MINI-CONFERENCES

[November, 09 - 09:00 - Room A]

MC1 - Motility, Invasion and Virulence of *Toxoplasma gondii*

L. D. Sibley

Efficient cellular invasion is responsible for the widespread success of a group of important pathogens in the phylum Apicomplexa including *Toxoplasma*, *Plasmodium*, and *Cryptosporidium.* Unlike most intracellular parasites that gain entry via host-mediated processes, these parasites use a novel system of adhesion-based motility called "gliding"to actively penetrate host cells. Gliding differs from other forms of motility and relies on the directional translocation of cell surface adhesions that are tethered to an actin-myosin motor beneath the parasite membrane. Recent studies reveal that gliding relies on a family of conserved adhesins on the surface of the parasite. This family of proteins contains a conserved von Willebrand Factor A domain, a series of thrombospondin type I repeats, a transmembrane region, and a cytoplasmic tail that links to the cytoskeleton. Thus, adhesins in this group of parasites share many similar domains and functions with mammalian integrins, despite their widely different evolutionary origins. The molecular process that powers gliding is comprised of a myosin motor (TgMYoA) that is anchored in the inner membrane complex. Assembly of actin filaments is crucial for motility, yet paradoxically actin remains almost exclusively monomeric in the parasite. We are exploring the factors that regulate actin turnover in the parasite in order to understand the regulation of motility. Interaction between parasite cell surface adhesins and the cytoskeleton facilitates parasite migration across cellular barriers, enables dissemination within tissues, and powers invasion of host cells.

[November, 09 - 09:00 - Room B]

MC2 - Gene silencing and antigenic variation in *Trypanosoma brucei*

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Silent genes encoding variant surface molecules have a striking sub-telomeric arrangement in several pathogens that undergo antigenic variation while telomeric DNA is known to induce local gene silencing in yeast, *Drosophila* and humans. We have explored the relationship between gene silencing and telomeric location in *Trypanosoma brucei.* These African trypanosomes use RNA-polymerase I for mono-allelic variant surface glycoprotein (*VSG*) gene transcription in bloodstream form cells. Other sub-telomeric *VSG* genes are heritably and reversibly repressed but no repressive DNA element has been identified in a trypanosomatid. We have now shown that telomeres repress Pol I-dependent gene expression in mammalian bloodstream and insect life-cycle stages while more extensive *VSG*-associated silencing is bloodstream stage specific. These data indicate that elaborated telomeric silencing could repress *VSG* expression. Newly acquired genome sequence data should facilitate identification of the silencing machinery in trypanosomatids. In other organisms a histone acetylation and methylation pattern is required for transcriptional silencing. Antisera specific for these modifications suggest that both are present in trypanosomatid chromatin and a search of the genome sequence data revealed a variety of putative acetyltransferases, deacetylases and methyltransferases. Several components of this system have been tagged and localised to the *T. brucei* nucleus and the genes have been disrupted or targeted using inducible RNA interference. We have identified two trypanosomatid proteins that disrupt telomeric silencing when expressed in yeast. To explore the relationship between histone modification and gene silencing in *T. brucei* we will use inducible RNAi to target nuclear modifiers in cells with a silenced sub-telomeric reporter. Other tools are under development to exploit genome sequence data and to facilitate identification and characterisation of the machinery responsible for regulating gene silencing and antigenic variation in *T. brucei.* See http://homepages.lshtm.ac.uk/ ipmbdhor/dhhome.htm for more details.

