ROUND-TABLES

[November, 2006-11-07 - 09h00 - ROOM A]

RT01A - Tousled-like kinase regulates cell division of *Trypanosoma brucei* through interactions with aurora-B kinase and chromatin assembly factors

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Faithful genome replication and proper chromosome segregation are mandatory for retaining genomic integrity and essential for cell proliferation among eukaryotes. The Tousled-like kinases comprise an evolutionarily conserved family of proteins that are implicated in chromatin assembly, DNA replication and chromosome segregation among the metazoa. They are substrates of the chromosomal passenger Aurora-B kinase and their phosphorylated form in turn activates Aurora-B kinase. These kinases are, however, absent from the yeast. We identified two closely related Tousled-like kinase homologs (TbTLK) from *Trypanosoma brucei*, and investigated their roles in regulating cell cycle progression. Depletion of TbTLK in the procyclic form resulted in an early S-phase defect and a subsequent G2/M arrest, suggesting that TbTLK may regulate DNA replication and chromosome segregation. The TbTLK-deficient bloodstream form was arrested in G2/M phase and lost the overall cell architecture to a round shape. Autophosphorylated TbTLK migrates to the putative centrosomes during mitosis. TbTLK interacts with the Aurora-B kinase TbAUK1 *in vivo* and can be phosphorylated by TbAUK1 *in vitro*. Procyclic-form cells deficient in both TbTLK and TbAUK1 assume similar cell cycle defects like the TbTLK-deficient cells, suggesting a role of TbTLK upstream of TbAUK1. TbTLK phosphorylates the chromatin assembly factors TbAsf1A and TbAsf1B, which play a redundant role in regulating S-phase progression in trypanosome. All together, we propose that the Tousled-like kinase in trypanosome performs a dual role in regulating cell cycle progression. It controls the TbAsf1′s in regulating DNA replication in the chromatin assembly pathway and interacts with TbAUK1 in regulating chromosome segregation during mitosis. It is the first time, to our knowledge, that a functioning Tousled-like kinase is identified in a eukaryotic microorganism.

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[November, 2006-11-07 - 09h00 - ROOM A]

RT01B - Subcelular and molecular dynamics during *Trypanosoma cruzi* cell cycle

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Eukaryotic cell cycle is characterized by progression of linked events that allow segregation of identical genetic material into the progeny. In kinetoplastid flagellated protozoa this includes the duplication of the single mitochondrion containing a network of DNA known as kinetoplast, the construction of a new flagellum, which growth from a cytoplasmic basal body through the flagellar pocket compartment before emerging from the cell and, as in all other organisms, the replication of genetic material. Although not yet characterized in Trypanosomas, DNA replication is a complex event involving several proteins and enzymes to ensure the accurate and timely duplication of genetic material. Replication occurs in replication foci assembled at the nuclear matrix, to where chromosomes go in the moment of duplication.
It will be showed the morphological events and the timing of these events during the cell cycle of the epimastigote form of *T. cruzi*, which concerns replication of DNA, mitosis as well as kinetoplast and flagellum pocket division, and growth of the new flagellum. It will also be discussed the chromosome movement that occurs during *T. cruzi* cell cycle and the patterns of PCNA localization that we found in the nucleus of epimastigote forms. PCNA is considered a marker for replication factories, it is a highly conserved protein and has no know enzymatic activity, but is nevertheless a central and essential factor for DNA replication. A possible nuclei organization that direct or - together with molecular bases- help to control the duplication of genetic material in this parasite will also be commented.
These precisely coordinated cell cycle events described here may conserve the epimastigote morphology with a single nucleus, a single kinetoplast, and a single flagellum status of the interphasic cell.

[November, 2006-11-07 - 09h00 - ROOM A]

RT01C - Cyclin- related protein kinases (CRKs) and putative partners in the cell-cycle control of *Trypanosoma cruzi*

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In *Trypanosoma cruzi* two CRKs genes TcCRK1 and TcCRK3 have been cloned. TcCRK1 levels and localization do not vary during the cell cycle, being highly concentrated in the kinetoplasto, suggesting a putative function in this organelle. TzCRK3 expression and activity was present throughout three life cycle stages of the parasite. In synchronized epimastigotes with hydroxyurea, TzCRK3 activity peaked at G2/M boundary while the kinase associated to p13suc1-beads increased at the same time point remaining high until late G2/M. TzCRK3 expression was constant

during the cell cycle showing the common pattern of CDK regulation. The results allow us to postulate that CRK3 shares functional homology with CDK1, and that it has a role controlling the G2/M transition in *T. cruzi*. The activity of CDKs is regulated by multiple regulatory mechanisms. At least four distinct posttranslational mechanisms positively regulated CDKs function, including binding of cyclins, binding of p13 (CKS) proteins phosphorylation and dephosphorylation of the protein by specific kinases and phosphatases. Searching proteins that regulate the cdc2-activity, we identified Tcp12^{CKS1} a member of the CKS family in the parasite *T. cruzi*, similar to the *Leishmania* p12 (Mottram, 1996). *TcCKS1* is expressed in the three forms of *T. cruzi*. By using anti-Tcp12^{CKS1} antiserum, protein kinase activities were immunoprecipitated. The PK activity level varies depending on the stage analyzed, and thus suggesting that different stages have different CKS-CRK complexes. Immunoprecipitation and Western blot analyses demonstrated that in the epimastigote stage, p12^{CKS1} stably interacts with TcCRK1 and TcCRK3. In addition, Tcp12^{CKS1} was able to rescue the p13^{SUC1} null mutant of *S. pombe*. The functional complementation between the CKS proteins of two evolutionary distant organisms supports the role of Tcp12^{CKS1} as a key regulator in *T. cruzi* cell cycle.

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[November, 2006-11-07 - 09h00 - ROOM A]

RT01D - Searches for centromeric sequences reveal an element conferring mitotic stability in *Leishmania*

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The centromere is a specialized region of eukaryotic chromosomes, the site of kinetochore formation, spindle attachment and regulation of chromosome segregation during mitotic and meiotic cell divisions. To identify sequences which increase mitotic stability and/or act as potential centromeres in *Leishmania major*, we first generated libraries of *Leishmania* linear artificial chromosomes (LACs) bearing 30 kb inserts of randomly selected genomic DNAs. These were rendered linear, transfected in *L. major* and will behave as LACs inside parasite cell, as far as they carry sequences playing a centromeric role. Transfectants obtained with mass transfection experiments would be expected to be unstable in the absence of drug pressure. On the other hand, transfectants bearing sequences involved in accurate segregation should be more stable under the same conditions. The study of dynamics of maintenance, for the transfectants, was made simpler by the combination of two strategies: a modified limiting dilution assay, followed by an evaluation of cell growth under an ELISA reader. In total, 108 LAC-bearing transfectant clones were obtained. Recovered colonies were subsequently serially passed in liquid culture under no drug pressure for ten passages. At this time multiple subclones of each transfectant population were generated by a modified limiting dilution assay in 96-well plates. After 10 days in culture the original plate was replicated into two new ones containing the same media with or without G418. The process was repeated after another 10-day period and plates were incubated for 10 days before evaluation of the cell growth by OD reading. From this data we calculated the parameter "percent LAC maintenance" defined as the number of G418 resistant clones/total number of clones. This analysis revealed a small number of clones with high level of mitotic stability. On the second replica, more than eighty percent of the clones had lost their linear recombinant once transferred back into G418 medium. Nineteen per cent of the clones presented a higher frequency of stability and the number of cells that would still carry the linear molecule after the second replica varied between 10 and 46%. The highest percentage of maintenance of the exogenous molecule, 30 to 46%, was only observed in 6 (5.5%) of the LAC-bearing transfectants. Thus the strategy was successful in identifying sequences, which greatly improved stability of linear exogenous molecules transfected into *Leishmania*. Mapping and sequencing of one clone (cSC10), which confers the highest degree of maintenance, revealed the presence of a sequence that was found within another stable episome (cSC45), and is dispersed in the genome of *L. major*. Those stretches of sequence showing similarity to flanking regions of the *L. major* DHFRTS gene found in cSC10 were also identified by Dubessay and co-workers as potentially involved in mitotic stabilization of fragmented chromosomes of *L. major* (Dubessay et al., 2001). This element may account for the mitotic stability of the analyzed LACs.

[November, 2006-11-07 - 09h00 - ROOM B]

RT02B - Activation of TLR4 by synthetic and of TLR4 and TLR2 by native glycosylphosphatidylinositol anchors isolated from tachyzoites of Toxoplasma gondii

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Several microbial agonists have been shown to activate im-

mune responses via Toll-like receptors (TLRs). Previous results have shown that GPIs highly purified from T. gondii tachyzoites induced TNF alpha production in macrophages through the activation of the transcription factor NF-KB. The glycan cores and the lipid moieties prepared from the T. gondii GPIs were sufficient to stimulate the synthesis of TNF alpha by macrophages. However, a remaining issue concerns the mechanisms by which the T. gondii GPIs signal the host cells. We show that glycosylphosphatidylinositols (GPIs) isolated from T. gondii as well as a chemically synthesized GPI lacking the lipid moiety, activated a reporter gene in Chinese Hamster Ovary (CHO) cells expressing TLR4, but not CHO cells expressing TLR2. However, the core glycans and the lipid cleaved from the GPIs, activated both TLR4- and TLR2-expressing cells. Additionally, we measured the production of TNF alpha by peritoneal macrophages from MyD88-/-, TLR2-/-, TLR4-/- and CD14-/- mice with the C57BL/6 mice genetic background, after stimulus with T. gondii GPIs. We found that only MyD88 is absolutely needed to trigger TNF alpha production by macrophages exposed to parasite GPIs. In order to investigate the role of the GPIs in the context of infection, mice were challenged with cysts of ME49 strain of T. gondii. Again, from the above used mice, only MyD88-/- were more susceptible to death than control, when infected with T. gondii. The number of brain cysts counted after 40 days of infection in survival mice was not statistically different between control and TLR2-, TLR4-, and CD14-knockout mice. Spleen cells of infected TLR2-/- mice produced more IFN gamma than cells of control or other knockout mice in response to in vitro stimulation with T. gondii RH strain extracts enriched in GPI-linked surface proteins. Together, our results suggest that, although TLR2 and TLR4 are not essential for the host response in vivo, both receptors may participate in the host defense against T. gondii infection through their activation by the GPIs. As MyD88 is crucial for in vivo resistance to T. gondii infection, we hypothesize that other MyD88- dependent receptors, like other TLRs or even IL-18R or IL-1R, could work together with TLR2 and TLR4 to obtain an effective host response against T. gondii infection.

