MINI-CONFERENCES

[November, 2005-11-07 - 17h30 - ROOM A]

MC01 - The epigenetic control of antigenic variation in Plasmodium falciparum

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Much of what is known about antigenic variation in the human malaria parasite P. falciparum has been established by studying phenotypic changes at the surface of parasitized red blood cells. Although this has contributed to our fundamental understanding of immune escape nothing conclusive has been elucidated about the molecular mechanisms that determine activation and silencing of members of the antigenic variation var gene family. Variation at the surface of P. falciparum-infected erythrocytes is mediated by the differential control of a family of surface antigens encoded by var genes. Switching of var gene expression occurs in situ, mostly from telomere-associated loci, without detectable DNA alterations, suggesting that it is controlled by chromatin structure. We have identified chromatin modifications at telomeres that spread far into telomere-proximal regions including var gene loci (over 50 Kb). One type of modification is mediated by a protein homologous to yeast Sir2 called PfSir2, which forms a chromosomal gradient of heterochromatin structure and histone hypoacetylation. Upon activation of a specific telomere-associated var gene, PfSir2 is removed from the promoter region and acetylation of histone H3 and H4 occurs. Our data demonstrate that mutually exclusive transcription of var genes is linked to the dynamic remodeling of chromatin.

[November, 2005-11-07 - 17h30 - ROOM B]

MC02 - Drug discovery and development for trypanosomiasis and leishmaniasis: new and alternative strategies

Simon L. Croft *(Drugs for Neglected Diseases initiative)*

The past two decades have seen considerable advances in our knowledge of the biochemistry and cell biology of the three pathogens that cause leishmaniasis or trypanosomiases, culminating in the publication of the genome sequences of these organisms in July 2005. Many new potential drug targets are now accessible and more methods are available for the rapid validation and characterization of targets. There is also renewed interest in drug discovery and development in academia and industry. There are solutions to the previously identified problems of: (i) access to HTS, (ii) increased input from the disciplines of chemistry, pharmacology, toxicology and pharmaceutics to complement these advances in molecular biology, (iii) development of suitable disease models and (iv) methods for progressing leads and candidate drugs through pre-clinical studies. So why has there been such limited progress in drug R & D for trypanosomiasis and leishmaniasis in the past two decades? And does the situation look better for the future? A major barrier for drug R & D for trypanosomiasis and leishmaniasis has been both (i) the lack of economic incentives in the current, for-profit, model of drug development, for the pharmaceutical industry to be fully engaged, and (b) the absence of alternative models with the appropriate expertise. The estimates of the cost of development of a new chemical entity (NCE) $800 million US are prohibitive for neglected diseases, especially when only 10% of the current global heath research and development effort is directed to address the medical needs of 90% of the human population, a result of both market failure and inadequate public policies in endemic countries. The cost of development of drugs for tropical infectious diseases could be significantly lower and real estimates need to be made. Increased awareness of this situation has led to the formation of several not-for-profit product development partnerships (PDPs), such as the Drugs for Neglected Diseases initiative (DNDi), and the Institute for OneWorld Health (IOWH), which add to the efforts of WHO Tropical Diseases Research Programme (TDR) to address this misbalance in the world biomedical R&D effort. The PDPs propose alternative drug R&D models, fostering effective collaborations between the public and private sectors as well as including groups from endemic countries. The major requirement is sustainable funding over a 10-15 period to take drugs through the expensive stages of development and to make them available to patients.

[November, 2005-11-08 - 09h00 - ROOM A]

MC03 - Acquired resistence to P. falciparum malaria: a search for new antibody markers based on the cross reactivity with avian malaria.

