MC.01 – THE DNA REPAIR AND OXIDATIVE STRESS IN *Trypanosoma cruzi*

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Trypanosoma cruzi is the causative agent of Chagas disease, a malady of great economic importance in the Americas. As an obligatory intracellular organism with a digenetic life cycle, this protozoan comes across several sources of oxidative stress within its different host environments. It has not only to deal with oxygen species produced by its own or its host metabolism, as well as to survive ROS produced as part of the host immune response. Hence the reports that these parasites have limited ability to deal with reactive oxygen species, based on their lack of a full set of enzymes involved in handling oxidative stress, seem somewhat paradoxical. 8-hydroxy-2'-deoxyguanosine (8-OxoG) is one of the main mutagenic modifications induced in DNA by oxidative stress. To counteract the mutagenic effects of 8-oxoG, organisms have developed a multi-defense mechanism, the so-called GO system, composed by the products of *fpg, mutY* and *mutT* genes in *Escherichia coli* or by the functional homologues *OGG1, MYH* and *MTH1* in eukaryotes. We have been studding the GO system from the *T. cruzi* to better understand the mechanisms involved in the oxidative DNA damage response in this parasite. We are investigating the activity of *T. cruzi* by *in vivo* studies performed with parasites, bacterial and yeast cells overexpressing these genes. The data that we have been obtaining indicate the importance of these genes in the response to oxidative stress, treatment with benznidazole and in the infection of *T. cruzi.*
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MC.02 – LEISHMANIA PARASITES INDUCE AND ARE KILLED BY NETTING NEUTROPHILS

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Neutrophils are short-lived leukocytes that die by apoptosis, necrosis and by a recently described mechanism named NETosis. Upon death by NETosis, neutrophils release fibrous traps of DNA, histones and granule proteins named neutrophil extracellular traps (NETs), which can kill bacteria and fungi. Inoculation of the protozoan *Leishmania* into the mammalian skin causes local inflammation with neutrophil recruitment. Here, we are describing the release of NETs by human neutrophils upon their interaction with *Leishmania* parasites, as well as NETs' ability to kill this protozoan. The NET constituents DNA, elastase and histones were detected in traps associated to promastigotes by immunofluorescence. Electron microscopy revealed that *Leishmania* was ensnared by NETs released by neutrophils, and promastigotes trapped by NETs presented a thin, flat body with protrusions, indicative of cell damage. Moreover, *Leishmania* and its surface lipophosphoglycan induced NET release by neutrophils in a parasite number- and dose-dependent manner. Disruption of NETs by DNAse treatment during *Leishmania*-neutrophil interaction increased parasite survival, evidencing NETs' leishmanicidal effect. *Leishmania* killing was also elicited by NET-rich supernatants from PMA-activated neutrophils. Immunoneutralization of histone during *Leishmania*-neutrophil interaction partially reverted *Leishmania* killing, and purified histone killed the parasites. Meshes composed of DNA and elastase were evidenced in biopsies of human cutaneous leishmaniasis and nests of amastigotes were seen associated with NETs in these lesions. NET is an innate response that might contribute to contain the infection preventing systemic spreading and diminish parasite burden in the *Leishmania* inoculation site.

MC.03 – MITOCHONDRIA AND CELL DEATH IN *T. CRUZI*

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The coupling between respiration and oxidative phosphorylation is mediated by a proton electrochemical potential generated by the pumping of protons across the inner mitochondrial membrane when electrons flow through the respiratory chain. This provides the energy for ATP synthesis by the ATP-synthase but can also be consumed by electrophoretic influx of cations to the mitochondrial matrix or by the uncoupling proteins (UCPs) that promote protons return to the matrix, thus uncoupling respiration from ATP synthesis and dissipating the energy of the proton gradient as heat. The current understanding on these molecular mechanisms has provided new insights into processes involved in the pathophysiology of mitochondria-dependent programmed or accidental cell death. The same redox reactions that generate the proton electrochemical potential lead to the physiological generation of reactive oxygen species (ROS). In addition, multiple biochemical stimuli such as mitochondrial Ca^{2+} overload leads to overproduction of ROS that may cause mitochondrial dysfunction via opening of pores in the inner (permeability transition pore, PTP) and outer membranes (mitochondrial outer membrane permeabilization, MOMP). These pores are components of the cell death machinery that render mitochondria as attractive targets for chemotherapy. For a long time it was assumed that programmed cell death arose along with multicellular organisms, however, apoptosis-like cell alterations have also been reported in a variety of unicellular eukaryotes including several members of Kinetoplastida. In this presentation will be reported data on the Ca^{2+}-dependent mechanismsunderlying T. cruzi epimastigotes apoptosis or necrosis mediated by mitochondrion dysfunction induced by fresh human serum (Irigoin F et al., Biochem J 418: 595-604, 2009) or the lectin Cramoll 1,4 (Fernandes MP et al., J Bioenerg Biomembr 42: 69-78, 2010), respectively.