[November, 09 - 09:35 - Room A]

MC3 - Present Challenges of Malaria's Therapy in Brazil

Marcos Boulos
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[November, 09 - 09:35 - Room B]

MC4 - Choreography of transcription and replication in *Trypanosoma cruzi*

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In trypanosomes most of the genome is constitutively transcribed and gene expression is mainly controlled at the post-transcriptional level. Exceptions are the ribosomal genes, which are transcribed by RNA polymerase I, and the splice leader genes, which are transcribed by RNA polymerase II, all having defined promoters and transcription factors. A major feature of trypanosomes is that the 5'-end of the splice leader transcript is capped and *trans*-spliced to non-capped pre-messenger RNAs, usually synthesized as long polycistronic RNAs. Concomitant with the *trans*-splicing reaction, the pre-mRNAs are polyadenylated and exported to the cytoplasm. Therefore, we propose that transcription and RNA processing events might have a simplified spatial organization when compared to other eukaryotes, which present a complex pattern of gene expression control. To provide evidences for this hypothesis we decided to investigate the localization of the transcription and replication machineries in the nucleus of proliferating epimastigote forms of *Trypanosoma cruzi*. We found that the transcription machinery, as observed in immunofluorescence assays using an antibody prepared against the carboxy-terminus of the large sub-unit of RNA polymerase II, was always found in a spot close to the nucleolus. This labeling coincided with the localization of the splice leader genes detected by fluorescent in situ hybridization (FISH) and was sensitive to actinomycin D and α-amanitin, suggesting that it corresponds to actively transcribing RNA polymerase II. In addition we observed a less intense RNA polymerase II labeling in several dots distributed in all nucleoplasm. Incorporation of bromo-uridine in lysolecithin permeable cells confirmed this pattern of transcriptional events in the nucleus of *T. cruzi*. In parallel, we found that replication as seen by bromodeoxy-uridine incorporation is mainly located at the nuclear periphery. The chromosomes detected by satellite DNA FISH move to the nuclear periphery sites to replicate. We also found that the replication machinery, detected using antibodies directed to the *T. cruzi* proliferating cell nuclear antigen (PCNA) is formed at two opposed nuclear domains close to the nucleolus. When replication starts, the PCNA labeling moves toward the periphery and disperses only when the cells divide. We conclude that although being a highly dynamic structure, the nucleus of *T. cruzi* has defined domains for replication and transcription. While replication of DNA occurs mainly at the nuclear periphery, the transcription events are more centrally distributed with a concentrated transcription of the splice leader genes close to nucleolus. This unique distribution may be helpful to understand the mechanism that controls the nuclear organization in eukaryotic cells, as well as to provide insights about the transcription and replication apparatus of trypanosomes.
Supported by FAPESP and CNPq

[November, 09 - 16:00 - Room A]

MC5 - Insights into antimicrobial defense of ticks

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Arthropods have developed several structural barriers (e.g. the outer exoskeleton, the perithrophic matrix of the midgut, and the chitinous linings of the trachea as the first defense line against infection. Moreover, a complex interaction of cellular and humoral reactions is induced to eliminate invaders when these barriers are breached. Ticks are obligatory blood-sucking arthropods being one of the most important vectors of human and animal diseases. Although a vast number of microorganisms has been identified inside ticks, little is known about the mechanism that they use to escape from this vector. We have shown that hemocytes of the cattle tick *Boophilus microplus* exhibit a phagocytic activity and produce reactive oxygen species (ROS) following a microbial challenge (Pereira, L.S. et al., 2001 Exp Parasitol 99: 66). In addition, we have isolated and characterized two different antimicrobial peptides (AMP) from the tick hemocytes. One of those peptides, with a molecular mass of 4,285 Da and six cysteine residues, belongs to the insect defensin family. The other peptide (7,103 Da), designated ixodidin, possess ten cysteine residues. Besides showing antimicrobial activity, ixodidin also exhibits proteolytic inhibitory activity against two serine proteinases, elastase and chymotrypsin. It would be interesting to establish if the antimicrobial and inhibitory activities of ixodin are related and how this interaction occurs. A third peptide, with molecular mass at 10,204 Da polypeptide and six cysteine residues, was isolated from the cell free hemolymph. Because of its