[November, 2006-11-07 - 09h00 - ROOM B]

RT02C - Toll-like receptor cooperation in *Trypanosoma cruzi* infection

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Activation of innate immune cells by *Trypanosoma cruzi*-derived molecules such as glycosylphosphatidylinositol (GPI) anchors and DNA induces proinflammatory cytokine production and host defense mechanisms. In the present study, we demonstrate that DNA from *T. cruzi* stimulates cytokine production by antigen presenting cells in a TLR9-dependent manner and synergizes with parasite-derived GPI anchor, a TLR2 agonist, in the induction of cytokines by macrophages. Compared to wild-type animals, *T. cruzi*-infected TLR9-/- mice displayed elevated parasitemia and decreased survival. Strikingly, infected TLR2-/-TLR9-/- mice developed a parasitemia equivalent to animals lacking MyD88, an essential signaling molecule for most TLR, but did not show the acute mortality displayed by MyD88-/- animals. The enhanced susceptibility of TLR9-/- and TLR2-/-TLR9-/- mice was associated with decreased *in vivo* IL-12/IFN-gamma responses. Our results reveal that TLR2 and TLR9 cooperate in the control of parasite replication and that TLR9 has a primary role in the MyD88-dependent induction of IL-12/IFN-gamma synthesis during infection with *T.cruzi*.

[November, 2006-11-07 - 09h00 - ROOM B]

RT02D - CO-STIMULATION AND *Trypanosoma cruzi* INFECTION

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Full activation of T cells depends on two signals: recognition of the TCR/MHC/peptide complex and engagement of co-receptors. CD28 and ICOS are stimulatory co-receptors, required for adequate immune response against most pathogens, while CTLA-4 and PD-1 act as inhibitory co-receptors, being key factors in the maintenance of peripheral tolerance. Then, they might be involved in autoimmunity and even in immune evasion. In fact, *T. cruzi* induces the expression of CTLA-4 and PD-1, and the blockade of any of them leads to increased susceptibility to *T. cruzi* infection. However, they appear to play distinct roles during *T. cruzi* infection, since blockade of CTLA-4, induces elimination of the parasite, while the blockade of PD-1 showed a conversely augmented parasitemia. PD-1 is the last-described inhibitory co-receptor, and its deficiency leads to development of several autoimmune disorders in mice, including autoantibody-mediated dilated cardiomyopathy. However, the possible involvement of PD-1 as a possible facilitator of pathogen persistence or a facilitator of cardiac pathology remains unknown. Of note, patients with Chagas′ heart disease (CHD) develop antibody against several heart components, early described as anti EVI (Endothelium, vessels and Interstitium). There is no doubt about the existence of a parallel between these facts, lending us to hypothesize that PD-1 signaling is involved in the generation of altered cardiac tissue tolerance during *T. cruzi* infection (maybe with production of antibodies against these specific cardiac components). Therefore,

we aimed to investigate and characterize the involvement of PD-1 regulatory pathway in *T. cruzi* infection. Notably, we observed increased expression of PD-1 and its ligand PD-L1 in hearts of infected mice. Additionally, when treated with blocking antibody against PD-1 and its ligands (PD-L1 and PD-L2), the mice showed a slight increased parasitemia, and myocarditis but there was not difference in mortality. We demonstrated that PD-1 signaling is involved in the control of *T. cruzi*-induce mtocarditis, although it appears to be marginal. Additional studies are required in order to establish if PD-1 signaling can constitute a therapeutic or diagnostic tool during CHD. Supported by CNPq and FAPESP

[November, 2006-11-07 - 09h00 - ROOM C]

RT03A - Elements in malaria parasite Duffy binding-like domains necessary for erythrocyte invasion

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Malaria, caused by *Plasmodium* parasites affects $>$ 500 million and kills $\sim$ 2 million people annually. An essential step in the life cycle of malaria parasites is the invasion of host erythrocytes by merozoites. This process is dependent on interactions between erythrocyte receptors and parasite ligands. Both *Plasmodium vivax* and *Plasmodium knowlesi* use the Duffy antigen as a receptor to invade human erythrocytes. Host receptor binding regions are cysteine-rich regions called Duffy-binding-like (DBL) domains. We have performed analysis of the overall three-dimensional architecture of *P. vivax* and *P. knowlesi* DBLs based on biochemical and biophysical techniques. Our experiments indicate that these domains are built of multiple subdomains and the central region is responsible for receptor recognition.

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[November, 2006-11-07 - 09h00 - ROOM C]

RT03B - HEMATOLOGICAL ASPECTS OF THE SEVERE VIVAX MALARIA IN THE WESTERN BRAZILIAN AMAZON

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Vivax malaria is the major type of malaria found in the Americas, but curiously, the less studied. Since the early 90′s, Brazil has reported more cases of *Plasmodium vivax* as opposed to *Plasmodium falciparum*. In Manaus, the capital of the state of Amazonas, vivax malaria is responsible for 80% of the total number of reported cases. In parallel to this increase in number of cases, we also observed a proportional increase in the number of hospitalizations due to vivax malaria. Despite of a low fatality rate, vivax malaria has killed more people than *P. falciparum*, at least in the Amazonas state, from 1998 to 2002. The clinical presentation of vivax malaria was considered to be benign so far, but the increase of infections due to this parasite, in the Brazilian Amazon, seems to be responsible for a wider clinical spectrum. Four-hundred twenty-six patients were followed in Manaus, between 1998 and 2000, and a high percentage of clinical complications was identified, the most frequent being anemia and thrombocytopenia. One patient died with disseminated intravascular coagulation, which seems to be rare in the literature, even for *P. falciparum* infections (Alecrim, 2000). Recently a case of severe rhabdomyolysis induced by P. vivax was observed. Applying the criteria for severe malaria from WHO (2000), usually used for severe falciparum malaria, from 2001 to 2003 we have detected 43 patients with severe vivax malaria (12.8% of 336 patients hospitalized with vivax malaria in our institution). The major complications were hyperbilirubinemia, spontaneous bleeding, severe anemia, acute renal failure, lung edema and shock. Severe thrombocytopenia, non-immune status and low age are associated with these severe cases of vivax malaria (Alexandre, 2004).
In a retrospective analysis of autopsies performed in a tertiary care health center in Manaus, from 1995 to 2005, of 659 autopsies performed, 6 were done in falciparum malaria patients and 16 in vivax malaria patients. The major findings in these patients with vivax malaria were lung edema and renal failure, similar to the findings of falciparum patients. Similar cases have also been reported in Southeast Asia (Kochar, 2005). Usually these cases are confirmed by PCR, in order to rule out the mixed infections.
Most of the clinical complications related to vivax malaria are however hematological complications, such as severe anemia and severe thrombocytopenia. In a study performed with 69 children with acute vivax infection, 89.9% had hemoglobin below 11.5g/dL, and most of the cases had normocytic and normocromic anemia. This anemia has no relation to the peripheral parasitemia suggesting the involvement of immunological mechanisms (Oliveira, 2004).
Thrombocytopenia is observed in 65% of the patients with vivax malaria and 19% develop severe thrombocytopenia (platelets $<$ 50.000/mm^3). So far there is no clear understanding of the precise pathogenesis of such hematological event, as well as its clinical management. One patient followed in Manaus in 2000 had the diagnosis of immune thrombocytopenic purpura (ITP) after *P. vivax* infection, which is also considered rare in the literature (Lacerda *et al.*, 2004). In our patients, there was a negative correlation between platelet count and peripheral parasitemia and the patients had normal platelet count by the end of the first week after the beginning of anti-malarial treatment. That points out to severe thrombocytopenia as an indirect sign of high para-

sitemia throughout the vivax infection, what is unusual since this parasite penetrates only in reticulocytes. There is a reasonable compensation by the bone marrow, because MPV increases in the same proportion of the platelet count fall. There is also a negative correlation between platelet count and circulating immune complexes (isolated from the serum by PEG). However, differently from patients with HIV-1 who develop ITP (Karpatkin, 2000), IgG isolated from these immune complexes do not bind to normal platelets, suggesting that molecular mimicry is not involved in the destruction of these platelets. The activation of platelets during the acute infection may justify the low occurrence of clinical spontaneous bleeding. Actually *in vitro* platelet aggregation tests showed that the total lysate of *P. vivax* is a potent platelet aggregator, what could also happen *in vivo*.
More attention should be devoted to the study of the pathogenesis of *P. vivax*, since its study has been neglected, probably because of its known low fatality rate.

[November, 2006-11-07 - 09h00 - ROOM C]

RT03C - Molecular basis of virulence in reticulocyte-prone non-lethal malaria

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In spite of major efforts, malaria continues to be the most devastating parasitic disease worldwide. Our group identified a subtelomeric multigene family, named *vir*, in the human malaria parasite, *Plasmodium vivax*, implicated in immune escape. Results from single-cell RT-PCR and laser confocal microscopy demonstrated that expression of vir genes and their encoding variant proteins is not clonal, excluding a role in the strict sense of antigenic variation. Moreover, the absence of the spleen does not influence the expression of variant genes in either *P. vivax* or *P. chabaudi* in experimental infections in *Aotus* monkeys or Balb/C mice, respectively. Our present working hypothesis is that *P. vivax* escapes spleen macrophage clearance by citoadhering to barrier cells in the spleen. To advance this hypothesis, we are using the lethal *P. yoelii* 17X strain, as well as the non-lethal *P. yoelii* 17XL and *P. chabaudi* AS strains in BALB-c mice to identify spleen macrophage receptors specifically activated or suppressed in experimental infections. To this end, we performed real time PCR analysis with material obtained from spleens at different times of infection. Spleen macrophage receptor expression patterns of BALB-c mice infected with *Toxoplama gondii* and *Leishmania amazonensis* are being used as controls of specificity of receptor expression. Significantly, during *P. chabaudi* AS malaria infection, no macrophage receptor of the spleen was significantly over-expressed as compared to results obtained with *P. yoelii*. Presently, we are performing global expression analysis of the spleen upon infection with the *P. yoelii* lethal and non-lethal strains to get a comprehensive view of expression in these contrasting murine malaria infections. The implications of these findings with regard to the establishment of chronic infections and vaccine development in *P. vivax* will be discussed.

