ROUND-TABLES

[November, 2007-11-06 - 09h00 - ROOM A]

RT01A - Intracellular signaling events mediated by IL-4 in CD8+ T cells

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IL-4, a member of the gamma-chain receptor cytokine family, has distinct effects on the differentiation and functional properties of CD8+ T cells. It has been shown its critical role to the development of protective responses against tumors and infections by *Leishmania* and *Plasmodium* parasites, sustaining a long living memory cell population. In CD4+ T cells, the IL-4 effect depends upon signaling through a receptor complex consisting of the IL-4Ralpha chain and the common gamma chain, resulting in a series of intracellular phosphorylation events which have been extensively studied. However, the nature of IL-4-induced signaling on CD8+ T cells has not been characterized. In this study, we demonstrate that IL-4 presents an anti-apoptotic effect in both naïve/activated and differentiated CD8+ T cells through a mechanism that involves the activation of at least two distinct intracellular signaling cascades: the Jak1/STAT6 and the insulin receptor substrate/PI-3K/protein kinase B pathways. We also found that IL-4 induces the Jak3-mediated phosphorylation and nuclear migration of STAT1, STAT3, and STAT5 in naïve/activated as well as differentiated, IFN-γ-producing CD8+ T cells. The induction of this broad signaling molecules in CD8+ T cells coincides with a transcriptional activity of suppressors of cytokine signaling genes (SOCS), which negatively control cytokine pathways of cell differentiation. In comparison to CD4+ T cells, CD8+ T cells display greatly diminished transcriptional levels of SOCS genes.

[November, 2007-11-06 - 09h00 - ROOM A]

RT01B - Towards understanding Leishmania braziliensis infection: lessons from a murine model

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Leishmania spp. cause a broad spectrum of diseases collectively known as leishmaniasis. Leishmania braziliensis is the main etiological agent of American cutaneous leishmaniasis (ACL) and mucocutaneous leishmaniasis. We have developed an experimental model of infection that closely resembles ACL caused by L. braziliensis. Using this model, we observed that BALB/c mice infected in the ear dermis with 10e5 parasites displayed parasite expansion in the ear dermis and developed of an ulcerated dermal lesion which healed spontaneously, as seen by the presence of a scar. Intracellular staining showed an up-regulation in the production of gamma interferon and in the frequency of gamma interferon-secreting CD4 and CD8 T cells. In terms of the inflammatory immune response, we observed that neutrophils were constantly recruited to the lesion site, throughout the infection period. Interestingly, when mice were depleted of neutrophils, we observed an increase in lesion size and in parasite load when compared to controls. On the contrary, animals co-inoculated with live neutrophils and L. braziliensis, displayed significantly smaller lesions, parallaled by a lower parasite load, indicating that neutrophils are essencial in the initial elimination of L. braziliensis in BALB/c mice. Using this dermal model of infection, we also observed that parasites are able to remain at draining lymph nodes, regardless of the development of an effective immune response as seen by lesion healing. We are currently investigating the role of regulatory T cells in parasite persistence and in development of concomitant immunity to reinfection. Lastly, data will also be presented on the role of an anti-saliva immune response in the development of ACL in this experimental model. Collectively, we can conclude that the dermal model of infection with L. braziliensis is able to reproduce important aspects of the natural infection and indicate that it is useful to address questions related to development of immunity against L. braziliensis.

[November, 2007-11-06 - 09h00 - ROOM A]

RT01C - The paradox of conventional Th1 cells: effectors and regulators of immunity during intracellular protozoan infections

Jankovic, D. *(NIAID, National Institutes of Health)*; Sher, A. *(NIAID, National Institutes of Health)*

Although IFN-γ secretion is essential for control of most intracellular pathogens, host survival often also depends on the expression of IL-10, a cytokine known to counteract IFN-γ effector functions. An important example of this phenomenon occurs in IL-10-deficient mice infected with protozoan parasite *Toxoplasma gondii*. We have analyzed the source of the CD4 T lymphocytes derived IL-10 that prevents *T. gondii*-induced acute mortality. Unexpectedly, IFN-γ-secreting T-bet$^+$Foxp3- Th1 cells were found to be the major producers of regulatory IL-10 in these animals. Further analysis revealed that the same IFN-γ^+IL-10$^+$ population displayed potent effector function against the parasite while paradoxically also inducing profound suppression of IL-12 production by APC. The latter finding suggests that IL-10 production by CD4$^+$ T cells can override the enhancing effect of costimulation on IL-12 synthesis. Although at any given time point only a fraction of the cells appeared to simultaneously produce IL-10 and IFN-γ, IL-10

production could be stimulated in IL-10-IFN-γ^+ cells by further activation in vitro, indicating that IFN-γ^+IL-10$^+$ population does not represent a specialized Th subset. In addition, experiments with *T. gondii*-specific IL-10$^+$IFN-γ^+ CD4 clones revealed that while IFN-γ expression is imprinted and triggered with similar kinetics regardless of the state of Th1 cell activation, IL-10 secretion is induced more rapidly from recently activated than from resting cells. Thus, IL-10 producing Th1 lymphocytes have a distinct profile of immunoregulatory properties that allows their expansion in the context of strong Ag-specific Th1 priming and serve the primary purpose of limiting collateral host damage while avoiding sustained suppression of effector function. We identified a similar population of CD4$^+$ T cells co-expressing IL-10$^+$IFN-γ^+ in mice acutely infected with *Trypanosoma cruzi* and speculate that this type of cells is present during wide variety of different infections and to be particularly prominent in those triggering extensive and highly polarized Th1 responses. Taken together, our findings indicate that IL-10 production by CD4$^+$ T lymphocytes need not involve a distinct regulatory Th population, but can be generated in Th1 cells as part of the effector response to intracellular pathogens.

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[November, 2007-11-06 - 09h00 - ROOM A]

RT01D - Mechanisms of immunodominance during CD8 T cell immune responses to *T. cruzi*.

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Interference or competition between CD8+ T cells restricted by distinct MHC-I molecules can be a powerful means to establish an immunodominant response. However, its importance during infections is still questionable. Here, we describe that following infection of mice with the human pathogen *Trypanosoma cruzi*, an immunodominant CD8+ T cell immune response is developed directed to an H-2K^b restricted epitope expressed by members of the *trans*-sialidase family of surface proteins. To determine whether this immunodominance was exerted over other non H-2K^b-restricted epitopes, we measured during infection of heterozygote mice, immune responses to three distinct epitopes, all expressed by members of the *trans*-sialidase family, recognized by H-2K^b, H-2K^k or H-2K^d-restricted CD8+ T cells. Infected heterozygote or homozygote mice displayed comparably strong immune responses to the H-2K^b-restricted immunodominant epitope. In contrast, H-2K^k or H-2K^d-restricted immune responses were significantly impaired in heterozygote infected mice when compared to homozygote ones. This interference was not dependent on the dose of parasite or the timing of infection. Also, it was not seen in heterozygote mice immunized with recombinant adenoviruses expressing *T. cruzi* antigens. Finally, we observed that the immunodominance was circumvented by concomitant infection with two *T. cruzi* strains containing distinct immunodominant epitopes, suggesting that the operating mechanism most likely involves competition of T cells for limiting APCs. This type of interference never described during infection with a human parasite may represent a sophisticated strategy to restrict priming of CD8+ T cells of distinct specificities, avoiding complete pathogen elimination by host effector cells, and thus favoring host parasitism.

[November, 2007-11-06 - 09h00 - ROOM B]

RT02A - The role of TGF-β in *Trypanosoma cruzi* cell cycle

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Transforming growth factor-β (TGF-β) is a cytokine that plays various functions in the control of *Trypanosoma cruzi* infectivity and in the progression of Chagas disease. The immunostaining of *Trypanosoma cruzi*-infected cardiomyocytes (following either in vivo or in vitro infections) for TGF-β, revealed a stronger immunoreactivity in parasites than in host cells. TGF-β immunoreactivity evolved during parasite cycle progression: intense staining in amastigotes versus very faint staining in trypomastigotes. TGF-β was present on the surface of amastigotes, in the flagellar pocket and in intraparasitic vesicles as revealed by electron microscopy. However, no ortholog TGF-β gene could be identified in the genome of *Trypanosoma cruzi* by in *silico* analysis or by extensive PCR and RT-PCR studies. Immunoreactive TGF-β was most probably taken up by the parasite from the host cell cytoplasm since such a binding and internalization process of biotinylated TGF-β could be observed in axenic amastigotes *in vitro*. These observations represent the first example of a novel mechanism by which a primitive unicellular protozoan can use host cell TGF-β to control its own intracellular cycle. In this context, the use of SB- 431542, an inhibitor of the TGF-β type I receptor (ALK5), during *T. cruzi* cycle in cardiomyocytes, demonstrated a strong reduction in the number of intracellular amastigotes per infected cells (163 versus 58 parasites in control and SB-431542 treated cells, respectively at 96h), and most importantly induced a great inhibition in the trypomastigote differentiation and release (106 versus 19 parasites in control and SB-431542 treated cells, respectively). Taken together, these data further confirm the major role of the TGF-β signaling pathway in *T. cruzi* cell cycle completion. It is now evident that TGF-

β participates directly and/or indirectly in the proliferation and differentiation steps of *T. cruzi* life cycle, inducing cellular expression of proteins or factors that are involved in the parasite biology.

[November, 2007-11-06 - 09h00 - ROOM B]

RT02B - Apoptosis-like death in Trypanosoma cruzi and effects of aromatic diamidines

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Apoptosis is one type of programmed cell death with morphological and biochemical specific features, such as phosphatidylserine exposure, cell retraction, nuclear condensation, DNA nicking, disruption of the mitochondrial membrane potential and caspase activity, among others. Although initially reported in multicellular organisms, later it has also been largely implicated in different aspects of the cell biology of single-celled organisms, including protozoan parasites. Our group has been investigating several aspects regarding the interaction of Trypanosoma cruzi and host cells, both in vivo as well as in vitro models. In these assays we investigated the molecular pathways implicated in the death of cardiomyocytes during T. cruzi invasion and found that some of them undergo apoptosis during both in vitro and in vivo infections, presenting diverse death rates and onsets related to the parasite stocks. In addition we also found that during their intracellular cycle, part of the parasites displayed apoptotic-like characteristic, presenting varied extents depending on the T. cruzi stock. In addition, we also found that apoptosis in T. cruzi can be elicited by pharmacological stimuli, as already reported by others authors and in other pathogens. Our results showed aromatic diamidines, an important class of DNA binder that display antiparasitic activity towards different parasites including T. cruzi, induce apoptosis-like death in trypomastigotes of T. cruzi and promastigotes of Leishmania (L.) amazonensis, which presented DNA fragmentation, cell retraction, decreased of the mitochondrial membrane potential and phosphatidylserine exposure. The treatment of the parasites with DB569, a phenyl-substituted analog of furamidine (DB75), a compound that induced higher parasite death rates, also lead to higher apoptotic levels as compared to the treatment with the reference drug, DB75. These results, reporting for the first time that the treatment of trypanosomatids with the aromatic diamidines induces apoptosis-like death, bring important contributions to the understanding of aromatic diamidines activity mechanisms. In resume, although some apoptotic-like features have been observed in trypanosomatids, it still remains to be clearly determined whether the apoptotic pathways operating in these microorganisms are identical or not as in metazoans. Then, additional studies are decisive to further characterize effectors and regulatory molecules involved in trypanosomatids death program, which can provide the identification of new targets for future chemotherapeutic drug development and therapeutic interventions.

