cell nuclear antigen PCNA is a homotrimeric protein that is found associated around double stranded DNA to form a ring like structure. PCNA interacts with DNA polimerases, enhancing the processivity of DNA replication at the fork. PCNA also serves as anchoring point for proteins that are involved in DNA replication, repair, and recombination as well as proteins involved in the regulation of the cell-division cycle. Here we identified the PCNA gene of *Trypanosoma cruzi* wich is the etiological agent of Chagas disease, and we characterized its expression and localization during the cell cycle of this protozoa parasite. PCNA encoding mRNA is detected throughout cell cycle but increases during the S-phase dropping to basal levels at the time of cell division. The same variation occurs for the protein, which is always found in the nuclear space. At the beginning of S phase PCNA is constrained in two opposed domains surrounding the central nucleolus. When DNA replication is maximal PCNA is constrained to the nuclear periphery, and is found disperse into nucleus when replication is complete. As observed by BrdU incorporation assays, we detected replications sites at nuclear periphery. These data here reported indicate the occurence of storage pools of replication factories at nucleolar periphery. During DNA synthesis there is an increment in PCNA amounts together with the transition of PCNA molecules to nuclear periphery. Supported by FAPESP

### BC10 - Transcription architecture and gene expression in *Plasmodium falciparum*

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Despite its medical importance, the molecular control of gene expression is very poorly understood in *Plasmodium falciparum*. In recent years, whole-genome transcriptional studies revealed that gene expression patterns in *P. falciparum* are remarkable complex, with about 80% of genes being cell cycle and, therefore, developmentally regulated, with a single peak of expression during the erythrocytic cycle. This complex pattern paradoxically contrasts with the low number of putative transcription factors encoded by the parasite genome. Because of that, post-transcription control and, more recently, epigenetic mechanisms have been proposed to play a substantial role in *P. falciparum* control of gene expression, and indeed epigenetic factors have been shown to be important for var genes regulation. In mammalian cells, genes are transcribed in discrete nuclear structures known as transcription sites, and developmentally co-regulated, functionally related genes have been shown to share transcription sites. In this context, we decided to investigate whether transcription organization plays a role in regulation of developmentally co-regulated genes in *P. falciparum*.
To this aim, we have labeled the transcription sites with BrUTP in *P. falciparum* erythrocytic forms. 3D models, constructed from deconvoluted images using a tailored algorithm, show that *P. falciparum* nascent transcripts are synthesized in discrete dots scattered throughout the nucleoplasm, resembling the organization of transcription sites found in higher eukaryotes. In *P. falciparum*, transcription sites are also found at the nuclear periphery, a region proposed to be transcriptionally silent in this parasite. Statistical analysis is being currently performed in order to determine the average number and size of transcription sites throughout nuclear zones.
We are also undertaking FISH and Chromosome Conformation Capture (3C) assays to investigate genes position before and after their activation and, most important, if functionally related, co-regulated genes, such as virulence factors, share transcription sites. We believe that the questions addressed are fundamental for better understanding *Plasmodium* gene regulation and pathogenesis.

### BC11 - *Trypanosoma cruzi* histone H1 nuclear localization depends on its phosphorylation state

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Phosphorylation of histone H1 is intimately related to the cell cycle progression in higher eukaryotes, reaching maximum levels during mitosis. This phosphorylation seems to release the histone H1 from chromatin, which was shown to prevent histone H3 phosphorylation, a key step in promoting chromatin condensation. We have previously shown that *Trypanosoma cruzi* histone H1 is phosphorylated in a single site during mitosis. We now present data describing the localization of non-phosphorylated and phosphorylated histone H1 at different phases of the cell cycle, aiming to understand the role of this modification in this protozoan, which does not condense the chromatin. By using laser confocal microscopy and specific antibodies against phosphorylated (anti-H1p) and non-phosphorylated (anti-H1t) we observed a different speckled pattern of fluorescence not associated with the bulk of DNA. Anti-H1t, as anti-H1p specific an-

tibodies stained the nuclear interior in places where chromatin is less densely packed in all the cell cycle phases. This contrasts with labeling with anti-histone H4 antibodies. In addition, there was only a partial colocalization of the two forms of histone H1. When the parasites were permeabilized by detergent prior fixation, all phosphorylated histone H1 was released. These results indicate that histone H1 is not always associated with chromatin and that its phosphorylation potentiates its release from the chromatin. Supported by FAPESP

## BC12 - Specific nuclear localization of acetylated H4 histone residues and bromodomain proteins in *Trypanosoma cruzi*.

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Trypanosomatids have peculiar mechanisms controlling gene expression and little is known about chromatin structure and transcriptional regulation. Their histones are among the most divergent known so far, differing in sequence, charge, and/or size when compared to their eukaryote orthologs. Histone tails provide sites for a variety of post-translational modifications, recruiting protein factors that alter the structure of the chromatin and its distribution in the nucleus, gene regulation control and DNA repair. Antibodies against the acetylated lysines 4, 10, and 14 from histone H4 were raised and used in indirect immunofluorescence assays for ultrastructural immunocytochemistry aiming to detect the nuclear localization of these modifications. Immunofluorescence analysis show a different patterns of nuclear labeling for the K4 compared to K10 and K14. At the ultrastrutural level H4 K4-acetylation is found in dense chromatin regions whereas acetyl-K10 and acetyl-K14 are present in boundaries of dense/non-dense chromatin regions, known to be sites of transcription, replication and DNA repair. Bromodomain-containing proteins bind acetylated lysine residues and this motif is present in many transcriptional regulators, participating in chromatin structure remodeling and transcription control. In another study, the *T. cruzi* bromodomain factor 2 (TcBDF2) was shown to interact with histone H4. Here we demonstrated that it co-localizes with histone H4 acetylated at lysine 10, and 14. The results suggest that K10 and K14 may have a specific role in nucleus of this parasite. Supported by FAPESP and CNPq.

## BC13 - Eukaryotic translation initiation factor 2 (elF2$\alpha$) has a punctuated distribution in the cytosol of trypanosomes

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In eukaryotic cells, protein synthesis is controlled by a variety of different signaling pathways sensitive to growth conditions, such as the availability of nutrients, or to cellular stress, such as misfolding of proteins. The phosphorylation of the a subunit of initiation factor 2 (eiF2$\alpha$) by specific kinases is a key step in this control. To evaluate whether this factor is modified during parasite life cycle, we have raised antibodies to *Trypanosoma brucei* recombinant eiF2$\alpha$ and against a synthetic peptide corresponding to the phosphorylation site (Thr169). We found that eiF2$\alpha$ has a punctuated cytoplasmic distribution in epimastigote forms of *Trypanosoma cruzi*, as well as in procyclic and bloodstream forms of *T. brucei*. We are now characterizing the specificity of anti-elF2$\alpha$-P and will show the labeling with these antibodies in parasites submitted to different stress conditions and at different life cycle stages of these parasites. Supported by FAPESP and CNPq

## BC14 - The membrane-associated eIF2$\alpha$ kinase of trypanosomatids

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Regulation of protein synthesis by the phosphorylation of the alpha subunit of the translation initiation factor 2 (eIF2 $\alpha$) is central to stress-induced programs of gene expression, from yeast to mammals. In yeast there is only one eIF2 $\alpha$ kinase, GCN2, activated upon amino acid starvation. Mammals have four eIF2 $\alpha$ kinases, GCN2, HRI, activated by low heme, PKR, activated by dsRNA and PERK, an endoplasmic reticulum transmembrane protein, activated in the unfolded protein response (UPR). Activation of these kinases occurs by autophophosphorylation events brought about by their dimerization and/or relief of inhibitory domains. Trypanosomatids express three eIF2 $\alpha$ kinases (eIF2K1-K3). We have previously shown that the *T. brucei* eIF2K2 (TbeIF2K2) phosphorylates TbeIF2 $\alpha$. TbeIF2K2 amino acid sequence suggests it is a type I transmembrane protein. Using antibodies raised against the N-terminal putative regulatory domain of TbeIF2K2, we have shown that TbeIF2 $\alpha$K2 is localized to the flagellar pocket of both procyclic and bloodstream forms. The regulatory domain would then reside in the lumen of the pocket, and the kinase domain in the cyto-

plasm. This topology then suggests a potential role of this protein in transducing an extracellular signal to the protein synthesis machinery. To further study the topology of this protein, we have raised antibodies to the kinase domain and the characterization of these antibodies will be presented. Interestingly, we found that TbeIF2K2 is downregulated in monomorphic bloodstream parasites grown to a density of 4 x $10^{6}$ cells/ml, when cell death ensues. *Trypanosoma cruzi* and *Leishmania* encode orthologs of TbeIF2K2, and antibodies raised against the regulatory domain of the *T. brucei* protein recognize a protein of the expected size in *T. cruzi*, but not in *Leishmania*. Antibodies against the *T. cruzi* protein were also obtained. Localization studies of TceIF2K2 employing these antibodies are underway.

## BC15 - *In vitro* translation of *Triatoma infestans* tryalisin

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The *Triatoma infestans* (Hemiptera: Reduviidae) is an exclusive hematophagous insect that transmit Chagas' disease in South America. Its saliva has anticoagulants, anti-platelet aggregation factors, apirases, proteases, sodium channel inhibitor and a pore forming protein, called trialysin that has antimicrobial activity. Trialysin has characteristics of amphipathic antimicrobial lytic peptides. It is produced as an inactive precursor form in the *T. infestans* salivary gland and it is activated after saliva ejection. Due to its strong lytic activity, the expression of trialysin or its precursor in bacteria or yeast was not successful. Here we demonstrated that trialysin can be expressed *in vitro* by using Wheat Germ Extract Plus (Promega). This system provides enough proteins to perform biological and structural studies of the protein function. Supported by FAPESP and CNPq

## BC16 - The isolated symbiont is capable of synthesizing phospholipds after isolation from *Crithidia deanei*

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Some trypanosomatids harbor a symbiotic bacterium, which maintains a mutualistic relationship with the host, constituting an excellent model to study organelle origin and cellular evolution. The endosymbiont is enclosed by two unit membranes, where sterols are absent and the cardiolipin is the main phospholipid, followed by phosphatidilcholine (PC), and phosphatidilethanolamine (PE). PC is synthesized through different pathways: the Kennedy pathway, predominant in eukaryotes, produces PC from free choline via the intermediates choline-phosphate and CDP-choline; the Greenberg pathway, predominant in prokaryotes, produces PC after three successive methylations of PE; the PCS pathway, present in prokaryotes closely associated to eukaryotes, produces PC in one single step from free choline. Symbiont and mitochondrion fractions were incubated with $^{32}$Pi for 1 and 3 hs. Differently from mitochondria, symbionts were capable of synthesizing phospholipids after isolation from the host cell. The major phospholipid produced was PE, followed by phosphatidylinositol (PI) and PC after 1 h; and after 3 h, an increase in the phospholipid content was observed. Our next step, was the incubation of isolated endosymbionts with $^{3}$H-choline for 1 and 3 h in order to verify if the bacterium could uptake the tracer from the medium to construct PC. No incorporation was observed, indicating that the symbiont lacks the PCS pathway. Recently, we observed that the mitochondrial fraction consumes higher rates of $O_2$ when compared to the symbionts, suggesting that although viable after the cell fractioning, this organelle is unable to synthesize phospholipids after isolation. Taken together, these results suggest that the symbiotic bacterium may obtain part of its PC, or even PC precursors, from the host trypanosomatid. The full genome sequencing of the symbiotic bacterium, as well as the use of specific inhibitors to PC biosynthesis enzymes, might thus clarify new aspects of symbiosis in trypanosomatids. Supported by: CAPES and FAPERJ.

