ROUND-TABLES

[October, 2008-10-28 – 14h30 - RUBI ROOM]

RT01A - New concepts in the pathogenesis of Chronic Chagas disease cardiomyopathy: myocardial gene and protein expression profiles

Cunha-Neto E[1,2,3],Nogueira LG[1,2,3],Teixeira PC.[1,2,3], Bilate AM[3,4], Honorato R[1], Ianni B[1], Fiorelli A[1], Mady C[1], Stolf N[1],Jorge Kalil[1,2,3].*

[1]Instituto do Coração, e [2]Disciplina de Imunologia Clínica e Alergia, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brasil; [3]Instituto de Investigação em Imunologia/Institutos do Milênio, São Paulo, Brasil. [4]New York University, New York, USA
Correspondencia: edecunha@gmail.com

In spite of the recent advances in triatomine vector control, the millions of chronically *T. cruzi*-infected patients that have developed or will develop chronic Chagas disease cardiomyopathy (CCC) still lack appropriate therapies. Clinical progression and survival are significantly worse in CCC patients as compared with patients with dilated cardiomyopathy (DCM) of non-inflammatory etiology. In CCC, the local cytokine production profile is consistent with a T1-type response, with interferonγ-induced chemokines, and CCC patients display upregulated production of IFNγ and TNFα in comparison to asymptomatic *T. cruzi*-infected individuals (1). In chronically *T. cruzi*-infected Syrian hamsters, intensity of the inflammatory infiltrate correlates with ventricular dilation (unpublished observations). Together, results suggest that chronic myocardial inflammation plays a major role in CCC pathogenesis and progression. High-throughput methods such as DNA microarrays and proteomic analysis disclose global cell/tissue activity, allowing for the identification of novel pathogenic mechanisms. Gene expression profiles of human explant tissues of CCC and DCM were characterized using a the 10,368 element cDNA microarray "Cardiochip", as well as real-time RT-PCR analysis. Immune-response, lipid metabolism, and mitochondrial oxidative phosphorylation genes were selectively upregulated in CCC heart tissue, with the IFNγ pathway playing a central role; 15% of CCC-specific up-regulated genes are IFNγ-inducible. Treatment of cultured cardiomyocytes with IFNγ alone or in the presence of the chemokine CCL2/MCP1 substantially increased the expression of of atrial natriuretic factor (ANF), a gene involved in the cardiomyocyte hypertrophy/heart failure pathway (1). Proteomic analysis of myocardial samples from CCC patients with 2D electrophoresis and MALDI-ToF mass spectrometry to identify proteins based on their tryptic digest spectra has allowed the identification of 300+ proteins (2). Proteins involved in apoptosis, immune system and stress/oxidative stress processes were identified. Protein expression of isoforms of several enzymes involved in the generation or translocation of ATP (ATP synthase α and β chains, aconitase, mitochondrial creatine kinase, creatine kinase M) are reduced in CCC myocardium as compared to normal donors or DCM. Cardiomyocytes from CCC patients may thus have a less eficient energy metabolism, resulting in depletion of cytoplasmic ATP. Significantly, IFNγ has been reported to downmodulate expression of creatine kinase, aconitase, and reduce mitochondrial ATP production in cardiomyocytes. Results support the hypothesis that inflammatory cytokines/chemokines may be involved in regulation cardiomyocyte gene and protein expression, leading to hypertrophy, apoptosis, oxidative stress, and reduced ATP production, which could play a role in the worse prognosis of CCC patients. For that matter, recent results from our group identified functional polymorphisms in genes associated to the control of the inflammatory response that are associated to progression to CCC (3-7). Cytokines/chemokines are thus attractive therapeutic targets in CCC. We tested this hypothesis in the chronically *T. cruzi*-infected hamster model(8). Hamsters were treated for 2 months with the TNF-alpha-blocking agent Etanercept starting 8 mo post- infection. To our surprise, instead of showing an improvement, treated hamsters showed a dramatic worsening of ventricular function and heart failure (9). The use of high-throughput functional genomics and proteomics analysis can speed up the discovery process and lead to the identification of novel pathogenic pathways in CCC. However, the translation of these findings to therapeutics is more complex than originally found.

Supported by CNPq and FAPESP.

References

1. Cunha-Neto E, et al. *Am. J. Pathology*, 2005;167: 305
2. Teixeira PC, et al. *Braz. J Med and Biol Research* 2006;39:1549
3. Drigo SA, et al. *Microbes Infect.* 2006;8:598
4. Ramasawmy R, et al. *J Infect Dis.* 2006;193:1394

5. Ramasawmy R, et al..*Clin Infect Dis*. 2006;43:305
6. Ramasawmy R, et al.*J Infect Dis.* 2007;196:1836
7. Ramasawmy R, et al. *Mol Immunol*. 2008;45:283
8. Bilate AM, et al. *Microbes Infect*. 2003;5:1116
9. Bilate AM, et al.*Microbes Infect*. 2007;9:1104

[October, 2008-10-28 – 14h30 - RUBI ROOM]

RT01B - IMMUNOREGULATORY NETWORKS IN HUMAN CUTANEOUS LEISHMANIASIS: BALANCING ACT FROM CYTOKINES TO SIGNALING

Dutra, W.O.[1], Keesen, T.S.L.[2], Carvalho, E.M.[3], Guimaraes, L.H.[3], Bacellar, O.[3], Machado, P.R.L.[3], and Gollob, K.J.[1]

[1]Universidade Federal de Minas Gerais, Dept. of Morphology; [2]Universidade Federal de Minas Gerais, Dept. of Biochemistry-Immunology, Belo Horizonte, Brazil; [3]Universidade Federal de Bahia, Salvador, Brazil.

Human cutaneous leishmaniasis is a disease caused by the infection with *Leishmania spp*. following a bite from the sandfly, the natural vector of this disease. Tens of millions worldwide are currently infected with *Leishmania* and no effective vaccines have been developed to date. It is clearly important to study the immunoregulatory mechanisms that are induced in humans naturally infected by this parasite if we hope to develop effective vaccines and immunotherapeutic treatments in the future. Cellular immune responses directed against protozoan parasites are key for controlling pathogen replication and disease resolution. However, an uncontrolled, or improperly controlled, response can be deleterious to the host in terms of both allowing for the establishment of pathology, as well as less effective establishment of memory responses. Our laboratory has focused, over the years, on the study of the local and systemic T cell response during the first episode of cutaneous leishmaniasis suffered by individuals before they undergo antimony treatment. Our most recent studies concerning the dichotomy between alpha/beta TCR and gamma/delta TCR expressing, CD4-CD8- (double negative-DN) T cells in the context of a balanced immune response against *Leishmania* have added this population to the list of biologically active T cells that can greatly affect the overall cytokine environment in human leishmaniasis. Moreover, we have shown that the potentially leishmanicidal and pathogenic cellular immune response, characterized by production of IFN-gamma and TNF-alpha in cutaneous disease is accompanied by a regulatory component, characterized by the production of IL-10. Elucidation of the cellular sub-populations responsible for the production of these cytokines, and the activity that these cytokines have on the overall immunoregulatory=-07y1 network is a key component of our work. Understanding the biological activity that these cytokines have on cell populations in human cutaneous disease will aid in our understanding of which components are critical for the formation of a protective immune response with long lasting memory, and what may go wrong in the formation of immune responses that fail to control the pathogen, or lead to excessive pathology. We will present work showing the immunoregulatory networks established in human cutaneous disease, along with the activation state of cytokine induced signaling pathways in human cutaneous disease. Finally, we will discuss the implications of these findings toward our understanding of human leishmaniasis, and possible clues towards intelligent vaccine development based on our knowledge obtained from these and other studies.