primary structure originality this peptide was named microplusin. The cDNA cloning established that microplusin is synthesized as a prepeptide while the hemocyte defensin is synthesized as a prepromolecule. Interestingly, despite the fact that microplusin and defensin have been isolated from different tissue components, their gene expression was found to have similar tissue distribution (Fogaça, A.C. et al., 2004 Devel. Comp. Immunol. 28, 191). A fourth AMP was purified from the gut contents. Interestingly, this peptide (Hb33-61) is not synthesized by the tick itself, but corresponds to a proteolytic product of the bovine hemoglobin α-chain (Fogaça, A.C. et al., 1999 J. Biol. Chem. 274, 25330). The synthetic peptide was active against Gram-positive bacteria and fungi at micromolar concentrations, being able to disrupt the bacterial membrane. To gain insights on its mechanism of action, the structure of amidated Hb33-61 bound to SDS micelles was determined by 2D-NMR analysis and by molecular modeling (Pertinhez, T. A. et al., 2004 unpublished). This peptide possess an α-helix spanning most of the C-terminal half, and a series of turns distributed throughout the first half of the peptide. With the aim of determining the structural requirements for Hb 33-61 activity, N- and C- terminally truncated fragments were synthetized and their conformational behavior evaluated by CD and NMR spectroscopies (Miranda, M.T.M et al., 2004 unpublished). The results suggest that the helicity is important, but no crucial, for the expression of antifungical activity of the Hb 33-61. The distinct structures and diversity of tissue distribution of the AMPs linked to the phagocytic activity of hemocytes suggest that these defense components might act in a co-operative way to prevent infections in the tick. In order to understanding the importance of these AMPs for tick defense, we intend to silence their gene expression in a cell line from *B. microplus* (BME26) and evaluate its survival following a microbial infection.

[November, 09 - 16:00 - Room B]

MC6 - Kinin-driven inflammation and its modulation by the angiotensin converting enzyme: a molecular pathway linking innate and adaptive immunity in experimental Chagas' disease

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Kinins (eg. bradykinin or lysyl-bradykinin) are short-lived vasoactive peptides liberated by proteolysis from an internal segment from the plasma proteins high or low-molecular weight kininogens (HK/LK). The pro-inflammatory effects mediated by the released kinins (eg. increase vascular permeability) are mediated by two pharmacologically distinct subtypes of G-protein coupled receptors. B_2R is constitutively expressed in several cell types (eg. endothelial cells, smooth muscle, neurons and cardiomyocytes) while B_1R is induced in inflamed tissues. Of relevance to immunology, we recently reported that immature dendritic cells (iDcs) express B_2R. Using model antigens