Antoniana Ursine Krettli *(Centro de Pesquisas René Rachou-FIOCRUZ, Belo Horizonte, MG)*

Repeated malarial infections or sporozoite-vaccination induce a strong protection against clinical malaria. Although temporary, in both cases, and requiring repeated boosters, it results in decreased morbidity and lethality to *Plasmodium falciparum (PF)* which, otherwise, kills 2-3 millions children yearly. Several anti-malaria vaccines with recombinant antigens, parasite DNA, live attenuated sporozoites are used in ongoing human trials but face several problems. Probably, the most important is the absence of a reliable test to evaluate protection prior to challenge. The only test to measure protective antibodies requires live *PF* sporozoites that, upon incubation with protected vaccines sera significantly decrease infectivity to host cells in vitro (HEP-G2). This test is complex and risky requiring PF infected mosquitoes, thus a practical test to measure protection is needed and should also help investigations on mechanisms underlying human protection to malaria. It is still controversy whether a long term exposure or age are responsible for acquired resistance to malaria. In previous work we showed that sporozoites of *Plasmodium gallinaceum (PGspz)* and PF, evolutionarily related, have crossreactive antigens and that human sera from malaria endemic areas or from PF vaccinated and protected subjects react with PGspz by immunoblot (WB) tests. By

indirect immunofluourescence (IIF) the overall IIF positive was 6- 71% in sera anti- *PF* and -*P. vivax*, highest (73-90%) in groups exposed to intense PF transmission for ≥20 years to transmission. All subjects denying clinical malarial ≥10 years were positive but around 200 anti-*P. vivax* sera and normal sera were negative. By WB analysis ˜90% of sera testing positive by IIF recognized the circumsporozoite (CS) protein of PGspz and a polypeptide of 57 kDA, also recognized by protected subjects vaccinated with live PFspz. We next investigated whether a recombinant CS of PGspz would be useful in an ELISA tests, after cloning the corresponding carboxiterminal (rCSC) and the amino-terminal (rCSA) of the PGspz parasite. Although both recombinants strongly reacted with the human malaria sera, more intense ELISA reactivity occurred with rCSC; the control normal sera also tested positive, at lower titers. The fact that the number of samples tested from protected individuals was small hampers our present conclusion. Thus, we aim now to test higher number of sera from subjects with an unquestionable strong acquired protection, may be from Africa regions to offer a conclusive result for the in vitro assay to measure protective antibodies to human malaria. In support to our hypothesis are preliminary data showing that protective MAb able to neutralize the infectivity of PGspz in vivo and in vitro react better with the rCSC than with the rCSA tested in parallel (with Cristiana FA Brito et al). In addition, like the protective Mab to PGspz, sera from protected individuals, after exposure or vaccination with PF spz were able to neutralize the infectivity of PGspz to the chicken host, another evidence that the crosseactivity between human and avian malaria should be further explored. *Financial support from CNPq and FAPEMIG.* - email akrettli@cpqrr.fiocruz.br

[November, 2005-11-08 - 09h00 - ROOM B]

MC04 - GENETIC APPROACHES FOR MALARIA CONTROL

Marcelo Jacobs-Lorena *(Johns Hopkins School of Public Health)*

Malaria kills millions of people every year and even more worrisome, the number of deaths is steadily increasing. Control of malaria is difficult because of resistance of the parasite to the most effective and inexpensive drugs, resistance of the mosquito vectors to insecticides and the lack of an effective vaccine despite great efforts that have been devoted to develop one. Clearly, new strategies for malaria control are urgently needed. The advent of techniques to insert genes into the mosquito genome (transgenesis) suggested a new strategy to interfere with transmission namely, the introduction into the mosquito of genes that interfere with the development of the malaria parasite. The lecture will review the advances made in the engineering mosquitoes resistant to the malaria parasite, including consideration of transformation techniques, promoters to drive the expression of transgenes, effector proteins (proteins that interfere with parasite development) and fitness of transgenic mosquitoes. Presently, a major roadblock is the development of means to spread transgenes into mosquito populations in the field. An alternate approach of expressing effector proteins from bacteria that live in the mosquito midgut (paratransgenesis) will also be considered. Present research is expected to lead to the development of a novel strategy that can be used in combination with traditional control strategies (drugs, insecticides, vaccines) to combat malaria.