[October, 2008-10-28 – 14h30 - RUBI ROOM]

RT01C - NEUROPEPTIDES AS ENDOGENOUS ANTIPARASITIC AGENTS

DELGADO, M; ANDERSON, P; GARCIA-SALCEDO, J.A; CARO, M; GONZALEZ-REY, E

Instituto de Parasitologia y Biomedicina "Lopez-Neyra",
Consejo Superior de Investigaciones Cientificas (C.S.I.C)

Trypanosoma brucei is the causative agent of African sleeping sickness. Available treatments are ineffective, toxic, and susceptible to resistance by the parasite. Here we show that various endogenous neuropeptides act as potent anti-trypanosome agents. Neuropeptides exerted their trypanolytic activity through an unusual mechanism that involves peptide uptake by the parasite, disruption of lysosome integrity and cytosolic accumulation of glycolytic enzymes. This promotes

an energetic metabolism failure that initiates an autophagic-like cell death. Neuropeptides-based treatment improved clinical signs in a chronic model of trypanosomiasis by reducing the parasite burden in various target organs. Of physiological importance is the fact that hosts respond to trypanosome infection producing neuropeptides as part of their natural innate defense. From a therapeutic point of view, targeting of intracellular compartments by neuropeptides suppose a new promising strategy for the treatment of trypanosomiasis.

[October, 2008-10-28 – 14h30 - SAFIRA ROOM]

RT02A - Oxidative stress and mitochondrial dysfunction in Chagas disease

Nisha, N.J.[1] , Wen, J.J[1], Monisha, M. [1], Shivali, S. [1], Galina, V. [1]

[1]Microbiol & Immunol, University of Texas Medical Branch, Galveston,

Chagas disease continues to pose a serious threat to health in Latin America and Mexico, and is the most important emerging parasitic disease in developed countries. Our studies have provided a new framework for understanding the initiation and progression of Chagas disease. Using animal models of T. cruzi infection and disease development, we have discovered that infection by T. cruzi induces activation of inflammatory cells (macrophages, neutrophils) that release cytotoxic reactive oxygen species (ROS)/reactive nitrogen species (RNS) for control of the parasite. Different tissues respond to acute inflammatory oxidative stress by activation of an antioxidant defense that prevents oxidative damage. Mitochondrial injuries in the heart resulted in decreased respiratory chain activity and ATP formation. Inhibition of CIII complex was particularly deleterious, as it maintained an ongoing process of electron leakage and ROS formation, resulting in consistent cellular oxidative damage in the heart and peripheral blood. Phenyl t-Butyl Nitrone (PBN, antioxidant and anti-inflammatory agent) prevented the cellular and mitochondrial oxidative damage in infected host, and preserved the cardiac LV function that otherwise was severely impaired during chronic phase. In other studies, we have shown that mitochondrial dysfunction and oxidant/antioxidant status are increased in the peripheral blood of seropositive human subjects, and these oxidative responses are more prominent in chagasic subjects than in subjects with cardiomyopathy of other etiologies. Together, these data allow us to propose that mitochondrial and cellular oxidative damage contribute to the initiation and severity of Chagas disease, to be discussed in this presentation.

[October, 2008-10-28 – 14h30 - SAFIRA ROOM]

RT02B - *Trypanosoma cruzi* explores CD8 T cell sialylation to dampen Ag-specific CD8$^+$ T cell responses

Adriane Regina Todeschini[1], Leonardo Freire-de-Lima[1], Frederico Alisson Silva[1], Fanny Tzelepis[2], Maurício Martins Rodrigues[2], Christina Maeda Tackyia[3], George Alexandre DosReis[1], José Osvaldo Previato[1], Lucia Mendonça-Previato[1].

[1]Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde-Bloco G, Universidade Federal do Rio de Janeiro, 21944-970, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, Brasil.
[2]Centro Interdisciplinar de Terapia Gênica and Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, Rua Mirasol 207, São Paulo, Brazil.
[3]Instituto de Ciências Biomédicas, Centro de Ciências da Saúde-Bloco G, Universidade Federal do Rio de Janeiro.

Despite a massive and specific CD8$^+$ T cell response to *Trypanosoma cruzi* epitopes, parasite handle to survive within the host, establishing a chronic disease. Our previous studies have shown that co-stimulation of T cells during *T. cruzi* infection can be regulated by a family of sialic acid-binding proteins known as *trans*-sialidase (TS). The active member of TS family (aTS) preferentially catalyzes the transfer of a sialic acid from sialylated donor substrates to acceptors containing a terminal □-galactopyranose moiety. TS activity is capable of sialylate host cell glycomolecules modulating cell invasion and host immunoresponse. Using an inactive analog of aTS, which competes for the same epitopes of its active analog impairing sialic acid transference, and manipulating cell surface sialylation *in vitro*, we have demonstrated that decreased activity of CD8$^+$ T cells from *T. cruzi* infected mice results from increased cell surface sialylation due the *trans*-sialidase activity: (i) CD8$^+$ T cells from *T. cruzi*-infected mice treated with aTS were

$CD44^{high}PNA^{low}$, contrasting with reported data showing that activated splenic $CD8^+$ T cells present a $CD44^{high}PNA^{high}$ phenotype. (ii) Flow cytometry analysis demonstrated that binding of anti-CD43 mAb S7, which binds to sialic acid containing epitopes on the 115-kDa isoform of CD43, was augmented on $CD8^+$ T cell from infected mice treated with aTS, indicating that CD43 is the sialic acid acceptor for TS activity during *T. cruzi* infection. (iii) Cytotoxic activity of antigen experienced $CD8^+$ T cells against the immunodominant synthetic peptide IYNVGQVSI was decreased upon aTS mediated sialylation *in vitro* and *in vivo*. Taken together these results demonstrate, for the first time, that a parasite explores sialylation to attenuate $CD8^+$ T cell rage for peptide/MHCI ligand, implying that sialylation of $CD8^+$ T cell might represent a sophisticated strategy employed by *T. cruzi* to ensure lifetime host parasitism.