## BC17 - Effect of kinase inhibitors in the encystation of *Giardia lamblia*

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*Giardia lamblia* is a protozoon parasite which inhabits the upper small intestine of mammals and causes giardiasis. This early diverged parasite is very different from the other eukaryotes. Its life cycle comprises two forms: the sugar and protein wall protected infective cyst and the replicative flagellated trophozoite. The transformation of the trophozoite into cyst is named encystation, which is crucial to the parasite, promoting its life outside the host. The signaling pathways concerning this transformation are still not clear. In that matter, what signalling pathways were crucial to *G. lamblia* differentiation? We adressed the question using Genistein - a tyrosine kinase inhibitor and LY294002 - a phosphatidylinositol-3 kinase inhibitor. We observed a 83,94% reduction in encystation rates when using 50$\mu$M Genistein and a 77,96% reduction using 10$\mu$M LY294002. The transmission electron microscopy images of 50$\mu$M Genistein treated cells showed membrane disorganization with many concentric membranes in the cytoplasm of the parasite. The 10$\mu$M LY294002 treated cells showed intense disorganization of peripheral vesicles, which became distributed not only at the periphery, but in the whole cell. In order to investigate when the signalling pathways investigated were important to cell differentiation, we induced the parasites to encyst and after 6 or 18 hours we added the drug to the culture, collecting the parasites when they completed 48 hours

of encystation. The 10$\mu$M LY294002 addition after 6 hours of encystation showed greater inhibition of encystation rates, approximately 97%. The 50$\mu$M Genistein treatment showed greater inhibition when added at the moment of encystation. The tyrosine kinase pathways seems to be involved in the early steps of encystation while the PI3K pathways may be involved in the formation of the clefs which accumulate the cyst wall material before the formation of encystation specific vesicles. Support: CAPES, CNPq, FAPERJ, Pronex

### BC18 - Localization of TOR-like kinases in *Trypanosoma brucei*

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TOR (Target of Rapamycin) are evolutionary conserved protein kinases that coordinate the balance between protein synthesis and protein degradation in response to environmental signals including nutrients, stress and mitogens in eukaryotic cells. Studies with mammalian and yeast cells signaling pathways showed that nutrient starvation inhibits TOR activity, which results in G1 cell cycle arrest, and triggers a stress response program leading to a blockade of translation initiation. The same stress response can be observed in cells treated with rapamycin, an imunosupressant drug, which binds to FKBP12 prolyl-isomerase forming a complex with the TOR kinase. By searching *T.brucei* genomic database we found two candidates for TOR (TbTOR1 and TbTOR2). RNA interference of these kinases in *T. brucei* showed a slow growth phenotype. We have obtained purified antibodies against recombinant TbTOR1 and TbTOR2 and by immunofluorescence assays we observed a different cytoplasmic localization for these kinases. While TbTOR1 is found in foci in all cytoplasm, TbTOR2 is found in interconnected structures. The localization of these kinases will be correlated with specific markers for cytoplasmic organelles like mitochondria, Golgi apparatus and endoplasmic reticulum. Supported by FAPESP and CNPq

### BC19 - Absence of flagellum-cell body attachment alters the endocytic pathway pattern of *Trypanosoma cruzi*

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Two surface domains of *Trypanosoma cruzi* epimastigotes are involved in the acquisition of nutrients, the flagellar pocket (FP) and, mainly, the cytostome (De Souza, Curr. Pharm. Des. 8:269, 2002). Okuda and cols. (Exp. Parasitol., 92:223, 1999) demonstrated that the cytostome is physically connected with the flagellar complex. We decided to study the role played by this association in the endocytic pathway, using gp72 null mutant epimastigotes of the Y strain of *T. cruzi*, where the flagellum-cell body attachment region is absent (Rocha *et al.*, J. Struct. Biol., 154:89-99, 2006). Wild type and gp72 epimastigotes were pre-incubated with albumin conjugated with Alexa 488 or 5nm gold particles for different periods. We measured by flow cytometry a reduction of 20.54% of albumin uptake in the null mutant after 30 min, although the pathway and uptake kinetics observed by fluorescence microscopy are the same as wild type parasites. Transmission electron microscopy of wild type and gp72 epimastigotes that had uptaken albumin-gold confirmed the fluorescence experiments. Moreover, after albumin capture, cargo is still found along the cytopharynx as well as inside vesicles and tubules in both mutant and wild type forms. Comparing microscopy and flow cytometry results we conclude that the dynamics of the process was the same and so fast as in wild type. However, mutant epimastigotes that are devoid of the flagellum-cell body attachment region were not able to capture the same amount of the tracer as wild type parasites under the same condition, suggesting that the physical association between cytostome and flagellar complex plays a role in endocytosis efficiency. Supported by: FAPERJ, FINEP, CNPq and CAPES.

### BC20 - Comparison of the cellular composition, cytokines and granzyme expression during progression of human cutaneous leishmaniasis

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Human infection with *Leishmania braziliensis* leads to the establishment of cutaneous leishmaniasis (CL). Previous studies have shown that circulating T cells from "early"CL patients (E-CL - approximately 15 days of illness, non-ulcerated lesion) displayed a down-modulated Th1-type response as compared to cells from "late"CL patients (L-CL - approximately 60 days of illness, ulcerated lesion). The aim of this study was to compare the cellular composition and cytokine expression of lesions between individuals with E-CL (n=6) and L-CL (n=14). Histopathological analysis showed that lesions from L-CL had a more exuberant inflammatory infiltrate as compared to E-CL, compatible with lesion progression. Although both E-CL and L-CL lesions were predominantly composed of mononuclear cells, lesions from patients with E-CL presented higher neutrophils (p=0.04), eosinophils (p=0.01) and macrophages percentages (p = 0.01), when compared to lesions from L-CL. We also determined the frequency of CD4+ and CD8+ T cells and cytokines in E-CL and L-CL lesions using confocal microscopy. We observed a higher CD4+/CD8+ ratio in E-CL than L-

CL lesions. While the percentage of IFN-$\gamma$+ cells was similar, the percentage of CD4+IFN-$\gamma$+ cells was lower in E-CL as compared to L-CL (25% versus 46%, respectively). The frequency of IL-10+ cells was similar in both groups. The percentage of granzyme A+ cells was higher in L-CL, as compared to E-CL (53% versus 10%, respectively). These results suggest involvement of polymorphonuclear cells during E-CL, preferential recruitment of CD4+ T cells in E-CL and an association of lesion progression with a higher frequency of CD4+IFN-$\gamma$+ cells. Moreover, increased granzyme A expression may be associated with higher tissue destruction observed in L-CL. Financial support: CNPq/PADCT, CAPES, PRONEX, WHO and TMRC/NIH.

## BC21 - Changes in transcription levels and ultrastructural analysis of *Trypanosoma cruzi* nucleus, kinetoplast and flagellum during in vitro metacyclogenesis

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Metacyclogenesis can be induced in vitro by incubating *Trypanosoma cruzi* epimastigote forms in TAU3AAG, a chemically defined medium that mimics triatomine urine (Contreras et al., 1985), thus allowing biochemical and molecular analyses of differentiating cells in vitro. Gene expression in T. cruzi is largely controlled at the post-transcriptional level. Genes are transcribed polycistronically by RNA polymerase II (RNA Pol II) and subsequently cleaved into functional mRNAs, by polyadenylation and trans-splicing of a capped 39-nucleotide leader RNA derived from a short transcript, the spliced leader (SL) RNA. As described previously, T. cruzi RNA Pol II is found concentrated in a domain close to the parasite nucleolus and containing the spliced leader genes in actively transcribing parasite stages (Dossin& Schenkman, 2005). The remaining RNA Pol II is diffusely distributed in the nucleoplasm and metacyclics forms transcribe much less than epimastigotes (Elias et al. 2001). In the present work we have investigated when transcription decreases during metacyclogenesis. From 24 to 48 h after metacyclogenesis induction we could visualize by transmission electron microscopy and immunofluorescence analysis the intermediate forms with a kinetoplast that migrates as the nucleus became elongated and RNA Pol II still associated with the SL body. RNA Pol II labeling and the transcriptional activity only decrease after complete cell transformation, when a spherical kinetoplast is at posterior end of the parasite. These results indicate that the differentiation process requires transcriptional activity and that a reduction in this activity is a feature only of completely differentiated cells. Supported by FAPESP and CNPq

## BC22 - Expression and cellular localization of molecules of the gp82 family in *Trypanosoma cruzi* metacyclic trypomastigotes

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A member of *Trypanosoma cruzi* gp82 family, expressed on metacyclic trypomastigote surface and identified by monoclonal antibody 3F6, plays a key role in host cell invasion. Apart from the gp82 defined by MAb 3F6, no information is available on members of this protein family. From cDNA clones encoding gp82 proteins sharing 59.1% sequence identity, we produced the recombinant proteins J18 and C03, the former containing and the latter lacking the epitope for MAb 3F6. Polyclonal antibodies to J18 and C03 proteins were generated and used, along with MAb 3F6, to analyze the expression and cellular localization of gp82 family members in metacyclic forms of CL and G strains, which belong to highly divergent genetic groups. By two-dimensional gel electrophoresis and immunoblotting, molecules of 82 to 86 kDa, focusing at pH 4.6 to 5.4, and molecules of 72 to 88 kDa, focusing at pH 4.9 to 5.7, were visualized in CL and G strains, respectively. Flow cytometry and microscopic analysis revealed in both strains a similar expression of MAb 3F6-reactive gp82 in live and permeabilized parasites, indicating its surface localization. The reaction of live parasites of both strains with anti-J18 antibodies was weaker than with MAb 3F6, and was increased by permeabilization. Anti-C03 antibodies bound predominantly to flagellar components in permeabilized G strain parasites, but in the CL strain the flagellum was not the preferential target for these antibodies. Host cell invasion of metacyclic forms was inhibited by J18 protein, as well as by MAb 3F6 and anti-J18 antibodies, but not by C03 protein or anti-C03 antibodies. Work supported by Fapesp and CNPq.