MC.04 – THE CELL BIOLOGY OF LEISHMANIA - SAND FLY INTERACTIONS

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The spectrum of clinical outcomes associated with Leishmania infections, ranging from localized cutaneous to disseminated visceral disease, is accounted for by the remarkable diversity of Leishmania species, which are themselves transmitted by an even greater species diversity of sand fly vectors. The identification of parasite- and sand fly-derived molecules that play a role in the development of transmissible infections in the fly has been facilitated by the fact that vector competent sand fly species are in some cases only permissive to the complete development of the species of Leishmania that they transmit in nature. Thus it has been the identification of species- or strain-specific parasite molecules, and the generation of Leishmania mutants specifically deficient in these molecules, that have led to most of the advances in our understanding of the molecular basis of vector competence. Cell surface and secreted phosphoglycans protect the parasite from the proteolytic activities of the blood-fed midgut. The surface lipophosphoglycan (LPG) mediates attachment to the gut wall so as to maintain infection during excretion of the digested bloodmeal. Developmentally regulated modifications in LPG explain the ability of infectious stage, metacyclic promastigotes to position themselves for efficient transmission by bite. LPG also displays inter- and intra-species polymorphisms in their phosphoglycan domains that in most cases can account for species- and strain-specific vector competence. The polygalactose eptiopes expressed by the LPG of most *L. major* strains are required for midgut attachment and promastigote persistence within their natural vector, *P. papatasi*. High-throughput sequencing of a midgut cDNA library of *P. papatasi* identified a novel galectin gene homologue termed *PpGalec* that is used by *L. major* as a receptor for mediating species-restricted binding to the midgut. The possibility that Leishmania diversity is generated, at least in part, by genetic exchange is supported by the first formal demonstration of a sexual cycle during parasite development in the sand fly vector. Co-infection of sand flies with parental lines bearing distinct drug resistance markers yielded hybrid parasites that were resistant to both drugs, and that inherited a full set of chromosomes from each parent. The frequency, timing, and anatomical location of genetic excahnge within the sand fly midgut, and the existence of a haploid, gamete stage are currently being explored.

MC.05 – ON CYTOADHESION OF *Plasmodium vivax*-INFECTED ERYTHROCYTES

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Plasmodium falciparum and *P. vivax* are responsible for most of the global malaria burden. While accentuated pathogenicity of *P. falciparum* occurs due to sequestration of the mature erythrocytic forms in microvasculature, this phenomenon was not yet noted in *P. vivax*. The rising number of severe manifestations in *P. vivax* infections, similar to those observed for severe falciparum malaria, suggests that key pathogenic mechanisms (e.g. cytoadherence) might be shared by the two parasites.Mature *P. vivax*-infected erythrocytes (Pv-iE) were isolated from blood samples collected from 34 infected patients. Pv-iE enriched on Percoll gradients were used in cytoadhesion assays to human lung endothelial cells (HLEC), to *Saimiri* brain endothelial cells (SBEC), and to placental cryosections.Pv-iE were able to cytoadhere under static and flow conditions to cells expressing endothelial receptors known to mediate cytoadhesion of *P. falciparum*. Although Pv-iE cytoadhesion levels were 10-fold lower than those observed for Pf-iE, the strength of the interaction was similar. Cytoadhesion of Pv-iE was in part mediated by VIR proteins, since specific antisera inhibited the iE-endothelial cell interaction. These observations prompt a modification of the current paradigms of malaria pathogenesis, and open the way to investigate the pathophysiology of *P. vivax* infections. Financial support: CAPES, CNPq, FAPESP, The National Institute for Vaccine Development and Technology (CNPq-INCTV) and Rede Malaria (CNPq).