[October, 2008-10-28 – 14h30 - SAFIRA ROOM]

RT02C - *Plasmodium*-Host interactions: approaching it from the host side.

Maria. M. Mota

Instituto de Medicina Molecular, Lisboa-Portugal

A potential approach to malaria control is to target mechanisms crucial for the development of *Plasmodium* and/or the pathology caused by its infection. This requires detailed knowledge of the complex host cell-*Plasmodium* interactions. The overall goal of our lab is to elucidate the role of host components in the establishment of a malaria infection (liver stage) as well as in the development of pathology (blood stage).
Both the strong tropism and obligate nature of the events that take place during the liver stage of infection suggest an essential requirement for hepatocyte-specific factors in enabling this complex lead-up to the blood stage. It is therefore of primary interest to identify and characterize the role of such host factors, as these may contribute to the design of rational interventional strategies for the development of novel prophylactic agents. To this end, we have applied a series of microarray and RNA interference screen analysis. We carried out a RNAi screen of the entire human kinome and associated signaling molecules. This strategy identified at least 5 kinases whose down-regulation leads to a marked decrease in infection. We also screened lipoprotein-related host factors, and showed that the class B, type I scavenger receptor (SR-BI) is the strongest regulator of *Plasmodium* infection among these, affecting both sporozoite invasion and intracellular parasite development. Besides the siRNA screens, a microarray-based analysis of host cell genes altered during the course of sporozoite infection identified several host genes that seem to play pivotal roles in the early establishment of a malaria infection. One of those is heme-oxygenase-1 (HO-1). Indeed, abolishing HO-1 activity by gene deletion or siRNA, results in a significant decrease in the liver parasite burden (Epiphanio *et al*., 2008. *Cell Host & Microbe* **3**: 331). Interestingly, we have previously shown that HO-1 plays a crucial role in the control of malaria pathology during the blood stage of infection, as HO-1 expression controls susceptibility to cerebral malaria in mice (Pamplona *et al.*, 2007. *Nature Medicine* **13**: 703). Thus, to our knowledge, HO-1 is the first host molecule to be identified as strongly influencing both the establishment of liver stage malaria and the development of pathology during the blood stage of infection, constituting therefore, an interesting way of the parasite hijacking the host organism.

[October, 2008-10-28 – 14h30 - ESMERALDA ROOM]

RT03A - The changing epidemiological patterns of American Cutaneous Leishmaniasis in Brazil

Shaw, J. J.

University of São Paulo

Identification of *Leishmania* using molecular tools does not require parasite isolation. This is a great step forward and means that field specimens from patients; suspected reservoirs and vectors can be collected using simple methods, thus allowing the collection of more detailed information from isolated regions. Coupled with these technological advances the notification of cases has also been more reliable in recent years. The total number of cases notified in Brazil decreased from 30,030 in 1996 to 22,264 in 2006 and the number per 100,000 inhabitants fell from 19.0 in 1996 to 11.9 in 2006. However, this trend was not uniform in the different regions. Similarly the incidence of the different parasites was not consistent between different macro regions. For instance in eastern Amazonia cases of *L. (Leishmania) amazonensis* are more frequent than in western Amazonia while

other species such as *L.(Viannia) lainsoni* are commoner in western Amazonia. Given the complexity of each epidemiological cycle and the absence of control measures it is only possible to hypothesize that what is driving these differences are such factors as: ecological niche differences in association with global warming that are affecting vector and reservoir populations and changes in man's habits and distribution within the regions.

[October, 2008-10-28 – 14h30 - ESMERALDA ROOM]

RT03B - Polymorphisms on *Leishmania chagasi* DNA – Genomic DNA or kDNA as a molecular marker for population genetics?

Diego Peres Alonso[1], Carlos Henrique Nery Costa[2], Hiro Goto[3] and Paulo Eduardo Martins Ribolla[1]

1- Departamento de Parasitologia – Instituto de Biociências, UNESP, Botucatu, São Paulo.
2- Instituto de Doenças Tropicais Natan Portela, Teresina, Piauí.
3- Instituto de Medicina Tropical, USP, São Paulo, São Paulo.

Leishmania chagasi is the agent of American Visceral Leishmaniasis (AVL) and nowadays is spreading over different regions in Brazil. A typical rural pathogen is now occurring inside populated cities and appearing in regions that were traditionally free of AVL. This change of AVL epidemiology is of great importance and need to be known in order to guide new strategies of control. One important point to be addressed is the occurrence of different *L. chagasi* lineages and the correlation between them and virulence, drug resistance and new cases. Our laboratory is in a crusade to distinguish polymorphisms on *L. chagasi* DNA that could be used as molecular markers of different lineages. Two different kinds of DNA had been surveyed, nuclear DNA and kynetoplast (kDNA). From nuclear DNA we were able to detected some polymorphic microsatellites and SNPs in different genes that are been used to differentiate *L. chagasi* isolates. From the 24 microsatellites described to amplified *L. donovani* DNA, 18 also generate products on *L. chagasi* and, at least, 04 of them are polymorphic in sympatric samples from Teresina, Piaui. Macrophage inhibitor factor (MFI) gene is been surveyed for the presence of SNPs and could represent an interesting target to differentiate epidemiological relevant lineages.

By the other way, kDNA, particularly mini-circle DNA, seems to be highly polymorphic and represent a potent tool to differentiate the isolates. PCR-RFLP of whole mini-circles with RsaI, HpaII and HaeII enzymes showed the occurrence of several polymorphic products from sympatric samples. Sequence of cloned PCR products showed that mini-circle population seems to change depending on parasite life form. This event could be linked to different necessities of the parasite to control different genes, by RNA editing, depending on the life cycle. Another interesting event is the occurrence of mini-circles sequences into nuclear DNA, demonstrating that this molecule could represent a transposon-like molecule and also could be related to nuclear RNA editing.