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[November, 2006-11-07 - 09h00 - ROOM C]

RT03D - Population Structure and Transmission Dynamics of Plasmodium vivax in Rural Amazonia

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Understanding the genetic structure of malaria parasites is essential to predict how fast some phenotypes of interest originate and spread in populations. Antigen-coding loci have revealed extensive diversity in Plasmodium vivax, but this may reflect the combined effects of population history and natural selection. Here we use the markers of choice for large-scale population genetic studies of eukaryotes, highly polymorphic microsatellites, to analyze 75 Plasmodium vivax isolates collected during cross-sectional and longitudinal surveys in an area of low malaria endemicity in Brazilian Amazonia. We compare our findings with microsatellite diversity data for a sympatric P. falciparum population and explore the temporal dynamics of genetically diverse P. vivax haplotypes. Plasmodium vivax populations are more diverse and more frequently comprise multiple-clone infections than local P. falciparum isolates, but these features paradoxically coexist with high levels of inbreeding leading to significant multilocus linkage disequilibrium. Moreover, we found high rates of microsatellite haplotype replacement during 15 months of follow-up, most likely as a result of random genetic drift and migration. We conclude that the small-area genetic diversity in P. vivax populations under low-level transmission is not severely constrained by the high rates of inbreeding, with clear evolutionary and public health implications.

[November, 2006-11-07 - 16h00 - ROOM A]

RT04A - Caspase inhibition reduces lymphocyte apoptosis and improves host immune responses to *Trypanosoma cruzi*

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Apoptosis has been described as a phenomenon correlated with the pathogenesis of experimental Chagas′ disease. CD4+ and CD8+ T lymphocytes from *Trypanosoma cruzi* infected mice are more susceptible to die by apoptosis, favoring parasite persistence. (LOPES et al., J. Immunol. 154: 744-752, 1995). Phagocytosis of apoptotic cells by *T. cruzi* infected macrophages leads to the release of anti-inflammatory mediators, suppressing the trypanocidal activity of those cells and increasing parasite replication (FREIRE- DE-LIMA et al., Nature,403:199-203, 2000). We have examined the influence of the pan caspase inhibitor (zVAD) in blocking the immunosuppressive effects of apoptosis during *T. cruzi* acute infection in mice. Treatment with zVAD inhibited the expression of the active form of caspase 3 and increased the number of viable T and B cells. Injection of zVAD in acutely *T.cruzi* infected mice resulted in reduced parasitemia and of T cell apoptosis. In addition, the inhibition of caspase activity by zVAD leads to an increase of T and B lymphocytes in spleen and lymph nodes, without affecting inflammatory infiltrate in the heart. We also found an increase of activated CD4+ and CD8+ T cells, and IFN-gama production by splenocytes stimulated with parasite antigen. Caspase inhibition increased microbicidal activity of peritoneal macrophages, which produced higher levels of nitric oxide and TNF-alfa following in vitro activation with reduced parasite loads. We also assessed the effect of zVAD in the viability of cell derived trypomastigotes forms in vitro. Our results suggest that the pro-apoptotic functions of caspases exert a negative effect in the control of infection and that inhibition of caspases functions could be used for immunomodulation in *T. cruzi* infection. Financial support: FAPERJ, CNPq, WHO, HHMI.

[November, 2006-11-07 - 16h00 - ROOM A]

RT04B - APOPTOTIC MIMICRY: MECHANISMS INVOLVED IN PS EXPOSURE BY AMASTIGOTES OF *Leishmania (L) amazonensis.*

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Amastigote forms of *Leishmania (L) amazonensis* purified from mouse lesions expose phosphatidylserine (PS) on their surface. Recognition of this molecule by host macrophages mediates parasite internalization and modulates the production of anti-inflammatory cytokines, contributing to the enhancement of amastigote infectivity. Parasites purified from susceptible BALB/c mice expose higher amounts of PS than those purified from resistant C57Bl/6 mice and the amount of exposed PS correlates with the lesion size developed by those two different mouse strains. Now we show that amastigotes purified from *in vitro* cultures of BALB/c and C57Bl/6 macrophages infected with stationary phase promastigote forms do not display quantitative differences in PS exposure, suggesting that the differences observed in amastigotes isolated from *in vivo* lesions are dependent on differential macrophage activation in the host. During promastigote to amastigote differentiation inside the parasitophorous vacuole (PV) there is an increase in PS exposure by parasite with a peak at 72-96 hours of infection. At this time no significant parasite proliferation was observed. The PS exposure by intracellular parasites correlates with an enlargement of the PV and an increase in macrophage macropinocytic activity. We have previously shown that the recognition of PS on the surface of amastigotes at the time of infection signals for macrophage macropinocytic activity, which increases infection by the parasite. Our results suggest that PS molecules on the surface of the intracellular parasite are capable of stimulating macrophages from inside the PV membrane. Macropinocytic vesicles containing Lucifer Yellow fuse with the vacuole membrane suggesting that the increased macropinocytic activity by macrophages can provide nutrients for intracellular parasites. This comprises a mechanism of interaction between *Leishmania* and host cell in which amastigote PS signaling from PV mediates acquisition of nutrients thus contributing to the establishment of the infection by this parasite. Financial support: CNPq, INCA/FAF

[November, 2006-11-07 - 16h00 - ROOM A]

RT04C - True altruism enables *Leishmania* disease development

Ger van Zandbergen, . *(University of Lubeck)*

The obligate intracellular pathogen *L. major* survives and multiplies in professional phagocytes. The evasion strategy to circumvent killing by host phagocytes and establish a productive infection is poorly understood.
Here we report that the virulent inoculum of *Leishmania* promastigotes contains a high ratio of Annexin A5-binding apoptotic parasites.

This subpopulation of parasites is characterized by a round body shape, a swollen kinetoplast, nuclear condensation and a lack of multiplication and represents dying or already dead parasites. After depleting the apoptotic parasites from a virulent population, Leishmania do not survive in phagocytes in vitro and lose their disease inducing ability in vivo. TGF-β induced by apoptotic parasites is likely to mediate the silencing of phagocytes and lead to survival of infectious Leishmania populations. The data demonstrate that apoptotic promastigotes, in an altruistic way, enable the intracellular survival of the viable parasites.

[November, 2006-11-07 - 16h00 - ROOM A]

RT04D - Phosphatidylserine exposure: apoptosis signaling or escape mechanism of protozoa parasites?

Seabra, S. H. *(Centro Universitário Estadual da Zona Oeste)*; de Souza, W. *(UFRJ/UEZO)*; DaMatta, R. A. *(Universidade estadual do Norte Fluminense)*

Phosphatidylserine (PS) exposure is a main event that indicates apoptosis. This exposure is fundamental for the Transforming Growth Factor-beta1 (TGF-β1) signaling that induced an anti-inflammatory response during phagocytosis of apoptotic cells. Some protozoan parasites expose PS, inhibit macrophage inflammatory activity by mimicking the uptake of apoptotic cells. *Leishmania spp.* are etiologic agent of Leishmanioses. These protozoan parasites are able to evade the killing activity of phagocytes and establish themselves as obligate intracellular parasites. Amastigotes of Leishmania, similar to apoptotic cells, inhibits macrophage activity by exposing PS. Exposed PS participates in amastigote internalization. Recognition of this phospholipid by macrophages induces TGF-β1 secretion and IL-10 synthesis, inhibits nitric oxide (NO) production, and increases susceptibility to intracellular leishmanial growth. Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii*. Activated macrophages control *T. gondii* growth by NO production. However, *T. gondii* active invasion inhibits NO production, allowing parasite persistence. We show that the mechanism used by *T. gondii* to inhibit NO production persisting in activated macrophages is similar to what Leishmania uses depending on PS exposure. Masking *T. gondii* PS with annexin-V, activated macrophages abolished NO production inhibition and parasite persistence. NO production inhibition depended on a TGF-β1autocrine effect confirmed by the expression of Smad 2 and 3 in infected macrophages. TGF-β1 led to inducible nitric oxide synthase degradation, actin filament depolymerization, and lack of nuclear factor-kappaB in the nucleus. All these features were reverted by TGF-β1 neutralizing antibody treatment. Thus, mimics the evasion mechanism used by Leishmania amazonensis and also the anti-inflammatory response evoked by apoptotic cells. *Trypanosoma cruzi* is the agent of Chagas diseases that infects about 16 to 18 million individuals in Latin America with 120 million people being at risk of infection. *T. cruzi* presents basically three different forms during its life cycle: amastigotes, epimastigotes and trypomastigotes. The latter initiates the infection in humans; thus, it has to deal with the innate immune system, especially macrophages. Our group has shown that trypomastigotes, from blood, supernatant of infected Vero cells, and after metacyclogenesis in chemically defined medium, is the only *T. cruzi* form that exposed PS at their surface. Thus, *T. cruzi* have the capacity to subvert the normal microbicidal system of activated macrophages involving PS exposure, which induced TGF-β1 signaling pathway causing disappearance of iNOS similar to Leishmania and *T. gondii*. In addition, Wanderley JL *et al.*, (2006) describes that a macropinocytic process correlates with the surface density of PS molecules. A similar result was obtained by our group showing reduction of the invasion capacity of *T. gondii* and fusion of the parasitophorous vacuole with lysosomes after the PS blockage by Annexin-V during the interaction with macrophages. It seams that PS exposure is a common feature of intracellular parasites that have to deal with macrophages. If this exposure is a prelude of apoptosis or only an adaptation for survives during interaction with macrophages is still an open question that is being addressed.
Supported by: CNPq, FAPERJ, PRONEX

[November, 2006-11-07 - 16h00 - ROOM B]

RT05A - Role of intracellular trafficking modulators in *Trypanosoma cruzi* cell invasion: a comparative study between distinct infective forms