[November, 2007-11-06 - 09h00 - ROOM B]

RT02C - Structural organization of the flagellum of *Trypanosoma cruzi*

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Flagellum is an intricate cytoskeletal structure conserved from bacteria and protozoans to mammals. In trypanosomes, its function has been associated with motility, cell-adhesion and, more recently, with the control of cell morphogenesis and division in *Trypanosoma cruzi*. To perform its several functions, the flagellum is exactly positioned at the cell surface and anchored to the cell cytoskeleton by mechanisms that remain poorly understood. Studies on the ultrastructural aspects of this structure are, therefore, important to further understand the functional role of this organelle in the cell biology of *T. cruzi*. In this presentation, we introduce the Atomic Force Microscopy (AFM) as a new tool to study the ultrastructure of the flagellum of epimastigote form of *T. cruzi*. Previous attempts to analyze the organization of pathogenic protozoa using AFM did not significantly contribute with new structural information. Here, AFM images of the flagellum of *T. cruzi* were compared with those obtained using Field Emission Scanning Electron Microscopy (FESEM) of critical point dried cells and Transmission Electron Microscopy (TEM) of negative stained and deep-etching replicas. AFM images of epimastigote forms showed a flagellum furrow separating the axonem from the paraflagellar rod (PFR) present from the emergence of the flagellar pocket to the tip of the flagellum. At high magnification, a row of periodically organized structures, which probably correspond to the link between the axonem, the PFR and the flagellar membrane were seen along the furrow. In the origin of the flagellum, two basal bodies were identified. Beyond the basal bodies, small periodically arranged protrusions, positioned at 400 nm from the flagellar basis were seen. This structure seems to be formed by 9 sub-structures distributed around the flagellar circumference and may correspond to the flagellar necklace. Altogether, our results demonstrate the importance of modern microscopy techniques and the potential of AFM to the ultrastructural characterization of the surface components of protozoan parasites and their cytoskeleton. Supported by: FAPERJ, FINEP, CNPq and CAPES.

[November, 2007-11-06 - 09h00 - ROOM B]

RT02D - Toll-like receptor 4 (TLR4)-dependent proinflammatory and immunomodulatory properties of the glycoinositolphospholipid (GIPL) from Trypanosoma cruzi.

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We have demonstrated recently that the glycoinositolphospholipid (GIPL) molecule from the protozoan Trypanosoma cruzi is a TLR4 agonist with proinflammatory effects. Here, we show that GIPL-induced neutrophil recruitment into the peritoneal cavity is mediated by at least two pathways: one, where IL-1β acts downstream of TNF-α, and a second, which is IL-1β- and TNFRI-independent. Moreover, NKT cells participate in this proinflammatory cascade, as in GIPL-treated CD1d(-/-) mice, TNF-α and MIP-2 levels are reduced significantly. As a consequence of this inflammatory response, spleen and lymph nodes of GIPL-treated mice have an increase in the percentage of T and B cells expressing the CD69 activation marker. Cell-transfer experiments demonstrate that T and B cell activation by GIPL is an indirect effect, which relies on the expression of TLR4 by other cell types. Moreover, although signaling through TNFRI contributes to the activation of B and $\gamma\delta(+)$ T cells, it is not required for increasing CD69 expression on $\alpha\beta(+)$ T lymphocytes. It is interesting that T cells are also functionally affected by GIPL treatment, as spleen cells from GIPL-injected mice show enhanced production of IL-4 following in vitro stimulation by anti-CD3. Together, these results contribute to the understanding of the inflammatory properties of the GIPL molecule, pointing to its potential role as a parasite-derived modulator of the immune response during T. cruzi infection.

[November, 2007-11-06 - 09h00 - ROOM C]

RT03A - Role of the ABC transporter PRP1 in pentamidine resistance in *Leishmania*

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Pentamidine is a second-line agent in the treatment of leishmaniasis whose mode of action and resistance is not well understood. In this study, we used a genetic strategy to search for *loci* able to mediate pentamidine resistance when overexpressed in *Leishmania major*. A shuttle cosmid library containing genomic DNA inserts were transfected into wild-type promastigotes and screened for transfectants resistant to pentamidine. Two cosmids identifying the same *locus* were found, which differed from other known *Leishmania* drug resistance genes. The gene involved in pentamidine resistance was mapped by deletion to an open reading frame belonging to the ABC (ATP-binding cassette) transporter family that we named Pentamidine Resistance Protein 1 (PRP1), recently classified as ABCC7 (Leprohon et al., 2006). The predicted PRP1 protein encodes 1807 amino acids with the typical dimeric structure involving transmembrane domains and the two nucleotide-binding domains. PRP1 can mediate pentamidine resistance and the resistance could be reversed by verapamil in promastigotes of *Leishmania*. PRP1 overexpressors showed cross-resistance to trivalent antimony but not to pentavalent antimony (glucantime). The role of this ABC transporter in the intracellular stage of the parasite showed that it can also mediate pentamidine resistance in axenic and intracellular amastigotes. Its cellular localization showed to be intracellular and located at the tubulovesicular element responsible in part for the exocytic and endocytic pathways. Considering its localization, PRP1 would confer resistance by sequestering pentamidine in vesicles that would be exocytosed by the cell throughout the flagellar pocket. We also generated *L. amazonensis* resistant mutants to pentamidine. After characterization of these mutants, we checked whether *PRP1* gene is involved in pentamidine resistance. Our data indicated that this ABC transporter is not associated to pentamidine resistance in these lines generated by drug pressure through mechanisms of amplification or overexpression of this gene.

[November, 2007-11-06 - 09h00 - ROOM C]

RT03B - A high-throughput cell-based assay to screen for drugs affecting the main *P. falciparum* virulence factor

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Malaria is a leading cause of death in the world and new drugs are urgently needed due to the emergence of multi-drug resistant parasites. Malaria can be caused by different species of *Plasmodium*, among which *P. falciparum* is the most deadly one. Much of the pathogenesis of *P. falciparum* malaria is due to the parasite ability of binding to several different receptors on host endothelial cells. This phenomenon, known as sequestration or cytoadhesion, is determined by the main virulence factor of the parasite, the *P. falciparum* erythrocytic membrane protein 1 (PfEMP1), and the biding phenotype depends on the sequence of the expressed PfEMP1. The cytoadhesion is a very important mechanism of immune system evasion because once bound to the endothelium, the parasites can escape spleen clearance and the subsequent destruction of infected red blood cell (iRBC). The purpose of our work is to develop a cell-

based assay to screen for drugs that block PfEMP1 assembly on the iRBC membrane by blocking either synthesis or transport to RBC surface. Parasites are pre-selected for a CSA-biding phenotype and subsequently cultivated in 384-well plates. Several images of each well are acquired in an automated, high-troughput manner using a confocal microscope platform. The images are subsequently analysed by an in house-built algorithm that concomitantly determines the parasitemia and the stage of development of the paraites, as well as detect the presence of PfEMP1 at the iRBC surface of CSAiRBCs. As a probe for PfEMP1-CSA biding phenotype, we use rhodamine-conjugated CSA. An anti-malarial drug acting on this PfEMP1 transport or assembly on the RBC surface will disable a major parasite escape mechanism and thus prevent the development of the most severe forms of malaria.

[November, 2007-11-06 - 09h00 - ROOM C]

RT03C - The contribution of field and laboratory studies for the understanding of putative resistance mechanisms to artemisinin derivatives in malaria parasites

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Malaria is the most devastating disease of mankind, claiming more than one million lives every year and taking a huge economic toll in developing countries. Malaria is both a cause and a consequence of poverty. Additionally, the human malaria parasite Plasmodium falciparum has become resistance to all drugs except artemisinin derivatives. It is widely accepted that the eventual emergence of artemisinin resistance could lead to a catastrophic scenario in terms of the destructive consequences of malaria. It is therefore extremely pertinent to direct efforts towards protecting the long-term efficacy of these compounds. In this context, we have adopted a multifactorial approach to investigate the genetic components of resistance to artemisinin and its derivatives before resistance emerges and becomes common. We have made use of the rodent malaria model Plasmodium chabaudi, in vitro cultures and field studies of P falciparum. In P. chabaudi, stable artemisinin- and artesunate-resistant parasites were generated though continuous drug exposure These parasites were used to identify the genes underlying resistance using a genome-wide approach denoted Linkage Group Selection (LGS), which analyses the uncloned progeny of a genetic cross (between resistant parasites and a genetically distinct sensitive clone) before and after treatment with artemisinin or artesunate. A genetic linkage group on chromosome 2 was selected under artemisinin and artesunate treatment. Within this locus, we identified two different mutations (both valine to phenylalanine) in a gene encoding a de-ubiquitinating enzyme (UBP-1). Each mutation maps to a critical residue in a homologous human de-ubiquitinating protein (HAUSP) structure and is predicted to give a loss of enzyme activity. We believe that the demonstration of mutations in UBP and its deep involvement in central cellular processes such as responses to oxidative damage and other stresses may encourage conceptual developments in our understanding of artemisinin resistance and its genetic basis. In the human malaria parasite we have successfully derived by means of selection, artemisinin resistant lines of P. falciparum. The multi-drug resistant, but artemisinin-sensitive clone P. falciparum Dd2 was grown in vitro in the presence of increasing concentrations of artemisinin. We compared the number of PfATPase6 and Pfmdr1 gene copies and transcript abundance in the resistant parasite, Dd2-ARTmut, and its sensitive counterpart, by Real-time quantitative PCR. We found that whilst PfATPase6 remained as a single copy in Dd2-ARTmut, the resistant parasites had gained three extra copies of the Pfmdr1 gene during the artemisinin selection procedure. These observations showed a causal effect between artemisinin resistance and a particular genetic alteration. We are undertaking a multicentric survey embracing P. falciparum endemic regions in South America (Brazil), Africa (Rwanda and São Tomé) and Southeast Asia (Thailand). Our preliminary results indicate towards interesting trends in the in vitro efficacy of artemisinin derivatives among the different areas and have highlighted region-specific patterns of mutations in the candidate resistance genes PfATPase6 and PfUBP-1.

[November, 2007-11-06 - 09h00 - ROOM C]

RT03D - Genomic and proteomic analysis for the understanding of drug resistance in *Trypanosoma cruzi*

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Here we present the genomic and proteomic analysis of *T. cruzi* populations and clones susceptible and with in vitro-induced (17LER) or *in vivo*-selected resistance (BZR) to benznidazole. Results from the *T.cruzi* BZ-resistant strains and clones were compared with their susceptible counterparts. Differentially expressed genes were selected by differential display (DD) and representation of differential expression (RDE). Using both methodologies, we identified 40 *T. cruzi* genes upregulated in *T. cruzi* populations and clones resistant to BZ. Twenty genes were highly similar to genes encoding hypothetical proteins and have orthologs

in the *Leishmania major* and/or *T. brucei* genomes. We also found 20 differentially expressed genes encoding proteins associated with drug stress (heat shock proteins), enzymes involved in antioxidant defence, transport proteins, surface proteins, and others. Confirmation of differentially expressed genes is being undertaken by determining mRNA levels, gene amplification and protein expression. For the proteomic analysis, *T .cruzi* total proteins were fractioned in bi-dimensional gel and stained. Differentially expressed proteins (spots) were determined by using a software to compare gels from *T.cruzi* BZ-susceptible and -resistant strains and clones. The proteins were considered differentially expressed when the same protein in different strains or clones presented a difference in spot intensity ≥ 2.5 fold. The spots were excised from the gel and the proteins were identified by mass spectrometry. A number of 56 differentially expressed proteins were identified: 36 (64%) in *T. cruzi* BZ- resistant populations and clones, 9 (16%) in susceptible ones and 11 (20%) in both. Database searches showed that differentially expressed spots corresponded to proteins involved in the metabolism of carbohydrates, amino acids, purine and, pirimidine, structural proteins, protein synthesis, stress response/chaperones or detoxification. Comparative analysis of genomic and proteomic results showed a weak association between the genes and the proteins differentially expressed, selected by both methodologies. Further studies are under way to investigate the involvement of those proteins with drug-resistance phenotype in *T. cruzi*.