[October, 2008-10-28 – 14h30 - ESMERALDA ROOM]

RT03C - IMMUNE RESPONSE AGAINST VARIANT ANTIGENS OF *PLASMODIUM FALCIPARUM* IN BRAZILIAN SETTINGS: NO CLEAR RELATION TO ASYMPTOMATIC INFECTION OUTCOMES

Alessandra Fratus[1], Letusa Albrecht[1], Márcia Melo Medeiros[1], Catarina Castinheiras[1], Simone L. Andrade[1], Rosemeire dalla Martha[2], Luiz H. Pereira da Silva[2], Fabiana P. Alves[1], Erney FP Camargo[1] and Gerhard Wunderlich[1]

[1]Department of Parasitology, Institute for Biomedical Sciences, USP, [2]CEPEM, Porto Velho

In many regions of the Brazilian Amazon, infections with *Plasmodium falciparum* show relatively mild malaria outcomes when compared to holoendemic settings such as sub-Saharan Africa. In order to elucidate if parasite related factors play a role in this markedly different picture, we have sequenced approximately 3000 plasmid clones containing fragments of the most important virulence-associated gene family (*var*) of *P. falciparum*. In contrast to other locations, the *var* gene family seems limited and we estimate that only 300 different *var* genes may be present in parasites from the Amazon. Many *var* genes were shared over time and in isolates from differing geographic origins. Specific *var* tags seemed even shared in their chromosomal position as revealed by pulsed field gel electrophoresis and a significant number of tags were found in up to 25 from 55 analyzed isolates. In order to measure the response against the *var* encoded PfEMP1s – previously associated with protection – we

expressed highly shared sequences as recombinant proteins and measured the humoral response against them. In general, all individuals reacted only weakly against the variant proteins while most sera recognized a control merozoite antigen run in parallel. Coincidently to what is found in previous studies from Africa, asymptomatic individuals reacted more against the variant proteins, while non-infected or oligosymptomatic individuals from the same area reacted poorly. In contrast, sera from patients with full-blown malaria reacted more than any other group. Additionally, a significant number of non-symptomatic subjects did not react against any antigen, suggesting that the response against variant antigens may be dispensable for an effective immune response in natural infections in the Brazilian Amazon.

Support: Fapesp

[October, 2008-10-29 – 14h30 - RUBI ROOM]

RT04A - POLY-DINUCLEOTIDE IN TRITRYP GENOMES

DUHAGON, M.A.[1], SMIRCICH, P.[1], PASTRO, L.[1], FORTEZA, D.[1], NAYA, H.[2], GARAT, B.[1]

[1] Laboratorio de Interacciones Moleculares, Facultad de Ciencias, Montevideo, Uruguay; [2] Bioinformatics Unit, Institut Pasteur de Montevideo, Montevideo, Uruguay

The human parasitic pathogens, *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania major* (Tritryp) constitute a very early branch in eukaryotic evolution, presenting several remarkable deviations from standard eukaryotic paradigms. In fact, though in most eukaryotes transcription initiation constitutes the major level of regulation of gene expression, Tritryps show little control of transcription initiation for protein coding genes and the *cis*-elements governing the basal transcription machinery assemble are still elusive. Trypanosome genes are arranged in large co-directional clusters, probably transcribed from the strand switch region between two divergent gene clusters. Alternatively, transcription could result from initiation at random sites along the co-directional cluster through transient changes favoring the open complex formation. In this context, regional DNA conformational dynamics could have particular relevance in the molecular mechanisms driving transcription. Local conformational changes are also involved in other DNA processes such as replication, recombination and repair. Dinucleotides repeats are highly frequent in intergenic regions; they can adopt different structural conformations and several relevant biological roles have been ascribed to them, including regulation of gene expression. Here we present an analysis of the frequency, length and distribution of poly-dinucleotides in the Tritryp genomes. We could establish that most types of poly-dinucleotide are significantly more abundant than what is expected by chance. We also found an asymmetrical distribution between coding and non-coding strands for some complementary dinucleotide repeats, which could implicate a role of these sequences in directional nucleic acid processes. Then, we addressed the localization of the dinucleotide repeats along the chromosome analyzing both the strand switch regions and the distribution within the co-directional clusters. The distribution of the poly[dT-dG].poly[dC-dA] sequences suggested its putative role as a signal in gene expression. The validity of such proposal was studied by experimental approaches in *T. cruzi*. The evaluation of the ability to establish interactions with *trans*-acting factors was followed by electrophoretic mobility shift assays. Specific complexes exhibiting significant affinity for each of the complementary single stranded poly-dinucleotide probes were found. In addition, transfection assays using episomal vectors showed that the insertion of each complementary poly-dinucleotide at the untranslated regions of reporter genes significantly affected the reporter gene expression..

[October, 2008-10-29 – 14h30 - RUBI ROOM]

RT04B - DEVELOPMENTALLY REGULATED SPHINGOLIPID SYNTHESIS IN AFRICAN TRYPANOSOMES

SUTTERWALA, S.S.[1], HSU, F.F.[2], SEVOVA, E.S.[1], SCHWARTZ, K.J.[1], ZHANG, K.[2], KEY, P.[2], MENON, A.K.[3], TURK, J.[2], BEVERLEY, S.M.[2] AND BANGS, J.D.[1]

[1]University of Wisconsin School of Medicine and Public Health, Madison, WI, USA [2]Washington University School of Medicine, St Louis, MO, USA, [3]Weill Cornell Medical College, New York, NY, USA

Sphingolipids are essential structural components of eukaryotic membranes, and also function in many cellular processes, including cell signaling

and protein trafficking. Sphingolipid synthesis begins in the endoplasmic reticulum (ER) with the condensation of serine and palmitate to form long chain base. Subsequent *N*-acylation and desaturation in the ER generates ceramide, which is delivered to the Golgi for conversion to complex sphingolipids by addition of polar head groups, either phosphoryl compounds from glycerolphospholipid donors, or hexoses from sugar nucleotide donors. Many unicellular eukaryotes, including kinetoplastid protozoa, are thought to synthesize exclusively inositol phosphorylceramide (IPC) as their major phosphosphingolipid. In contrast mammalian cells synthesize primarily choline phosphorylceramide (sphingomyelin), and consequently there is considerable interest in targeting parasite sphingolipid metabolism for novel therapeutics. Consistent with major differences between kinetoplastids and mammals, *Leishmania* promastigotes only require sphingolipid synthesis as a precursor for the production of ethanolamine for anabolic purposes. Pharmacological inhibition of the first enzyme of the sphingolipid pathway (serine palmitoyltransferase) is lethal in cultured African trypanosomes (*Trypanosoma brucei*), and this lethality can be rescued with exogenous long chain base, but unlike *Leishmania* cannot be rescued with ethanolamine. Thus African trypanosomes require de novo synthesis of sphingolipids for purposes other than generation of essential ethanolamine. To further investigate this issue we have characterized complex sphingolipid composition and synthesis in *T. brucei*, and a trypanosome sphingolipid synthase gene family (*TbSLS1-4,* Tb09.211.1030-Tb09.211.1000) that is orthologous to *Leishmania* IPC synthase (LmjF35.4990). Using ESI mass spectrometry we find that procyclic trypanosomes contain IPC, but also sphingomyelin, while surprisingly bloodstream stage parasites contain sphingomyelin and ethanolamine phosphorylceramide (EPC), but no detectable IPC. In vivo fluorescent ceramide labeling confirmed stage specific biosynthesis of both sphingomyelin and IPC. Transgenic expression of *TbSLS4* in *L. major* promastigotes resulted in production of sphingomyelin and EPC indicating that the *TbSLS* gene family has bi-functional synthase activity. In situ epitope tagging indicates, as expected, that the TbSLS4 gene product is located in the Golgi. The *TbSLS* gene family is expressed constitutively in the *T. brucei* life cycle, and pan-specific RNAi silencing in bloodstream stage parasites led to rapid growth arrest and eventual cell death, confirming the need for complex sphingolipid synthesis in African trypanosomes. Ceramide levels were increased >3-fold by silencing suggesting toxic downstream effects mediated by this potent intracellular messenger. Interestingly trafficking of glycosylphosphatidylinositol anchored proteins was unaffected. Topology predictions support a revised six transmembrane domain model for the orthologous kinetoplastid sphingolipid synthases consistent with the proposed mammalian SM synthase structure. This work reveals novel diversity and regulation in sphingolipid metabolism in this important group of human parasites, highlights significant differences among kinetoplastid protoza, and raises intriguing questions concerning the evolution of sphingolipid synthesis in eukaryotic taxa.