(eg. ovalbumin) and synthetic bradykinin we show evidence that (i) kinin peptides may act as endogenous "danger"signals, i.e., they activate iDCs (loaded with captured antigens) through B_2R, stimulating their migration to draining lymph nodes and (ii) bradykinin-induced maturation of DCs resulted in upregulated production of IL-12, this response being coupled to Th1-polarization of Ovalbumin-specific T cells (Aliberti et al., J. Immunol., 2003). We now demonstrate that these mechanistic principles operate in the context of an infectious disease. The mouse model of *T. cruzi* infection seemed appropriate to test this mechanistic hypothesis because tissue trypomastigotes (TCT) processes HK/LK, liberating kinins through the activity of the major cysteine protease, cruzipain (Del Nery et al., JBC,1997; Lima et al., JBC,2002). Motivated by this biochemical finding, we first studied the role of the kinin-signaling pathway in parasite invasion. As previously reported, studies with primary human endothelial cells and murine cardiomyocytes revealed that cruzipain-mediated release of the kinin agonist evoked [$Ca2^+$]i-inducing responses through their cognate GPCRs, thereby stimulating parasite infectivity in vitro. (Scharfstein et al., J Exp.Med, 2000;Todorov et al., FASEB J, 2003, reviewed by Scharfstein, J.2003). We then demonstrated that TCTs are able to induce edematogenic inflammation by sequentially engaging endothelium B_2R and B_1R, (Todorov et al, FASEB J., 2003). More recently, we performed intravital microscopy studies in the hamster cheek pouch to characterize the dynamics of kinin-mediated microvascular leakage. Our results, combined to studies in mice with genetic deletions of B_2R, Toll-like 2 receptor (TLR2-KO) and TLR4-KO, revealed that trypomastigotes indeed activate the kinin system in vivo by a cruzipain-dependent pathway. Analysis of the molecular mechanisms underlying pathogen-evoked increases in vascular permeability indicated that it is initiated by Toll-like 2 receptors (TLR2) stimulation by mucin-linked glycosylphosphatidylinositol anchors of tissue culture trypomastigotes, previously identified as potent activators of TLR2 (Almeida and Gazzinelli, J Leukoc Biol. 2001). Microvascular leakage is then rapidly amplified by neutrophile-dependent influx of plasma-bone kininogens into the infection site. Acting further downstream, cruzipain liberates kinins from processed kininogens, further augmenting local inflammation. In agreement with our hypothesis, analysis of APCs isolated from the draining lymph nodes indicated that $CD11c^+$ DCs upregulated IL-12 production in response to kinin stimulation and this innate immunity response to the parasite was linked to upregulated IFN-γ production by antigen (*T. cruzi*) specific T cells. Importantly, alterations in kinin homeostasis observed at early stages of infection modulated host susceptibility to acute infection as well as disease severity in the chronic phase (heart pathology). Of note, kinin-degrading peptidases, such as Angiotensin Converting Enzyme (ACE) attenuate the pro-inflammatory responses initiated through the TLR2-B_2R axis, thereby counterbalancing pathogen-induced activation of innate/adaptive immunity. In short, our studies suggest that tGPI/cruzipain may exert dual roles in the pathogenesis of Chagas' disease. On one hand, the pathogen may invade cells more efficiently by activating kinin-receptors. On the other hand, the increased liberation/accumulation of kinins in sites of inflammation leads to upregulation of type 1 immunity. Under extreme conditions, excessive kinin production may favor exacerbated T cell responses, increasing risks of associated chronic pathology.
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[November, 09 - 16:35 - Room A]

MC7 - DIGESTION OF PROTEINS IN TRIATOMINES

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Triatomines differ in many aspects from other hematophagous insects, especially in the digestion of proteins. They digest their blood meal over the course of several days using cysteine and (or) aspartic proteases (Lehane, 1994, Kollien and Schaub, 2000). This is consistent with a neutral/alkaline or acid pH of their midgut lumen, respectively. Two different proteases (cathepsins D and B), as well as lysosomal carboxypeptidase B and aminopeptidase have all been identified and characterized in the posterior midgut of triatomines using synthetic substrates and inhibitors (Houseman and Downe, 1983). However, the exact location, i.e. luminal versus intracellular, and the molecular biology of proteolytic digestion in triatomines remains poorly studied. The nucleotide and deduced amino acid sequences of just a single cathepsin L-like protease gene in *R. prolixus* have been described (Lopez-Ordoñez *et*