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[November, 2007-11-06 - 16h00 - ROOM D]

RT04A - A New Frontier to Chagas Disease: Amazon Region

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Chagas disease has been known as an enzootic disease of wild animals since 1924, when Carlos Chagas confirmed that a parasite isolated from the monkey *Saimiri sciureus* (described as *Crysotrix sciureus*) in Belém do Pará, Brazil, was *Trypanosoma cruzi*. Today, this disease can be considered to be an anthropozoonosis: a disease transmitted from animals to humans when they invade sylvatic ecotopes and/or when wild triatomines invade human dwellings. In the Amazon region, Chagas infection is predominantly transmitted orally, through food contaminated by feces and urine from triatomines infected with *T. cruzi* or by urine and odoriferous glandular secretions from marsupials that are also infected by *T. cruzi*. At least 24 species of wild triatomines are known in the Amazon region, and most of them are infected with *T. cruzi*. Several foci of *Rhodnius prolixus* that have adapted to human dwellings have been found in the Colombian and Venezuelan parts of the Amazon region. *Panstrongylus geniculatus* has also been found in the State of Pará, Brazil, and *Panstrongylus herreri* has a tendency to adapt to human dwellings in the Peruvian Amazon region. *Triatoma maculate* has been found adapted to human dwellings in Roraima, Brazil, Colombia and Venezuela. *Triatoma rubrofasciata* a cosmopolitan species that is a vector for *T. lewisi* in rodents; it can also be infected by *T. cruzi* and has adapted to human dwellings in the port cities of Maranhão and Pará in Brazil and in French Guyana.

The risks of endemic Chagas disease in the Amazon region are related to: 1) uncontrolled human migration from areas that are endemic for Chagas disease to the Amazon region; 2) disorganized colonization and uncontrolled deforestation of the region by human actions; 3) presence of several species of wild mammals (marsupials, bats, rodents, edentates, carnivores and primates) that are infected with *T. cruzi*; 5) possible adaptation of sylvatic triatomines to human dwellings in that region; 6) migration of individuals infected with *T. cruzi* from areas that are endemic for Chagas disease to the Amazon region, taking with them domestic animals and triatomines that can be also infected by *T. cruzi*.

Several acute cases and outbreaks of human Chagas disease have been reported from the Amazon region. In the localities where this disease has been reported, the chronic form of this disease is considered to present low endemicity. The first two acute cases were reported by Floch and Camain (1941) from French Guyana. Shaw et al (1969) described another four acute cases in Belém, the capital of the State of Pará, in northern Brazil. Since then, more than three hundred acute cases have been reported, most of them in the States of Pará, Amapá and Amazonas, Brazil (Valente et al 1993, 1999; Pinto et al 2004, 2006). Serological surveys and cross-sectional studies carried out by Fundação Nacional de Saúde from 1975 to 1980 in different states in the Brazilian Amazon region and by Coura et al from 1971 to 2002 in the State of Amazonas showed prevalences ranging from 2.4% to 13.2% (Camargo et al 1984; Coura et al 1999, 2002). Sporadic acute cases of Chagas disease have also been reported from almost all the countries in the Amazon region, but very few chronic cases have been reported by our group from the Brazilian Amazon. (Albajar et al. 2003; Xavier et al. 2006).

Key words: Chagas disease - Trypanosoma cruzi - Amazon region. Risks of endemicity.

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[November, 2007-11-06 - 16h00 - ROOM D]

RT04B - Asymptomatic *Plasmodium* infection: clinical and epidemiological aspects.

Suárez-Mutis, M.C. *(Instituto Oswaldo Cruz (Fiocruz))*; Coura, J.R. *(Instituto Oswaldo Cruz (Fiocruz))*

Asymptomatic *Plasmodium* infections are commonly observed in holo and hyper endemic areas of sub-Saharan Africa and some regions in the Southeast Asian. In America, this phenomenon is relatively new. In the last years, several articles with more evidences about asymptomatic carriers have been published, the most part of then, from the Amazon region. Different studies in high endemic areas have shown prevalences between 4.2 and 38.5% when thick smear is used as diagnostic method and between 10.6 and 64.8% when PCR is used. In South America, the first study that showed this situation was done in Mato Grosso, Brazil, where 14 of 20 (70%) individuals with positive *Plasmodium* thick smear were asymptomatic (Andrade et al, 1995). The prevalence of asymptomatic infections detected by thick smears was 7.2% in Apiacás Garimpo MG, (Fontes, 2001), 4.1-16.9% in Portochuelo and Ji-paraná RO, (Alves et al, 2002), 21.6% in Apaporis river, Colombia, (Suárez-Mutis et al ,2000), 4.9-38.5% in the frontier between Venezuela and Brasil (Marcano et al, 2004), 1-3.6% in the Padauiri river, middle rio Negro, (Suárez-Mutis et al, 2007). The prevalence of asymptomatic *Plasmodium* infections detected by PCR was of 27.3% in Apiacás, 6.4%-64.8% in Portochuelo e Ji-Paraná, 31.3% in the Jau National Park, rio Negro (Ladeia-Andrade, 2005), 8.2%-24% in the Padauiri river, 10.6-19.2% in Vila Candeias RO (Tada et al 2007), 29.4% in Acre (Silva Nunes & Ferreira, 2007). The high proportion of asymptomatic *Plasmodium* infection occurs in individuals with 28 years old or more (Fontes 2002) or in adults (26,5 vs. 14%, Alves et al 2002). Marcano et al (2004) found a minor prevalence of malaria in individuals older than 10 years which had high frequency of asymptomatic infection (38.5%). Laserson et al (1999), Alves et al (2002), Marcano et al (2004), Ladeia-Andrade (2005) and Suárez-Mutis et al (2007) observed differences in the malaria prevalence and asymptomatic *Plasmodium* infection along the few kilometers within the researches areas. Diverse malaria situations can be observed in a small geographical space limiting the control strategies. Alves et al (2002) could not find a season pattern in the epidemiology of asymptomatic infection. Suárez-Mutis et al, in the Padauiri river, showed the existence of most cases of asymptomatic infection in the dry season (5.5 in thick smear and 28,1% in PCR) comparing with rainy season (1,2% in thick smear and 7,8% in PCR). The number of parasites in asymptomatic *Plasmodium* carriers is too low. The thick smear is not a good method for detecting the parasite in these patients because the low sensibility (Suárez-Mutis & Coura, 2007). Laserson et al showed a mean of 489 parasites/mm3 in symptomatic patients comparing with 124 parasites/mm3 in asymptomatic *P. vivax* infections. Alves et al found less than 500 parasites/mm3. The manifestations of *Plasmodium* infection is so wide ranging from asymptomatic infection until dead for severe malaria; in semi-immune patients could be found intermediate levels of disease. In a study in Acre, only 52.6% of confirmed symptomatic malaria diagnosed was associated with fever perceived as "intense"; no fever was reported in 19.1% of such episodes, although other symptoms were present. In the Padauiri river, only 33,7% of patients with symptoms referred intense malaria. Currently, the asymptomatic *Plasmodium* infection is a challenge for control programs. Alves et al showed that asymptomatic carriers, with very few parasites, can transmit the disease to human hosts. Still there are many questions and few answers.

[November, 2007-11-06 - 16h00 - ROOM D]

RT04C - Urbanization of Leishmaniasis in Brazil

Romero, G. A. S. *(Universidade de Brasília)*

Leishmaniasis are zoonotic diseases associated at least with eight *Leishmania* species in Brazil. Seven species are related to cutaneous and mucosal involvement and one species is responsible for the visceral leishmaniasis syndrome. The official numbers of reported cases to the Ministry of Health are approximately 35,000 new cases of the tegumentary form and 3,500 new cases of visceral leishmaniasis, annually. These numbers could not represent the real disease burden because underreporting is important. Recent estimates obtained through database linkages of the Mortality Information System (SIM), the Hospitalization Authorization Form System (AIH) and the National System of Notifiable Diseases (SINAN) showed that the real number of cases of visceral leishmaniasis could be twice the official ones. The geographic expansion of both disease syndromes during the last decades has followed the migratory trend of the Brazilian population to the cities and the development of urban slums with poor sanitary conditions. During the past decades bigger cities such as Belo Horizonte, São Luiz, Teresina, Campo Grande, and others were progressively affected by the expansion of visceral leishmaniasis with high lethality. The risk factors associated with the involvement of the urban scenario are poorly understood. Some studies have showed significant association between the poor sanitary conditions such as lack of sewage system and rubbish collection and higher risk of the disease. Furthermore, the association is important also for the type of dwelling, suggesting that there is a possibility of interventions not necessarily directed against the vectors or reservoirs. The visceral leishmaniasis vector, *Lutzomya longipalpis*, has been suffered adaptation to suit the modified environment, keeping the zoonose in contact with the urban population. In fact the poor sanitary conditions experienced in urban slums favor vector proliferation and generally the reservoir population is abundant in that scenario. The urbanization of zoonotic cutaneous leishmaniasis is more controversial. Some authors suggest that vector adaptation to modified environment is less notable than that observed with *Lu. longipalpis*. In fact, some vector species such as *Lu. umbratilis* has shown poor capacity to survive and proliferate

in modified spaces, causing the limitation of the expansion of cutaneous leishmaniasis due to *L. (Viannia) guyanensis* to people who invade forested areas in the Amazon basin. Other vectors such as *Lu. withmani* and *Lu. intermedia* have been found inside houses of rural modified spaces and they probably represent the best example of adaptation which could result in increased risk of transmission of *L. (V.) braziliensis* parasites to urbanized populations. The role of domestic animals as reservoirs of cutaneous leishmaniasis in Brazil has not been yet demonstrated and the evidences in neighbor countries such as Peru are controversial. In Brazil, control measures against tegumentary leishmaniasis are based on the early detection and adequate treatment of cases. No consistent measure against vectors and potential reservoirs are applied, therefore, the disease expansion is comprehensible and it depends mainly on the intensity of occupational exposition to the zoonotic cycle. The urban cases have been poorly studied and in some situations the risk of urban population is related to the proximity with forested areas in the periphery of the cities. The anthroponotic cycle of transmission of *L. infantum* among intravenous drug users reported in Europe has not been confirmed in Brazil, however the studies on *Leishmania* spp./HIV co-infection are still scarce in Brazil. It is remarkable the poor impact of the application of control measures directed against vectors and reservoirs of visceral leishmaniasis in Brazil. Studies specially designed to evaluate the impact of specific interventions are urgently needed, and the inclusion of observations in urban scenarios should be highly recommended as an essential component of the applied research in that field.