[October, 2008-10-29 – 14h30 - RUBI ROOM]

RT04C -GENE EXPRESSION AND DNA REPAIR IN TRYPANOSOMA CRUZI: IMPLICATIONS IN GENETIC DIVERSITY AND THE PATHOGENESIS OF CHAGAS DISEASE

Campos PC[1], Machado-Silva A[1], Araújo PR[1], DaRocha WD[1],.Cerqueira GC[1], Almeida RM[1], Furtado C[1], Pais FS[1], McCulloch R[3], Bartholomeu DC[2], Machado CR[1] and Teixeira[1] SMR.

[1]Departamento de Bioquímica e Imunologia e [2]Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil; [3]Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow, U.K.

T. cruzi, the protozoan that causes Chagas disease, has a heterogeneous population which is divided into three main lineages, named *T. cruzi* I, II and III. Such division are based upon extensive polymorphism analyses of various loci, including *msh2,* a gene encoding a key component of the mismatch repair pathway (MMR). Molecular phylogeny, including analysis of multigene families, indicates that Tc II and hybrid strains present greater genetic variability when compared to Tc I strains. Since the MMR pathway is one of several mechanisms affecting the levels of genetic diversity within a given population, we investigated the role of MSH2 and the existence of differences in MMR among *T. cruzi* strains. Treatment with genotoxic agents, as well as *in vitro* studies with recombinant *Tc*MSH2 protein, suggest that strains belonging to Tc II present a less efficient MMR when compared to Tc I strains. Attempts to knock out both *T. cruzi* msh2 alleles were unsuccessful,

suggesting that the Tcmsh2 gene is essential and may be multifunctional. Also unexpectedly, we were not able to complement the MMR pathway in *Trypanosoma brucei* msh2-null mutants through heterologous expression of MSH2 from different *T. cruzi* strains. However, the sensitivity to oxidative stress phenotype could be reverted in *T. brucei* transfectants. Taken together, our results suggest that trypanosome MSH2 may be directly involved in the response to oxidative damage in a manner that is independent of MMR. They also support the hypothesis that a less efficient MSH2 from Tc II results in greater genetic variability in these strains. Since epidemiological studies indicate a preferential association of *T. cruzi* strains II with Chagas disease, differences in genetic variability, determined in part by differences in the DNA repair machinery, may constitute one of the factors that have contributed to the adaptation of these strains to the new human host. We have also studied differences in gene expression during the parasite's life cycle in order to better understand the molecular basis of host-parasite interaction. It is well established that, in trypanosomatids, gene regulation occurs almost exclusively at the transcriptional level. To characterize the regulatory elements involved in controlling mRNA levels, first we investigate the sequence requirements involved in *T. cruzi* mRNA processing by mapping all available ESTs and cDNAs containing poly(A) tail and/or the Spliced Leader sequence to genomic intergenic regions using the *T. cruzi* genome database. These analyses will allow in silico prediction of the *T. cruzi* mRNA processing products and the identification of a large number of untranslated regions in T. cruzi mRNAs, for which no EST/cDNA is currently available and which could be developmentally regulated. Next, we developed a transfection vector that allowed expression of two reporter genes which may be linked to sequences derived from constitutive as well as from stage-specific mRNAs. Using alfa-tubulin, amastin and MASP genes as gene models that are up-regulated in epimastigotes, amastigotes and trypomastigotes, respectively, we showed that sequences present in the 3' UTR of these genes are responsible for the stage-specific expression regulation of these abundant *T. cruzi* proteins.

[October, 2008-10-29 – 14h30 - RUBI ROOM]

RT04D - "Domestication" of extinct degenerate retroposons for the control of post-transcriptional gene expression in the parasitic protozoan *Leishmania*

PAPADOPOULOU, B[1], MÜLLER, M[1], SMITH, M[1], ROCHETTE, A[1], BRINGAUD, F[2]*

[1]Research Centre in Infectious Disease, CHUL Research Centre and Department of Medical Biology, Laval University, Quebec, Canada, G1V 4G2

[2]Laboratoire de Résonance Magnétique des Systèmes Biologiques, Université Victor Segalen, UMR-5536 CNRS, Bordeaux Cedex, France

Leishmania belongs to a group of unicellular parasitic protozoa that cause important diseases in humans and exhibit several interesting features regarding genome organisation and regulation of gene expression. In the absence of transcriptional control, regulation of gene expression occurs exclusively post-transcriptionally in these parasites, and 3'-untranslated regions (3'UTRs) are major players in this mode of regulation. Using state-of-the-art bioinformatic analyses, we have recently identified a new family of extinct short degenerate retroposons (~2,000 copies) named SIDERs (**S**hort Interspersed DEgenerate Retroposons) in the genomes of several *Leishmania* species but not of other protozoa parasites. SIDERs constitute the largest family of transposable elements described so far in the trypanosomatid genomes and can be divided into 2 subfamilies (SIDER1 and SIDER2) depending on their primary sequence. SIDER elements are quite uniformly dispersed throughout all analyzed *Leishmania* genomes and their distribution is greatly syntenic. The majority of SIDERs are located within 3'UTRs, yet 30-40% of strand-switch regions that characterize a changing orientation of directional gene clusters contain a SIDER. Several lines of evidence indicate that SIDERs participate in large RNA networks, which can be distinct for SIDER1 and SIDER2. DNA microarray analyses, reporter gene assays and polysome profiling showed that members of the SIDER1 subfamily can increase mRNA translation efficiency in a stage-specific manner (*Leishmania* cycles between extracellular promastigotes in the insect vector and intracellular amastigotes in the vertebrate host). On the other hand, members of the SIDER2 subfamily promote mRNA destabilization. Interestingly, several SIDER2-containing transcripts are low abundant and short-lived. Further investigations on the mechanism by