## BC23 - Effects of Actin and Tubulin Inhibitors on Growth and Ultrastructure of *Trypanosoma cruzi*.

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Microfilaments were never observed in the cytoplasm of *T. cruzi*. However, actin, the main component of microfilaments, was identified in *T. cruzi* by immunofluorescence microscopy and by biochemical studies. Microtubules, the most important citoskeletal component of trypanosomatids, are distributed along all the cell body immediately under the plasma membrane (subpellicular microtubules). We report here the effects of three inhibitors of cytoskeleton components, jasplakinolide (irreversible F-actin stabilizer), cytochalasin D (microfilament depolimerizer) and nocodazole

(microtubule depolimerizer), on growth and ultrastructure of epimastigotes *T. cruzi* (Y strain). The IC50 were: jasplakinolide: 3,46 uM; cytochalasin D: 7,48 uM; and nocodazole: 7,29 uM. Jasplakinolide at 15 and 20 uM was the drug that caused greater growth inhibition of the parasites. Nocodazole had almost no effect on growth of treated epimastigotes. Parasites treated with the three drugs presented an incomplete cytoplasm division and multiflagellated and multinucleated forms suggesting interruption of the cellular division. Parasites treated with jasplakinolide and nocodazole presented concentric membranes inside the mitochondrion with complete disorganization of its internal cristae. Other important morphological effects caused by cytochalasin D were: (a) formation of large autophagosomes; (b) loose flagellar membrane; (c) exocytosis of sacks full of shedded membranes close to the flagellar pocket. Many cells presented the formation of a vacuole closely associated to the flagellar pocket and the presence of numerous vesicles close to the Golgi apparatus and between this organelle and the flagellar pocket. Nocodazole also caused alterations of the subpellicular microtubules of the treated parasites. There was a space between the cellular membrane and the cytoplasm, and the subpellicular microtubules were not juxtaposed to the membrane. The flagellar membrane was also affected by this drug. The flagellar membrane was also affected by the drug. This work was supported by the Papes IV (Fiocruz / CNPq), European Commision, PRONEX, FINEP, CNPq and FAPERJ.

## BC24 - Phenotypic analysis of two isolates of *Acanthamoeba polyphaga*: a way to decode pathogenicity *in vitro*.

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*Acanthamoeba* is a genus of free-living amoebae, widely dispersed in all types of environment, which occasionally can cause serious human infections, like granulomatous amoebic encephalitis, a lethal brain infection; amoebic keratitis, a sight-threatening corneal infection among other disseminated infections. The mechanisms of amoebic pathogenicity, as well as correlations between taxonomic status and pathogenicity are complex, controversial, and not entirely known subjects. In order to decipher the relation between amoebae origin and pathogenicity, the objective of this work was to examine biological characteristics of two distinct isolates of *A. polyphaga*: one isolated from human cornea of a keratitis patient (highly cytopathic in vitro) and the second, a freshwater, environmental isolate, in order to determine pathogenicity determinants among amoebae cell physiology. We were able to observe that corneal-isolated amoebae possess a group of phenotypic characteristics totally distinct from environmental amoebae. Corneal amoebae grow faster in culture medium, transform more rapidly in cysts and also it is able to destroy LLC-MK2 cell monolayers. Amoebae isolates possess morphological differences, in the amount of spiny-like projections (acanthopodia) and microfilament organization, with the presence of evident locomotory structures in the corneal isolate. Adherence on plastic varies between both isolates, where environmental amoebae were encountered highly attached on plastic surfaces. Zymography demonstrated that keratitis isolate possess highly proteolytic activity and alsowere more invasive on collagen matrices than freshwater amoebae. Altogether, we conclude that there is a group of phenotypic characteristics, related to mechanisms of tissue invasion, which are strongly related to *A. polyphaga* pathogencity in vitro. Financial support: FAPERJ, CNPq and FUJB-UFRJ

## BC25 - LACK OF PURIGERGIC $P2X_7$ RECEPTOR DOES NOT PREVENT LYMPHOID CELL PROLIFERATION IN CUTANEOUS LEISHMANIASIS, BUT IS IMPORTANT FOR THEIR HOMING TO THE INFECTION SITE.

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The importance of purinergic receptor $P2X_7$ ($P2X_7R$) in the growth control of intracellular microorganisms is already known for *Chlamydia trachomatis* and *Mycobacterium tuberculosis*. In Leishmania, we have shown that the phagocytosis of latex beads and adhesion/internalization of promastigotes of *L.amazonensis* is decreased in macrophages lacking the $P2X_7R$. Here, we compared the role of $P2X_7R$ in cutaneous leishmaniasis using mice deficient in $P2X_7R$. Thus, C57Bl/6 $P2X_7R^-/-$ and control $P2X_7R^+/+$ mice were infected in the footpad with $2x10^6$ promastigotes of *L. amazonensis*-GFP, and on days 7 and 78, the footpads and draining lymph nodes were excised, weighted and grinded in medium. On day 7, the popliteal lymph nodes from $P2X_7R^-/-$ mice were double as heavier than $P2X_7R^+/+$ controls, consistent with their increased cell numbers ($15 \times 10^6 \pm 2,2 \times 10^6$ and $5,9 \times 10^6 \pm 1,5 \times 10^6$, respectively). On the other hand, half the number of total cells was observed in the infected footpads of $P2X_7R^-/-$ mice, as compared with controls. In spite of that, the parasite loads in the footpads as measured by limiting dilution analysis, were found to be similar in both groups. On day 78, the lymph nodes of $P2X_7R^-/-$ mice remained more enlarged and with more cells than $P2X_7R^+/+$ controls. Their lesions were even larger, but not due to increased parasite loads, which were curiously similar in both groups. These results indicate that the deficiency in $P2X_7$ receptor does not prevent lymphoid cell activation in the lesion-draining lymph nodes in cutaneous leishmaniasis, but is important for their homing to the infection site. Moreover, it appears that the cells that normally migrate to the infection site are not effective, as the parasite loads were similar in both $P2X_7R^-/-$and $P2X_7R^+/^+$ groups. Financial support: CAPES

## BC26 - INFECTION WITH Toxoplasma gondii EXTENDS THE SURVIVAL OF VERO CELL CYTOPLASTS

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The survival of cells enucleated (cytoplasts) by centrifugation in the presence of a cytochalasin depends on the cell type, history, and physiological condition; on the method used for enucleation, and on the conditions of maintenance of the cytoplasts. Our laboratory looks for agents or maintenance conditions capable of extending the survival of cytoplasts. As first reported by Glasgow virologists, we found that the estimated 50% survival of Vero cell cytoplasts was 18h at 37°C and 32h at 34°C (Ketna Khusal). The mechanisms involved in the decay and death of the cytoplasts have not been characterized, although over-expression of anti-apoptotic Bcl-2 protects them from apoptosis induced by different agents. It has also been shown that infection of nucleated cells by *Toxoplasma gondii* increases their resistance to apoptosis inducers. We ask here if the survival of Vero cell cytoplasts can be extended upon infection with *T. gondii* (RH strain). Infected or non-infected cytoplasts prepared from Vero cells were kept at 34 or 37°C, fixed at different times between 4 and 48h, and counted after with Giemsa. Two hours after enucleation, cytoplasts were infected with *T. gondii* for 4h, washed and incubated in fresh medium. In uninfected cultures kept at 37°C, only 20-30% of the cytoplasts survived for 24h. In contrast, in infected cultures, whereas 70-80% of infected cytoplasts survived at 24h, only 20-30% of the uninfected controls did survive in the same preparations. At 34°C the effects of temperature and of infection were synergistic. The mechanisms involved in the increased survival of *T. gondii* infected cytoplasts are under investigation. Acknowledgement: UENF for scholarship for CSC; FAPESP and UNIFESP for support for MR.