[November, 2007-11-06 - 16h00 - ROOM D]

RT04D - Clinical Evaluation of the Autonomic Nervous System as Marker of Chagas Disease Evolution

Correia,, D. *(Universidade Federal do Triângulo Mineiro)*; Junqueira JR, L.F. *(Universidade de Brasília)*; Molina, R.J. *(Universidade Federal do Triângulo Mineiro)*; Resende, L.A.P. *(Universidade Federal do Triângulo Mineiro)*; Prata,, A. *(Universidade Federal do Triângulo Mineiro)*

Chagas disease caused by the protozoan *Trypanosoma cruzi* and transmitted by the triatomid bugs is highly prevalent in almost all Latin and Central American countries affecting between 11-13 million people. Chagasic megaesophagus and megacolon usually produce typical clinical symptoms but the heart involvement through the chagasic cardiomyopathy is, by far, the most severe presentation of the disease and the main cause of death. Despite nearly a century of intense research, the pathophysiology of the disease is still not fully understood. In addition to inflammatory and degenerative lesions of the myocardial, myoenteric fibers and the cardiac conducting tissue, a remarkable involvement of the intrinsic autonomic innervation and neurotransmitters receptors is noted in a great number of cases of chronic Chagas heart and digestive disease. Evidence from studies in animal models and also in humans point out to four major pathogenetic mechanisms to explain the heart involvement in Chagas disease: cardiac disautonomia, microvascular disturbances, parasite-dependent myocardial damage and immune-mediated myocardial injury. Parasite persistence and immunological mechanisms seem to be strongly related in the myocardial aggression during the chronic phase of Chagas disease. Carlos Chagas and Eurico Vilella reported in 1922 a blunted chronotropic response to atropine in chagasic patients. From the evaluation of acute heart rate responses through different methods has been possible demonstrate variable impairment of parasympathetic and sympathetic modulation in a high number of chagasic patients with isolate or combined cardiac form without heart failure and in individuals with the digestive form alone. Time- and frequency-domain analysis of short- or long-term heart interval variability has also demonstrated autonomic dysfunction with sympathovagal imbalance in chagasics patients with indeterminate or cardiac clinical forms of the disease. Heart rate variability assessment in the clinical forms of Chagas disease has shown controversial results. Autonomic dysfunction in chagasic patients may be detected before the development of ventricular dysfunction and in all phases of the disease, even in the indeterminate and digestive forms. Concerning the indeterminate form, the findings are apparently conflicting; some authors have observed subtle or overt autonomic dysfunction while others have not. Looking for better markers of risk than a depressed left ventricular ejection fraction (LVEF$\leq$30%), several candidates have been used: frequent ventricular ectopy, nonsustained ventricular tachycardia, heart rate variability (HRV), positive late potentials and recently a new marker of autonomic imbalance called heart rate turbulence (HRT). This term is used to describe the sequence of a ventricular premature complex (VPC) with a compensatory pause, there is known to be an initial acceleration and a later deceleration of sinus rhythm. HRT is thought to be a measure of the autonomic response to perturbations of arterial blood pressure invoked by a ventricular premature complex. Some authors pointed out that HRT is blunted in patients at high risk of mortality after myocardial infarction and dilated cardiomyopathy. Concerning Chagas disease, abnormal values of heart rate turbulence indices (turbulence onset - TO, turbulence slope - TS) have been noted, but their prognostic value remains to be determined. Lately heart rate variability has been used to evaluate autonomic function in several clinical conditions such as coronary artery disease and arrhythmias. In patients with atrial fibrillation after electrical cardioversion the recurrence rate was higher in patients with impaired sympathovagal balance (LF/HF >2). In sudden syncope and syncope with latency sympathovagal modulation impairment has also been detected. Recently studies showed sympathetic and parasympathetic dysfunction in patients with moderate to severe obstructive sleep apnea, but the clinical prognostic value is still unknown.

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[November, 2007-11-06 - 16h00 - ROOM A]

RT05A - Zoonotic visceral Leishmaniasis: Immunogenicity of proteins released by Leishmania infantum promastigotes

Santos-Gomes, GM *(Instituto de Higiene e Medicina Tropical)*; Marques, C *(Instituto de Higiene e Medicina Tropical)*; Roos Rodrigues, O *(Instituto de Higiene e Medicina Tropical)*; Rosa, R. *(Instituto de Higiene e Medicina Tropical)*

Members of the genus *Leishmania* are sandfly-transmitted protozoan parasites that cause leishmaniasis. The domestic dog is the main reservoir of *Leishmania infantum*, the etiologic agent of zoonotic visceral leishmaniasis (ZVL). This parasite causes human leishmaniasis with visceral manifestations and canine leishmaniasis with visceral and cutaneous clinical signs. The advances in the development of new compounds with therapeutic activity against leishmaniasis have been insufficient and the antimonial drugs, used since the 1940s, still are the first line of drugs used to treat leishmaniasis. Although treatment failures have been associated to resistance in human population, classic therapeutics have always presented a limited efficiency in achieving sterile cure in dogs. In fact, some of the treated dogs can transmit the parasite. Therefore, chemotherapy does not seem to be sufficient to control ZVL and extensive evidences indicate that vaccination should be considered. Different approaches have been used toward the history of vaccine research, resulting vaccine candidates able to induce variable levels of protection. *Leishmania* infection is not always synonymous of disease. Whereas a large proportion of infections progress to disease, some cases remain asymptomatic. The factors responsible for the variation in the clinical outcome are not well known. Young age, malnutrition and immunodeficiency due for example to HIV co-infection predisposes to disease. An important role of genetic background has also been suggested. However the understanding of the immunological mechanisms involved in resistance/susceptibility is fundamental for the development of immunoprophylactic tools. Resistance has been associated to cellular immunity and requires the intervention of $CD4^+$ and $CD8^+$ T cells producing IFN-gamma and IL-12, inducing macrophage activation and parasite killing. Antigenic macromolecules secreted by *Leishmania* play important roles in the establishment of immunological and physiological interactions with the host. Although their role on the host immune response still needs further clarification it is known that some of these proteins contribute to parasite dispersion while others modulate host immune response. Taking this knowledge into account, it is feasible to hypothesize that an effective vaccine should mimic the parasite antigens delivered in a natural infection. The immunogenic effects of three proteic fractions released by cultured *L. infantum* promastigotes were studied. These fractions were able to modulate mice immunity, exerting different effects on splenic leukocytes depending on host genetic background and on the magnitude of parasite burden. Early during infection, fractions directed towards activation of $CD4^+$ and $CD8^+$T cells subsets, increased the production of IFN-gamma and IL-12, inducing a Th1 response with IL-12 involvement, required for the maintenance of memory cells. The immunization of mice with the proteic fractions LiRic1 (>75kDa) and LiRic2 (<37kDa) gave partial protection to *L. infantum* infection and a predominant $CD4^+$ response which leads to the activation of leishmanicidal machinery by host macrophages and the subsequent control of infection. LiRic2 induced the production of IL-12 by $CD8^+$T cells, giving evidence of the participation of this cell subset in protective immune response. The results obtained with two proteic fractions released by *L. infantum* promastigotes encourages further research on the immunogenecity of antigenic macromolecules secreted by the parasite.

[November, 2007-11-06 - 16h00 - ROOM A]

RT05B - Recombinant adenoviruses induce strong T and B cell responses and are efficient tools for prime-boost immunization against intracellular (*Plasmodium, Leishmania, Toxoplasma and Trypanosoma*) parasites.

Bruna-Romero, O *(UFMG/ICB e FIOCRUZ/IRR)*

Our main efforts during the last years have been devoted to the development of experimental recombinant tools for vaccination against intracellular pathogens. For this, we chose antigens of four parasites with intracellular stages (CS/*Plasmodium*, A2/*Leishmania*, SAG1,2,3/*Toxoplasma* and TS-ASP2/*Trypanosoma*) that had been described to induce protective immune responses. Side-by-side comparisons showed that recombinant adenoviral vectors encoding some of these antigens were the most efficient vaccines both in terms of immunogenicity and protective levels after a single immunization. However, complete protection after a single inoculation with a given adenovirus was achieved in only a minority of cases. Thus, we have also studied possible prime-boost protocols that could improve primary vaccine-induced immune responses by using repeated administration of those adenoviruses or combining them with other recombinant vectors. By doing this, a much higher degree of protection could be elicited in all cases against a challenge with the corresponding live parasites. Not only the numbers of IFNγ-producing cells or the antibody titers, but also the in vivo cytolytic capacity of the vaccine-induced T lymphocytes was augmented in this situation. Curiously, although not an isolated finding when considered recent reports found in the literature, the prime-boost protocol that induced the highest levels of Th1-related immune memory was the one that not only induced IFNγ and TNFα but also induced the highest levels of interleukin 4 (IL-4) after the boost. The conclusions drawn from these experiments and their future applicability in a real clinical setting will be discussed during our presentation.

[November, 2007-11-06 - 16h00 - ROOM A]

RT05C - Experimental immunization with recombinant influenza virus encoding SAG2 antigen of *Toxoplasma gondii*

Machado, A.M.V *(Centro de Pesquisas René Rachou - FIOCRUZ, Belo Horizonte, Minas Gerais, Brazil)*; Polidoro, R.B. *(Centro de Pesquisas René Rachou - FIOCRUZ, BH, MG, Brazil)*; Caetano, B.C. *(Centro de Pesquisas René Rachou - FIOCRUZ, BH, MG, Brazil)*; Rabelo, R.H. *(Centro de Pesquisas René Rachou - FIOCRUZ, BH, MG, Brazil)*; Bruña-Romero, O. *(Dpto de Microbiologia-UFMG, BH, MG, Brazil)*; Gazzinelli, R.T. *(Centro de Pesquisas René Rachou - FIOCRUZ, BH, MG, Brazil)*

Toxoplasmosis caused by the protozoan parasite *Toxoplasma gondii* is a serious public health concern in Latin America, leading to abortion, congenital malformation and serious disease in immuno-compromised patients. Currently, there is no licentiate vaccine to be used in human beings.

Recombinant viruses are promising tools aiming the development of a toxoplasmosis vaccine. Different studies have shown that strong immune response is elicited when two different viral vectors are used during priming and boosting immunizations (heterologous prime-boost strategy). In the present study, we explored such approach by using a recombinant influenza virus encoding SAG2 protein during priming, followed by a boosting immunization with another recombinant virus also encoding the SAG2 protein. Therefore, recombinant influenza viruses were generated by reverse genetics.

Our studies performed in mice showed that priming with recombinant influenza viruses followed by a boost with another recombinant virus elicited antibodies levels that were significantly lower than those obtained following two sequential immunizations with recombinant adenoviruses. In spite of the lower antibody titers, in experimentally challenged animals, similar levels of protective immunity were achieved as could be assessed by the number of brain cists in mice subjected to the heterologous prime-boost protocol when compared to those obtained in animals immunized twice with recombinant adenoviruses encoding the SAG2 proteins (homologous prime-boost protocol). Such results give support to the potential use of recombinant influenza viruses in heterologous prime boost protocols aiming the development of vaccines against *Toxoplasma gondii*.

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[November, 2007-11-06 - 16h00 - ROOM A]

RT05D - Are sand fly salivary proteins viable components for an anti-parasite vaccine?

Valenzuela., J.G. *(Laboratory of Malaria and Vector Research, NIAID, NIH)*

Parasite-vector and vector-host interactions are fundamental for a successful pathogen transmission in vector-borne diseases. In vector-host interactions sand fly salivary proteins have been shown to play a major role in *Leishmania* transmission. In naïve animals saliva was shown to exacerbate *Leishmania* infections while animals previously exposed to sand fly salivary gland homogenate or uninfected sand fly bites were protected against *Leishmania* infection.
The protective effect conferred by sand fly salivary proteins was shown to be related to the presence of a strong cellular immune reaction in a form of a delayed type hypersensitivity response (DTH) to the salivary proteins and not to a neutralization effect of an exacerbating salivary molecule. The current hypothesis suggests that the protective effect is an indirect killing of the parasite due to the inhospitable environment created by the DTH to a sand fly salivary protein and not due to specific anti- *Leishmania*immunity.
We will discuss the challenges facing the implementation of sand fly salivary proteins as vector based vaccines. This includes the identification of the potential vaccine candidate from different sand flies, the responses in different hosts and salivary protein variability. Besides addressing the viability of salivary vaccine candidates in rodents we aspire to move forward and be able to test these proteins in large animals including humans and key reservoirs such as dogs. Furthermore we will discuss how a multidisciplinary approach based on cDNA sequencing, proteomics, bioinformatics, functional genomics, entomological and veterinary studies are helping us to address some of these challenges.