which SIDER2 elements degrade mRNA indicated that the signature sequence, common to all trypanosomatid retroelements, (first 160 nt of SIDER2) is necessary and sufficient for mRNA destabilization. Degradation of SIDER2-containing transcripts is deadenylation-independent and involves most likely a novel mechanism of mRNA decay likely to be mediated through an endonucleolytic cleavage. SIDER2-mediated degradation can be blocked when providing SIDER2 in an antisense orientation allowing the formation of RNA duplexes. We are currently investigating the molecular mechanisms of SIDER-mediated gene expression in *Leishmania*. The considerable expansion of SIDERs within 3'UTRs and their high diversity supply *Leishmania* species with novel genetic material, which could be exapted into diverse regulatory functions, and consequently help the parasites gain an evolutionary edge.

[October, 2008-10-29 – 14h30 - SAFIRA ROOM]

RT05A - BENZNIDAZOLE TREATMENT OF PATIENTS IN THE CHRONIC PHASE OF CHAGAS DISEASE: A 10-YEAR PROSPECTIVE STUDY

ELIANE DIAS GONTIJO[1], GLÁUCIA ANDRADE[2], SILVANA ELOI-SANTOS[3], LÚCIA M. C. GALVÃO[4]

[1]Departamento de Medicina Preventiva e Social, Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG); [2]Departamento de Pediatria, Faculdade de Medicina, UFMG; [3]Departamento de Propedêutica Complementar, Faculdade de Medicina, UFMG; [3]Departamento de Parasitologia, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Minas Gerais, Brasil

The etiological treatment of the chronic phase of Chagas disease is justified by the expectation that through the eradication of the parasite the progression to Chagas heart disease is reduced. Since 1997, researchers from the Chagas Disease Clinic of the Hospital das Clínicas of the Federal University of Minas Gerais, following recommendations of the Ministry of Health of Brazil, have been conducting a prospective study to test the hypothesis that specific treatment can impact the evolution of the disease. In addition, this study was also aimed at evaluating the efficiency of parasitological and serological methods as criteria of cure and characterizing the immunological responses of the treated patients. In a cohort of 162 adult patients, 86 (53%) were treated with Benznidazole (BZ) and the remaining were used as controls. Adverse side effects were observed in 42.8% of the treated individuals, mostly cutaneous reactions (56%) and polineuropathy (13.5%). Four patients presented leukopenia (total leukocytes < 3,900); in two of these patients systemic alterations were observed (one individual with 1,600 leukocytes, 400 neutrophils and angina; and the other with 2,700 leukocytes and furunculosis). In general the manifestations were mild and reversible. Only 2.4% were considered more serious. So far a reliable technique to assess cure of Chagas disease is lacking. The efficacy of BZ treatment has been evaluated mostly by hemoculture and serology. However, hemoculture has a limited sensitivity in detecting circulating parasites. In fact, hemocultures were positive in the basal time in 54% of the treated patients and 57% of the controls. Five years after treatment, hemocultures were negative in 86% of the treated individuals. In 31 treated patients cure was monitored simultaneously by hemoculture, PCR to kDNA, lysis mediated by complement (LMCo) and recombinant complement regulatory protein (rCRP) ELISA. Below we present the results of the four tests performed before treatment (BT) and four years after treatment (AT). Hemoculture: BT, 51.6% - AT, 0%; PCR: BT, 77.4% - AT, 50%; LMCo: BT, 100% - AT, 33.4%; rCRP ELISA: BT, 100% - AT, 33.4%. The data show that each test has a different sensitivity. Evaluation of cure by conventional serology (CS) presents a certain degree of difficulty since CS remains positive for many years after treatment in a large percentage of patients, including those who have repeatedly negative parasitological results. We employed flow cytometry to monitor serological titers in 16 patients five years post-treatment. In 4 patients we verified the decrease in antibody titers, suggesting possible cure, whereas CS only detected the negative seroconversion in two cases. Taken together, the data indicate that the finding of a test that can fulfill the role of evaluating the efficacy of Chagas disease treatment continues to be a major challenge. Immunologic events that occur in Chagas disease are multiple and include diverse effectors and regulatory processes, which result in a balance between resistance and pathogenesis. We investigated the effect of specific therapy on the immune state of the patient. The analysis of phenotypic markers of the T lymphocyte population and CD4+ and CD8+ subpopulations did not shown significant variation after treatment, nevertheless higher activation status was observed

in innate immune cells. The temporary or persistent decrease of parasitemia induced by BZ-treatment seems to be able to modify lymphocyte activation, a phenomenon involved in the pathogenesis of Chagas disease. The evaluation of the impact of specific therapy on preventing disease evolution is in progress and needs extended clinical follow-up. Nevertheless the persistence of negative parasitemia in chronic treated patients strengthens the beneficial effect of the etiological treatment.

[October, 2008-10-29 – 14h30 - SAFIRA ROOM]

RT05B - A MECHANISM FOR CROSS-RESISTANCE TO NIFURTIMOX AND BENZNIDAZOLE IN TRYPANOSOMES

WILKINSON, S.R.

Queen Mary University of London, London EC1 4NS UK

Nifurtimox and benznidazole are the front line drugs used to treat Chagas disease, the most important parasitic infection in the Americas. These agents function as pro-drugs and must be activated within the parasite to have trypanocidal effects. Despite more than 40 years research, the mechanism(s) of action and resistance have remained elusive. Here, we report that in trypanosomes, both drugs are activated by a NADH-dependent, mitochondrially-localised, bacterial-like, type-I nitroreductase (NTR), and that down-regulation of this enzyme readily explains how resistance may emerge. Loss of a single copy of this gene in *Trypanosoma cruzi*, either through in vitro drug selection or by targeted gene deletion, is sufficient to cause significant cross-resistance to a wide range of nitroheterocyclic drugs. In *Trypanosoma brucei*, loss of a single NTR allele confers similar multi-drug resistance without affecting parasite growth rate or the ability to establish an infection. This potential for drug-resistance by a simple mechanism has important implications, since nifurtimox is currently undergoing phase III clinical trials against African trypanosomiasis.