## BC27 - Co-infection with Trypanosoma cruzi protects mice against early death by neurological or pulmonary disorders induced by Plasmodium berghei ANKA

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Multiple infections by parasitic agents that cause diverse clinical manifestations occur frequently and increase or decrease of overall pathogenic impact can be influenced by synergistic or competitive interactions between parasite species. The objective of this study was to investigate whether the infection of C57BL/6 mice by *Plasmodium berghei* ANKA, which causes severe malaria, was modulated by co-infection with *Trypanosoma cruzi*. Groups of C57BL/6 mice were infected either with *P. berghei* ANKA, *T. cruzi* strain G, or with both parasites. Parasitemia was checked by microscopic examination of blood samples. Symptoms of neurological or respiratory disorders, as well as mortality, were registered. Breakdown of the blood brain barrier was determined by injecting the dye Evans blue. Histological sections of the lung were prepared and stained with hematoxilin-eosin. Co-infection with *T. cruzi* protected mice against the early death by malaria infection, by partially preventing either the breakdown of the blood brain, and cerebral malaria as a consequence, or the pulmonary oedema. All mice infected only with *P. berghei* ANKA died within 7-11 days post-infection, either with symptoms of cerebral malaria or with respiratory abnormalities. The animals co-infected with *T. cruzi* strain G survived longer, without any of the referred to symptoms. Protection against the early death by severe malaria was effective when mice were given *T. cruzi* 15 days before *P. berghei* inoculation. Breakdown of the blood brain barrier and extensive pulmonary oedema, caused by malaria parasites, were much less pronounced in co-infected mice. The degree of protection to severe malaria and early death, conferred by co-infection with *T. cruzi*, was comparable to that conferred by treatment with anti-CD8 antibodies. Supported by CNPq and FAPESP

## BC28 - Autophagy interferes *L. amazonensis*-macrophage interaction

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CBA/J mouse macrophages express different genes in response to *L. amazonensis* and *L. major* infection. *L. amazonensis* enhanced the expression of rab24 in relation to control non-infected macrophages. Rab24 participates in autophagocytic processes. Autophagy is essential in regulation of cell growth, maintenance of homeostasis and energy generation during cell stress. It was recently described that autophagy may contribute to a protective response against bacterial infection. On the other hand, few studies evaluated the autophagic phenomenon in protozoa infection. This study aims to analyze the participation of autophagy in *L. amazonensis* macrophages *f*ninfection. Macrophages were infected with *L. amazonensis* or exposed to yeast or zymosan, previously or after autophagy induction. The previous induction of autophagy by nutrient deprivation or by rapamycin treatment reduces the macrophage phagocytic capacity. Macrophage phagocytic capacity was partially recovered when autophagy was reverted by nutrient addition to cell cultures previously subjected to nutrient deprivation. In addition, there was an enhancement in zymosan-FITC binding to phalloidin-

labelled cells, previously subjected to nutrient deprivation, at 4$^o$C. This temperature only allows binding, without particle internalization. These data indicate that phagocytosis inhibition is a direct consequence of autophagy and occurs in a downstream step after particle binding. Moreover, incubation in nutrient deprived medium or treatment with rapamycin after infection induced a marked reduction in the percentage of *L. amazonensis*-infected macrophages. The data herein suggest the role of autophagy in reducing macrophage *f*nphagocytic capacity and intracellular *L. amazonensis* survival.
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## BC29 - INTERACTION BETWEEN *Leishmania amazonensis* AND HOST CELL: PHOSPHATIDYLSERINE EXPOSURE BY INTRACELLULAR PARASITE MODULATES MACROPHAGE ACTIVITY

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Amastigote forms of *Leishmania (L) amazonensis* purified from mouse lesions expose phosphatidylserine (PS) on their surface, a phenomenon described as Apoptotic Mimicry, and the amount of exposed PS is modulated by the host (Current Biology 11:1870-1873,2001; J. Immunol.176:1834-1839,2006). Recognition of this molecule by host macrophages mediates parasite internalization by macropinocytosis, modulates the production of anti-inflammatory cytokines and inhibition of Nitric Oxide (NO) synthesis, contributing to the enhancement of amastigote infectivity. Now we show that, different from the *in vivo* model, amastigotes purified from *in vitro* cultures of BALB/c and C57BL/6 macrophages do not display quantitative differences in PS exposure. Amastigotes purified from lesions in BALB/c NUDE mice expose less PS on surface than that from BALB/c mice. These results suggest that the differences observed in amastigotes isolated from *in vivo* lesions are dependent on differential macrophage activation in the host and are T cell dependent. During promastigote to amastigote differentiation inside the parasitophorous vacuole (PV) there is a time-dependent increase in PS exposure by the parasite. As the PS exposure increases there is a concomitant increase of macropinocytic activity, synthesis of TGF-$\beta$ and inhibition of NO production by infected macrophages. PV alkalinization inhibits intramacrophagic signal and macropinocytic activity of infected macrophages. Our results suggest that PS molecules on the surface of the intracellular *L. amazonensis* are capable of stimulating macrophages from inside the PV membrane. This comprises a mechanism of interaction between parasite and host cell that mediates down-regulation of macrophage anti-parasite activity and thus contributes to the establishment of the infection.

## BC30 - Comparative analysis of the macropinocytic activity of macrophages infected with *Leishmania amazonensis* and *Leishmania major*

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As described for apoptotic cells, amastigotes of *Leishmania amazonensis* expose phosphatidylserine (PS) in the external leaflet of their plasma membrane, a mechanism described as Apoptotic Mimicry (Current Biology 11:1870-1873, 2001). Recognition of exposed PS by macrophages induces parasite internalization by macropinocytosis, production of IL-10, TGF-$\beta$, and inhibition of nitric oxide synthesis (J. Immunol. 176:1834. 2006), facilitating infection and parasite intracellular proliferation. These mechanisms are dependent on the amount of PS exposed by the parasite. In macrophages infections with *Leishmania major*, amastigotes reside in individual tight vacuoles. However, amastigotes of *L. amazonensis* reside in large vacuoles, usually harboring more than a single amastigote. With the goal of defining if PS exposure is important in the genesis of the large macropinocytic vacuoles, we compared the PS exposure by *L. amazonensis* and *L. major* amastigotes purified from *in vitro* infected macrophages, and looked for correlations with the observed vacuoles size. During *L. amazonensis* infections, an increase in PS exposure occurs during the intracellular promastigote to amastigote differentiation, with maximum values at 72 or 96 hours post-infection. At this moment, an enhanced macropinocytic activity by the infected macrophage can de observed. During *L. major* infections, PS exposure on amastigote surface is lower than that observed on *L. amazonensis* amastigotes, and no relevant macropinocytic activity can be detected. The induction of macropinocytic activity during infections with *L. amazonensis* suggests that PS on the parasite surface signals from within the parasitophorous vacuole, contributing to the enlargement of the vacuoles containing the amastigotes. The low PS exposure by *L. major* amastigotes is probably not sufficient for the induction of macropinocytic activity, resulting in tight vacuoles around the amastigotes. The differences in PS exposure and consequent induction of macropinocytic activity by these species suggests different parasite-host cell relationship mechanisms between New and Old World species of *Leishmania*.

## BC31 - Intraspecific fusion of Leishmania (L.) amazonensis parasitophorous vacuoles in mouse macrophages

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*Leishmania (L.) amazonensis* amastigotes reside in

macrophages within spacious parasitophorous vacuoles (PVs) which may contain numerous parasites. After sporadic fusion events were detected by time-lapse cinemicrography, PV fusion was examined in two different models. In single infections, it was inferred from the reduction in PV numbers per cell. In a reinfection model, macrophages infected with unlabeled amastigotes were reinfected with carboxyfluorescein diacetate succinimidyl ester-labeled parasites; fusion was detected by the colocalization of labeled and unlabeled amastigotes in the same PVs. The main findings were: 1) as expected, fusion frequency increased with the multiplicity of infection; 2) most fusion events took place in the first 24h of infection or reinfection, prior to the multiplication of incoming parasites; 3) resident and incoming parasites multiplied at similar rates in fused PVs. The model should be useful in studies of parasite and host cell factors involved in PV fusogenicity.

## BC32 - Reactive oxygen intermediates production during innate immune response of CBA/J mouse macrophages infected *in vitro* by *Leishmania major* or *Leishmania amazonensis*.

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We previously demonstrated that CBA/J macrophages control *L. major* infection and are permissive to *L. amazonensis*, indicating an important role for macrophages on determination of Leishmania infection outcome. CBA/J macrophages stimulated by IFN-gamma and infected with *L. major* or *L. amazonensis* show similar nitric oxide production. Preliminary proteomics analyses showed that CBA/J macrophages infected by *L. major* or *L. amazonensis* express catalase and antioxidative enzymes, such as thioredoxin reductase, glutathione-S-transferase and casein kinase in response to *L. major*. It is well established that *Leishmania* spp. are destroyed by reactive oxygen intermediates (ROI) inside macrophages and could also inhibit ROI production by those cells. Our hypothesis is that although CBA/J macrophages liberate ROI and reactive nitrogen intermediates (RNI) in response to either *L. major* or *L. amazonensis* infection, only *L. amazonensis* is resistant to these toxic radicals. We analyzed cytochrome c oxidation to measure indirectly the superoxide production and there were no differences between ROI productions by CBA/J macrophages infected by *L. major* or *L. amazonensis*. Pick's technique was used to determine hydrogen peroxide ($H_2O_2$) production, and no differences were observed in $H_2O_2$ production by infected cells. Due to the low sensibility of those techniques, it was used a more sensitive approach based on photon counts of lucigenin-enhanced chemiluminescence and it was not detected any differences in superoxide production between *L. major*- or *L. amazonensis*-infected cells. However, $H_2O_2$ formation, measured by monitoring luminol and microperoxidase chemiluminescence, showed that *L. major* induces higher levels of $H_2O_2$ production by CBA/J macrophages when compared to *L. amazonensis*-infected cells. These results suggest that in CBA/J macrophages, *L. major* destruction occur in dependence of $H_2O_2$ production. Current experiments goals to evaluate comparatively *L. major* and *L. amazonensis* sensibility to ROI and RNI free radicals. Financial support: CAPES, CNPQ 478804/2004-0 & FAPESB ET64/2004.

## BC33 - Participation of macrophage membrane rafts in *Trypanosoma cruzi* invasion process.

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The establishment of the infection by *Trypanosoma cruzi*, the ethiologic agent of Chagas' disease, depends on a series of events involving adhesion of the parasite to surface receptors of the cell, recruitment of additional receptors to the infection site, a re-organization of the membrane and, in particular, the formation of a parasitophorous vacuole. Distinct microdomains in the plasma membrane are responsible for the invasion of some virus, bacteria and protozoan. Membrane rafts are small and dynamic regions enriched in sphingolipids, cholesterol, ganglioside GM1 and protein markers like flotillins 1 or 2, forming flatter domains, or caveolins 1, 2 or 3, which are characterized as stable flask shape invaginations. We explored whether membrane rafts participate on the entry of *T. cruzi*'s trypomastigotes into murine macrophages. Transient depletion of macrophage membrane cholesterol with methyl beta cyclodextrin and treatment with filipin caused a reduction of trypomastigote adhesion, internalization and affect the *T. cruzi*'s survival. Treatment with crescent concentration of cholera toxin B that binds GM1, demonstrated the ability to inhibit the adhesion and invasion of trypomastigotes and amastigotes forms. The same has not been viewed with epimastigote forms. By immunofluorescence we observed a colocalization of GM1, flotilin 1 and caveolin 1 in *T. cruzi*'s parasitophorous vacuole. Together our results suggest that membrane rafts are involved on the process of *T. cruzi* invasion and survival of macrophages. Supported by CNPq, FAPERJ and CAPES.