al., 2001). Since these specific enzymes may offer an opportunity to identify species or populations of triatomines (domiciliary, peridomiciliary, silvatic) and since the development of *T. cruzi* is affected by the conditions in the alimentary tract, here we investigate this topic and report cathepsin B- and cathepsin L-like activities in gut extracts of the bug *Triatoma infestans* by using specific substrates and inhibitors. Activities decreased during the first two days after feeding but increased to a maximum value at 5 and 10 days post feeding. Cysteine protease activities after feeding were present at the anterior part of the midgut wall and in the posterior midgut; in the latter, activities were higher in the lumen than in the wall. In adults of *Triatoma brasiliensis*, activity of cathepsin B and L was higher on the third day after feeding, while for cathepsin D the highest activity was detected nine days after a blood meal. *In situ* hybridizations on whole guts of *T. infestans* with probes labeled with digoxigenin showed that, after feeding, the cathepsin B-like protease mRNA was strongly expressed in the small intestine. With increasing time after feeding, concentrations of mRNA became strongly reduced in the posterior part of the midgut, but still persisted in the anterior part. We cloned and sequenced two cathepsin B-like and one cathepsin L-like gene from *T. infestans.* When compared to the deduced amino acid sequences of other cathepsins (Sajid and McKerrow, 2002), all three showed the characteristics shared by cathepsins B and L (Kollien *et al.*, 2004). The occluding loop, characterizing B-like cathepsins, was present only in two sequences. The slightly modified ERFNIN and GNFD motifs occurred in the third deduced pre-proenzyme, defining it as cathepsin L-like. One of the two cathepsin B-like genes was expressed at low, constitutive levels in unfed and fed *T. infestans.* The deduced 332, 333 and 328 amino acid sequences showed high levels of identity (50-80%) to other insect cathepsin B-like and L-like proteinases, respectively. The identity between the two cathepsin B-like protease genes was 80%; the identity of the third to cathepsin L-like proteinases of *Rhodnius*, 68%. After identification of cathepsin B-like protease genes of other triatomine species (*T. vitticeps, T. sordida, T. dimidiate*), a comparison of the amino acid sequences showed about 80% identity between any two triatomines. In *T. brasiliensis* two cathepsin D-like genes were sequenced. *T. infestans* hydrolyzes polypeptides using at least two glutamyl-aminopeptidases, of which two gene fragments have been sequenced. In addition, this species possesses a serine carboxypeptidase. In Real Time PCR, the concentration of mRNA of the carboxypeptidase was reduced during the first two days after feeding and increased thereafter.

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[November, 09 - 16:35 - Room B]

MC8 - Immunoregulatory dendritic cells controlling the outcome of *Leishmania donovani* infection.

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Experimental infection of mice with *Leishmania donovani* is followed by distinctive, organ-specific outcomes. In the liver of most mouse strains, early parasite expansion is eventually brought under control, with the concomitant development of a granulomatous tissue response, parasite clearance and local protection from re-infection. In contrast, in the spleen and bone marrow, parasite growth is initially slow, but then increases dramatically with the establishment of long term persistence. Granulomatous inflammation is absent and the spleen is characterised by extensive splenomegaly, an ordered breakdown in the lymphoid microenvironment and high level expression of cytokines, notably IL-10 and TNFα. TNFα plays an important role in directing tissue remodelling, whereas IL-10 acts to regulate APC function. Although the breakdown in lymphoid architecture plays a role in limiting the effectiveness of local immune responses, it is clear that there are also a number of other immunoregulatory changes that occur in the spleen during the onset and maintenance of chronic infection. In particular, there are important qualitative and quantitative changes that occur in the dendritic cell (DC) population. Two of these will be discussed in detail. First, we have shown for the first time that splenic stromal cells induce myeloid progenitor cells to differentiate into dendritic cells with potent capacity to induce regulatory IL-10-producing Tr-1 type $CD4^+$ T cells in vitro and to induce antigen-specific tolerance in vivo. This capacity of stromal cells to induce regulatory DC is enhanced during chronic infection. As an increase in progenitor cell number is also evident during chronic infection, these two events turn the spleen of infected mice into a highly effective factory for the differentiation of regulatory DC. Second, adoptive transfer experiments have been used to demonstrate that DC therapy can have diverse effects on

the outcome of established infection. Thus, LPS-activated DC can enhance host protection, whereas DC isolated from mice with chronic infection cause disease exacerbation. Some, but not all, of the effects of DC transfer are correlated with changes in the frequency of Tr-1 cells. Furthermore, DC from infected mice are heterogeneous, with respect to surface phenotype, cytokine production and the capacity to induce T cell differentiation in vitro. Together, these studies serve to illustrate the high degree of complexity that is evident within the DC compartment during chronic infectious diseases. Further understanding of these cells during infection is critical to understand the potential impact of a variety of proposed immunotherapies for visceral leishmaniasis.