[October, 2008-10-29 – 14h30 - SAFIRA ROOM]

RT05C - *TRYPANOSOMA CRUZI* INFECTION DOES NOT CORRELATE WITH PARASITE DRUG SENSITIVITY

BIANCA ZINGALES

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brasil

Benznidazole (BZ) is one of the two drugs used to treat Chagas disease. Nevertheless, in a number of patients the treatment is inefficient. Although natural resistance of *T. cruzi* to nitroderivatives is described as an important factor to explain therapeutic failures in humans, this hypothesis was never demonstrated. To investigate such possibility, we standardized an *in vitro* test to quantify the drug activity. For twelve *T. cruzi* laboratory strains, the IC_{50}, corresponding to the drug concentration which inhibits parasite growth by 50%, varied from 7 to 127 µM. In parasites sub-cultured during four months the IC_{50} value showed minimal fluctuations. We found no correlation between the IC_{50} values and the phylogenetic subdivision in the two *T. cruzi* major groups. The test was then applied to isolates retrieved from seven chronic patients submitted to BZ therapy in 1999: four females and three males, of age between 25 and 51-years old. All the patients had positive hemoculture and positive serology when treatment was started; all of them completed treatment and neither of them traveled to endemic areas during the follow-up. On different times after treatment, hemocultures were performed and the presence of parasites and anti-*T. cruzi* antibodies was scored. The IC_{50} of the pre-treatment isolates from three patients considered cured by several criteria varied from 19 to 35 µM. Similarly, pre-treatment isolates from four non-cured patients showed IC_{50s} of 15.6 to 51 µM. We compared BZ susceptibility of the pre- and post-treatment isolates of the four non-cured patients. The isolate from one patient maintained the IC_{50} value, whereas in two patients a 2-fold decrease and in one patient a 3-fold increase in BZ susceptibility was observed in the post-treatment strains. Possible selection of parasite sub-populations during or after treatment was ruled out based on the profile of nine microsatellite *loci*. Nevertheless, we can not exclude the possibility that the original infecting strain was composed by sub-populations of the same genetic make-up but with different susceptibilities to BZ, or that specific mutations or amplification/deletions of genes related to

increased/decreased BZ resistance occurred. A few genes were described to be involved in *in vitro*-induced BZ resistance, but not in the natural resistance phenotype. To identify the differential expression of genes in naturally resistant and susceptible strains, we employed DNA microarrays covering ~8,000 CL Brener ORFs (PFGRC). Among the genes up-regulated in the resistant strain, we chose the gene that codes for one ABC-transporter for further studies. This gene shows high similarity with homologous genes of *Leishmania* species and *T. brucei.* The transcript abundance of the ABC-transporter was, on average, three-fold higher in five naturally resistant strains as compared to three sensitive strains. The relative abundance of ABC-transporter transcripts in parasites isolated from patients submitted to BZ treatment and who showed therapeutic failure was also determined. The data indicate a direct correlation between the ABC-transporter RNA abundance and the IC_{50} values of the isolates obtained before and after treatment. In conclusion, our results show for the first time that the susceptibility to BZ of the infecting *T. cruzi* population is not predictive of cure and support the notion that, in addition to the direct role in parasite clearance, the effect of BZ on the host immune response may determine chemotherapy efficacy. We also propose that this ABC-transporter may be one of the components involved in natural drug resistance in *T. cruzi*. Support: FAPESP; Edital MCT/CNPq/MS-SCTIE-DECIT 25/2006 - Estudo de Doenças Negligenciadas.

[October, 2008-10-29 – 14h30 - SAFIRA ROOM]

RT05D - IMMUNOLOGICAL INSIGHTS BEYOND THE BENEFITS OF BENZNIDAZOLE TREATMENT OF CHAGAS DISEASE

SATHLER-AVELAR, R.[1,2], VITELLI-AVELAR, D.M.[1], DE LANA, M.[3], DIAS J.C.P.[1], GONTIJO E.D. [2], ELÓI-SANTOS, S.M.[2], TEIXEIRA-CARVALHO, A.[1], MARTINS-FILHO, O.A.[1]

[1]CPqRR-FIOCRUZ, [2]FM-UFMG [3]NUPEB-EF-UFOP

Specific chemotherapy is recommended for the treatment of Chagas disease applying the general assumption that the earlier the specific treatment is initiated the greater the chance of parasitological cure and lower is the disease progression to heart-related pathology. At present, Chagas disease chemotherapy in Brazil has been restricted to the use of Benznidazole, which is recommended for the treatment of acute, congenital and the initial stage of the indeterminate form, usually seen in children and adolescents. Despite the well known role of immunological mechanisms in the pathogenesis of Chagas disease, little has been reported about the impact of Bz-treatment on the host immune response. It has been demonstrated that after Bz-treatment, cured patients produce high levels of IFN-γ. However, as IFN-γ may also favor the development of pro-inflammatory response, it is possible that a fine balance of pro- and anti-inflammatory cytokines could be the key to control morbidity following Bz-treatment. In order to confirm this hypothesis, we have performed a short-term longitudinal investigation to evaluate the impact of Bz-treatment on the host immunological status during early (E-IND) and late (L-IND) indeterminate Chagas disease. Our results demonstrated that in E-IND, despite the Bz-treatment was associated with higher frequency of activated monocytes ($CD16^+CD14^+$ and HLA-$DR^{High}CD14^+$ cells), there was a negative correlation between these activation phenotypes and the frequency of $IL\text{-}12^+CD14^+$ monocytes. Moreover, Bz-treatment triggered high frequency of circulating $CD3^-CD16^+CD56^-$ NK cells, besides high NK-cell activation status ($CD69^+CD16^+$ cells), but associated with a type 1-modulated cytokine pattern (IFN-γ^+ and IL-4^+ NK-cells). In the adaptive immunity compartment, the Bz-treatment induced substantial T and B-cell activation status associated with an overall IL-10 modulated type-1 cytokine profile. These results suggest that despite the increased number of activated leukocytes in the peripheral blood, Bz-treatment of E-IND may also involve a qualitative change in their functional capacity that drives their activation state toward a modulated cytokine profile. Furthermore, our findings gave evidence that NK cells and $CD8^+$ T lymphocytes are the major sources of IFN-γ in Bz-treated E-IND. Moreover, our data have also brought additional information, pointing out IL-10 production mainly by $CD4^+$ cells and B lymphocytes, as the putative key element for parasite clearance in the absence of deleterious tissue damage. Therefore, the presence of activated leukocytes in the peripheral blood would not be a limiting factor for the etiological treatment of E-IND, considering the overall type-1 modulated cytokine profile induced by Bz-treatment. Aiming to substantiate whether this phenomena may also occur following Bz-treatment of L-IND, we evaluated the cytokine pattern of circulating leukocytes of L-IND before and after Bz-treatment. Our data pointed out that in the control cultures, all

circulating leukocytes subpopulations from Bz-treated L-IND displayed an overall down-regulation of both inflammatory and regulatory cytokine. However, upon *in vitro* stimulation with *T. cruzi* antigens, the peripheral blood leukocytes from Bz-treated L-IND displayed a shift toward an overall type-1 modulated cytokine pattern, with increased levels of IL-12^{+}CD14^{+}, TNF- α^{+}CD14^{+}, TNF-α+ and IFN-γ^{+}CD16^{+} cells and IFN-γ^{+}CD8^{+} T-cells counterbalanced by higher levels of IL-10 from monocytes and IL-4 from NK cells. Taken together, our findings pointed out that regardless the particularity of the immune response observed in E-IND and L-IND after Bz-treatment, the ability NK and CD8^{+} T-cells to produce IFN-γ^{+} represent a general event following BZ-therapeutic intervention of Chagas disease but however, highlighted that a non-polarized cytokines pattern is the hallmark of Bz-treated patients during both, early and late chronic Chagas disease..