Fernandes, M.C. *(UNIFESP)*; L'Abbate, C. *(UNIFESP)*; Mortara, R.A. *(UNIFESP)*

Previous studies have shown that *Coxiella burnetii*, an intracellular bacterium that resides within acidified vacuoles with secondary lysosomal characteristics, is an effective modulator of the intracellular traffic of trypomastigote forms of *Trypanosoma cruzi*. In addition, vacuolar and cellular pH are related to fusion events that result in doubly infected phagosomes. *T. cruzi*, the etiological agent of Chagas′ disease, occurs as different strains grouped in two major phylogenetic lineages: *T. cruzi* I, associated with the sylvatic cycle, and *T. cruzi* II, linked to the human disease. In this work we compared extracellular amastigotes (EA), metacyclic trypomastigotes (MT) and tissue culture derived trypomastigotes (TCT) belonging to *T. cruzi* I or *T. cruzi* II in their ability to invade and escape from their parasitophorous vacuole (PV), in Vero cells or Vero cells harboring the bacteria. Distinct invasion patterns were observed between different infective stages and between infective forms of different strains. Studies on the transference kinetics revealed that pH modulates the intracellular traffic of each infective stage but this influence is not exclusive for each phylogenetic group. Endosomal to lysosomal sequential labeling with EEA1 and LAMP-1 of the PV formed during the entry of each infective form revealed that the phagosome maturation processes are distinct but not strain-dependent. Due to their low hemolysin and transialidase activities, MTs are retained for longer periods in LAMP-1 positive vacuoles. Our results thus suggest that

despite the contrasting invasion capabilities, parasites of distinct phylogenetic group behave in similar fashion once inside the host cell.
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[November, 2006-11-07 - 16h00 - ROOM B]

RT05B - Lysosomes: *Trypanosoma cruzi* anchors in host cells

Andrade, L. O. *(Universidade Federal de Minas Gerais)*; Andrews, N. W. *(Yale University)*

T. cruzi is capable of invading a wide variety of cell types, and the invasion process involves attachment to cell surface molecules, various cell signaling events and ultimately internalization. It was shown before that *T. cruzi* entry was dependent on lysosomal exocytosis, with the lysosomes providing the membrane for the formation of the parasitophorous vacuole. More recently, an alternative mechanism for *T. cruzi* entry was proposed, where parasites would first reside in a plasma membrane-derived vacuole. That work showed that inhibition of PI-3 kinase greatly reduces the lysosomal pathway of parasite invasion. In order to determine whether the pathway of entry would influence survival inside host cells, we infected primary mouse embryonic fibroblasts with *T. cruzi* in the absence or presence of a PI3-kinase inhibitor. In this cell type, 30 to 50% of parasites were found in lysosomal compartments shortly after internalization, indicating that the remainder intracellular parasites invaded by plasma membrane derived vacuoles. After 10 minutes all parasites were inside lysosomes. As expected, treatment with a PI-3 kinase inhibitor (wortmanin) reduced both cell infection and lysosome associated intracellular parasites right after infection. However, treated cells showed a marked reduction in the number of intracellular parasites post infection, which continued to decay until all remaining internalized parasites were inside lysosomes. Further experiments indicated that 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 appear to be essential not only for formation of the parasitophorous vacuole, but also for retaining the parasites inside host cells. Now we are searching for the mechanisms by which lysosomes function as anchors for *T. cruzi* in the host cells.

[November, 2006-11-07 - 16h00 - ROOM B]

RT05C - Malaria parasite senses the environment. Searching the mechanism to couple signal transduction and parasite cell cycle.

Garcia, C.R.S *(niversidade de São Paulo Departamento de Fisiologia Instituto de Biociências)*; Bagnaresi, P. *(IB-USP-Dpto de Fisiologia)*

Malaria is the most devastating parasitic disease in humans, afflicting 300-500 million people worldwide and being responsible for 1.5-2.7 million deaths annually. The rational development of a malaria vaccine and new therapies for the disease is urgently required, but its success is highly dependent of a better understanding of molecular details of parasite cell biology. Our lab have searched for the molecular mechanisms used by malaria parasites to recognize environmental cues and modulate its cell cycle. In mammalian cells it has become clear that Ca^{2+} is a key controller of the life span of the cells, in modulating not only cell division, but also cell death. We -and other groups as well- have reported that calcium handling mechanisms and cAMP signal transduction pathways are operative in malaria parasites to survive inside the inhospitable intracellular environment of red blood cells (Gazarini, M.L., et al.. 2003.*J. Cell Biol.* 161:103-110; Beraldo, F.H., et al.. 2005. *J. Cell Biol.* 170:551-557). On the other hand, the upstream signaling pathway components, i.e. membrane receptors, of *Plasmodium* still remain unknown. The question then arises: how would the parasite sense the environment to activate or to silence its signaling machinery for the control of their growth and/or differentiation? By searching *Plasmodium* genome we have identified four serpentine receptors in malaria parasites and propose that these proteins, which are currently annotated as hypothetical, constitute novel members of the largest class of membrane receptors widespread in living organisms, the class of G protein-coupled receptors (GPCRs), more generally called serpentine or heptahelical receptors (Madeira et al, unpublished). We have also investigated rather melatonin is able to modulate the rodent malaria parasite *P.berghei* cell cycle, and have found that the hormone neither elicits an elevation of intracellular calcium concentration in these parasites or alter their cell cycle. This data strengths the hypothesis that melatonin synchronizes malaria parasites, and in *P. berghei*, the infection presents an unsynchronized behavior due to lack of response to melatonin. We have also investigated that *in vivo* alteration of parasite synchronous development, using luzindole, a melatonin receptor antagonist, increases the antimalarial activity of chloroquine. The data provide additional strong evidence that the parasite uses the cell cycle synchrony as a strategy to evade the immune system, a concept that can be explored in alternative treatments models. Finally by using real time PCR we have been able to explore the molecular machinery involved in melatonin-signal transduction pathways in *Plasmodium falciparum*. Moreover analysis of gene expression will aid us to elucidate the downstream mechanisms for cell cycle modulation.

[November, 2006-11-07 - 16h00 - ROOM B]

RT05D - TRYPANOSOMATID DRUG RESISTANCE: SOME CELLULAR FACETS

Vannier-Santos, M.A. *(CPqGM-FIOCRUZ-BA)*; Borges, V.M. *(CPqGM-FIOCRUZ-BA)*; Yong, V. *(IBCCF-UFRJ)*; Lima, A.P.C.A. *(IBCCF-UFRJ)*; Scharfstein, J. *(IBCCF-UFRJ)*

Understanding endocytic pathways and intracellular trafficking may be required for the effective design of better drug delivery systems for parasitic diseases caused by intracellular protozoa (*e.g.* Jones *et al.*, 2003). Cellular traffic may regulate drug resistance in different ways and this phenomenon relies on mechanisms distinct from p-glycoprotein activity and associated with physiological events in parasites of the genus *Leishmania* (Ponte-Sucre, 2003). We have previously shown that the leishmanicidal effects of ergosterol biosynthesis inhibitiors largely rely on the enhanced fusogenicity of membrane-bounded organelles as well as on the disrupture of such compartments (Vannier-Santos *et al.*, 1995; 1999). Both mechanisms may be brought by altered membrane physical properties, such as viscosity and lead to loss of cytoplasm compartmentation and impaired cellular trafficking. Understanding the parasite cell biology may be useful in the elucidation of drug resistance (Vannier-Santos *et al.*, 2002), as underlying mechanisms may be more complex than initially supposed. Besides p-glycoprotein activity, the multidrug resistance (MDR) phenotype may be produced by microscopically detectable alterations. The selection of MDR phenotypes with vinblastine may be associated to the rise of cytoskeletal abnormalities which may affects not only drug susceptibility but the parasite architecture (Borges *et al.*, 2005). Recently we observed that the selection of *Trypanosoma cruzi* epimastigotes resistant to cysteine proteinase inhibitors (Yong *et al.*, 2000) also leads to the development of parasites with altered deficient endocytic pathway (Lima *et al.*, submitted). Taken together these data indicate that the cellular/ultrastructural approach may optimize drug design and therefore chemotherapy strategies.
Supported by CNPq, CAPES, FINEP and FAPESB

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[November, 2006-11-07 - 16h00 - ROOM C]

RT06A - The proteolytic armament and protease inhibitors in the tick gut

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Ticks differ from other hematophagous parasites in intracellular localization of hemoglobin proteolysis. Hemoglobin digestion in ticks is a critical process for two reasons: It provides primary energy resources, and the generated hemoglobin fragments function as antimicrobial peptides. We have analyzed the peptidase spectrum in the gut of the hard tick *Ixodes ricinus*, a vector of Lyme disease and tick-borne encephalitis. The substrate/inhibitor based profiling demonstrated endo- and exopeptidases of cysteine and aspartic class in the tick gut homogenate. The screening of gut-specific cDNA by PCR amplification was performed with primers derived from the conserved regions of the detected peptidases. It resulted in identification of genes coding for (i) cysteine peptidases: asparaginyl endopeptidase designated as **IrAE** (legumain), **cathepsin B1, B2** and **L**, and **cathepsin C** (dipeptidyl peptidase I), and (ii) aspartic peptidase **cathepsin D**. The whole proteolytic machinery in the tick gut closely resembles the digestive system of *Schistosoma* blood flukes. Since the schistosomal asparaginyl endopeptidase (AE) plays a pivotal role in the hemoglobin digestion by *trans*-activation of other high-performance cysteine and aspartic peptidases (Caffrey et al., Trends Parasitol. 20: 241-8, 2004), we performed at first a detailed characterization of the IrAE in the tick gut. Biochemical characteristics of recombinant IrAE, comprising the strict preference for asparagine at P1 position, substrate/inhibitor specificity, pH optimum and stability, were fully in accord with other known AEs/legumains of the CD clan of cysteine peptidases (family C13). The recombinant IrAE pro-enzyme was capable to self-activate, *trans*-process the schistosomal cathepsin B1 and digest hemoglobin at pH 4.5, corresponding to the acidic milieu in the digestive vesicles of tick gut cells. Immunolocalization of IrAE in the tick gut sections surprisingly demonstrated that IrAE is present not only in the digestive vesicles, but is also markedly enriched extracellularly, within the perithrophic matrix. These findings provoke a tempting speculation about a possible role of IrAE in hemoglobin pre-processing prior to its uptake by digestive cells. We have also characterized two cysteine proteinase inhibitors of cystatin family in the African soft tick *Or-*

nithodoros moubata. **Cystatin-1** was expressed specifically in the tick gut whereas **cystatin-2** was also present in other tissues including salivary glands. Both cystatins were significantly down-regulated after blood-meal. In the tick gut, the cystatins were found to be associated with the residual bodies formed by aggregated heme, suggesting their possible function in the process of heme detoxification. The elevated level of cystatins in the gut containing partially or fully digested blood-meal indicates also a defense role of these inhibitors against undesired proteolytic action of cysteine peptidases released from the digestive cells to the gut contents.