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[November, 2007-11-06 - 16h00 - ROOM B]

RT06A - A novel kinase involved in translational control localizes to the flagellar pocket in *Trypanosoma brucei*

Moraes, M. C. S. *(Universidade Federal de São Paulo)*; Jesus, T. C. *(Universidade Federal de São Paulo)*; Schenkman, S. *(Universidade Federal de São Paulo)* Castilho, B. A. *(Universidade Federal de São Paulo)*

Translational control mediated by phosphorylation of the alpha subunit of the eukaryotic initiation factor 2 (eIF2α) is central to stress-induced programs of gene expression. Mammals encode four eIF2α kinases: GCN2, the only eIF2 kinase present in all eukaryotes, is activated by amino acid starvation; PERK, a type I transmembrane protein of the endoplasmic reticulum in metazoans, is activated by conditions that elicit the accumulation of unfolded proteins in the ER;

PKR, by dsRNA and cytotoxic stresses; and HRI by the lack of heme in erythrocytes. While increased levels of eIF2α(P) result in global protein synthesis inhibition, the translation of specific messages, such as GCN4 in yeast and ATF4 in mammals, is induced. These proteins are transcriptional factors that activate a downstream response aimed at promoting the recovery of the cells from the initial stress. Trypanosomatids, important human pathogens, display differentiation processes elicited by contact with the distinct physiological milieu found in their insect vectors and mammalian hosts, likely representing stress situations. The paucity of transcriptional factors in these organisms suggests that they rely heavily on translational control to regulate gene expression in order to adapt to their different hosts. *Trypanosoma brucei* encodes three potential eIF2α kinases (TbeIF2K1-K3) that are also found in *T. cruzi* and *Leishmania*, suggesting that phosphorylation of eIF2α plays a relevant role in these parasites. We show here that the catalytic domain of TbeIF2K2 phosphorylates yeast and mammalian eIF2α at Ser51. It also phosphorylates the highly unusual form of eIF2α found in trypanosomatids specifically at residue Thr169, that corresponds to Ser51 in other eukaryotes. *T. brucei* eIF2α, however, is not a substrate for GCN2 or PKR in vitro. TbeIF2K2 thus presents a new substrate specificity regarding other known kinases of this class. TbeIF2K2 is a type I transmembrane glycoprotein expressed both in procyclic and bloodstream forms, representing thus the only known membrane-associated eIF2α kinase in unicellular eukaryotes. In both procyclic and bloodstream form parasites, TbeIF2K2 is mainly localized in the membrane of the flagellar pocket, an organelle that is the exclusive site of exo- and endocytosis in these parasites. It can also be detected in endocytic compartments but not in lysosomes, suggesting it is recycled between endosomes and the flagellar pocket. TbeIF2K2 location and predicted topology, with an extracellular regulatory domain and a cytoplasmic catalytic domain, suggest its relevance in sensing the endocytic cargo in *T. brucei* and signaling to the protein synthesis machinery. Supported by FAPESP

[November, 2007-11-06 - 16h00 - ROOM B]

RT06B - GENOME-WIDE CHARACTERIZATION OF *Trypanosoma cruzi* RNA BINDING PROTEINS REGULATED mRNAs.

Dallagiovanna, B.D. *(FIOCRUZ- IBMP)*; Morking, P. *(FIOCRUZ- IBMP)*; Perez-Diaz, L. *(Universidad de la Republica)*; Probst, C.M. *(FIOCRUZ-IBMP)*; Holetz, F. *(FIOCRUZ-IBMP)*; Smircich, P. *(Universidad de la Republica)*; Buck, G.A. *(Center for the Study of Biological Complexity)*; Fragoso, S.P. *(FIOCRUZ-IBMP)*; Goldenberg, S.G. *(FIOCRUZ-IBMP)*; Goldenberg, S. *(FIOCRUZ-IBMP)*; Krieger, M.A. *(FIOCRUZ-IBMP)*

Gene expression in trypanosomes differs from that of higher eukaryotes and involves unusual mechanisms, such as polycistronic transcription. No canonical RNA polymerase II promoter has yet been identified and there is no clear evidence of transcriptional regulation for protein coding genes. As the individual genes present in a given polycistronic unit may show different temporal expression patterns, the regulation of gene expression in trypanosomes is thought to be post-transcriptional. Posttranscriptional regulation involves specific interactions between regulatory transacting factors and conserved cis elements present in the 5‘ and 3‘ untranslated regions (UTR) of the transcripts. RNA binding proteins (RBPs) bind to sequence specific and/or structural elements in the UTR regions of functionally related mRNAs, modulating their expression. The PUF (Pumilio/FBF1) family of posttranscriptional regulators is widespread among eukaryotes. PUF proteins regulate mRNA stability and translation by enhancing the deadenylation and subsequent degradation of mRNAs or repressing translation initiation. Several conserved PUF proteins have been identified in the genomes of kinetoplastida. In Trypanosoma cruzi, the PUF protein family has ten members. We have previously described and characterized TcPUF6, a member of this family. T. cruzi PUF6 protein is produced constitutively, throughout the parasite‘s life. The protein is present in the cytoplasm of different forms in multiple discrete foci, a characteristic pattern also observed for members of the yeast PUF family. We used a ribonomic approach to identify the putative target mRNAs associated with TcPUF6. Ribonomics has been defined as the en masse identification of mRNAs regulated by RBPs. We characterized the putative mRNA targets of TcPUF6, using a strategy combining an analysis of overall gene expression in the transfected parasites and TAP-TAG affinity purification of the TcPUF6-associated mRNAs. Ribonucleoprotein complexes formed by mRNAs and the tagged TcPUF6 protein were purified from soluble protein lysates prepared from transfected exponentially growing epimastigote forms by affinity chromatography on IgG-Sepharose columns. Nine probes were selected based on increased signals obtained in at least two of three experiments. Microarray analysis of affinity-purified transcripts and whole expression of the transfected parasites showed that mRNAs associated with TcPUF6 were downregulated in parasites overexpressing TcPUF6. It has been suggested that PUF proteins regulate the half lives of associated mRNAs by interacting with the Ccr4p-Pop2p-Not protein complex and other specific proteins, such as the Dhh1 helicase, to the mRNA. We observed an interaction between TcPUF6 and the decapping activator TcDhh1, supporting the notion that TcPUF6 might target mRNAs for degradation. Functionally related mRNAs are regulated in ribonucleoprotein complexes and degraded or stored for subsequent translation in defined cytoplasmic regions, defining what has been termed ‘post-transcriptional operons”. TcPUF6 regulated mRNAs, though not functionally related, have in common a stage specific expression in metacyclic forms. We have expanded this ribonomic approach to other RBPs with different binding motifs like CCCH zinc fingers and RRMs. We were able to identify target mRNAs for these proteins which were also functionally related. The zinc finger protein TcZFP2 regulate genes involved in endocityc pathways while the RRM containing protein TcRBP19 regulates amastigote specific genes. Our

results show that this genome-wide approach could be used to unravel the complex posttranscriptional gene circuits that control gene expression in kinetoplastid parasites. Financiamento: NIH, PRONEX/ Fundação Araucária, and CNPq/ PROSUL.

[November, 2007-11-06 - 16h00 - ROOM B]

RT06C - Global control of translation during heat shock in trypanosomes.

Kramer, S *(University of Cambridge)*; Ramarao, R *(University of Cambridge)*; Webb, H *(University of Cambridge)* Carrington, M *(University of Cambridge)*

The primary level of regulation of gene expression in yeast and metazoa is at the initiation of transcription. In contrast, in trypanosomes there is little regulation of transcription by RNA polymerase II and most regulation of gene expression depends on modulation of mRNA half life and/or translation. Regulated mRNA instability is the norm for genes silenced in particular developmental stages. The pathways for eukaryotic mRNA turnover have been characterised in detail using budding yeast and subsequently similar and additional pathways have been shown to be present in metazoa. Several of the pathways including mRNA turnover, RNA interference and microRNA-mediated silencing, are centred on P-bodies which are discrete ribonucleoprotein particles located in the cytoplasm. In yeast, the pathway of mRNA turnover starts with shortening of the 3' polyA tail and movement of the mRNA to a P-body. The 5' cap is removed from the mRNA by the products of the DCP2 and DCP1 genes and the process is regulated by the products of several other genes including DHH1, PAT1, EDC1 to EDC3 and LSM2 to LSM8. Decapping commits the mRNA to degradation by a 5' to 3' exonuclease encoded by the XRN1 gene. In yeast, many of the genes involved in decapping are not essential and null mutants are viable as the 5' to 3' degradation pathway is redundant with a 3' to 5' degradation pathway catalysed by the exosome. In trypanosomes the pathways of mRNA turnover use several components identified in yeast and metazoa. P-bodies in trypanosomes contain SCD6 and DHH1. However, in the trypanosome genome, there is no obvious orthologue of DCP2, the catalytic subunit of the decapping enzyme or of LSM1, the key component of the LSM1 to 7 complex and the LSM2 to 8 complex is primarily located in the nucleus. Several other components, such as EDC1 to 3, PAT1 and DCP1 are not well enough conserved to identify orthologues using conventional bioinformatics. Here, we describe the behaviour of the P-bodies during heat shock and show that: (1) translation initiation is inhibited and (2) there is an accumulation of translation initiation factors at discrete loci that also contain P-body components.

[November, 2007-11-06 - 16h00 - ROOM B]

RT06D - Understanding protein synthesis in trypanosomatids: multiple eIF4F homologues display distinct properties which might reflect different activities associated with translation control.

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In Trypanosomatids the lack of transcriptional control for the synthesis of the mRNA coding genes has implicated the initiation stage of protein synthesis, or translation, as a likely major step for regulation of gene expression. However, little is known about protein synthesis in these pathogenic protozoans. In mammals, yeast and plants translation initiates with the binding of the heterotrimeric translation initiation complex eIF4F - eIF4E, eIF4A and eIF4G - to the monomethylated cap present on the 5‘ end of mRNAs, a step which is highly regulated. Subsequently eIF4F recruits the small ribosomal subunit which then scans the mRNA 5‘UTR until it finds the translation initiation codon and starts protein synthesis. The eIF4E subunit is the cap binding protein which interacts directly with the 5‘ cap nucleotide, eIF4A is a highly conserved RNA helicase and eIF4G acts as a scaffold for the complex with binding sites for both eIF4E, eIF4A and other translation factors such as eIF3 and the poly(A) binding protein (PABP). Both eIF4E and eIF4G can have their activity regulated through phosphorylation and other means. The main focus of our work deals with the study in Trypanosomatids of the various homologues of eIF4F subunits previously identified by our group in Leishmania major and Trypanosoma brucei: 4 eIF4Es, 2 eIF4As and 5 eIF4Gs. The genes encoding most of these homologues from the two organisms were cloned and used for protein expression and antibody production. The recombinant polypeptides and antibodies were then used to study the various proteins biochemically. In vivo assays in T. brucei were also performed to further characterise their biological role. Here we summarize results obtained so far, focusing mainly on recent data, which identified, for the various proteins under study, significant differences in properties such as overall gene expression, requirements for viability, subcellular localisation and RNA/protein binding affinities. So, for the two eIF4As, they were seen to differ in abundance, subcellular localization, sensitivity to RNA interference and inhibition by a dominant negative mutant. The biochemical data plus comparative sequence analysis and molecular modelling confirmed the first eIF4A homologue as a true translation initiation factor (4AI) whilst implicating the second protein as

an orthologue to mammalian eIF4AIII, only described previously in metazoans and which is part of the exon-junction complex in the nucleus. The four eIF4Es can be grouped into two distinct classes. The 4E1-2 proteins are expressed at low levels (particularly so for 4E2), localize both to the nucleus and cytoplasm and so far were not seen to be required for viability in T. brucei. In contrast, 4E3-4 are much more abundant, strictly cytoplasmic and are both required for viability in T. brucei bloodstream forms although in procyclic cells only 4E3 seems to be required. The latter two proteins were also observed as two distinct bands, indicative of post-translational modification such as phophorylation. For the various eIF4Gs, we have concentrated our study on the 4G3-4 proteins since they share unique similarities outside the HEAT domain, common to all 5 homologues, and were the only ones implicated in translation through their ability to bind to 4AI. The two proteins were required for cellular viability indicating non-redundant roles in the parasite. Both are cytoplasmic and can also be detected as two isoforms. Furthermore 4G4 binds strongly and specifically to 4E3 whilst 4G3 binds weakly to different 4E homologues and also to a Leishmania homologue of PABP. Overall all results are compatible with the initiation of protein synthesis in trypanosomatids being a complex process with at least two eIF4F complexes acting non-redundantly in a manner dissimilar to what is seen in vertebrates.