[November, 2005-11-08 - 09h35 - ROOM B]

MC06 - VL in the Sudan: A centenary of a serial killer

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The living memory of diseases of epidemic nature in east Africa tells of no match to visceral leishmaniasis, probably one of the greatest killer diseases of all times. **The culprit** is a protozoan parasite discovered at the turn of last century first in India and then in the Sudan. Both countries are now among the major world foci of visceral leishmaniasis. In the Sudan, the identity and classification of the causative agent remained for some time a point of conjecture, a matter reflecting on the nomenclature of the parasite, mainly due the wide and intriguing spectrum of clinical presentation ranging from simple cutaneous diseases to a deadly acute illness. Thanks to the new **molecular tools** that a pile of information have accumulated during the past decade, which promises to solve some of the major conundrums surrounding the epidemiology of visceral leishmaniasis. Phylogeography, based on mitochondrial DNA analysis of a global sample of *Leishmania* parasite, suggests that parasites causing human visceral Leishmaniasis went out of Africa accompanying early migration of *Homo sapiens* and co-evolved as man went to explore and adapt to new environments and terrains. Such co-evolution may have been marked by increased resistance to VL among particular human populations with ensuing appearance of protective polymorphisms due to natural selection. The distribution of the diseases in the Sudan suggested early on both an ethnic and geographic elements in the epidemiology of diseases. Genetic studies have focused thereafter on some of the most vulnerable populations and the emerging results are starting to shed light on some of the possible mechanisms that might determine susceptibility to *L. donovani* infection. Since the clinical disease pattern is complex, it is likely that a plethora of genes that regulate innate and adaptive immunity might be involved. Leishmaniasis outbreaks is believed to be of cyclical nature where the diseases **strikes** every ten years, this may be influenced by a multitude of factors including herd immunity, human migration and cross protection with other prevalent species of *Leishmania* . Nearly one hundred years after the discovery of the causative agent of visceral leishmaniasis a more comprehensive portrait of a killer disease is beginning to take shape and with it raises the hopes of being able to establish better and potent control measures

[November, 2005-11-09 - 09h00 - ROOM A]

MC07 - Immunopathology of Cutaneous and Mucosal Leishmaniasis: Lessons for Therapy

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Control of leishmania infection is dependent of macrophage activation by IFN-γ produced by T cells. Impairment in Th1 cell response is associated with high parasite load and dissemination of the disease. In cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML) a very strong type 1 immune response is documented but patients develop cutaneous and mucosal ulcers respectively. Evidences that the immune response participates in the pathology of leishmaniasis are: 1) Lymphocytes from individuals with CL or ML produce high amounts of IFN-γ and TNF-α, two important pro-inflammatory cytokines, in peripheral blood and tissue; 2) The lesions are characterized by infiltration of lymphocytes and plasma cells and parasites are rare or absent at the lesion sites; 3) During the initial stages of CL (lesions with less than 20 days old), granulomatous vasculitis precedes the appearance of the ulcer; 4) There is a correlation between the frequency of inflammatory cytokine producing T cells and lesion size; 5) Drugs that down-modulate the immune response associated with antimony therapy increase the cure rate and decrease the healing time of cutaneous and mucosal ulcers. In order to identify factors that may be involved in the biased immune response in these patients leading to an exacerbated immune response, we have compared the immune response of patients will CL and ML. In both diseases the main source of IFN-γ is CD4 T cells and macrophages, CD4 and CD8 T cells contribute to TNF-α secretion. Expression of co-stimulatory molecules is similar in antigen presenting cells and in T cells and no difference in the frequency of apoptopic cells was documented. The frequency of cells expressing T cells activating markers (CD69, CD28 $^-$, CD62L $^-$) are higher in ML than in CL. In both disease addition of CTLA-4 suppressed very little IFN-γ production while in PPD stimulated cultures of healthy subjects CTLA-4 suppressed 98% of the IFN-γ production. While IL-10, anti IL-2 and anti IL-15 suppress IFN-γ in CL, these molecules are not or less effective in down regulate IFN-γ in ML. Moreover the number of cells expressing IL-10 receptor is lower in ML than in CL. Another important evidence that immune response is key in the pathogenesis of leishmaniasis is the documentation that antimony therapy early in the disease do not prevent the appearance of the ulcer. Based on this finding and because T cell response may be involved in the pathogenesis of leishmaniasis and an exaggerated T cell response is observed mainly in ML but also in CL, we decided evaluate the efficacy of antimony associated with molecules that modulated the immune response in the treatment of cutaneous and mucosal leishmaniasis. GM-CSF is not only able to modulate the immune response but also kill leishmania. Pentoxyfilline is a vasodilator drug that inhibits TNF-α production. In two independent randomized double blind studies we have been able to show that GM-CSF associated to antimony are more effective than antimony alone and also accelerate ulcer healing in CL. Regarding mucosal disease in a open trial with ML patients refractory to antimony therapy, and in a randomized double blind trial with ML without previous therapy, we observed that pentoxyfilline associated to antimony is more effective than antimony alone in ML. These studies show impairment in the modulation of the immune response in CL and ML. Moreover, association of antimony with drugs that down modulate immune response increase the cure rate and accelerate healing of cutaneous and mucosal ulcers. – e-mail edgarufba.br and imunoufba.br