### BC34 - A novel 21 kDa secreted component from *Trypanosoma cruzi* is involved in mammalian cell invasion

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We have recently identified by microarray screening a 21 kDa component from T. cruzi (P21) that is involved in mammalian cell invasion by *T. cruzi* infective forms. P21 amino acid sequences from CL and 588 strains are highly conserved and similar to the sequence from CL- Brener clone from T. cruzi genomic database. On the other hand, P21 amino acid sequence from G strain displayed only 86.3 % similarity. Recombinant protein (His6-P21) was cloned from genomic DNA from G strain. Western blotting from parasite whole extracts and supernatants revealed that P21 is a secreted and ubiquitous protein. Immunofluorescence from infected Vero cells harboring intracellular amastigotes revealed that P21 is diffusely distributed in the cytosol of formaldehyde fixed and saponin permeabilized amastigotes from G, CL and 588 strains. In addition, some parasites showed a polar P21 distribution and in others the protein localized around the nucleus. The recombinant protein was used in assays to verify the ability to adhere to host cell and to trigger calcium signaling. The results showed that although His6-P21 adhered to HeLa cells in a dose dependent manner, it failed to trigger calcium signaling in NRK cells. In invasion assays it was verified that His6-P21 inhibited cell invasion by extracellular amastigote from G, CL e 588. Conversely, treatment with His6-P21 increased cell invasion by metacyclic trypomastigotes. Pre-treatment of the parasites with the monoclonal antibody 2C6 produced against His6-P21 inhibited invasion from G and 588 strains but not from CL strain. We are now checking the in vitro and in vivo effect of P21 super expression in metacyclic trypomastigotes and extracellular amastigotes from G and CL strains. Financial support: FAPESP, CAPES, CNPq.

### BC35 - Invasion mechanism of Trypanosoma dionisii in non-phagocytic mammalian cells involves lysosome recruitment

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*Trypanosoma* (Schizotrypanum) *dionisii* is a bat trypanosomatid related to *Trypanosoma cruzi*, the etiological agent of Chagas disease. During its life cycle *Trypanosoma dionisii* alternates throught different developmental forms between vertebrate and invertebrate hosts: epimastigotes and metacyclic trypomastigotes in the invertebrate host, and bloodstream trypomastigotes and amastigotes in the vertebrate host. In vitro *T. cruzi* metacyclic trypomastigotes are able to invade and replicate within a large number of mammalian cells. When *T. cruzi* metacyclic trypomastigotes invade a cell, lysossomal markers are found in parasitophorous vacuoles (PV) and for efficient infection, parasites need to escape from these compartments, differentiate into amastigotes and replicate in the cytosol of the cell. One of the earliest steps of parasite entry involves lysossomal recuitment to the region of parasite/cell adhesion. Previous studies showed that *T. cruzi* acid compartment residence is essential for a successful infection. In this work we have shown that *T. dionisii* metacyclic trypomastigotes also recruit host cell lysosomes during host cell invasion and reside inside Lamp1 positive PV for many hours. Cos7 cells were infected with *T. dionisii* metacyclic trypomastigotes and left for 4 to 5 days. In these cell, most of parasites had escaped from Lamp1 positive vacuoles to the cytoplasm and multiplied; however, a small proportion remained inside Lamp1 PV where they transformed into amastigotes. Ours results indicate that the formation of lysosomal-enriched compartments is part of a cell-invasion mechanism retained by related trypanosomatids and indicate that residence and further escape from a lysosomal compartment may be a common requisite for successful infection.

### BC36 - Investigation of the binding on *Tritrichomonas foetus* and *Leishmania (L.) amazonensis* to laminin-1 and its role in the cell-parasite interaction.

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The interaction between pathogens and the host extracellular matrix is an important task for the infection success. Nervertheless, little is still known on such subject. The extracellular parasitic protozoan *Tritrichomonas foetus* is the causative agent of bovine trichomonosis, a urogenital disease of veterinary relevance. Since it is an extracellular parasite its recognition of the host millieu is crucial to the parasite surveillance into host. The intracellular parasitic protozoan *Leishmania (L.) amazonensis* is the causative agent of cutaneus leishmaniosis. *L. amazonensis* is an intracellular parasite adaptated to survive and multiply inside of the acidic vacuoles of macrophages. Thus, to reach macrophages promastigotes and amastigotes forms of *L. amazonensis* have

to cross the host extracellular matrix during the infection course. This work is concerning on analyses of the interaction between *T. foetus* or *L. amazonensis* and immobilized laminin-1 (LMN-1). Either *T. foetus* or each one of the both forms of *L. amazonensis* were capable to recognize LMN-1. Following parasite-LMN-1 interaction we did observed a drastic morphological transformation among *T. foetus* which became mostly ameboid. A requirement for divalent cations to the interaction of both protozoa parasites to the immobilized LMN-1 was observed. The involvement of laminin binding proteins (LBPs) present to the surface of *T. foetus* and *L. amazonensis* was also observed. Five LBPs seem to exist in *T. foetus*. On the other hand, promastigotes of *L. amazonensis* seem to possess four laminin binding molecules or four subunits of the same LBP. Related to the role played by the binding of each one of the parasites to immobilized LMN-1 we demonstrated that such interactive process enhanced *T. foetus* toxicity to HeLa epithelial cells. In the case of *L. amazonensis*, LMN-1 seems to play a functional role of a molecular bridge between promastigotes and macrophages. (Supported by PIBIC-UFRJ/CNPq, FAPERJ and FUJB-UFRJ).

## BC37 - Lysosomal components LAMP-1 and LAMP-2 are not essential for efficient mammalian cell invasion by *Trypanosoma cruzi* amastigotes and metacyclic trypomastigote forms

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It has recently been demonstrated that mammalian cell invasion by *Trypanosoma cruzi* primarily involves the formation of a host cell plasma membrane vacuole in coordination with lysosome exocytosis and it has been proposed that fusion with lysosomes is required for parasite retention inside host cells. Recently, knockout mice for LAMP-1 and LAMP-2 proteins (Lysosome Associated Membrane Proteins) have been generated. Since deletion of both *lamp* genes causes embryonic lethality, mouse embryo fibroblasts were immortalized to generate cell lines. These cells made it possible to demonstrate that LAMP proteins are important components in the fusion between late endosomes and lysosomes. We are now investigating the role of these proteins in the cell invasion by infective forms of two phylogenetic distinct *T. cruzi* strains. Surprisingly, we have found that invasion rates in knockout cells were consistently higher in comparison to wild type cells, in infection assays with either extracellular amastigotes or metacyclic trypomastigotes of G and CL strains. Moreover, when we followed the course of infection for 72 hours, we observed a 2-fold and 4-fold increase in the number of intracellular amastigotes in knockout cells when previously infected with metacyclic trypomastigotes and extracellular amastigotes, respectively. The same results were observed after cell infection with parasites of both phylogenetic lineages. We are now performing experiments in order to investigate the possible mechanisms underlying the higher invasion and replication rates verified in *lamp-1/2* knockout cells. Financial support: FAPESP, CNPq & CAPES

## BC38 - Lysosome proteins (LAMP1 and LAMP2) and its influence in host cell invasion by *T. cruzi* tissue culture trypomastigotes

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Chagas disease caused by the protozoan parasite *T. cruzi*, is a serious debilitating illness with a variable clinical course that affects millions of people throughout America. During vertebrate host colonization, *T. cruzi* is able to invade a wide variety of cell types, in a process that requires attachment to the host cell, host and parasite cell signaling and recruitment of lysosomes for the formation of the parsitophorous vacuole. Recently we have shown that during the process of invasion, tissue culture trypomastigotes can enter cells transiently by invagination of the plasma membrane, but are able to slide back out again - unless fusion with lysosomes occurs, resulting in a stable intracellular vacuole. Lysosomes then appear to be essential not only for formation of the parasitophorous vacuole, but also to work as an anchor for parasites inside host cells. Lysosome internal surface is almost completely covered by two major membrane integral proteins, LAMP (Lysosomal Associated Membrane Protein) -1 and -2. We used fibroblasts derived from mice Knocked out for these two proteins (LAMP-1/2 KO) in invasion assays with *T. cruzi* tissue culture trypomastigotes from Y strain. Lack of these two proteins led to a reduction of 50% in cell invasion by this infective form. This reduction was maintained when we used different MOIs (50:1/100:1), showing it is a stable phenotype. On the other hand, preliminary data indicate that parasite intracellular development is higher in LAMP-1/2 KO cells. Parallel work with different infective forms from other strains has also shown a better intracellular development in LAMP1/2 KO cells. We are now on the course of investigating the invasion with tissue culture derived trypomastigotes from other strains, as well as determining the mechanisms by which LAMP interferes in this process.

## BC39 - Platelet- activating factor (PAF) modulates protein phosphorylation and cellular proliferation in *Phytomonas serpens* and *Leptomonas wallacei*

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*Leptomonas wallacei* is a trypanosomatid parasite that was first isolated from the intestinal tract of the plant-sucking insect *Oncopeltus fasciatus*, which has been widely used as a model for studies of the interaction between trypanosomatids and its midgut. *Phytomonas serpens* is a protozoan that parasitize plants of great economical importance causing diseases over a wide range of geographical areas. 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. Previously we have shown that PAF stimulates cell differentiation of *Trypanosoma cruzi* and *Herpetomonas muscarum muscarum*, triggering a signal transduction pathway that activates a casein kinase 2 (CK2), via protein cinase C (PKC), in *H. m. muscarum*. In the present study, we demonstrated an increase of cell proliferation and changes in the protein phosphorylation profile promoted by PAF in *Phytomonas serpens* and *Leptomonas wallacei*. The parasites were grown in the absence or in the presence of $10^{-}6$ M PAF and $10^{-}9$ M PAF and the cell growth was determined by counting the number of parasites in a Neubauer chamber. PAF promoted a reduction in the generation time of *Leptomonas wallacei* and *Phytomonas serpens* in a concentration-dependent fashion. PAF also promoted an augmentation in the intensity of the overall protein phosphorylation in these parasites. These results are suggestive that PAF is a modulator of some signaling pathways that may culminate in cellular proliferation of *Phytomonas serpens* and *Leptomonas wallacei*. Supported by: CNPq, CAPES, FAPERJ.