MC.06 – FROM PROLINE TO GLUTAMATE: SEVERAL ROLES FOR A BIOCHEMICAL PATHWAY

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The flagellated parasite *Trypanosoma cruzi* uses proline and glutamate, among other amino acids, as energy source. It was established that proline is involved in the metacyclogenesis, the process occurring in the insect vector, in which replicative non-infective epimastigotes differentiate into infective, non-replicative metacyclic trypomastigotes. It was also demonstrated the participation of proline in the differentiation between the intracellular epimastigote-like and the trypomastigote stages. More recent studies describing a proline racemase presenting a mitogenic activity contributed also to highlight the possible roles of proline in the biology of *T. cruzi.* Together, these data led us to deepen the study of the metabolism of this amino acid. Our work evidenced that *T. cruzi* is equipped with genes coding for actives proline dehydrogenase (PRODH) (EC 1.5.1.2) and a pyrroline-5-carboxylate dehydrogenase (P5CDH) (EC 1.5.1.12) which are able to oxidize L-proline into glutamate. TcPRODH and TcP5CDH genes complemented null mutants of *Saccharomyces cerevisiae* for the genes PUT1 or PUT2, demonstrating the activity of their products. Yeasts complemented with the TcPRODH gene showed lower free intracellular proline levels which correlated with enhanced sensitivity to oxidative stress. The ratio GSS/GSH confirmed the relationship between PDH activity, free intracellular proline levels and sensitivity to oxidative stress. The role of free proline in the resistance to oxidative stress was also confirmed in epimastigote forms. Since proline metabolism seems to be a central pathway for *T. cruzi*, we approached its study by looking for inhibitors among several structural analogues of proline and glutamate. Due to their performance, L,4 thiazolidine carboxylic acid (T4C) was choose for further *in vivo* studies. T4C behaved as a specific competitive inhibitor of the proline transport systems in *T. cruzi*. The treatment of epimastigotes with this analogue reduced their intracellular free proline content, making them more sensitive to oxidative stress. Besides, treated parasites were more sensitive to metabolic and thermal stress. All these data point the proline - glutamate pathway as part of a resistance mechanism against stresses that challenge the parasite along its natural life cycle, and prompt its component as interesting targets for drugs against *T. cruzi* infection.
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MC.07 - ADAPTTIVE ESTRATEGIES IN THE INTERACTION OF *LEISHMANIA* WITH THE CELL HOST: MODULATION OF NF-KB DIMERIZATION AND PKR ACTIVATION