[November, 10 - 09:00 - Room A]

MC9 - Parasite and host contributions to the pathogenesis of amebic colitis

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In 1903, Schaudinn named *Entamoeba histolytica*, the enteric ameba that causes amebic dysentery and liver abscesses, for its remarkable ability to invade and destroy host tissues. Understanding the mechanism of this tissue destruction has been the focus of work by numerous investigators, and cumulative in vitro and in vivo studies form the basis for an emerging model of pathogenesis. It is now clear that tissue destruction results both directly from amebic factors and from the host's inflammatory response. Step-wise invasion of the colonic mucosa by *E. histolytica* begins with excystation and adherence of the motile trophozoites to colonic mucins. Amebic proteases then degrade mucins and facilitate penetration. Direct contact with epithelial cells leads to apoptotic and necrotic killing of host cells by amebae, as well as activation of an epithelial cell acute inflammatory program that culminates in recruitment of neutrophils and immune mediated tissue damage. The resultant epithelial damage enables penetration of amebae deep into the mucosa followed by lateral spread. Immune evasion by *E. histolytica* may then facilitate persistent infection, as is suggested by the sparse inflammation that is typically present during chronic disease. This talk will highlight selected studies that form the basis for this model of pathogenesis, as well as major unanswered questions in the field.

[November, 10 - 09:00 - Room B]

MC10 - Mitochondria: ATP, heat and the regulation of cell death.

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Until 40 years ago, mitochondria were thought to be the site of some metabolic pathways (citric acid cycle, fatty acid beta oxidation, and aminoacids oxidation), oxidative phosphorylation and nonshivering thermogenesis. The latter is exclusively found in the brown adipose tissue. By this time, Peter Mitchell introduced the concept of coupling between respiration and phosphorylation through a transmembrane proton electrochemical potential. This potential is generated by the pumping of protons across the inner mitochondrial membrane when electrons flow through the respiratory chain. The proton pumping makes the matrix alcaline and negatively charged relative to the intermembrane space and provides the energy for ATP synthesis by the ATP-synthase. This electrochemical potential can also be consumed by electrophoretic cation influx to the mitochondrial matrix or by the uncoupling proteins (UCPs) that facilitate protons return to the matrix, thus uncoupling respiration from ATP synthesis and dissipating the energy of the proton gradient as heat. Since 1995, several isoforms of the uncoupling protein were discovered in animal, plants, fungi, and protozoa. Unlike UCP from brown adipose tissue, the new isoforms seem to be unrelated to thermogenesis but they may play a role in the control of reactive oxygen species (ROS) production and regulation of the proton electrochemical potential. Current understanding on the molecular mechanisms of ATP synthesis, electron transfer reactions and ion transport across the inner mitochondrial membrane have provided new insights into other processes involved in the pathophysiology of mitochondria-dependent cell death. The pivotal role of the proton electrochemical potential in energy transduction revealed the importance of the inner membrane impermeability to protons and other charged solutes for mitochondrial physiology and cell viability. The loss of this impermeability comprises a key event in the process of programmed or accidental cell death in many pathologic conditions. The same redox reactions that generate the proton electrochemical gradient driving oxidative phosphorylation leads to the formation of ROS that may cause cellular injury when the antioxidant systems are depleted. ROS may contribute

to the decline of mitochondrial bioenergetic capacity with advancing age. High levels of matrix Ca^{2+} stimulate ROS production in mitochondria. Ca^{2+}-accumulation in the matrix occurs during sustained elevation of its concentration in the cytosol due to failure in intracellular Ca^{2+} homeostasis or due to a regulated release from endo(sarco)plasmic reticulum. Both situations can induce opening of pores on the inner (permeability transition pore, PTP) and outer membrane (mitochondrial outer membrane permeabilization, MOMP). PTP opening permits the entrance of molecules up to 1.5 kDa and water causing large amplitude mitochondrial swelling. This process causes elimination of the proton electrochemical potential and, consequently, of oxidative phosphorylation. When PTP opens in a large number of mitochondria, cell death may occur due to the lack of ATP (necrosis). MOMP induces cell death through the release of molecules that promote both caspase-dependent (apoptosis) and caspase-independent cell death. MOMP itself can occur via processes dependent or independent of PTP opening. In the latter case, the MOMP opening is likely to be mediated by members of the Bcl-2 protein family. The antiapoptotic members of Bcl-2 family inhibit the opening of this pore, while the proapoptotic members promote it. Parasitic protozoa such as *Leishmania* and *Trypanosoma* possess mitochondria with basic energy transduction processes similar to vertebrates. A form of programmed cell death mediated by depolarization of proton electrochemical potential, cytochrome c release, protease activation, exposition of phosphatidylserine, loss of plasma membrane integrity, and DNA fragmentation also occur in protozoa.