[November, 2006-11-07 - 16h00 - ROOM C]

RT06B - Is the heme detoxification associated with acaracide resistance in the tick Boophilus microplus?

Lara, F.A. *(ICB-UFRJ)*

The cattle tick *Boophilus microplus* ingests 100 times its own weight in blood. The digest cells of the midgut are responsible for the endocytosis of blood components and huge amounts of heme are released during hemoglobin digestion. Heme is a pro-oxidant molecule, able to peroxide lipids, proteins and nucleic acids. Most of this heme is detoxified by accumulation into a specialized organelle, the hemosome. The main point of this work is the identification of heme transport mechanisms located in hemosome membranes.
Cerium chloride, a soluble electron dense lanthanide, is converted to insoluble deposits of cerium phosphate when in the presence of a functional ATPase. We have exposed midguts of engorged females in the second day after blood meal to cerium chloride, and verified by transmission electron microscopy ATPase activity sensitive to cyclosporine located in membranes of digest cells hemosomes.
We propose that such activity is involved in heme transport by hemosomes, since Sn-Protoporphyrin IX uptake is also cyclosporine sensitive, as observed by reversed phase HPLC analysis. Others amphipathic xenobionts such as chloroquine and amitraz were also captured by hemosomes and this transport was also modulated by cyclosporine.
The transfer of toxic amphipathic xenobionts to the hemosome interior is not only a way to remove a toxic molecule from the cytosol, but also to oxidize it. Cyclosporine is a strong modulator of glicoprotein P (PgP) related ATPases, a class of ABC-transporters. ABC-transporters are ATPases involved in resistance to multiple drugs phenotypes, which should be present in hemosome membranes, transferring amphipathic molecules such as heme and different acaricides to its interior, detoxifying them.
As conclusion, we propose that the hemosomes are involved not only in the physiologic detoxification of heme excess from the diet, but also as a mechanism of dismutation of toxic amphipathic xenobionts, being directly involved in the increase of resistance against tick drugs.

[November, 2006-11-07 - 16h00 - ROOM C]

RT06C - Boophilus microplus protease inhibitors: structural and physiological studies.

Tanaka, A.S. *(Universidade Federal de Sao Paulo)*; Sasaki, S. D. *(Universidade Federal de Sao Paulo)*; Lima, C. A. *(Universidade Federal de Sao Paulo)*; Torquato, R. J. S. *(Universidade Federal de Sao Paulo)*

The hard tick *Boophilus microplus* infests bovine cattle causing heavy losses in meat, milk and leather productions. This ectoparasite is also the vector of bovine diseases as babesioses and anaplasmosis. The tick control is still a challenge for the cattle producers because of the high costs and less tick physiology information. In attempt to contribute in the tick biochemistry and physiology knowledge several proteinase inhibitors were identified and characterized in tick *Boophilus microplus*. Among them, the BPTI-Kunitz type serine proteinase inhibitors (BmTIs) were more abundant but TIL (trypsin inhibitor like rich in cysteine residues) (BmSI) and cysteine protease inhibitor (Bmcystatin) such as cystatin were also characterized. The BmTIs were found in egg and larva development stages, they inhibited bovine trypsin, human neutrophil elastase (HNE) and human plasma kallikrein (HuPK) with apparent dissociation constant (Ki) in nM range. BmTI-A was the first molecule to be described as a BPTI-Kunitz type inhibitor in *B. microplus*, later we purified and characterized three others BPTI-Kunitz type inhibitors, BmTI-D, BmTI-2 and BmTI-3. BmTI-D proved to be the best HuPK inhibitor, while BmTI-3 was more efficient for HNE with dissociation constants (Ki) of 12 and 0.5 nM, respectively. In an immunological trail using partial purified BmTIs, *Bos indicus*, Nelore breed calves, previously sensitized with BmTIs and challenged with tick larvae (20.000 larvae/animal), showed 72.8% efficacy to interfere in tick development. Due to this data, recently we expressed and characterized a three Kunitz-type domain (Bm 6) and a synthetic BmTI (BmTIsint). By using RNA interference of Bm6 gene in engorged female *B. microplus* the results showed decreasing in egg production and trypsin inhibitory activity. The BmTIsint was able to produce immunological response in mice but not in bovines. More recently, we also characterized a cystatin like inhibitor from *B. microplus* fat body specific to cathepsin L; its gene expression site suggests a possible role in control of proteases involved in embryogenesis. Financial supported by FAPESP, CNPq and PRONEX-FAPERJ.

[November, 2006-11-07 - 16h00 - ROOM C]

RT06D - Chemical and structural requirements for the biological activity of the peptide derived from the a-chain of bovine hemoglobin isolated from the tick Boophilus microplus

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Peptides derived from endogenous hemoglobin play important biological roles in a variety of living systems. We first identified a hemoglobin fragment with antimicrobial activity, fragment 33-61 of bovine alpha-hemoglobin (Hb33-61), in the gut contents of the cattle tick *Boophilus microplus*. Its synthetic amidated form, Hb33-61a, turned out to be very active against some strains of Gram-positive bacteria and fungi. Recognizing that one of the key steps to study new compounds with potential pharmaceutical application is to identify the structural elements essential to express functionality, we determined its structure in the presence of SDS micelles. Knowing that a low hemolytic activity and a reduced molecular size are prized for pharmaceutical development, we also examined whether Hb33-61a was hemolytic, lethal to a *Candida albicans* strain and able to permeabilizes its plasma membrane. Also, we designed, synthesized and studied a number of fragments of Hb33-61 in their carboxyl-free and amidaded forms. The results obtained showed that Hb33-61a preserves the fold it has in the native bovine hemoglobin alpha-chain, it is lethal to *C. albicans* (6.25-50 micromolar), it permeabilizes its plasma membrane (62.5 micromolar) and, quite important, it produces hemolysis lower than 15% (up to 50 micromolar). While Hb40-61a exhibited the same MIC as Hb33-61a, Hb33-52a and Hb48-61a were considerably less active. The C-terminus amidation produced a 4-fold potentiation of the activity. While we associate such increase to a preferred structural and spatial organization of the C-terminal region favored by amidation, the data obtained showed that the most active peptides exhibit the concomitant presence of an helical structure in the C-terminus and of a beta-turn containing N-terminal region; these two structured regions, by fluctuating independently in the lipid environment, are expected to act in a synergic fashion disrupting the yeast plasma membrane.

[November, 2006-11-08 - 09h00 - ROOM A]

RT07A - The Fas-death pathway controls CD8 T cells and cytokine responses to *Trypanosoma cruzi* infection

Guillermo, L. V .C *(UFRJ)*; Silva, E. M. *(UFRJ)*; Ribeiro-Gomes, F. L. *(UFRJ)*; De Meis, J. *(UFRJ)*; Pereira, W. F. *(UFRJ)*; Yagita, H. *(Juntendo University School of Medicine)*; DosReis, G. A. *(UFRJ)* Lopes, M. F. *(UFRJ)*

T cells play a key role in the immune responses to *Trypanosoma cruzi*. T cells secrete type-1 and type-2 cytokines that regulate macrophage microbicidal activity and control resistance to *T. cruzi* infection (1). T cells die by apoptosis during *T. cruzi* infection (2), and apoptotic lymphocytes increase parasite loads in vitro and in vivo (3). We have previously shown that CD4 T cells from *T. cruzi*-infected mice undergo Fas-induced apoptosis upon activation, whereas CD8 T cells die spontaneously in vitro (2). To understand how Fas affects T cell death and cytokine production, we blocked Fas/Fas ligand (FasL) interactions with anti-FasL antibody in vitro and in vivo, during *T. cruzi* infection. Here we show that anti-FasL, but not anti-TRAIL or anti-TNF- α, blocked CD8 T-cell death and, to a lesser extend, apoptosis in activated CD4 T cells from *T. cruzi*infection. CD8 T cells rescued from apoptosis were not anergic and proliferated as well as T cells from normal mice. CD8 T cells expressed FasL and upregulated Fas expression earlier than CD4 T cells in the course of *T. cruzi* infection. In addition, a higher proportion of CD8 T cells were activated and expressed IFN-γ compared to CD4 T cells. Treatment with anti-FasL reduced parasitemia and CD8 T-cell apoptosis in vivo, and increased the recovery of CD8 T cells from spleen and peritoneum. Injection of anti-FasL also increased the recovery of B cells, CD4 and CD8 T cells from lymph nodes. Peritoneal macrophages from mice injected with anti-FasL produced nitric oxide spontaneously and controlled better endogenous infection. Splenocytes produced increased levels of IFN- γ, but also IL-4 and IL-10 in response to *T. cruzi* antigens. Treatment with anti-FasL also increased secretion of IL-4 and IL-10 by activated CD4 T cells in vitro. These results suggest that both CD8 and Th2 effector T cells die through the Fas-death pathway in the course of *T. cruzi* infection. In agreement with this conclusion, increased Th2 response was previously observed in FasL-deficient *gld* mice and correlated with a higher susceptibility to *T. cruzi* infection (4). Therefore, Fas plays a major regulatory role, by controlling CD8 T cell expansion and both Type-1 and Type-2 cytokine responses to parasite infection. Support: TDR-WHO, CNPq, FAPERJ, PRONEX, Howard Hughes Medical Institute. 1.Tarleton, R.L., Grusby, M.J., Postan, M., and Glimcher, L.H. 1996. *Trypanosoma cruzi*infection in MHC-deficient mice: further evidence for the role of both class I- and class II-restricted T cells in immune resistance and disease. Int. Immunol. 8:13-22. 2.Lopes, M.F., da Veiga, V.F., Santos, A.R., Fonseca, M.E., and DosReis, G.A. 1995. Activation-induced CD4+ T cell death by apoptosis in experimental Chagas' disease. J. Immunol. 154:744-752. 3.Freire-de-Lima, C.G., Nascimento, D.O., Soares, M.B., Bozza, P.T., Castro-Faria-Neto, H.C., de Mello, F.G., DosReis, G.A., and Lopes, M.F. 2000.