## BC40 - Platelet-activating factor (PAF) promotes an increase on peritoneal mouse macrophage infection by *Leishmania chagasi*

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In the New World, the visceral form of leishmaniasis is caused by *Leishmania chagasi*, whose symptoms are similar to those observed for the Old World species. 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. Previous study from our group showed that PAF modulates the interaction of peritoneal mouse macrophages with *Leishmania amazonensis*. PAF also stimulates cell differentiation of *Trypanosoma cruzi* and *Herpetomonas muscarum muscarum*, triggering a signal transduction pathway that activates a protein kinase CK2, in *H. m. muscarum*. PAF effects in trypanosomatids occur through membrane receptor and intracellular signaling, via protein kinase C (PKC). In this work, we describe the effects of PAF on the interaction of peritoneal mouse macrophages with *Leishmania chagasi*. Prior to the infection, the parasites and/or the macrophages were treated for four hours with PAF and/or one of the following modulators: WEB 2086 (antagonist of PAF receptor), BIS I (PKC inhibitor), TBB (CK2 inhibitor), KT 5720 and H89 (PKA inhibitors). The interaction was inhibited when the macrophages or both the parasites and the macrophages were treated with PAF, but PAF-treated parasites promoted a two-fold increase in the association indices. The antagonist of PAF receptor, WEB 2086, as well as the protein kinase inhibitors abrogated PAF effects in both systems. We also showed that PAF stimulated the nitric oxide production when the macrophages or both the parasites and the macrophages were treated with this phospholipid. Supported by: CNPq, FAPERJ, CAPES

## BC41 - Experimental infection of trypanosomatid-free *Oncopeltus fasciatus* with *Phytomonas serpens*

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*Phytomonas* species, parasites of both plants and insects, have recently begun to attract attention due to their role as agricultural parasites. The hemipteran *Oncopeltus fasciatus* is not only the natural host for *Phytomonas elmasiani*, but it is also capable of hosting different species of trypanosomatids by experimental infection. The life cycle of *Phytomonas* spp in the insect host involves the colonization of the intestinal tract, the passage to the haemolymph, and the infection of salivary glands. In the present study we observed the experimental infection of *O. fasciatus*, which was "cured"from other trypanosomatids that naturally infect its midgut, with *P. serpens*. The distribution of this parasite along the intestinal tract of the insects was monitored for a month. This study was carried out by means of light and scanning electron microscopy. In the first 24 hours we observed the presence of a great number of parasites in the first and second ventricles (V1 and V2) of the midgut and the absence of parasites in the third and fourth ventricles (V3 and V4). However, after 48 hours, it was observed the opposite, the absence of parasites

in the two first ventricles and the third and fourth were filled with flagellates. The infection of V3 and V4 was persistent for 15 days, when the parasites began to reach the hindgut. In the following days, live parasites were observed in fresh feces. FAPERJ, CNPq and CNPq/PIBIC-UFRJ. F.A. Dias is recipient of "FAPERJ Nota 10"Fellowship.

## BC42 - TRYPANOCIDAL AND CITOTOXITY STUDY OF NAPHTOQUINONES ON *TRYPANOSOMA CRUZI* AND VERO CELLS

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Naphtoquinones are compounds obtained from Bignoniaceae and Verbanaceae trees, which are known by its huge biological activities and have been studied regarding their inhibition on topoisomerases. Chagas disease is caused by an infective protozoa called *Trypanosoma cruzi*, affecting million of people in South America, but has not yet drug treatment with no collateral effects. So, the aim of the present work is to investigate the trypanocidal effect of naphtoquinones derivates on epimastigotes forms of *Trypanosoma cruzi* and investigate the citotoxic effect of these substances on Vero cells. Methods- Vero Cells- Analysis of the drug effect on the Vero cells were done as reported by Margis et al, 1989 (Anal Biochem 181:209-211). A stock solution of the substance MDVDCACLN 3 was prepared in DMSO (1.0%). The concentrations of substances used were: 12,5; 25 and 50 $\mu$M (group control was treated with DMSO 1.0%). Parasite- *Trypanosoma cruzi* was kept in liver infusion BHI-medium. The Trypanocidal Assay was done with the substances MDVCACLN 3, ACI 103, ACI 07, CT 16, and Mannich Bases. The toxic effects of these substances were investigated by counting in an optical microscopy. The concentrations used were 2,5; 12,5 and 50 $\mu$M to MDVCACLN 3 and 50 $\mu$M to the others. Results- The performance of the substances CT 16 and ACI 103 was similiar after 3 days of incubation. After 7 days of incubation, CT 16 showed higher trypanocidal effect. MDVCACLN 3 showed high trypanocidal effect after 3 days of incubation and did not show cytotoxity. Mannich Bases did not show high trypanocidal effect after 3 days. The base R 261 R showed higher Trypanocidal effect. Conclusion- CT 16, R 261 R and MDVCACLN 3 presented the most significant activity against epimastigotes forms of *Trypanosoma cruzi*. Besides that, MDVDCACLN 3 did not show cytotoxicity on Vero cells.

## BC43 - IS *Plasmodium vivax* CAPABLE OF CYTOADHESION?

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*Plasmodium vivax* is the most prevalent parasite outside the sub-Saharan Africa affecting 90 - 150 million people annually. In Brazil, it has been reported 500,000 - 600,000 malaria cases in 2004 - 2005, 80% of those caused by *P. vivax* infections. Despite the fact that several bodies of evidence have associated malaria fatalities to the ability of mature forms of *P. falciparum*-infected erythrocytes in adhering to various host receptors expressed in the surface of endothelial cells and in the placental syncytiotrophoblast; recent studies, although scanty, have focused on the growing number of cases reporting severe forms of the disease caused by *P. vivax*; leading to cerebral, pulmonary and placental complications. Nevertheless, mechanisms involved in severe P. vivax infections are still unknown and analyses of *P. vivax* adhesive properties to host endothelial receptors have been neglected. To verify the cytoadherence of *P. vivax*-infected erythrocytes (PvIE) we collected blood parasites from patients from Manaus (3.09S, 59.58W), located in an area of unstable transmission in the west Brazilian Amazon, with an occurrence of 40,000 cases and an annual parasite index of 19.7 in 2006. Then, PvIE were enriched by Percoll gradient and parasite adherence were tested in static conditions to placenta cryosections and to endothelial cells from human lung (HLEC) or *Saimiri* brain (SBEC). Preliminary data revealed that PvIE significantly adhere to endothelial cells, which are probably associated to the presence of mature forms of the parasite. Moreover, adhesion seems to be independent of age, sex and number of malaria infections, and possibly mediated by trypsin resistant antigens. Taken together, these data suggest that PvIE might also present adhesive capacities and open perspectives for a better understanding of the pathological phenomenon related to severe *P. vivax* malaria, including the discovery of novel parasite ligand(s) and host receptor(s).

## BC44 - A novel and potent antimalarial molecule active against *Plasmodium falciparum* chloroquine resistant, TAQ, mobilizes calcium ( and protons from intracellular compartments of the parasite.

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TAQ, a new synthetic antimalarial molecule (Boechat et al, Patent PCT BR 2005/000049), is a hybrid between two antimalarials, mefloquine (MEFLO, a quinoline methanol) and

artesunate (ART, a trioxane from *Artemisia annua* plants). TAQ aims to replace ART+MEFLO combinations presently used in areas of chloroquine-resistant *P. falciparum* (PfCR) parasites. Although ART induces a shorter parasite clearance time than any other antimalarial, it does not clear all parasites from the infected patients. We now show that TAQ is more active in vitro against PfCR strain W2 with lower IC50 values (1.0ng/ml) than the combination ART+MEFLO tested in parallel (IC50 values of 2.2, 1.8 and 2.3ng/ml for the mass proportions 1:1, 1:2 and 2:1, respectively). TAQ is a strong candidate to treat human malaria provided it has no toxicity to the vertebrate host. The target of TAQ was studied by confocal microscopy using fluorescent probes (Fluo-4AM, for Ca2+ and BCECF-AM for H+) for PfCR trophozoites in synchronous culture of infected red blood cells with W2 clone. Dynamic images show that 1.0ng/ml of TAQ increases the cytoplasmic Ca2+, regardless of EGTA, thus it exhibits an intracellular action. In the presence of thapsigargin, an inhibitor of Ca2+-ATPase pump from the endoplasmic reticulum (ER), TAQ still increases cytoplasmatic Ca2+ suggesting another intracellular target. To investigate the action of TAQ on the digestive vacuole (DV), trophozoites were loaded with BCECF-AM that accumulates on acidic compartments of eukaryotic cells. TAQ was able to mobilize protons from the DV, altering the pH gradient. However, in the presence of Bafilomicyn A1 (1$\mu$M) an inhibitor of H+ pump from acidic compartments of eukaryotic cells, TAQ had no action on DV. In conclusion, ER and DV are intracellular targets for TAQ on PfCR, suggesting two modes of action of this new antimalarial. Financial support from CNPq, PDTIS-FIOCRUZ

## BC45 - Do *Trypanosoma cruzi* mixed infections modulate the biological behavior of infective forms from strains belonging to different phylogenetic groups?

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Recent characterization of *T. cruzi* phylogenetic lineages established two major groups. *T. cruzi* I strains that are commonly associated with the sylvatic cycle and *T. cruzi* II strains that are found mainly in patients and vectors in human dwellings. Several laboratories have confirmed the observation that *T. cruzi* infective forms from different strains display distinct infectivities towards cells and animals. It was previously established that metacyclic trypomastigotes from CL strain (*T. cruzi* II), were usually much more infective when compared to G strain (*T. cruzi* I). On the other hand, amastigotes forms from both strains showed an opposite behavior of the related metacyclic trypomastigote. Biochemical and molecular markers have been demonstrated the occurrence of mixed infection in human, vectors and reservoirs. Thus, the goal of this study is to evaluate if mixed infections with infective forms from strains belonging to different phylogenetic groups would modulate the invasiveness of the parasites in comparison with single infections. For this purpose we used G and CL strains transfected with the vectors pTREX-GFP and pTREX-DsRed respectively. Regardless, parasites harboring the transfected constructs as either episomes or stable chromosomal integrations showed high-level expression of fluorescent proteins. We are now expanding the parasites cultures in order to perform the biological assays. Financial support: CNPq, CAPES, FAPESP.