[November, 2007-11-06 - 16h00 - ROOM C]

RT07A - STUDY OF *RHODNIUS PROLIXUS* IMMUNE SYSTEM DURING FUNGAL CHALLENGES.

Machado, E.A. *(Universidade Federal do Rio de Janeiro)*

Insect immunity is based in physical, cellular and humoral constituents. The first is the protection provided by the cuticle and midgut that avoids invasion by pathogens. The second involves the phagocytosis and destruction of antigens by hemocytes. The last constituent comprises mainly the synthesis of antimicrobial peptides and proteins secreted in the hemolymph. Our goal is to access the physiology of the immune system of *Rhodnius prolixus* during fungal challenges and to isolate peptides with antifungal activity from infected animals. As models for stimulating immune response we injected the fungus *Aspergillus niger* and Zymosan (a sterile preparation of fungi cell wall) into the insect hemocoel. The parameters analyzed during challenge were: phenoloxydase and pro-phenoloxydase activities; protein concentration in the hemolymph; reproductive fitness. Our results showed a different profile on the hemolymph of control and infected animals, with no substantial changes on the protein concentration in the haemolymph. There was a loss of reproductive fitness, with an important reduction in ooposition during immune challenge, as described in other models. It was also observed an increase on the phenoloxydase activity durin fungal challenge. Finnaly, there was observed a pronuncied antifungal activity in the low molecular weight fractions (<10kDa), probably associated to peptides. The isolation of these antimicrobial peptides is still in course.

PRONEX, ICGEB, CNPq, FAPERJ

Machado, E.A. Programa de Biologia Celular e Parasitologia, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

RT07B - Fitness studies of transgenic malaria-resistant mosquitoes

The introduction of genes that impair Plasmodium development into mosquito populations is a strategy being considered for malaria control. The effect of the transgene on mosquito fitness is a crucial parameter influencing the success of this approach. We have previously shown that anopheline mosquitoes expressing the SM1 peptide in the midgut lumen are impaired for transmission of Plasmodium berghei. Moreover, the transgenic mosquitoes had no noticeable fitness load compared with nontransgenic mosquitoes when fed on noninfected mice. We have shown that when fed on mice infected with P. berghei, these transgenic mosquitoes are more fit (higher fecundity and lower mortality) than sibling nontransgenic mosquitoes. In cage experiments, transgenic mosquitoes gradually replaced nontransgenics when mosquitoes were maintained on mice infected with gametocyte-producing parasites (strain ANKA 2.34) but not when maintained on mice infected with gametocyte-deficient parasites (strain ANKA 2.33). These findings suggest that when feeding on Plasmodium-infected blood, transgenic malariaresistant mosquitoes have a selective advantage over nontransgenic mosquitoes. This fitness advantage has important implications for devising malaria control strategies by means of genetic modification of mosquitoes

[November, 2007-11-06 - 16h00 - ROOM C]

RT07C - IMMUNITY IN *Lutzomyia longipalpis*: PUTATIVE GENES AND IDENTIFICATION OF A NONSPECIFIC ANTIVIRAL RESPONSE

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One prospective to control arthropod-born diseases relies on the possibility of controlling the vector or interfering with parasite-insect interaction, for which an understanding of the vector‘s immune system is critical. *Lutzomyia longipalpis* is the major vector for visceral leishmaniasis in Brazil. In an effort to identify molecular events that might be important

in vector-pathogen interaction we have used two major approaches: 1) EST sequencing of cDNA libraries made from RNAs of *L. longipalpis* gut (6 and 72 hours after blood feeding (ABF), and 72 hours after artificial infection with *Leishmania chagasi*), identification and expression analysis of putative immunity related genes, and 2) the use of RNAi in *L. longipalpis* cell line LL5 which led us to the identification of a non-specific antiviral response. After clusterization and annotation of ESTs, 1504 sequences were identified. In general, 21% of the sequences (317) had no Blast hit against the used data bases (GenBank, Pfam, Conserved Domain Database (CDD), Refseq), which represents putative species-specific sequences. Many of the identified sequences have a potential role in insect immunity. Sequences related to apoptosis, defense, oxidation, antigen and cell adhesion were found mostly in the infected library. Sequences related to blood digestion were predominantly derived from the 6 hours ABF library. The expression profile of some immunity important genes was analyzed individually. A defensin gene is expressed only after de 4^{th} larval stage, with an increase in adults, and is modulated by blood meal. Females 6 and 12 hours ABF have higher levels of defensin expression than females without feeding. In the final stages of blood digestion defensin RNA levels are reduced. Cactus is responsible for the activation of several immune-related genes in adult insects. Although there is a small decrease of cactus RNA expression in pupae, during the earlier developmental stages this gene demonstrates a constitutive profile and its regulation might be happening at the protein level. MAP-Kinase which is involved in the initial steps of defense peptide synthesis, is highly expressed in eggs and, after eclosion, the mRNA levels of 1^{st} larval stage decrease. On 2^{nd} and 3^{rd} stages transcription increases again but on the 4^{th} stage it decreases, and pupae have only a basal expression. In adults, MAPK is highly expressed, especially in females. VATPase with a possible role in midgut acidification, could be related to metacyclogenesis of *Leishmania* in the insect gut. This gene has high expression levels in early larval stages, but in the 4^{th} stage the expression level decreases and remains at basal levels in pupae. In adults the expression levels increase mainly in females, probably as a consequence of diet. RNAi has recently arisen as a convenient way of performing functional studies in insects. To establish RNAi assays in *L. longipalpis*, we have transfected cultured cells with double stranded RNAs (dsRNA), using West Nile virus-virus like particles (VLPs) expressing luciferase as model. Luciferase dsRNA caused a lowered production of VLPs as expected. Surprisingly, we found that various unrelated dsRNAs, that included *L. longipalpis* gene sequences, but also the *E. coli* β-galactosidase sequence, diminished the production of VLPs. A similar response was seen in shrimp, but this is the first report on non-specific anti-viral response triggered by ds-RNA in an insect cell line. Preliminary experiments, submitting naïve cells to conditioned medium from cells treated with dsRNA followed by VLP infection, indicate the presence of soluble factors involved in the anti-viral response. Further characterization of these putative factors is underway. It will be of great interest to identify the mechanisms by which LL5 cells recognize dsRNA, and the signaling pathway that produces the antiviral response. Supported by PAPES IV-Fiocruz, CAPES and CNPq

[November, 2007-11-06 - 16h00 - ROOM C]

RT07D - Double stranded RNA and antiviral immunity in a marine invertebrate

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Immune systems of both vertebrates and invertebrates seem to have evolved the capacity to sense viral infections through the recognition of double stranded RNA (dsRNA), a conserved virus-associated molecular pattern. In invertebrates, the best understood antiviral pathway that is initiated by dsRNA relies on the RNA interference (RNAi) machinery. However, the presence of viral dsRNA is likely to trigger other immune responses, as it does in vertebrates, where dsRNA recognition initiates a complex set of antiviral mechanisms, chiefly directed by the action of Interferons. The studies reported here in marine shrimp support the notion that dsRNA recognition can lead to innate antiviral responses other than RNAi in invertebrates. Specifically, the injection of dsRNA confers shrimp with broad-spectrum antiviral immunity in a manner that is largely independent of the sequence and base composition of the dsRNA. The molecular mechanisms that mediate this immune response are presently unknown, but high throughput gene expression profiling points to the potential involvement of RNA helicases, lectins, chaperones, and proteins associated with intracellular trafficking or secretion. In shrimp, dsRNA not only induces innate non-specific antiviral immunity, but it also can direct potent antiviral protection when virus-specific dsRNA is injected, presumably through the engagement of the RNAi machinery. These findings demonstrate the coexistence of both sequence-independent and sequence-specific antiviral pathways in an invertebrate, and open the possibility of utilizing shrimp as a model to study the functional interactions between antiviral RNAi and classical innate immune pathways activated by dsRNA.

[November, 2007-11-07 - 09h00 - ROOM A]

RT08A - FLY, a conserved sequence from the gp85/TS family of *T. cruzi*, enhances parasitemia and modulates host cell mitogen - activated protein kinase pathway.

Tonelli, R.R. *(Universidade de São Paulo - Instituto de Química)*

Invasion of mammalian cells by *Trypanosoma cruzi* causes Chagas' disease, but the mechanisms of parasite entry are still unclear. Our group has previously shown that the con-

served FLY domain (VTVXNVFLYNR), present in all members of the gp85/trans-sialidase glycoprotein family coating the surface of trypomastigotes, binds to the surface of $LLC-MK_2$ epithelial cells, possibly through cytokeratin 18 (CK18), and significantly increases parasite entry into these cells (Magdesian et al. 2001. JBC 276, 19382). In order to characterize the mechanism involved in FLY potentiation of cell infection by *T. cruzi*, different signaling cascades have been analyzed in mammalian cells exposed to that domain. Binding of FLY, present on the surface of trypomastigotes or on latex beads, to $LLC-MK_2$ cells promotes activation of the ERK1/2 signaling cascade and dephosphorylation of CK18, culminating in an increase of approximately 9-fold in the number of parasites/cell. Inhibition of ERK1/2 phosphorylation completely blocks the adhesion of FLY to cells and blocks by 57 % host cell infection by *T. cruzi* (Magdesian et al. 2007. Exp. Cell Res. 313, 210). The relevance of FLY during the acute phase of *T. cruzi* infection *in vivo* was investigated. Mice primed with FLY one week before infection with bloodstream trypomastigotes show a 2-fold increase in parasitemia and mortality rates as compared to infected mice primed with control peptide (FAY) or PBS. Moreover, an increase in the number of amastigote nests in the heart, bladder and small intestine was observed in FLY-primed mice. Concomitantly, a decrease in NO production and in the pro-inflammatory cytokine IFN-γ by spleen cells was detected in these animals. Taken together, the results herein reported suggest that the conserved FLY domain is an important tool, among others, that trypomastigotes use to modulate the immune system in order to ensure a successful infection. Supported by FAPESP. RRT is a FAPESP post-doctoral fellow.

[November, 2007-11-07 - 09h00 - ROOM A]

RT08B - Lysophosphatidylcholine and vector saliva are enhancers of Trypanosoma cruzi transmission.

Silva-Neto, M.A. *(Universidade Federal do Rio de Janeiro)*

Lysophospholipids are powerful modulators of cell signaling in mammalian cells. We have previously shown (Golodne et al., J. Biol Chem. 2003 Jul 25; 278(30): 27766-71) that salivary glands of the blood-sucking bug **Rhodnius prolixus** store lysophosphatidylcholine (LPC) during their growth. **Trypanosoma cruzi**, the etiological agent of Chagas disease is transmited by bug feces which are deposited on host skin after a blood meal on human skin. Parasite infection occurs by the wound produced by insect mouth parts or by a later contact of the feces with host mucosas. Here, we show that LPC is also a component of bug feces. Therefore, in both routes of infection invading parasites face a tissue environment previously stimulated by LPC. In the present work we tested the role of both bug saliva and LPC on parasite transmission. Firstly, the ability of bug saliva to induce cell chemotaxis was determined by three different approaches: pleurisy assays, skin histology and cell migration in Boyden chambers. In all tested techniques a massive amount of neutrophils was found associated to the inoculated sites of saliva and LPC. Secondly, pre-incubation of peritoneal macrophages with either 1/300 salivary gland pair or LPC doubled the association of **T. cruzi** with these cells. Such effect is concomitant with the induction of an increase in intracellular free calcium concentration. Finally, injection of either saliva or LPC on mouse skin in the presence of the parasite induces a three to six fold increase on blood parasitemia during the course of infection. Such effect is probably caused by the ability of LPC to downregulate the production of nitric oxide and proinflammatory cytokines in **T. cruzi** infected macrophages. In conclusion, we have gathered evidence that bug saliva and specially LPC controls host intracellular signaling pathways and enhance **T. cruzi** transmission. This is the first demonstration of involvement of a vector-derived molecule as a transmission enhancing factor in Chagas disease.