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[November, 2006-11-08 - 09h00 - ROOM A]

RT07B - Natural Regulatory T cells in skin lesions of patients with cutaneous leishmaniasis

Campanelli, A.P. *(FMRP)*; Roselino, A.M *(FMRP)*; Cavassani, K.A. *(FMRP)*; Mortara, R.A, *(UNIFESP)*; Brodskyn, C.L *(cENTRO DE PESQUISA GONÇALO MUNIZ)*; Barral-Netto, M. *(CPQGM-BA)*; Barral, A. *(CPQGM)* Silva, J.S. *(FMRP)*

The long-term persistence of pathogens in a host is a hallmark of certain infectious diseases, including tuberculosis and leishmaniasis. The ability of pathogens to establish latency in immune individuals often has severe consequences for disease reactivation. In experimental leishmaniasis, the equilibrium established between effectors and regulatory T cells ($CD4^+CD25^+$) in sites of infection might reflect both parasite and host survival strategies. *Leishmania(Viannia) braziliensis* is the main agent of cutaneous leishmaniasis (CL) in Brazil, and is also responsible for the more severe mucocutaneous form. Here we investigated the possible involvement of Treg in the control of the immune response in human skin lesions due to *L. (V.) braziliensis*. We show that functional Treg cells can be found in skin lesions from CL patients. Those cells express phenotypic markers of Treg such as CD25, CTLA-4, Foxp3 and GITR and are able to produce large amounts of IL-10, and TGF-β. Furthermore, $CD4^+CD25^+$ T cells purified from skin lesions of three out of five patients, at a 1:10 ratio, significantly suppressed PHA-induced T cell proliferative responses of allogeneic PBMC from normal individuals. These findings suggest that functional $CD4^+CD25^+$ T cells accumulate in sites of *Leishmania* infection in humans and can contribute to the local control of effector T cell functions and/or disease outcome.
Supported by CNPq

[November, 2006-11-08 - 09h00 - ROOM A]

RT07C - Immune responses to recombinant proteins representing the *Plasmodium vivax* merozoite surface and apical membrane proteins in human exposed to malaria: implications for vaccine development

Soares, I.S. *(Faculdade de Ciências Farmacêuticas, Universidade de São Paulo)*

Plasmodium vivax is responsible for more than 40 millions cases of malaria annually and unfortunately no effective means of prevention is available. In the last years, we have studied several aspects of the immune responses against *P. vivax* merozoite antigens in individuals from malaria endemic areas of Brazil. Our study aims at the determination of highly immunogenic regions of these antigens that can be expressed as recombinant proteins and used for the development of recombinant vaccines. Overall, we found that a high frequency of individuals had immune responses to certain recombinant proteins expressing portions of the Merozoite Surface Protein (MSP)-1, MSP-3 alpha or beta, and Apical Membrane Antigen-1 (AMA-1) of *P. vivax*. The immune response to MSP-1 was mainly directed to recombinant proteins representing the 19 kDa C-terminal region (1). In the case of MSP-3 alpha and beta, recombinant proteins representing the C-terminal regions were found to be the most immunogenic during natural infections (2). In our most recent work, we evaluated the immunogenicity of recombinant proteins representing different regions of AMA-1 ectodomain. We found that recombinant proteins containing the domain II were the most immunogenic to humans (3,4). Based on these results, we are currently developing immunizations protocols in non-human primates aiming at the development of high levels of immunity. Initial immunizations of *Callithrix jacchus jacchus* showed a strict dependency of the adjuvant used in the levels of antibodies after immunizations with a recombinant protein containing the $MSP1_{19}$ and two human $CD4^+$ T-cell epitopes (5). REFERENCES: 1) Rodrigues, M.H.C. et al., 2003, Malar. J. 2:39; 2) Jimenez, M.C.S. et al., 2006, this meeting; 3) Rodrigues, M.H.C. et al., 2005, Int. J. Parasitol. 35:185; 4) Múfalo, B.C. et al., 2006, this meeting ; 5- Rosa, D.S. et al., 2006, Microbes Infect, in press. Supported by FAPESP and CNPq (Instituto do Milênio-IMTEV).

[November, 2006-11-08 - 09h00 - ROOM A]

RT07D - Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses

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Resistance to several prevalent infectious diseases requires both cellular and humoral immune responses. T cell immunity is initiated by mature dendritic cells (DCs) in lymphoid organs, whereas humoral responses to most antigens require further collaboration between primed, antigen specific helper T cells and naïve or memory B cells. To determine whether antigens delivered to DCs in lymphoid organs induce T cell help for antibody responses, we targeted a carrier protein, ovalbumin (OVA), to DCs in the presence of a maturation stimulus and assayed for antibodies to a hapten, (4-hydroxy-3-nitrophenyl) acetyl (NP), after boosting with OVA-NP. A

single DC targeted immunization elicited long lived T cell helper responses to the carrier protein leading to large numbers of antibody secreting cells and high titers of high affinity anti-hapten IgGs. Small doses of DC targeted OVA induced higher titers and a broader spectrum of anti-NP antibody isotypes than large doses of OVA in alum adjuvant. Similar results were obtained when the circumsporozoite protein of *Plasmodium yoelii* was delivered to DCs. We conclude that antigen targeting to DCs combined with a maturation stimulus produces broad based and long-lived T cell help for humoral immune responses.

[November, 2006-11-08 - 09h00 - ROOM B]

RT08A - Fundamental nuclear factors complexes in *Trypanosoma cruzi*.

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Even though little is known about transcription in Trypanosomes, enough evidence has been achieved to show unique characteristics, different from those present in other eukaryotes. Due to the lack of true regulatory transcription factors, the assembly of the basal factor complexes, and its interaction with the RNA polymerases (RNPs), chromatin proteins and DNA, seems to be the major regulatory events at the transcription initiation level. Nevertheless, post-transcriptional control remains the most plausible fine tune regulatory mechanism. A number of ortologues of basal nuclear factors were detected experimentally and by data mining of the TriTryp genomes. In our laboratory, we amplified nearly twenty *T. cruzi* CDCs considered as basal factors implicated in RNAP II y RNAP III transcription. These sequences were cloned into pENTR vectors from the Gateway TM System (Invitrogen) and introduced by recombination to different destination vectors. Interactions among factors were assayed by Two-hybrid System. The most strong interaction detected was between TcTBP and TFIIB-related factor TcBFR. This interaction was mapped at the TcBRF C-terminal domain, and the first repeat of TcTBP. In contrast, TcTBP and TcTFIIB interaction probed to be weak. All currently known components of the SL-binding complex were also assayed for interaction by the same technique. TcTBP showed interaction with TcSNAP50 and TcTFIIA2. As expected, TcSNAP50 interacts with TcSNAP46. These results, with a number of low intensity interactions also detected, allowed us to propose a model for the architecture of the complex.

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[November, 2006-11-08 - 09h00 - ROOM B]

RT08B - A FORWARD GENETIC APPROACH TO STUDY THE SURFACE GLYCOCALYX OF AFRICAN TRYPANOSOME INSECT FORMS

Lee, S.Y. *(University of Glasgow, UK)*; Pena, P. *(University of Glasgow, UK)* Acosta-Serrano, A. *(University of Glasgow, UK)*

The surface of *Trypanosoma brucei* insect (procyclic) forms is covered by several millions of glycosyl phosphatidyl inositol (GPI)-anchored glycoproteins (e.g. procyclins) and free GPIs. The exact role of these molecules is unknown but evidence suggests that they may protect the parasite surface against the action of tsetse fly molecules. Structural analysis of GPIs from *in vitro* cultured procyclic parasites revealed that these molecules are substituted with an oligosaccharidic side chain that contains up to 10 N-acetyllactosamine repeats (some containing terminal sialic acid) and is unusually linked to the GPI glycan core via two beta-galactosyl residues. We have exploited the currently available forward genetic methodologies (i.e. RNAi library and mariner transposition) with the aim to identify genes and pathways involved in GPI modifications in procyclic trypanosomes. As an example, we isolated one mutant (N1-1) by mariner mutagenesis(1) followed by selection with Con A, which is completely unable to express procyclins and free GPIs on its surface. Cell-free system analyses using hypotonically lysed membranes and labeled sugar nucleotide precursors, showed that this mutant is defective in the transfer of *N*-acetylglucosamine into phosphatidyl inositol molecules, which corresponds to the first step of GPI synthesis. These partially ′naked′ parasites are unable to survive in the tsetse fly gut, suggesting that the GPI-surface coat is essential for survival and/or migration through the different compartments of the fly. The same lethal phenotype has been observed when tsetse flies were infected with GPI12$^{-/-}$ cells, which are deficient in GPI de-*N*-acetylation (second step of the GPI pathway)(2). Genetic analysis of N1-1 cells showed that three genes have been affected by mariner transposition. All three genes encode for hypothetical proteins and we are currently in the process of identifying the gene(s) responsible for the GPI phenotype in N1-1 cells. In this lecture, a summary of the results obtained after screening of the *T. brucei* RNAi library will be also presented, including the construction of a new library specifically designed for the identification of genes involved in the synthesis of trypanosome surface glycoconjugates.

(1)Leal, S, Acosta-Serrano, A, Morris, JC and Cross GA (2004), JBC
(2)Guther, MLS, Lee, SY, Tetley, L, Acosta-Serrano, A and Ferguson MA (submitted).

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[November, 2006-11-08 - 09h00 - ROOM B]

RT08C - Expression profile analysis of the mucin-associated surface protein (MASP) gene family of Trypanosoma cruzi

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A novel large family of hypervariable proteins was identified during the activities of the T. cruzi genome project (El-Sayed et al., 2005). Because most members of this family are located downstream of TcMUC II mucins, we have named them family mucin associated surface proteins or MASPs. A total of 1,377 genes were manually annotated. This family is characterized by conserved N- and C-terminal domains that encode a signal peptide and a GPI-anchor addition site, respectively, suggesting a surface location in the parasite. MASP can be subdivided into different subfamilies based on sequence similarity of the central hypervariable and repetitive region. Our analysis indicates that MASP is expressed preferentially in the trypomastigote forms, which is consistent with the proteome profiling results reported in Atwood III et al. (2005). Also, sequencing of cDNA libraries suggests a limited set of members is expressed at a given time in the parasite population. MASP pattern of expression, surface localization and variable nature suggest MASP may participate in parasite-host interactions. We speculate MASP may be involved in host cell attachment and/or invasion or in mechanisms of immune evasion. Analysis of the MASP flanking regions revealed that its $3'$ untranslated region is highly conserved. We are currently investigating the pattern of expression in individual parasites and the mechanisms involved in the temporal and stage-specific control of MASP expression.