## BC46 - Fibrosis and Hypertrophy Induced by Trypanosoma cruzi in a Three-Dimensional Cardiomyocyte Culture System

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Cardiac damages caused by in vivo infection with Trypanosoma cruzi are still not fully clarified. Here we describe for the first time an in vitro model of fibrosis, hypertrophy and remodeling induced by T. cruzi in cardiomyocyte spheroids (cardiac microtissues). In this new three-dimensional system, cardiomyocytes re-aggregated when cultured in a scaffold free environment and formed cardiac spheroids that presented spontaneous contractility, with typical cardiac morphology and production of extracellular matrix components. There was a 4 to 6-fold increase in respectively area and volume of T. cruzi-infected cardiomyocytes and whole microtissues, together with a 50% reduction of the cell population. Immunofluorescence showed increased expression of fibronectin, collagen IV and laminin in the microtissues 144 hours after infection. T. cruzi infection induced an increase in cellular area and extracellular matrix components in cardiac spheroids, which contributed to an increase in total microtissue volume, making this a powerful 3D model to study cardiac tissue hypertrophy, fibrosis and remodeling in vitro.

## BC47 - Trypanosoma cruzi-associated changes in cardiac connexin43

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The correct expression of gap junction proteins (connexins) in the heart is required for proper function of the tissue, since the channels formed by these proteins directly connect neighboring cells, allowing intercellular transmission of current carrying ions and signaling second messenger molecules. Previous studies demonstrated that rat neonatal cardiac my-

ocytes infected with trypomastigotes of *Trypanosoma cruzi* (Tulahuen Strain) lost of gap junctional communication and decreased automaticity. In contrast, the infection of cultured mouse embryonic cardiac myocytes with trypomastigotes (Y strain) resulted in increased rate of spontaneous activity among infected cells. We infected cultured cardiac myocytes with the Y strain and demonstrated by immunocytochemistry that there were alterations in connexin43 (Cx43) distribution one hour post infection. At 72 hours post infection there was an absence of Cx43 staining in the membranes of infected cardiac myocytes. Western blot analysis of infected cardiac myocyte cultures revealed an approximately 68% increase in Cx43 levels at 1hpi where the phosphorylated forms were most prominent. At 72hpi Cx43 levels were 61% decreased in infected cultures. Interestingly other combinations of host cell/ *T. cruzi* stocks resulted in unaffected Cx43 protein levels during different time points of infection. Western blot analysis of heart tissue obtained from mice during the acute phase of infection revealed a reduction in levels of Cx43 protein in both ventricles (24%) and atria (28%). However, there were no significant differences between abundance of phosphorylated vs unphosphorylated isoforms. Our results demonstrate that infection with the Y strain alters Cx43 expression in vitro and in vivo. These data suggest a mechanism for the genesis of dysrrhythmias in *T. cruzi* infection.

## BC48 - Heart histopathological evaluation of Beagle dogs during experimental acute and chronic Chagas disease

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*Trypanosoma cruzi* is a hemoflagelate parasite associated with heart dysfunctions causing serious problems in Central and South America. The aim of this work was to investigate the impact of infection with different *T. cruzi* strains (Be-78, Y or ABC) on heart histopathological lesions during acute and chronic experimental infection in Beagle dogs. Heart fragments from control and infected dogs were evaluated with hematoxilin-eosin, Masson Tricromic and immunohistochemistry using a policlonal rabbit anti-*T. cruzi* antibody to detect inflammation, fibrosis and parasites, respectively. No parasites were observed during chronic phase and in the acute phase immunohistochemistry evaluation showed: (A) 101 free amastigotes and 183 nests; (B) 52 amastigotes and four nests and (C) 20 amastigotes and 53 nests in dogs infected with Be-78, Y and ABC *T. cruzi* strains, respectively. In acute phase it was observed significant increase of the inflammatory process in animals infected with the Y and ABC strains and presence of fatty tissue in animals infected with Y strain. In dogs infected with Be-78 it was observed increase of the inflammatory process only in the right atria. The presence of collagen was observed in the left atria and sept of infected animals with the Y strain and in the left ventricle, sept and tip of dogs infected with ABC strain. In the chronic phase, animals infected with Be-78 showed inflammation, the infected with Y strain fibrosis, and the ones infected with ABC strain degenerative process significantly higher than the observed in controls. Altogether, the data here pointed out strongly suggest that there was no correlation between *T. cruzi* presence in the heart during acute phase and lesions during chronic phase, and that *T. cruzi* strain is important in the course of cardiac alterations of Chagas disease in dog model. Supported by FAPEMIG, CNPq and UFOP

## BC49 - CARDIOMYOCYTE SECRETION PATTERN DEPENDS ON THE *TRYPANOSOMA CRUZI* POPULATION

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Our previous studies showed an increase in the serum levels of nitric oxide (NO) and cytokines in rats infected with *Trypanosoma cruzi*. It is largely described the preferential tropism of this protozoa to muscle cells, in particular cardiac muscle fibers. However, the role of these cells in the response to the parasite has not been established. The present study evaluates the production of NO, IL-1$\beta$ and TNF-$\alpha$ by cardiomyocytes isolated from neonatal rats and infected either with the Y strain or the CL-Brener clone of *T. cruzi*. The cultures, at the concentration of $4{,}5x10^5$ cells/well, were infected at day 5 with 3-5 trypomastigotes/cell. The supernatants were collected at 24, 48 and 72 hours post-infection for NO (Griess), IL-1$\beta$ and TNF-$\alpha$ assay (ELISA). Control culture supernatants were also analyzed and showed non-detectable levels for both cytokines and NO. The infection with the Y strain resulted in an increase in the IL-1$\beta$ and TNF-$\alpha$ only after 24 hours. Infection by the CL-Brener clone also induced production of IL-1$\beta$ and TNF-$\alpha$ after 24 hours, but at lower levels when compared to those induced by the Y strain. Infection with the CL-Brener clone induced a higher production of NO after 24 and 48 hours when compared to the Y strain. Our data shows a change in the secretion pattern of cardiomyocytes induced by the infection by *T. cruzi*, and this change depends on the population of *T. cruzi*.

## BC50 - Recruitment of inflammatory cells to the diaphragm is modified by the blockade of endothelin receptors during acute Chagas disease

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Previous studies demonstrated that blockade of $ET_A$ and $ET_B$ endothelin receptors in *Trypanosoma cruzi*-infected rats enhances the parasitemia and paratism in the diaphragm without affecting the amount of inflammatory process. However a significant decrease in the serum levels of nitric oxide derivatives was detected. These results suggested a role for endothelin in the early control of *T. cruzi* infection in rats. Now we tested the effect of bosentan, an antagonist of both $ET_A$ and $ET_B$ receptors, on inflammatory cell types in the acute infection with the Y strain of *T. cruzi* (300,000 trypomastigotes/50g; i.p.) in juvenile male rats. The treatment (100 mg/Kg/day, by gavage) started one day before the inoculation and was maintained until the sacrifice at day 13 of infection, around the parasitemic peak. The rats comprised four groups: uninfected and infected rats treated with bosentan or with the vehicle. Using immunohistochemical methods we analyzed the amount of resident ($ED2^+$) and recruited ($ED1^+$) macrophages, helper ($CD4^+$) and cytolytic ($CD8^+$) T lymphocytes, NK cells and B lymphocytes in the diaphragm. Infected rats showed an increase of all cell types analyzed, excepting $ED2^+$ macrophages and NK cells, when compared with uninfected animals. Bosentan-treated infected rats showed decreased amount of all cell types analyzed excepting NK cells that occurred in higher amount. Our results indicate that endothelin receptor blockade modifies the recruitment of inflammatory cells to the diaphragm during the acute phase of *T. cruzi* infection.

## BC51 - Charcoal use for the evaluation of intestinal motility in a murine model of Trypanosoma cruzi infection

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Chagas disease, caused by Trypanosoma cruzi, is a serious health public problem in Latin America. It is estimated that 25 to 30% of infected subjects will progress to cardiac, esophageal or colonic damage. The destruction of the autonomic enteric innervations caused by the parasite leads to dysfunction of the digestive system. The prevalence of colopathy in endemic areas is unknown because of the practical difficulty of studying in the field. The main sign and symptoms that suggest a diagnosis of mega colon are associated with faeces and gas. Constipation is also the most frequent symptom in patients who seek medical care. Denervation in the myenteric plexus is evident and presumed to be the major contributory factor to the malfunction of motility and secretory mechanisms. Our goal was evaluate the intestinal motility through charcoal ingestion in mice during acute phase of T. cruzi infection. After infection with bloodstream forms of T. cruzi, male Swiss mice stayed three hours of food deprivation. Later, aqueous suspension of charcoal in water was orally administered to the animal by gavages. Charcoal was observed on the faeces using normal light or using a microscope to help the identification of the black spots. At dpi 15, infected mice showed a significant increase in charcoal elimination time in faeces (310 41 min), as compared with uninfected (109 16 min). Our results demonstrated that the charcoal use is an ethical and efficient procedure to evaluate the intestinal motility which is altered in murine model of T. cruzi infection.

## BC52 - Interaction with host factors increases Trypanosoma cruzi infectivity in vitro and in oral infection

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In recent years, outbreaks of severe acute Chagas′ disease acquired by oral infection, leading to death in some cases, have been reported. Using the mouse model, we investigated the basis of such virulence by analyzing a *Trypanosoma cruzi* isolate, SC, from a patient with severe acute clinical symptoms, who was infected by oral route. It has previously been shown that, upon oral inoculation into mice, *T. cruzi* metacyclic trypomastigotes invade the gastric mucosal epithelium by engaging the stage-specific surface glycoprotein gp82, whereas the surface molecule gp90 functions as a down modulator of cell invasion. Here we found that, when given orally into mice, metacyclic forms of SC isolate, which express high levels of gp90, produced high parasitemias and high mortality, in sharp contrast with the reduced infectivity in vitro. Upon recovery from the mouse stomach one hour after oral inoculation, the parasites had the gp90 molecule completely degraded, and their entry into HeLa cells, as well as into Caco-2 cells, was increased. The gp82 molecule was more resistant to digestive action of the gastric juice. Host cell invasion of SC isolate metacyclic trypomastigotes augmented in the presence of gastric mucin. No alteration in infectivity was observed in *T. cruzi* strains CL and G used as references, which express gp90 molecules resistant to degradation by gastric juice. Taken together, our findings suggest that the exacerbation of *T. cruzi* infectivity, such as observed upon interaction of the SC isolate with the mouse stomach components, may be responsible for the severity of acute Chagas′ disease that has been reported in outbreaks of oral *T. cruzi* infection. Supported by FAPESP and CNPq.