[November, 2007-11-07 - 09h00 - ROOM A]

RT08C - Expression of protein kinases in *Schistosoma mansoni* and their relation to host penetration

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In addition to the generation of new genetic networks and the diversification of the regulation of gene expression the development of specific cellular signalling pathways represents a central mechanism by which animal evolution has occurred. The platyhelminth *Schistosoma mansoni* represents not only a novel, lophotrochozoan model system for the study of these systems, but is also a medically important parasite. *Schistosoma mansoni* is one of the three main species of schistosomes that cause schistosomiasis, a disease that affects over 200 million people, with a further 600 million individuals at risk. The impact of the disease has been seriously underestimated in the past. Moreover, concerns about the development of resistance by the parasite to the only available drug, praziquantel, underline the necessity for the development of novel control strategies. Proteins implicated in cellular signalling, particularly those at the host-parasite interface, should provide specific and vital targets for novel chemotherapeutic strategies. The description of signal trans-

duction molecules and mechanisms are essential to elucidate *Schistosoma* host-parasite interactions and parasite biology. Like other developmental processes, the complex life-cycle of *Schistosoma*, including two hosts and six morphologically distinct forms, implies that the parasite requires permanent sensing of the environment, communication between cells of individual worms, between the worms and their hosts as well as between paired males and females, all of which involve signal transduction mechanisms. However, although a variety of signalling proteins have been identified in schistosomes, including cell surface receptor tyrosine kinases and cytosolic protein kinases, many components of the corresponding signalling cascades have yet to be characterised. We have previously identified and characterised the molecular structure of new PKs in *S. mansoni*, SmFes and SmPKC1. SmFes exhibits the characteristic features of Fes/Fps protein tyrosine kinase subfamily of which it is the first member described in helminths. PKCs are involved in a wide variety of signal transduction pathways that control various physiological processes. They are present in multimolecular complexes inside the cells and their activity is finely tuned by changes in the extracellular and/or intracellular milieu. The differential expression of SmPKC1 and SmFes mRNA and protein during the *S. mansoni* life cycle (adult worm, miracidium, sporocyst, cercaria, and schistosomulum) was demonstrated using quantitative RT-PCR, Western blotting and immunolocalisation studies. In skin stage schistosomula, SmPKC1 is expressed in the acetabular gland as well as a small amount in the tegument. It is probable that the SmPKC1 protein sequestered in the acetabular glands would be enzymatically inactive, in accord with previous observations of a translocation of enzyme activity to the tegument on transformation of cercariae into schistosomula. The presence of large amounts of protein in the acetabular gland may also suggest a role for SmPKC1 in secretions and possibly mediating direct interactions with host cells. This role may extend to interactions with the intermediate host since SmPKC1 was also present in glandular structures in primary sporocysts. On the other hand the SmFes protein was detected at all the main life-cycle stages and was most abundant in cercariae and newly-transformed schistosomula. However, no protein was detected in schistosomula maintained *in vitro* for 7 days. By immunolocalisation studies, we showed that SmFes is particularly concentrated at the terebratorium of miracidia and tegument of cercaria and skin stage schistosomula. The miracidial terebratorium is implicated in the attachment of these larvae to the snail intermediate host prior to penetration. The presence of large amounts of SmPKC1 protein in secretory glands of both sporocysts and cercariae and of SmFes at the terebratorium of miracidia and tegument of cercariae and schistosomula suggests a central function for these kinases in signalling pathways triggered by interaction with the host and in larval transformation after penetration into both the intermediate and definitive hosts.

[November, 2007-11-07 - 09h00 - ROOM A]

RT08D - ROLE OF PROTEIN PHOSPHORYLATION AND ACTIN IN EGRESS OF *Toxoplasma gondii* INDUCED BY CALCIUM IONOPHORE AT DIFFERENT TIMES POST-INFECTION

Caldas, L. A. *(Universidade Federal do Rio de Janeiro)*; Attias, M. *(Universidade Federal do Rio de Janeiro)*; de Souza, W. *(Universidade Federal do Rio de Janeiro)*

Toxoplasma gondii is the obligate intracellular parasite responsible for toxoplasmosis. This parasite is able to infect virtually all nucleated cells of warm-blooded animals. Its egress from host cells is still an obscure event. Because under natural conditions egress occurs eventually and asynchronously, calcium ionophores have been used to induce it in vitro. Calcium plays crucial roles in more than one important event in *T. gondii* life cycle, including motility, invasion and egress from the host cell. The permeabilization of host cell membrane caused by A23187 calcium ionophore administration results in a decrease of potassium, which leads, by a yet unknown cascade, to the increase of intraparasitic calcium concentration activating *T. gondii* egress. In this report, we describe the effect of kinase inhibitors immediately before *T. gondii* calcium ionophore induced egress at 2 and 24 hours post-infection (hpi) from LLC-MK2 cells. Wortmanine, staurosporine and genistein significantly reduced ionophore induced egress at 2hpi. The same occurred when the actin inhibitor cytochalasin D was used. At 24hpi, wortmanin and staurosporin slightly inhibited induced egress when compared to genistein and cytochalasin D. Inhibitors of protein kinases were already seen to interfere in the process of attachment and penetration in macrophages. Their participation in egress was first shown by the proposition that a raise in intracellular calcium concentration promotes egress by its activation. Taken together, our results show that protein phosphorylation is necessary for the egress to occur, since it is blocked in the presence of kinase inhibitors. We also believe that egress requires integrity of host cells microfilaments, since cytochalasin D has already been shown to block both *T. gondii* egress and invasion from different strains of host cells. Supports: CNPq, CAPES, PRONEX/FAPERJ.

[November, 2007-11-07 - 09h00 - ROOM B]

RT09A - *Trypanosoma cruzi*: parasite shed vesicles generate an intense inflammatory response and increase heart parasitism

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Infective cultured-derived trypomastigotes of *T. cruzi* release, into the culture medium, vesicles enriched in glycoproteins of the gp85/Trans-sialidase superfamily and α-galactosyl-containing glycoconjugates (Gonçalves et al., 1989). We have previously shown that *Trypanosoma cruzi-*

derived glycosyl-phosphatidylinositol (GPI) anchors strongly stimulate the host innate immunity via Toll like receptor 2 (TLR2). It is, however, poorly understood how these parasite membrane components become accessible to the host cognate receptor. Presently, we show that host cell-derived trypomastigotes release vesicles (TcVes) that strongly trigger proinflammatory response in murine macrophages via TLR2. We have further fractionated vesicles by gel-filtration and immunoaffinity chromatography, and obtained a major vesicular fraction enriched with highly immunogenic. By proteomic analysis, we have identified 37 proteins on vesicles, nine of them members of the trans-sialidase/gp85 superfamily, previously implicated in the pathogenesis of experimental Chagas' disease. Vesicles strongly induced proinflammatory cytokines and nitric oxide (NO) in LPS-low responsive murine macrophages, and activated TLR2- but not TLR4-transfected CHO/CD14 cells. Macrophages from TLR2-knockout mice did not respond to TcVes as measured by the production of proinflammatory cytokines and NO. We also demonstrated that Vesicles considerably enhanced parasite infectivity *in vitro*. We propose that Vesicles represent a novel *T. cruzi* virulence factor. The effect of these membrane fractions *in vivo* was therefore investigated. The parasitemia of BALB/c mice pretreated with purified membranes prior to infection with trypomastigotes did not differ from the control. However, 40% of membrane treated animals died at the 16^{th} day post-infection and 100% at day 20^{th} whereas untreated animals started dying only after day 30. Moreover, vesicles-treated animals developed severe heart pathology, with intense inflammatory reaction, higher number of intracellular amastigote nests. Analysis of the inflammatory infiltrates in the cardiac tissue of mice 15 days after infection showed a dominance of $CD4^+$ and $CD8^+$ T lymphocytes and macrophages (MI), as well as very few areas labeled with anti-iNOS monoclonal antibody as compared to the control and higher levels of IL-4 and IL-10 mRNAs transcripts when compared to the controls mice. The results strongly suggest that membrane vesicles released by *T. cruzi* can modulate infection *in vivo* by inducing the synthesis of IL-4 and IL-10 cytokines, thus playing a central role during the acute phase of the disease. Supported by FAPESP and CNPq.

[November, 2007-11-07 - 09h00 - ROOM B]

RT09B - Imaging motile parasites with electron and light microscopy

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We are applying a number of different microscopy techniques to study Plasmodium sporozoites. Sporozoites from in parasitic cysts at the midgut wall of Anopheles mosquitoes. They enter the hemolymph and from there the salivary gland of the mosquito. During a bite, sporozoites can be transmitted to a mammalian host and depend on their active motility to reach their destination, the hepatocyte, where they differentiate into thousands of merozoites (1, 2). We use a number of different imaging approaches to investigate the various stages of the sporozoite in vivo and in vitro. Our main focus is to elucidate the biophysical nature of parasite gliding. To this end we image sporozoites with total internal reflection microscopy (TIRFM), reflection interference microscopy (RICM) and traction force microscopy. TIRFM relies on the generation of a thin evanescent field above the coverslip on which an object rests (3). This allows the visualization of fluorescent dyes or proteins that are within about 100 nm distance from the top of the glass surface. RICM relies on the generation of interference patterns when polarized light is reflected from the glass surface and the object resting on the glass (4). The complex fringes that are generated allow the determination of distances from the glass surface and thus complement TIRFM when investigating object-surface contacts. Traction force microscopy uses flexible substrates such as gels made from polyacrylamid that are modified in a way that the pulling of a moving object can be imaged and traction forces can be calculated (5). These imaging techniques yielded insights into how sporozoites contact the surface on which they move, how dynamic adhesions regulate the velocity of movement and where the parasite applies forces during active movement. To gain more detailed insights, we investigate sporozoites on an ultra-structural level using cryo-electron tomography, which allows the generation of 3D images at a resolution near 5 nm. This revealed a number of new features including additional material at the luminal side of parasite microtubules that likely serves to stabilize these structures (6). The talk will aim at illuminating the use of these imaging techniques for our studies on Plasmodium sporozoites, in the hope that they could readily be transferred to other pathogens of interest. References: (1) Prudencio M, Rodriguez A, Mota MM, The silent path to thousands of merozoites: the Plasmodium liver stage. Nat Rev Microbiol. 4, 849-856, 2006. (2) Amino R, Thiberge S, Martin B, Celli S, Shorte S, Frischknecht F, Menard R. Quantitative imaging of Plasmodium transmission from mosquito to mammal. Nat Med. 12, 220-224, 2006. (3) Schneckenbuger H, Total internal reflection fluorescence microscopy: technical innovations and novel applications, Curr Opin. Biotechol. 16, 13-18, 2005. (4) Verschueren H, Interference reflection microscopy in cell biology: methodology and applications. J. Cell Sci 75, 279-301, 1985. (5) Beningo KA, Wang YL, Flexible substrata for the detection of cellular traction forces. Trends Cell Biol. 12, 79-84, 2002. (6) Cyrklaff M, Kudryashev M, Leis A, Leonard K, Baumeister W, Menard R, Meissner M, Frischknecht F. Cryoelectron tomography reveals periodic material at the inner side of subpellicular microtubules in apicomplexan parasites. J Exp Med. 204, 1281-1287, 2007.