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Leishmania species are obligate intracellular parasites that reside and multiply within mammalian host macrophages. Macrophage infection typically leads to the induction of numerous cellular genes, several of which encode cytokines capable of stimulating both pathogen resistance and an inflammatory response. Adaptive strategies have been evolved and ultimately tailored sophisticated mechanisms leading to the evasion of microbicide macrophage functions. In this work we present our results on the role of the transcription factor NF-kB and the double-stranded RNA-activated protein kinase (PKR) in the infection by *L.amazonensis.* The transcription factor NF-kB is activated by a number of pathogens, including *Leishmania* species, and regulate the expression of key immunological mediators. We have investigated the KF-kB activation and the NF-kB-dependent regulation of the Inductible Nitric Oxide Synthase (**iNOS**) expression by *L.amazonensis* in human and murine macrophages. We demonstrated that *L.amazonensis* infection determined a peculiar and specific activation of the NF-kB homodimer p50/50, which lacks the transactivator transcription domain. NF-kB expression luciferase-reporter assays with macrophages infected with *L.amazonensis* and treated with the LPS (a classical NF-kB inducer) revealed the repression of NF-kB-dependent expression induced by LPS. These results corroborate the notion that *L.amazonensis* activated- NF-kB -homodimer is a transcription repressor. We aimed to investigate the activity of the iNOS promoter which is regulated by NF-kB. Luciferase reporter assays confirmed that *L.amazonensis* infection repressed the activity of the iNOS promoter, containing nk-sites, induced by a mixture of LPS, TNF-α and IFN-γ. In addition, we also observed that during the infection there is an increase of nuclear histone deacetylase-1 levels (HDAC1) in macrophage nuclei accompanied by an augment of HDAC-activity. The double-stranded RNA-activated protein kinase (PKR) is activated during viral-infections and mediates the antivirus response leading to the expression of Type I interferon (IFN1) and other cytokines such as IL-10 and TNF. In addition, PKR may also be activated through TLR2 and TLR4 and plays a role in the innate immune response. To investigate the role of PKR during the infection by *L.amazonensis,* we infected murine and human macrophages with this parasite and treated with the synthetic RNA molecule Poly I:C. Strikingly, Poly I:C treatment enhanced the infection and this effect was abrogated by the PKR inhibitor 2-AP. Poly I:C treatment did not lead any significant effect in the infection of macrophages transfected with a dominant-negative PKR construction (DN-PKR). Strikingly, *L.amazonenis*, in the absence of exogenous PKR-inducer, was able to activate and increase PKR levels. In fact, PKR signaling was proven to be important for the success of the infection, since the infection index of macrophages of either PKR-KO mice or RAW 264.7 DN-PKR was dramatically reduced compared to wt cells. Interestingly, activation of PKR through the TLR4 ligand LPS also favored the infection in a PKR-dependent fashion. Moreover, we could demonstrate that IL-10 expression was one of the PKR determinant effectors in the infection. Current studies are in progress to investigate the role of TLR receptors in the PKR activation by *L.amazonensis*. We are also addressing the role of IFN1 in the infection and activity of the PKR promoter in this context.

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MC.08 – STRESS-INDUCED ACTIVATION OF THE *L. MAJOR* MAP KINASE LMAMPK7 INCREASES PARASITE RESISTANCE AGAINST THE LEISHMANICIDAL DRUG MILTEFOSINE

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During the infectious cycle, *Leishmania* is exposed to various environmental stress signals in insect and mammalian hosts, which trigger differentiation of the virulent metacyclic and pathogenic amastigote life cycle stages. Despite the importance of stress sensing and response in transmission and intracellular parasite infection, our understanding on mechanisms underlying these processes remain very poor. We previously used a transgenic strategy to gain insight into the functions of the mitogen-activated *Leishmania major* protein kinase LmaMPK7 in parasite virulence. Establishment of *L. major* and *Leishmania donovani* lines expressing episomal green fluorescent protein (GFP)-LmaMPK7 fusion proteins revealed environmentally-induced activity of this kinase in response to low pH and high temperature, and linked this kinase to amastigote growth regulation and translational control. Here we show that LmaMPK7 confers increased resistance to nutritional stress at stationary growth phase, and treatment with the anti-leishmanial drug miltefosine. Drug resistance was specific to LmaMPK7 as transgenic expression of related LmaMPK4 and LmaMPK10 had no effect. We showed by *in vitro* kinase assay that LmaMPK7 activity itself is induced during miltefosine treatment and thus identified for the first time a regulatory interaction between an anti-leishmanial drug and a parasite signaling pathway that results in enhanced *Leishmania* drug resistance. Transgenic parasites expressing an LmaMPK7 kinase dead mutant were susceptible to miltefosine, demonstrating a direct role of LmaMPK7 phosphotransferase activity and its downstream substrates in parasite drug resistance. Quantitative phosphoproteomics analysis of LmaMPK7 transgenic parasites correlated increased LmaMPK7 activity with enhanced phosphorylation of LmjF29.1240, a hypothetical protein with homology to TPR domain-containing chaperones implicated in stress response. Based on these results we hypothesize that LmaMPK7 regulates *Leishmania* stress resistance through chaperone phosphorylation, which may alter parasite susceptibility to anti-microbial drugs. Our data thus unravel a novel mechanism of drug resistance regulated at the post-translational level, and propose the *Leishmania* stress response in general and LmaMPK7 in particular as attractive targets for anti-leishmanial intervention.