## BC53 - ICP REGULATION OF *Trypanosoma brucei* CYSTEINE PEPTIDASES; INFLUENCE ON PARASITE TRAVERSAL OF THE BRAIN BARRIER.

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ICP is a chagasin family natural tight-binding inhibitor of papain-like cysteine peptidases (CPs) found in protozoa and bacteria. We have generated *Trypanosoma brucei* bloodstream form *ICP*-null mutants, $\Delta icp$, and detected a 4-fold increase in CP activity in $\Delta icp$ parasite lysates, which was restored to the levels of wild type by re-expression of the gene in the null mutant. $\Delta icp$ parasites reached higher parasitemia than wild type in infected mice, suggesting that ICP modulates parasite infectivity. Using a model of the human blood-brain barrier (BBB) consisting of human Brain Microvascular Endothelial Cells (BMEC), we have previously reported that bloodstream forms of *T. b. gambiense* cross the BBB at higher efficiency than *T. b. brucei* and that this ability may be due to differences in the levels of the cathepsin L-like CP, brucipain. We have found that $\Delta icp$ parasites traverse the BBB more efficiently than wild type, a phenotype that was reversed when parasites were pre-incubated with inhibitors to brucipain, but not with the inhibitor to cathepsin-B, CA074-Me. BBB crossing by wild type parasites was enhanced in the presence of secretion products derived from $\Delta icp$, and this effect was countered by synthetic CP inhibitors. $\Delta icp$ induced phosphorylation of Akt in BMECs and parasite traversal was impaired when cells were pre-incubated with wortmannin or with an inhibitor to MEK, but not with inhibitors of ERK1/2. In contrast, BBB permeability and parasite traversal were enhanced by BMEC pre-treatment with inhibitors to adenylate cyclase. Taken together, these data suggest that CPs of *T. b. brucei* bloodstream form are endogenously regulated by ICP and drive trans-endothelial migration in vitro by engaging PI3-kinase dependent signaling pathways.

## BC54 - Liver alterations in canine visceral leishmaniosis

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*Leishmania* species protozoa that alternately parasitize their sand fly vectors and mammalian macrophages. Parasites are deposited in the mammalian skin by infected sand flies and thereafter must interact with and overcome a variety of obstacles, including extracellular matrix and basement membrane proteins, to establish infection within macrophage. Structural and functional changes of the liver in canine disease have been studied by workers in rodent models of *Leishmania* infection, human visceral leishmaniasis, experimental and natural canine disease. We report histological alterations of hepatic tissue obtained from 30 mongrel dogs naturally infected with *Leishmania chagasi* sacrificed with 2.5% intravenous Thionembutal and T61. Liver samples were fixed in buffered formalin 10% solution. Tissue samples were dehydrated, cleared, embedded in paraffin, cut into 4-5 micrometers thick sections, and stained by Hematoxylin and Eosin. All liver fragments tissues were stained with Gomori$^s$ Ammoniacal Silver. The streptavidin-biotin immunohistochemical method was carried out for *Leishmania* detection. Liver sections stained with Gomori$^s$ Ammoniacal Silver were analyzed morphometrically to characterize the intralobular collagen deposition, excluding perivascular collagen.These analyses were carried out using an Axiolab light microscope (Zeiss) with $440x$ resolution. The images were transferred to a computer video screen by means of the software KS300 and relayed to a computer-assisted image analysis system (Kontron Elektronic/Carl Zeiss, Germany). The mainly histological alterations were the sinusoidal congestion (18/30), vacuolar hepatocyte degeneration (17/30), chronic portal inflammatory reaction (12/30), intralobular granulomas (11/30) and hemossiderin deposition (9/30). Symptomatic dogs showed higher parasitism, than asymptomatic animals ($p<0,05$). Infected dogs showed higher collagen deposition, than in liver samples non-infected animals ($p<0,001$). Symptomatic and asymptomatic dogs showed statistical difference too ($p<0,001$). Spearman rank Correlation among load parasite and collagen deposition showed positive correlation ($r=0,7671$). Besides, serum biochemistry revealed increase alkaline phosfatase, mainly, in symptomatic dogs. Our results indicate that *Leishmania chagasi* can induce serious liver injuries.

## BC55 - IMMUNOPATHOLOGICAL FEATURES ASSOCIATED TO CUTANEOUS LESIONS PRODUCED BY EXPERIMENTAL INFECTION WITH *Leishmania (Viannia) lainsoni* IN THE MONKEY *Cebus apella* (PRIMATES: CEBIDAE)

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Clinical and histopathological features of the evolution of infection produced experimentally by different species of *Leishmania*-inducing American cutaneous leishmaniasis (ACL) in the monkey *Cebus apella* (Primates: Cebidae) confirm its susceptibility to *Leishmania* infection and support previous indications that this monkey may be useful as experimental model for studies of ACL.

In order to characterize the immunopathological features associated to the evolution of experimental infection of *C. apella* with *Leishmania (V.) lainsoni*, biopsies of cutaneous lesions were sequentially collected at 30, 60 and 90 days post-infection (PI) from *C. apella* inoculated intradermally with 3 x $10^6$ promastigotes of *L. (V.) lainsoni*. The skin sections were stainned by H&E 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 inflammatory infiltrate in the dermis formed by parasitized macrophages, lymphocytes and plasma cells. The inflammatory process increased with the time of infection. The immunohistochemistry showed moderate presence of parasites in the lesions at $30^{th}$ and $60^{th}$ day decreasing at $90^{th}$ day PI; activation of macrophages characterized by positive reaction for lysozyme was moderate at $30^{th}$ and $60^{th}$ day and discrete at $90^{th}$ day PI. The CD3+ cells were present since $30^{th}$ day PI and it was increased with the time of infection. B cells were observed from $30^{th}$ day and it was mild during the time studied.

These results suggest that the increase of cellular immunity characterized by the presence of CD3+ cells could be responsible for the activation of macrophages and decrease of the parasite burden in the cutaneous lesions of *C. apella*.

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## BC56 - MODULATION OF HOST NEUTROPHIL ELASTASE BY ECOTIN-LIKE SERINE PEPTIDASE INHIBITORS (ISP) OF *Leishmania major*: INFLUENCE ON PARASITE INFECTIVITY AND SURVIVAL.

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Bacterial ecotins are potent competitive inhibitors of Clan PA, family S1 serine peptidases such as trypsin and neutrophil elastase (NE). Three ecotin-like genes, *ISP1*, *ISP2* and *ISP3*, were identified in the genome of *Leishmania major*. However, no Clan PA serine peptidases are encoded in the *Leishmania* genome, which raises the possibility that the inhibitors modulate the activity of host enzymes. We generated *L. major ISP2/ISP3* deficient mutants ($\Delta$*isp2/3*) and analysed their interaction with the mammalian host. $\Delta$*isp2/3*mutants were internalised by mice peritoneal macrophages more efficiently than wild type, a phenotype that was reversed by the re-expression of both genes in the mutant. The invasion of macrophages by $\Delta$*isp2/3* was reduced to wild type levels upon addition of aprotinin,recombinant ISP2 (rISP2) or NE synthetic inhibitors. The same phenotype was observed using the RAW macrophage cell line,suggesting that the increased levels of *Leishmania* uptake by macrophages promoted by NE did not require previous exposure of macrophages to neutrophils. The phagocytosis of fluorescein-coupled latex beads by macrophages was enhanced in the presence of $\Delta$*isp2/3* parasites, but not of wild type or the re-expression line. Phagocytosis returned to basal levels by incubation with aprotinin or rISP2, suggesting that up-regulation of macrophage phagocytic activity by serine peptidases promotes more efficient internalisation of *Leishmania*. We observed that, after 72h, intracellular $\Delta$*isp2/3* parasites failed to survive at high numbers and rISP2 restored parasite survival in resident macrophages. The ability of the transgenic parasites to cause lesions in susceptible mice was analysed by injection of promastigotes in the footpad. We observed that lesions provoked by $\Delta$*isp2/3* developed similarly to those caused by wild type during 40 days, but were significantly reduced thereafter. Collectively, our data suggest that ISPs play an important role in the maintenance of *Leishmania* infection in the host, possibly by modulation of neutrophil elastase activity.

## BC57 - The effect of PLA2 from *Crotalus durissus collilineatus* in experimental cutaneous leishmaniasis

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**Introduction and objective**. PLA2 enzyme is important in many parasitical diseases and it is present in many cell types. The PLA2 from snake venoms are used to investigate physiopathological processes, thus their structure are similar with mammalian PLA2. The aim of this work was evaluate the role of PLA2 in experimental cutaneous leishmaniasis. **Methods**. The PLA2 from *C.d.collilineatus* was purified according Toyama et al (2003) and its sequence was acquired according Toyama et al. (2005). The PLA2 (1 to 32$\mu$g/mL) was incubated with infected macrophages with *Leishmania(Leishmania)amazonensis*, after 24h the supernatant was collected to quantification of PGE2, IL-2, IL-4, IL-10 and IFN-gamma levels. The *in vitro* infection was evaluated by ELISA *in situ* technique. BALB/c mice were infected with treated promastigote with PLA2 (32$\mu$g/mL) during 45 days, when biopsies were collected for hystopathological evaluation. **Results**. The PLA2 sequence is similar with others PLA2 from snake venoms. Interestingly, this PLA2 has an active site analogous in 80% with rat and human PLA2, suggesting similar biological activities. In the infected macrophages (*in vitro*), the PLA2 increase the severity of infection and this data corroborated with *in vivo* experiments results. The PGE2 levels was increased twice in infected macrophages treated with PLA2 and the IL-2 levels was decreased in infected macrophages treated with PLA2, the others cytokines showed no relevant levels. **Conclusion**. This results showed that PLA2 should be a factor (from host or parasite) that can worst the infection, thus was capable of increase PGE2, a suppressor factor of macrophages in leishmaniasis, moreover, there was a decrease of IL-2, a cytokine related to Th-1 response. This study shows a possible target to block the leishmaniasis progression. Supported by FAPESP/LIM-50