[November, 2007-11-07 - 09h00 - ROOM B]

RT09C - Till death do them part: enucleated cells as hosts for intracellular pathogens

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Microbial pathogens, directly and indirectly, modulate gene transcription and translation, and affect a variety of cell functions. These responses may protect host cells and/or the infected host, favor the pathogens or may be neutral. Is the host cell nucleus necessary for infection with intracellular pathogens? Enucleation abolishes gene transcription, RNAs processing, and important nucleus-dependent signaling pathways. Classic studies have shown that cytoplasts can be infected with *Toxoplasma*, *Chlamydia* or *Rickettsia prowazekii*. However, the variety of infection stratagems employed by pathogens suggests that conclusions derived from experiments with a few pathogens cannot be generalized. Furthermore, the short life expectancy of cytoplasts limits assays to pathogens with relatively short doubling times. It should also be kept in mind that survival of cytoplasts depends on the kind and functional status of the cell that is enucleated, the procedure used for enucleation and the conditions of maintenance of the cytoplasts. We will discuss experiments with *Shigella flexneri* (D. Yamamoto et al., 2006), *T. cruzi* (V.C. Coimbra et al., 2007), and with *Toxoplasma gondii* (C. S. Carvalho, ongoing experiments). The field of cytoplast biology has been nearly abandoned for close to two decades, and remained largely immune to the rapid progress of cell physiology and molecular biology. We believe that, given the complexity of the control of gene expression, the enucleated cell model could again become an important tool for the biology of normal and infected cells (Supported by FAPESP, UNIFESP, UENF, CNPq).

[November, 2007-11-07 - 09h00 - ROOM B]

RT09D - Initial Discrete Macrophage Response to *Leishmania* is Decisive to Infection Destiny

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Murine experimental cutaneous leishmaniasis has been widely used to characterize the immune response against *Leishmania*. It is well documented that the ultimate response in resistance to L. major infection is associated with differentiation of $CD4^+$ Th1 lymphocytes, leading to parasite destruction by IFN-gamma-stimulated macrophages, in a TNF-alpha- and NO-dependent mechanisms. Alternatively, susceptibility to *L. amazonensis* was associated with Th2 response, characterized by IL-4 production and failure in IFN-gamma-induced macrophage activation. *L. amazonensis* induces chronically non-healing infections in several mouse strains such as C3H, C57BL/6 and C57BL/10, which are resistant to *L. major*. In addition, BALB/c and CBA/J mice are highly susceptible to *L. amazonensis*. We have shown that CBA/J mice resistance to *L. major* is associated with a Th1 response and susceptibility to L. amazonensis with a Th2 response. Early events after Leishmania infection determine the type of host immune response. *L. amazonensis* primary stimulated-T cells from CBA/J mice induced higher parasite burden associated to higher IFN-gamma and IL-10 productions without IL-4 in comparison to *L. major* stimulation. Furthermore, IFN-gamma up-regulation induced by IL-10 neutralization did not reduce parasite burden, indicating that *L. amazonensis* priming induces a response consistent with a regulatory phenotype and resists to be killed by mechanisms which normally destroy *L. major*. Macrophage plays an important role in the early events of Leishmania infection. We have also demonstrated that CBA/J macrophages control *L. major* infection and are permissive to *L. amazonensis*. To identify genes involved in macrophage response to *Leishmania*, short oligonucleotide microarray was used to characterize the mRNA expression profile of more than 12,000 mouse genes before and after macrophage infection with *Leishmania*. First of all, we characterized gene expression of non-infected *L. amazonensis*-susceptible CBA/J macrophages in comparison to expression by C57BL/6 macrophages. Several genes involved in early immune response are differential expressed by those macrophages such as CCR5, CCL3, Rhoc and IL-10 receptor. These differences were confirmed by qRT-PCR. These data indicate that previous to the encounter with *Leishmania*, macrophage express a set of genes which may contribute to infection outcome. Furthermore, we showed slight differences in gene expression of *L. amazonensis*- and *L. major*-infected macrophages. qRT-PCR confirmed higher expression of pro-apoptotic genes in *L. amazonensis* infection, such as BAD and LCN2. In *L. major* infection, higher expression of VAV a GTP exchange factor for the small GTP binding proteins such as Rho and Rac1, and of the scavenger receptor MARCO. These proteins are directly or indirectly involved in phagocytosis of microorganisms. These small differences in gene expression occur along with higher production of H202 by CBA/J macrophages, monitored by luminol and microperoxidase chemiluminescence, in response to *L. major* when compared to *L. amazonensis* infection. These data suggest that *L. amazonensis*, in vitro, inactivates and resists to innate macrophage killing mechanisms, whereas *L. major* is destroyed by macrophages. Proteomic approach is under analyses in order to identify differences at the proteome level and post-transcriptional modulations

which can occur during these infections. The data herein present evidences that *Leishmania* induces an initial discrete macrophage response that determines ultimate parasite infection.
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[November, 2007-11-07 - 09h00 - ROOM C]

RT10A - *Cryptosporidium hominis*: from the genome to a vaccine

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Background. *Cryptosporidium hominis* is a waterborne parasite that causes acute, self-limiting, gastroenteritis in normal humans and potentially fatal infections in immunocompromised patients. Currently, there is no specific treatment for cryptosporidiosis. The goal of our research is to apply genomic strategies to identify potential targets for immunoprophylaxis, immunotherapy, and chemotherapy in the *Cryptosporidium* genome. Herein, we describe our efforts to scan the genome for potential vaccine targets using bioinformatics, gene expression and proteomic analysis, and our examination of the immune responses induced by these antigens in murine models.

The genome of *C. hominis* is comprised of approximately 9.2 megabases in eight chromosomes and approximately 4,000 protein coding genes. It is highly streamlined and conserves only those genes the organism needs to survive in its limited environments. Using bioinformatics approaches to search for signal peptides, signal anchors, GPI anchors, or transmembrane regions, we identified several hundred *C. hominis* proteins that we judged to be likely to be surface associated. A DNA microarray containing a probe for each of the ∼4,000 C. hominis genes was developed and used to identify transcripts selectively expressed in the infective sporozoite phase of the parasite. Expression of these transcripts as proteins was verified by 2 D nano LC - MS/MS analysis. These analyses identified approximately 80 *C. hominis* proteins that were judged good potential vaccine candidates.

Ten of these antigens were selected for initial screening. Each was over expressed in three different bacterial systems incorporating a His-tag, a GST-tag, or a Nus-tag to identify the most efficient method for production of recombinant antigen. Each was also fused with the *Salmonella* ClyA hemolysin gene and expressed in *Salmonella* for use as a $'live'$ vaccine designed to induce mucosal immunity. Finally, each candidate was ligated into a DNA vaccine vector under the control of a CMV promoter for maximal expression in mammalian cells. Antibodies generated to these proteins were used to confirm their expression in sporozoites and their association with the parasite membrane.

We examined the ability of these recombinant antigens and the live vaccine to induce an immune response in three murine models: a murine gamma interferon knockdown model, an adult mouse model, and a neonatal mouse model. Our results showed that the antigen pools of up to four (4) antigens, delivered as a DNA vaccine and/or as purified recombinant proteins, induced a strong humoral response in the gamma interferon knockdown model. Moreover, antiserum from some of these mice blocked invasion of cultured HCT-8 mammalian cells by *Cryptosporidium* sporozoites. In addition, an immunization protocol consisting of intranasal administration of the live *Salmonella* expressing ClyA - fused recombinant antigen followed by a boost of intramuscular recombinant antigen provided the best immune responses. Thus, adult mice immunized according to this protocol showed high titers of antigen specific antibodies, strong cellular responses, and production of significant levels of gamma interferon. Experiments are now underway to explore the ability of these immunogens to protect animals from exposure to *Cryptosporidium*.

These results, taken together, illustrate the value of the reverse vaccinology approaches combining both *in silico* and *in vitro* technologies for screening and identification of potential new immunogens for protozoan pathogens.

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[November, 2007-11-07 - 09h00 - ROOM C]

RT10B - An Epigenomic map of the *Toxoplasma gondii* genome.

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The molecular mechanisms underlying the regulation of gene expression in the Apicomplexa are poorly understood. Nevertheless, expression studies have shown that the Apicomplexa have coordinated patterns of gene expression associated with different phases of their life cycle. As for *Plasmodium*, the *Toxoplama gondii* genome encodes few conventional transcription factors. To evaluate the role of chromatin modification in *T. gondii* gene expression, we investigated the distribution of acetyl-histone H4 and H3K9 and trimethyl-histone H3K4 on a 0.65 Mb fragment of chromosome Ib with coverage of one oligonucleotide every 50bp. By combining chromatin immunoprecipitation (ChIP on chip) with gene expression studies (cDNA hybridization), we showed that these modified histones are associated with gene expression and are landmarks for active promoters in *T. gondii*. To further characterize the *T. gondii* histone code, we have mapped the distribution of other methylated histones and have found unique differences in distribution when compared to H3K4me3, H3K9ac and H4ac. We are now extending our analysis to the whole *T. gondii* genome, identifying active promoters and corresponding tachyzoite-expressed genes. A preliminary analysis reveals that a large proportion of genes are expressed at the tachyzoite stage, but that others have no evidence of expression in tachyzoites. These unexpressed genes may correspond to pathways exclusively utilized at other stages of the life cycle. Further studies will clarify the role of histone modification and chromatin remodeling in regulation of gene expression in *T. gondii*. Finally, integration of our ChIP/chip and gene expression data improves annotation of the *T. gondii* genome allowing us to identify new genes including non coding RNA that are missed by conventional DNA sequence-based annotation efforts.

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[November, 2007-11-07 - 09h00 - ROOM C]

RT10C - How *Theileria* hijacks signalling pathways and host cell structures to induce uncontrolled proliferation.

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Several *Theileria* parasites possess the unique ability to transform the leukocytes they infect, inducing continuous, uncontrolled host cell proliferation and resistance to apoptosis. Unravelling the molecular mechanisms by which this intracellular protozoan parasite induces these phenotypic changes is one of the main goals of our laboratory. Schizonts of the *T. parva* and *T. annulata* are not enclosed in a parasitophorous vacuole but, instead, reside free in the host cell cytoplasm. From this location, *Theileria* schizonts interfere with host cell signalling pathways that regulate proliferation and cell survival. Parasite-induced activation of IKK, a multicomponent complex that regulates the transcription factor NF-κB, is a central event in the transformation process. NF-κB cannot only contribute to regulating DNA synthesis, it is also an important trans-activator of anti-apoptotic genes such as c-FLIP and cIAP. These can be expected to help protect the parasitised cell against death-receptor-mediated killing. In addition to activation of the Jun-NH2-terminal kinase, the PI3-kinase cascade is also constitutively activated in *Theileria*-transformed cells, and it could be shown that the latter is essential for maintaining continuous proliferation. Clonal expansion of the schizont-infected cell requires more than 'merely' inducing continuous proliferation and protection against destruction by apoptosis. To ensure its propagation, it is essential that the parasite be distributed between the two daughter cells each time host cell undergoes cytokinesis. Schizont surface proteins are perfectly positioned to interact with host cell structures involved in mitosis and cytokinesis. The genomes of both *T. parva* and *T. annulata* have been sequenced and the use of bioinformatics tools allowed us to identify and clone a gene encoding a GPI-anchored protein, gp34, which is expressed on the surface of the transforming schizont stage. At this stage, we are not able to interfere with parasite gene expression, but expression in mammalian cells allowed us to establish potential host cell targets that gp34 interacts with. When expressed in Cos-7 cells, epitope- , RFP- or GFP-tagged soluble forms of gp34 interact with host cell structures that participate in mitosis. Importantly, during mitosis, gp34 binds to the host cell central spindle and during cytokinesis, gp34 can be found at the midbody. Pull-down experiments show that gp34 can interact with chromosomal passenger complex proteins such as Aurora B, MKLP1 and also with Plk1. These kinases localise to the central spindle and the midbody where they contribute to the regulation of cytokinesis. Intriguingly, as cells enter mitosis, the parasite accumulates host cell Plk1 on its surface. This interaction lasts throughout mitosis and cytokinesis. In *Theileria*-transformed cells, overexpressed soluble gp34 blocks cytokinesis and induces multinucleation. These findings may reflect a potentially important role of this GPI-anchored schizont protein in parasite-host cell interactions that regulate the process of mitosis and cytokinesis.