[November, 2005-11-09 - 09h00 - ROOM B]

MC08 - ORAL INFECTION BY *TRYPANOSOMA CRUZI*: MECHANISM OF INVASION OF GASTRIC EPITHELIUM

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Oral infection is a mode of *T. cruzi* transmission that is possibly more prevalent than previously thought, and metacyclic trypomastigotes are the parasite forms that most efficiently establish infection through this route. One interesting aspect of oral infection by metacyclic forms is that they preferentially invade the gastric mucosal epithelium, where they differentiate and replicate as amastigotes. Intrigued by the low capacity of a *T. cruzi* isolate in invadig HeLa cells, while exhibiting high efficiency in infecting mice by oral route, we aimed at elucidating the molecular basis of such a discrepancy. As compared to metacyclic forms of CL isolate, ∼3-fold fewer parasites of isolates 573 and

587 entered epithelial HeLa cells, compatible with their surface profile. The three isolates expressed similar levels of gp82, the invasion-promoting molecule, whereas the expression of gp90, a down regulator of parasite internalization, was higher in isolates 573 and 587 than in CL isolate. However, when administered orally into mice, metacyclic forms of isolate 573 produced high parasitemia levels ($>10^6$ parasites/ml at the peak), killing $\sim$40% of animals, comparable to those produced by CL isolate, whereas inoculation of isolate 587 resulted in reduced parastiemias ($>10^6$ parasites/ml at the peak) and null mortality. On the fourth day post-inoculation, tissue sections of the mouse stomach stained with hematoxylin and eosin showed a 4-6 fold higher number of epithelial cells infected with isolate 573 or CL than with isolate 587. The rate of intracellular parasite development was similar in all isolates. Mimicking in vivo infection, parasites were treated with pepsin at acidic pH and then assayed for their ability to enter HeLa cells or explanted gastric epithelial cells. Pepsin extensively digested gp90 from isolate 573 and significantly increased invasion of both cells, but had minor effect on gp90 or infectivity of isolates 587 and CL. The profile of g82 digestion was similar in isolates 573 and 587, with partial degradation to a $\sim$70 kDa fragment, which preserved the target cell binding domain as well as the region involved in gastric mucin adhesion. Gp82 from CL isolate was resistant to pepsin. Assays with parasites recovered from the mouse stomach 2h after oral infection showed an extensive digestion of gp90 and increased infectivity of isolate 573, but not of isolate 587 or CL. Our data indicate that *T. cruzi* infection in vitro does not always correlate with in vivo infection because host factors may act on parasites, modulating their infectivity, as is the case of pepsin digestion of isolate 573 gp90. Work supported by FAPESP and CNPq.
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[November, 2005-11-09 - 09h35 - ROOM A]