[November, 10 - 09:35 - Room A]

MC11 - The control of T cell migration to the heart of mice infected with *Trypanosoma cruzi*

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Several hypotheses have been raised to explain the pathological lesions in the heart of *T. cruzi*-infected patients, including an essential role of autoimmunity and infection or persistence of parasite antigen at the inflammatory site. The understanding of the molecular events that underlie the initiation and maintenance of carditis, may shed light on the mechanisms responsible for controlling parasites and the inflammation associated with tissue damage during acute and chronic infection. The paradigm for the recruitment of leukocytes into tissues predicts an essential role for the activation of *G*-protein-coupled serpentine receptors on the surface of leukocyte. Chemokines can trigger such receptors and have been shown to mediate the recruitment and activation of a range of leukocyte subsets *in vitro* and *in vivo*. Soon after the infection of macrophages and cardiac myocytes by *T. cruzi* pro-inflammatory cytokines and chemokines are produced, which may activate these cells to produce nitric oxide to control parasite replication. In fact, CC chemokines produced by infected macrophages, especially MCP-1, enhance the ability of macrophages to kill *T. cruzi*. Interestingly, MCP-1 potentiates the IFN- γ-induced activation of iNOS, suggesting an important role of this molecule in the control of parasites in the early acute phase of infection. To understand how the parasites trigger myocarditis, we have investigated the mediators produced by *T. cruzi*-infected cardiomyocytes and macrophage, that induces leukocyte migration and leukocyte activation. Besides the cytokines TNF- α and IL-1 β, we found that these infected cells released several CC and CXC chemokines, which were able to induce activation of iNOS and trypanocidal activity. Although these cytokines and chemokines are also found in the heart of infected mice, we only have indirect evidence, mainly from experiments in knock out mice, that they are triggering migration of leukocyte to the site of infection. For example, in hearts of *T. cruzi*-infected mice deficient in IFN- γ (GKO) we did not find inflammatory cells, which correlated with absence of RANTES, Mig and IP-10. On the contrary, in p55KO mice we found inflammatory cells and the chemokines Mig and IP-10 in the heart. Moreover, cardiomyocytes from iNOS-/- mice produced higher levels of the chemokines than those from WT when infected or/and stimulated with cytokines. Accordingly, hearts of iNOS-/- mice infected showed a pronounced increase in the expression of mRNA for chemokines and more conspicuous cellular infiltrates. We also found message and protein for the CCR5 ligands CCL3, CCL4 and CCL5 in hearts of infected mice in association with $CD4^+$ and $CD8^+$ T cells. There was a high level of expression of CCR5 by $CD8^+$ T cells harvested from hearts of infected mice. Moreover, CCR5 expression was positively modulated by *T. cruzi* in $CD8^+$ T cells from spleen in vivo and in vitro. *T. cruzi*-infected CCR5-deficient mice had a dramatic decreased in the inhibited migration of T cells to the heart. These mice were also more susceptible to infection than their WT controls, as demonstrated by the higher parasitemia, heart parasitism and mortality. Altogether, the results suggest a central role for CCR5 and its chemokine ligands in controlling the influx of T cells in *T. cruzi*-infected mice. Although CCR5 may be relevant for parasite control in the acute phase of infection, interventions aiming at antagonizing this pathway in chronic Chagas disease may prevent myocarditis and protect from the development of severe disease. These data contributes to our understanding of the genesis and modulation of inflammatory reactions in *T. cruzi* infected mice. Supported by FAPESP, CNPq and CAPES.