[November, 2006-11-08 - 09h00 - ROOM B]

RT08D - Functional Genomics analysis in *Trypanosoma cruzi*

Krieger, M.A. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Probst, C.M *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Avila, A.R. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Pavoni, D.P. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Correa, A.D. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Dallagiovana, B.M. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Sotomaior, V. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Monteiro Góes, V. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Marchini, F. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*; Ozaki, L.S. *(Virginia Commonwealth University)*; Manque, P. *(Virginia Commonwealth University)*; Buck, G.A. *(Virginia Commonwealth University)*; Goldenberg, S. *(Instituto de Biologia Molecular do Paraná-IBMP-FIOCRUZ)*

Trypanosoma cruzi is an interesting biological model for cell differentiation studies because of host and cell-types alternation during its life-cycle. The metacyclogenesis process, which occurs inside the insect host, is the differentiation of the replicating, non-infective epimastigotes into the non-replicating metacyclic trypomastigotes which are infective to the mammal host. This process represents an excellent model to investigate the mechanisms acting on differentiation, infectivity and gene expression regulation of pathogenic trypanosomatids. We evaluated the mRNA levels of *T. cruzi* throughout the differentiation process using a microarray composed of 10,500 probes complementary to about 8,000 *T. cruzi* genes. The experiments were performed using three biological replicates of six differentiation time points, from two distinct transcriptome representations (total cellular RNA and polysome-associated RNA). This resulted in the selection of 2,000 probes ($\sim$20%) differentially expressed genes (DEG). The number of DEG was significantly higher in the polysomal mRNA fraction in comparison to total mRNA fraction and comprised genes participating from distinct biological pathways such as carbohydrate and protein transport and metabolism, surface proteins, replication, transcription, translation, intracellular transport, ubiquitin-proteasome complex, cellular signaling, and chaperones. However the majority of the DEGs are currently annotated as hypothetical proteins, an observation that strengthens the importance of their biological characterization. Aiming to get clues on the biological function of these differentially expressed hypothetical genes, we are presently cloning and expressing them in order to produce recombinant proteins for antisera production. Preliminary results corroborate the differential expression data from microarray analysis. In addition, hightroughput proteomic analysis indicates a high level of correlation between the RNAs associated to polysomes and synthesized proteins. In order to evaluate patterns of co-expressed genes we are performing functional genomic analysis of distinct biological processes such as cell differentiation (complete cell cycle), drug resistance, response to environment condition changes and exogenous gene transfer.

Financial Support CNPq, Pronex NIH, FIOCRUZ.

[November, 2006-11-08 - 09h00 - ROOM C]

RT09A - Paromomycin hydrophilic formulation and azitrhomycin: possible alternatives for the treatment of cutaneous leishmaniasis

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It is largely agreed that there is an urgent need for new treatment of cutaneous leishmaniasis (CL) and that the most promising approach at present is the drug combination. Oral and topical treatments would be especially useful. The activity of a hydrophilic formulation of paromomycin for the topical treatment of CL was evaluated in animals experimentally infected by *L. (L.) amazonensis* and *L. (Viannia) braziliensis*. After development of ulcerated lesions, 32 BALB/c mice infected with *L. (L.) amazonensis* were divided into 4 groups of 8 animals: 1) PA-gel group: lesions were covered with 50 μl of 10% PA-gel, twice a day, for 24 days; 2 and 3) antimonial groups: AP5 and AP20 were given 5 or 20 mg of meglumine antimoniate per kg/day, through intramuscular injection, for 20 days; 3) PA-gel/AP5 group: topical PA-gel and pentavalent antimony, 5 mg/kg/day, for 20 days and 4) placebo group: treated with the hydrophilic solution without paromomycin. After the lesion development, hamsters infected by *L. (V.) braziliensis* were divided into 3 groups of 8 animals: 1) PA-gel group: PA-gel applied on the lesions in an amount of 1 mg/mm^2, twice a day, for 20 days; 2) AP group: meglumine antimoniate, 20 mg/kg/day for 20 days; 3) placebo group: as above. During and after treatment, the size of lesions was determined weekly using a caliper. Animals were followed for an additional period of 70 days after the end of treatment. The topical treatment activity with PA-gel in BALB/c mice infected by *Leishmania (L.) amazonensis* was higher than that observed for parenteral antimony treatment, while the efficacy of these two regimes in hamsters infected by *L. (Viannia) braziliensis* was similar (Gonçalves et al. Acta Trop 93:161-7, 2005).The potential antileishmanial activity of azithromycin against three especies of *Leishmania* from the New World was assessed using *in vitro* models. In three repeated experiments, cell-free log-phase promastigotes (1x10^6/mL) triplicate cultures of *Leishmania (Leishmania) amazonensis* and *Leishmania (Viannia) braziliensis* were grown in Schneider′s insect medium in 96-well plates. After exposure to azithromycin, 50, 100, 200, 300, 500, 750, 1000, 1250 e 1500 μg/mL and amphotericin B 0.2 μg/mL (control), for 72 hours, parasite viability was determined by the colorimetric alamar Blue^{TM} assay. Activity was observed for all species tested, with ED_{50} values of 789.3 μg/mL for *L. (L.) amazonensis* and 458.7 μg/mL for *L. (V.) braziliensis*.To evaluate drug activity upon intracellular amastigotes, tioglycolate-eliceted mouse peritoneal macrophages were harvested from Balb/c mice, infected with 4x10^6/mL stationary-phase promastigotes *L. (L.) amazonensis* (for a macrophage/parasite rate of 1:10) were cultured with RPMI 1640 medium in 24-well plates with coverslips, at 37^{o}C with 5% CO2. Infection with *L. (V.) braziliensis* was performed with amastigotes-like at the same macrophage/parasite rate and same culture conditions. After 72 hours of exposure to the different concentrations of azithromycin and 0.2 μg/mL of amphotericin B (control), the coverslips were removed, stained with Giemsa, and the number of amastigotes counted in 100 macrophages. The number of intracellular amastigotes of the species *L. (L.) amazonensis* and *L. (V.) braziliensis* were significantly decreased, with ED_{90} 64.5 μg/mL and 43.9 μg/mL, respectively, as compared to the drug-free cultures. Based on the experimental data and on a limited clinical data, a clinical study aiming to evaluate the efficacy of oral azitrhomycin and paromomycin-gel alone and in combination for the treatment of cutaneous leishmaniasis will be carried out.

Support: CNPq, Fiocruz.

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[November, 2006-11-08 - 09h00 - ROOM C]

RT09B - The efficacy of Posaconazole and Benznidazole treatment of *Trypanosoma cruzi* murine infecton is dependent of cytokines and lymphocytes

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Chagas disease affects 18 million people approximately in Latin America. Benznidazole (BZ) is the major drug used for the treatment and it presents limited effectiveness in the chronic phase of the disease or in the treatment of individuals imunossupressed. In an attempt to identify new drugs for the treatment of Chagas disease, it was observed that *Trypanosoma cruzi* needs ergosterol for its survival and growth. The parasite cannot use the cholesterol of the host and it is sensitive to the ergosterol biosynthesis inhibitors (EBIs). The bis-triazoles are a new generation of EBIs, that were active in the acute and chronic phases of the experimental Chagas disease. Among them, Posaconazole (POS) that was highly active ′in vitro′ and ′in vivo′ against *T. cruzi*. POS has become a drug of choice for clinical trials in human Chagas disease. Here we have investigated the influence of cytokines IFN-γ, IL-12 and lymphocytes LT CD4+, LT CD8+ and LB on the efficacy of POS treatment of acute experimental infections with *T. cruzi*. Mice deficient (knockout - KO) either of cytokines or lymphocytes and non deficient (normal C57Bl/6) were infected with *T. cruzi* trypomastigotes of the Y strain and treated with POS or BZ. The parasitemia, cure and mortality were evaluated. Normal mice infected with *T. cruzi* and treated with POS or BZ had no parasitemia,

100% of survival and approximately 86% of cure. IFN-γ KO mice infected with *T. cruzi* and treated with BZ controlled the parasitemia during treatment but it relapsed after the treatment ended and had 0% survival. The IFN-
#61543; KO mice treated with POS controlled better the infection after the end of treatment and had 45% survival ($p<0.05$). IL-12 KO mice infected and treated with POS or BZ had intermediary results displaying enhanced parasitemia, decreased survival (77-83%) and reduced cure rates (36-39%) when compared with normal mice. The LT CD4+ were the host immune effectors that influenced most and equally the BZ and POS efficacy. LT CD4+ KO mice treated with POS or BZ presented 0% cure and 94% mortality. The LT CD8+ mice presented considerable differences between treatments. Mice treated with POS presented 31% cure and 6% mortality, whereas those treated with BZ presented 66% and 14%, respectively. LB KO mice treated with POS presented 71% cure and 0% mortality whereas those treated with BZ presented 22% cure and 33% of mortality. Our results demonstrate that the deficiency of cytokines and lymphocytes reduces the efficacy of POS or BZ in murine experimental model and suggest that both drugs are influenced by specific components of the immunological system. Altogether the results show that POS presented equal or better activity than BZ on treatment of the acute phase of immunodeficient mice infected with *T.cruzi*.
Supported by CNPq, CAPES and FIOCRUZ.

[November, 2006-11-08 - 09h00 - ROOM C]

RT09C - New Drug Leads Against Parasitic Protozoan. The Quest in Two Fronts: Bioprospecting and High Throughput Screening.