MC09 - Rhodnius prolixus immunity and interactions with trypanosomes

GARCIA E.S. *(Instituto Oswaldo Cruz)*; AZAMBUJA P. *(Instituto Oswaldo Cruz)*

The co-evolution of parasites and insects has promoted the development of a powerful and sophisticated strategy based on both physiological and biochemical vector and/or parasite mechanisms, which act to facilitate parasite development or its disruption in the invertebrate host. The debate concerning the Trypanosoma cruzi and T. rangeli-triatomine vector interactions results from their complexity and modes of parasite transmission. Many factors are believed to contribute to the infection of trypanosomes in the vectors. However, differences in the biological cycles between both parasites exist: T. rangeli, but not T. cruzi, invades the hemocele and barriers such as the gut membranes which are important to T. cruzi infection, but not to T. rangeli. It will be outline research on the developmental stages of T. cruzi and T. rangeli in the vector Rhodnius prolixus. Special attention is given to the interactions of these parasites with gut and hemolymph molecules and the effects of intestinal organization on the parasite development. The vector insect's permissiveness to T. cruzi, which develops in the vector gut, largely depends on the host nutritional state, parasite strain and molecular interactions with stomach lytic factors, lectins, hemoglobin fragments and resident bacteria in the gut as well as neuroendocrine system. T. rangeli invades the hemocele and once in the hemolymph can be recognized and activates the defense system of its vector. Several aspects of the humoral and cellular immune responses will be discussed, including lysozymes and trypanolytic activity, phenoloxidase system, phagocytosis, hemocyte microaggregation, hemolymph agglutination, superoxide, nitric oxide activity, and eicosanoid biosynthesis pathway. Finally, the mechanisms of these interactions and their significance for T. cruzi and T. rangeli transmissions indicate the complexity of trypanosome - triatomine relationships amd indicate that research into basic aspects of parasite - invertebrate host interactions can reveal subtle mechanisms for the establishment of parasite infections, that are transmitted by blood-feeding insects. Financial support: Papes (Fiocruz), CNPq and Faperj.

[November, 2005-11-09 - 09h35 - ROOM B]

MC10 - The immunobiology of the Amastigote Surface Protein 2 of *Trypanosoma cruzi*

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The Amastigote Surface Protein-2 (ASP-2) is an 83 kDa stage- and species-specific surface antigen expressed by amastigotes of *Trypanosoma cruzi*. Based on the predicted amino acid sequence of ASP-2 gene, this protein was assigned as a member of the sub-family II of the sialidase/trans-sialidase gene super-family. Although the parasite has a vast number of copies of *asp-2* in the genome, only a limited number of genes are expressed at the cDNA level. Comparative analysis of the sequences of different genes isolated from cDNA of infective *T. cruzi* I and II strains yield a dN/dS lower than 1 indicating that the ASP-2 gene is under a strong negative selection (Claser et al., 2005). The selective pressure to maintain the structure may imply a critical function for this molecule to the survival of amastigotes. Initial immunological studies showed that the purified antigen was specifically recognized by antibodies from chagasic individuals residing in geographically distant regions of South America (Pan & McMahon-Pratt, 1989). In addition to B-cell epitopes, ASP-2 contains multiple epitopes recognized by specific CD8 cytotoxic T lymphocytes from *T. cruzi* in-

fected mice and humans (Low et al., 1998, Wizel et al., 1998, Tzelepis et al., 2005). Recent vaccination studies in mice provided evidence that ASP-2 is an important target for protective immunity against *T. cruzi* infection. Mice vaccinated with plasmids or recombinant proteins develop strong cellular immune response mediated by CD4 Th1 and CD8 Tc1, thus surviving otherwise lethal acute infection. In addition to surviving acute infection, chronic phase pathologies of vaccinated mice were significantly reduced (Garg & Tarleton, 2002, Boscardin et al., 2003, Fralish & Tarleton, 2003, Vasconcelos et al., 2004, Araújo et al., 2005). Based on the evolutionary and immunological studies performed so far, we propose that ASP-2 is a critical *T. cruzi* protein/antigen with an unknown biological function that deserves much attention as a chemotherapeutic or immunological target. Supported by FAPESP, CNPq, FAPEMIG, NIH and CAPES.