[November, 10 - 09:35 - Room B]

MC12 - Involvement of SOD, OYE and HSP-70 genes in *Trypanosoma cruzi* resistance to benznidazole.

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Recently we have investigated the differential gene expression in *T. cruzi* populations and clones susceptible and resistant to benznidazole (BZ). From a panel of genes differentially expressed, we have characterized three genes in order to establish a possible involvement with the drug resistance phenotype in *T. cruzi.* Iron superoxide dismutase (TcFeSOD) and heat shock protein 70KDa (TcHSP70) genes were over expressed whereas old yellow enzyme (TcOYE) gene was under expressed in the in vitro-induced *T. cruzi* 17LER resistant population compared to its susceptible counterpart 17WTS. TcFeSOD is a central component in oxidative defense in most organisms and functions to remove excess superoxide radicals via dismutation to oxygen and hydrogen peroxide. TcHSP70 gene is over expressed under different chemical or physical stress. TcOYE catalyzes prostaglandin PGF2α synthesis in addition to the reduction of some of the trypanocidal drugs (Kubata et al. 2002). The northern blot profile of total RNA from drug-resistant and susceptible *T. cruzi* samples, hybridized with the respective gene probes revealed two transcripts, one of 1.2 and another of 1.6 Kb for TcFeSOD; one of 2.1 Kb for TcHSP70 and one of 1.68 Kb for TcOYE. 17LER expressed 3 and 4-fold more TcFeSOD and TcHSP70 mRNAs than 17WTS, respectively. Contrarily, TcOYE mRNA was 8-fold less expressed in the 17LER than in 17WTS. This difference on mRNA expression of the three genes was confirmed by real-time RT-PCR. Other *T. cruzi* populations and clones tested expressed the same mRNA levels for the three genes independently of their drug resistance phenotype. Copy number of the TcFeSOD and TcOYE genes was determined by real-time PCR and southern blot. The results show that TcFeSOD gene is 2-fold amplified, whereas the TcOYE gene presented 3 out of 4 copies deleted in 17LER compared to 17WTS. TcFeSOD gene is located in the chromosomal bands 775 and 880 Kb in 17WTS and 17LER. Both chromosomal bands were more intense in 17LER than in 17WTS. TcOYE is located in the 1.8 Mb band for all strains and also in the 1.9 Mb band for zymodeme B strains. The 1.8 Mb band in the 17LER was 4-fold less intense than in the 17WTS. Western blot analysis of *T. cruzi* protein extracts probed with rabbit polyclonal serum anti-recombinant TcFeSOD protein revealed a common band of 23 KDa for all *T. cruzi* strains. The intensity of this band was similar in all samples, except 17LER, which displayed a band 2-fold more intense. Antisera against TcHSP70 protein recognized two bands, one of 70 and another of 73 KDa for all *T. cruzi* strains. Each band had the same intensity and was common to all *T. cruzi* samples independently of their drug resistance phenotype. Although the TcHSP70 mRNA is 2-fold over expressed in the 17LER compared to 17WTS, the protein level is similar in both populations. Antisera against TcOYE protein revealed a unique band of 42 KDa for all *T. cruzi* strains. The intensity of this band was similar in all samples, except 17LER, which displayed a band nearly 7-fold less intense. In conclusion, our data show that TcFeSOD and TcHSP70 genes are up-regulated whereas TcOYE gene is down-regulated in the *T. cruzi* population with in vitro-induced resistance to BZ. Additional data suggest that the mechanism of drug resistance seems to be different between the *T. cruzi* populations with in vitro-induced and *in vivo*-selected resistance or naturally resistant to BZ. Supported by CNPq, FAPEMIG, PRONEX, PADCT and PAPES / FIOCRUZ. romanha@cpqrr.fiocruz.br