We will discuss our recent results on the search of natural and synthetic leads for the development of new drugs for parasitic protozoan diseases. Two complimentary strategies will be presented: prospecting the Brazilian biodiversity (Bioprospecting) using crude plant and fungal extracts in cell based assays, and high throughput (HTS) screening of synthetic compounds using molecular targets. The first approach is being carried out at CPqRR in collaboration with botanists and mycologists from several groups in Brazil. The bioprospecting approach involved the manual screening of more than 3,000 crude extracts in two cell based assays: a colorimetric assay using a transgenic *Trypanosoma cruzi* strain expressing the *E. coli* beta-galactosidase gene and a MTT based assay with the amastigote-like form of *Leishmania amazonensis* as well against the enzyme trypanothione reductase. The HTS was run as part of a WHO supported training at Serono Pharmaceutical Research Institute and included the enzymes PfSUB-1 and LmCPB, a subtilisin like protein from *Plasmodium falciparum* and a cysteine protease from *Leishmania mexicana*, respectively. About 45,000 compounds were screened against the PfSub in a robotized campaign and and 13,000 were manually tested against LmCPB. As a result of this endeavor, about 20 extracts from the bioprospecting were selected for bioassay-guided fractionation as they presented reproducible activity against the parasites but low toxicity to mammalian cell lines. From the HTS, four compounds active against PfSUB-1 were selected by WHO for secondary screening. Thirty-six hits were found in the primary screen with LmCPB and will be subjected to further investigation.

[November, 2006-11-08 - 09h00 - ROOM C]

RT09D - Squalene synthase, an important enzyme of the sterol biosynthesis, as a potential target for the development of a new chemotherapy in Leishmaniasis

Rodrigues, J. C. F. *(Universidade Federal do Rio de Janeiro)*; Fonseca de Souza, A. L. *(Universidade Federal do Rio de Janeiro)*; Meyer-Fernandes, J. R. *(Universidade Federal do Rio de Janeiro)*; Atella, G. C. *(Universidade Federal do Rio de Janeiro)*; Urbina, J. A. *(Instituto Venezolano de Investigaciones Científicas)*; Gilbert, I. H. *(University of Dundee)*; de Souza, W. *(Universidade Federal do Rio de Janeiro)*

Parasites of the *Leishmania* genus require for viability and growth the *de novo* synthesis of sterols such as episterol and 5-dehydroepisterol, because they are not able to use only the sterol found in their mammalian hosts, cholesterol. Squalene synthase catalyzes the first committed step in the sterol biosynthesis and has been investigated as a potential target in the treatment of human hypercholesterolemia. Leishmaniasis affects millions of people around the world and is associated with significant levels of morbidity and mortality in endemic countries. The chemotherapeutic approaches currently employed are very unsatisfactory and there is an urgent need for safer and more efficacious anti-*Leishmania* agents. In the last years we have investigated the effect of different squalene synthase inhibitors on *Leishmania amazonensis*, some of them presenting IC50 in the range of nanomolar in both forms of the parasite. The more active compound against the extracellular promastigote form was ER-119884, with an IC50 of 1.4 nM, while for E5700 the corresponding value was 11.2 nM. Against intracellular amastigotes grown in cultured macrophages ER-119884 at 50 nM caused total growth arrest after 48h and loss of cell viability after 72h without any effect in host cells. Other quinuclidine compounds as BPQ-OH and WSP 1267 were also effective with IC50 in submicromolar range. Differential interference contrast microscopy revealed the presence of promastigotes with several flagella and altered morphology, when cultivated in presence of only 1 nM of both compounds for 24h. The possible change in the cell cycle was confirmed using fluorescence microscopy with DAPI which revealed the presence of about 20 % of the cells with several nuclei and kinetoplasts. On the other hand, ER-119884, E5700, BPQ-OH and WSP 1267 induced different changes in the ultrastructure of the parasites as demonstrated by transmission electron microscopy, probably, indicating that these quinuclidine compounds have different targets within the cells. Biochemical studies using

approaches as thin layer chromatography indicated several changes in the fatty acid and phospholipids biosynthesis after the treatment with the quinuclidine compounds confirming the presence of different mechanisms of action. In addition, we also observed interesting changes in different ectoenzymes activities in promastigote forms indicating that alterations in membrane composition can affect the function of the membrane′s enzymes. With these results, we conclude that squalene synthase is a promising target for the development of new chemotherapeutic agents for the specific treatment of Leishmaniasis. **Financial support**: FAPERJ, CAPES, CNPq, Pronex and European Commission

[November, 2006-11-08 - 09h00 - ROOM D]

RT10A - ANALYSIS ON THE CYTOSKELETON OF *ENTAMOEBA HISTOLYTICA*

Batista, E.J. *(UFPA)*; de Souza, W. *(UFRJ)*

Entamoeba histoltyica is a pathogenic protozoan that displays intense ameboid movements and phagocytic activity. Both processes depend on modulation of the cytoskeleton organization and interaction with extracellular matrix proteins such as laminin and fibronectin. New morphological approaches as high resolution scanning electron microscopy, quick-freezing, freeze-fracture and deep-etching are being using to further analyze the organization of the cytoskeleton of *Entamoeba histolytica* trophozoites. These techniques have revealed new features of the cytoskeleton organization. Filamentous structures were observed in the cytoplasm of the trophozoites. In addition, these kinds of structures were also observed inside several vacuoles.

[November, 2006-11-08 - 09h00 - ROOM D]

RT10B - The *in vitro* cytotoxicity exerted by trichomononads and free-living amoebas: modulation by the extracellular milieu

Silva Filho, F.C. *(UFRJ)*

Trichomonas vaginalis and *Tritrichomonas foeuts* are protozoa usually found inhabiting the urogenital tracts of humans and bovine cattle, respectively. By contrast, *Acanthamoeba polyphaga* and *Balamuthia mandrillaris* have been considered free-living protozoa. However, under certain circumstances each one of the here cited protozoa may exhibit both adherence and toxicity to cultured mammalian cells. Studies toward focusing the underlying molecular mechanisms concerning cytoadhesion and cytotoxicity exerted by parasitic protozoa have been mainly centered their attention to the surface chemistry of the interacting cells and protozoa. Nevertheless, data accumulated during the last 15 years have bring at light that surface tribology, mechanotransduction, and the chemistry related to the interaction medium greatly influence, if not determine, the ability of a eukaryotic cell including protozoa to display adhesion and toxicity. The possible role played by some extracellular matrix glycoproteins (fibronectin and laminin-1, mainly) usually found in human tissues and cell tensigrity during interaction of each one of trichomonads and free-living amoebas are here discussed throughout.

Acknowledgements: MCT-IMBT and PRONEX, FAPERJ, CNPq, and FUJB-UFRJ.

[November, 2006-11-08 - 09h00 - ROOM D]

RT10C - Morpho-funcional organization of *Giardia lamblia* cytoskeleton

Campanati, L. *(UFRJ)*; Labati-Terra, L. *(UFRJ)*; Bittencourt-Silvestre, J. *(UFRJ)*; de Souza, W. *(UFRJ)*

Giardia lamblia, for many years, has been primarily described as the major cause of the intestinal disease named Giardiasis. Nowadays, with the increase in the study of the parasite itself, not only of the clinical aspects of Giardiasis, and the developments observed in areas such as molecular biology, this protozoon is being placed among the better-studied organisms. Several excellent reviews have been published in the past few years, concerning *Giardia* basic biology, how protein traffic takes place, how does the secretory system is organized and also about the cytoskeleton, the object of our study. Trophozoites of *Giardia lamblia* present an exuberant cytoskeleton composed of several microtubules-based stable structures: eight flagella, the ventral disk, median body and the funis. Besides, structures of unknown composition and function are also found, such as the laminated plates, lateral crest, dense rods and the banded collars. Despite several studies have been made, the exact functions of these structures (except for the flagella) are still controversial. The ventral disk, also known as adhesive disk, is regarded as the responsible for the attachment, although some researches believe that the lateral crest takes the main role in this process due to the presence of contractile proteins. The sheets of microtubules that make up the funis are involved in parasite dislocation and body movements and the median body, most likely, works as a source of microtubules. The other aforementioned structures are associated to the flagella and hints to their functions are being given by morphological studies. With the help of membrane extraction techniques associated to high resolution scanning microscopy, immunofluorescence, negative staining and transmission electron microscopy, our group is elucidating the organization of the cytoskeleton of *Giardia* trophozoites. The same strategy is being applied to the study of the modifications of the cytoskeleton during the encystation process.

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[November, 2006-11-08 - 09h00 - ROOM D]

RT10D - *Trichomonas vaginalis* e *T. foetus*: Cell-cell interaction with bovine cells.

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Tritrichomonas foetus is an extracellular parasite of the reproductive tract in cattle. The mechanism by which *T. foetus* causes abortion in cattle is largely unknown. There are no studies of infection in the cow oviducts, almost all published papers are related to vagina infection and few articles focusing on the uterus. A working model of bovine oviduct epithelial cells was established and several experimental approaches were conducted concerning to *Tritrichomonas foetus* and *T. vaginalis* interaction. Bovine oviducts were obtained from cows at a commercial abattoir and a primary cell culture was established. Sperm cells and oocytes from bovines were obtained and submitted to *T. vaginalis* and *T. foetus* interaction. Analyzes were made by scanning and transmission electron microscopy. The results reported here demonstrate that: (1) fresh whole oviducts can be used as a good model to study parasite-host cell interaction; (2) cow oviduct epithelium has been shown to consist of two cell types: ciliated and nonciliated secretory cells, and *T. foetus* displayed great specificity for the nonciliated cells localized in the deeper oviduct folds; (3) *T. foetus* adheres as single separate cells, and maintains the flagella externalized; (3) differently from *T. vaginalis*, *T. foetus* does not change its shape during the adhesion process; (4) the process of adhesion was initially by the posterior region of the cell, through filopodia, and digitopodia, in a different way of *T. vaginalis*; (5) *T. foetus* provoked a severe damage to BOECs in very short periods of time, leaving imprints in the epithelial cells, wide intercellular spaces, and large lesions; (6) BOECs exhibited characteristics of cell death after *T. foetus* adhesion; (7) steps of BOECs retraction, blebbing and detachment was followed; (8) clumps of trichomonads around of detached BOECs are seen as final destruction of the epithelium; (9) Whole, viable, strains K and 152, kill and phagocytize bovine oviduct epithelial cells. This cell killing occurs exclusively on direct contact; (10) trophozoite adherence to the epithelium is a crucial prerequisite for producing infection; (11) trichomonas exerts a cytolethal effect extracellularly and usually precedes cells phagocytosis; (12) oocytes are destroyed by *T. foetus*; (13) sperm cells are phagocytosed by trichomonads. The consequences of these observations for trichomonads pathogenesis are discussed.