[October, 2008-10-29 – 14h30 - ESMERALDA ROOM]

RT06A - Lipids Derived from Insect Vectors: Signaling Molecules and Metabolic Precursors.

Georgia C. Atella

In the last 10 years our group has focused the biological dynamics of the main lipid-transfer particle of insect hemolymph, Lipophorin (Lp). Lp-mediated lipid redistribution in insect hemolymph achieves the metabolic needs of each organ. However, insect hemolymph is also the site used for some specific phases of several pathogen´s life cycle. We demonstrated for the first time Lp uptake by a living parasite, *Trypanosoma rangeli*, while crossing the vector hemolymph in its way to salivary glands. We have also shown that malaria parasite hemolymphatic stages are also able to remove lipids from *Anopheles gambiae*-Lp. Pathogen transmission by blood-sucking arthropods may involve the invasion of salivary glands. Lp is also able to deliver lipids to such tissue. We have recently shown (Golodne et al., J. Biol Chem. 2003, 278:27766-27771) that *Rhodnius prolixus* salivary glands store lysophospholipids which are powerful modulators of cell signaling in mammalian cells. Lysophosphatidylcholine (LPC) isolated from either salivary glands or spit saliva blocks platelet aggregation and induces nitric oxide synthesis in cultured porcine endothelial cells. In vitro incubation of murine macrophages with saliva or isolated LPC increases the association of T. cruzi with these cells. In vivo both saliva and LPC increase *T. cruzi* blood parasitemia. Enhancement of host infection by vector-derived or pathogen-derived bioactive lipids is not confined to Chagas disease transmission. *Schistosoma mansoni* cercariae secrets LPC and a phospholipase A2 durings its transformation to schistossomula. In conclusion we have gathered evidences in the previous years that lipid dynamics and specially LPC biology act as multidirectional molecules which modulates either vector metabolism as well as host signaling pathways in several different cells and help pathogen infection. Detailed molecular investigation of such processes in incoming years may lead to novel lipid-based mechanisms to block disease transmission by blood-sucking arthropods.

[October, 2008-10-29 – 14h30 - ESMERALDA ROOM]

RT06B - NEUROPEPTIDES AND NUTRITIONAL REGULATION OF JUVENILE HORMONE SYNTHESIS IN MOSQUITOES

Noriega, F.G., Nouzova, M. Mayoral, J.G.

Florida International University, Miami, Florida, USA

Juvenile hormone (JH) is a major hormonal regulator in insects. In the female mosquito, JH signals the completion of the ecdysis to the adult stage, and initiates reproductive processes. The aims of our studies are 1) to understand the regulation of juvenile hormone levels in mosquitoes. 2) To understand how nutritional signals affect the activity of the neuroendocrine system. JH titer is essentially determined by the rate at which the corpora allata (CA) synthesizes JH. The rate of CA activity is, in turn, regulated by allato-regulatory peptides that exert either allatostatic (inhibitory) or allatotropic (stimulatory) activities. We have described that *Aedes aegypti* allatotropin (AT) stimulates and *Ae. aegypti* allatostatin-C (AS-C) inhibits JH synthesis; in addition we have showed that nutrients accumulated during the larval stages regulate the CA activity in newly emerged adults. Based on this work we propose that AT and AS-C released by the brain are essential for the activation and modulation of JH synthesis in adult female mosquitoes. The synthesis and release of these peptides is connected to nutritional signals. JH is therefore an important part of a transduction mechanism that connects changes in the nutritional status with activation of specific physiological events during reproduction. We are

using molecular and biochemical tools to investigate the rate limiting steps and regulatory points on JH synthesis, as well as the mechanism of action of allatoregulatory peptides.

[October, 2008-10-29 – 14h30 - ESMERALDA ROOM]

RT06C - Reactive oxygen species in the midgut of blood-sucking insects

Oliveira JHM[a], Leite MS[a], Conceição R[a], Masuda CA[a] Gandara AC[a], Lara FA[b], , Paiva-silva GO[a], Oliveira PL[a].

(a) Instituto de Bioquímica Médica, Programa de Biologia Molecular e Biotecnologia, Universidade Federal do Rio de Janeiro, CEP 2a94a-590, Rio de Janeiro, Brazil; (b) Instituto Oswaldo Cruz, Laboratório de Microbiologia Celular; FIOCRUZ, Rio de Janeiro, Brazil.

Besides the toxic effects of reactive oxygen species (ROS) it has been shown that these molecules have important roles in a wide range of physiological processes, from signal transduction, modulation of cellular response to stress and infection and microbial killing. Blood feeding insects generate high concentrations of heme in their midguts as a consequence of hemoglobin degradation. Heme is a pro-oxidant molecule, which redox activity depends on the interaction with ROS produced by oxidative cell metabolism (mitochondrial respiration, NADPH oxidase, among others). Concerning this scenario the aim of our work is to characterize the production of ROS in the midgut of *Ae aegypti* and determine its possible roles in the local immunity. Using redox sensitive dyes we showed that high ROS levels are found in the gut lumen of sugar-fed *Ae aegypti* females, which are immediately reduced after a blood meal. These oxygen radicals are reduced by DPI, a flavin-based oxidase inhibitor. A similar pattern was found in *Rhodnius prolixus*, an insect vector of Chaga's disease, with a reduction in radical formation in the posterior midgut, the segment of digestive apparatus where hemoglobin degradation takes place. In the cattle tick, *Boophilus microplus*, hemoglobin is taken up by midgut cells by means of receptor-mediated endocytosis and digestion is intracellular. In tick cells, however, intense reduction of the amount and respiratory activity of mitochondria – a major source of oxygen radicals – seems to parallel hemoglobin accumulation and digest cell maturation. Taken all together, our data suggest that the control of radical production is a major antioxidant mechanism that may be operating in the gut of hematophagous invertebrates, helping to ameliorate toxic effects of heme.