Samuel Pessoa Conference

SPC001 - SUCCESS UNDER ADVERSE CONDITIONS: THE *LEISHMANIA*

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The Leishmania are amongst the most successful group of parasites and have gained renown as being the etiological agent of a group of diseases that are classified as the 2nd most important Neglected Tropical Disease (NTD), loosing only to malaria. However, there is a very big difference between these two groups of diseases. Man is the primary reservoir of malaria, so theoretically it can be eliminated, as have some viral diseases such as smallpox. The reservoirs of the leishmaniases are, however, an incredible number of wild animals and so they can never be eliminated. If one had to choose a successful evolutionary path you would be unlikely to choose the one that the Leishmania decided to take: getting from one host to another by the bite of a fly and then invade and develop the very cells whose function is to remove pathogens and tell other cells what to do in the face of invasion. Parasitism has been defined as a form of symbiosis in which an organism (the parasite) benefits at the expense of another (its host). Measuring the degree of this expense is difficult and one of the principal misconceptions related to the Leishmania is that they are parasites that produce disease. The line between symbiosis and parasitism a very fine one and the Leishmania have crossed this line but only provoke disease when they enter a new hosts, such as men and dogs, that are not linked to their evolution. Recent studies on reservoirs of L.(Viannia) braziliensis indicate that occult infections, that are not detectable by any method including kDNA and SSUrDNA PCRs are infectious to sand flies. It is difficult to classify such infections as symbiotic since there is no evidence that the host gains anything but they are tolerated with no evidence of pathology. If we look at the records of other Leishania species in their natural hosts this is the rule rather than the exception. Could the Leishmania in some way be beneficial for the host? In man and dogs L.(L.) donovani and L.(L.) infanatum infections can be fatal. Loosing a few hosts while allowing the vectors to gain access to large numbers of parasites favors rapid expansion, especially when these host populations are large. Parasites that kill have been classified as poor parasites but they are not poorly performing parasites. In fact they are very successful ones and are taking the opportunity of expanding their populations in a very dynamic and positive fashion. There is, however, growing epidemiological evidence of occult L.infantum infections in man. So is L.(.L.)infantum becoming like other Leishmania in their natural reservoir hosts? The Leishmania evade elimination by tolerance strategies. Most immunological research concentrates on the mechanisms that cause pathology but in my opinion tolerance deserves more attention. The genetic diversification of the Leishmania also guarantees their survival as a group but each species survival depends on an intricate vector/host contact that is modulated by the reproduction of both. Leishmania readily cross mammalian orders in terms of their infectivity which suggests they are capable of adapting to different habitats. However, their real habitat is the immune system and it depends on their ability at handling its different components as how they survive when plunged into a new habitat by a vector. Specificity in the vector is not related to insect immunity but to interactions between parasite and gut surface molecules. In some parasite/vector combinations, such as L.major/P.papatasi this is specific. The advantage of this strategy is that there is no competition for this vector by another Leishmania, such as L.infantum. At the present time we do not know how widespread this phenomenon is amongst species of the two subgenera but we do know that if by luck it is taken up by a vector with universal gut receptors it may gain a new field to expand in. An example of this is L.infantum being transmitted by Lu.longipalpis in the Americas. The leishmania are continually battling with immune systems, trying to hold on to gut walls and trusting that the weather does not knock out their houses (hosts & vectors), but they are experts at surviving!

CO1 - RECOGNITION OF PROTOZOAN PARASITES BY INTRACELLULAR INNATE IMMUNE RECEPTORS

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Parasites of the Leishmania genus are the causative agents of leishmaniasis in humans, a disease that affects more than 12 million people worldwide. The parasites replicate intracellularly in macrophages, and the primary mechanisms underlying host resistance involve the production of nitric oxide (NO). In this study, we showed that the Nlrp3 inflammasome is activated in response to infection and is critical for the restriction of parasite replication both in macrophages and in vivo, as demonstrated through the infection of inflammasome-deficient mice with L. amazonensis, L. braziliensis and L. infantum chagasi. The inflammasome-driven IL-1β production was critical for host resistance to infection, as signaling via IL-1R/MyD88 was necessary and sufficient to induce the NOS2-mediated production of NO. In this study, we elucidate the major signaling platform for the host resistance against Leishmania spp. infection and describe the molecular mechanisms underlying Leishmania-induced NO production by macrophages. **Supported by:**FAPESP

CO2 - THE IMPACT OF IMMUNITY TO SAND FLY SALIVARY PROTEINS ON LEISHMANIASIS: FROM BASIC SCIENCE TO TRANSLATIONAL RESEARCH.

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Every time a sand fly attempts to get a blood meal it injects saliva into the skin of the host. It is remarkable that only few nanograms of injected salivary proteins are sufficient to disarm the host hemostatic system, including vasoconstriction, platelet aggregation and the blood coagulation cascade. Importantly, this small amount of salivary protein is sufficient to induce a systemic humoral and cellular immune response in animals and consequently protect rodents against leishmaniasis. Using a multidisciplinary approach based on transcriptomics, biochemical and immunological assays we aimed to understand the basis of this protective effect and to identify the protective salivary protein. We observed a protective effect of immunity to sand fly salivary proteins in rodents, dogs and non-human primates and this protection correlates to the development of a TH1-biased delayed-type hypersensitivity response (DTH) to the salivary molecule/s and to the development of an accelerated robust Leishmania-specific immune response with minimal pathology. Importantly, immunity to a TH1-DTH-inducing salivary protein protects against both cutaneous and visceral leishmaniases suggesting, as expected, that it exert its influence early after an infected bite while the parasites are in the skin and at their most vulnerable stage. During this presentation I will show evidence of the protection in the various models, allude to the mechanism of protection and discuss the realistic prospect for a sand fly salivary protein as a component for a Leishmania vaccine. **Supported by:**DIR, NIAID, NIH

CO3 - TEN YEARS SINCE THE GENOME SEQUENCE; EVOLUTIONARY, MOLECULAR AND CELLULAR BIOLOGY OF AFRICAN TRYPANOSOMES

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The availability of a genome sequence for Trypanosoma brucei almost ten years ago led to an improved understanding of molecular processes present in the cell but also enabled an acceleration in research when combined with emerging technologies. Here, the knowledge acquired from the genome and the implication for how the cell functions will be described. Research output over the last ten years has vastly increased knowledge of the evolution of pathogenicity, and cell and molecular processes that regulate growth and form. Selected highlights will be discussed to illustrate what has been discovered and the questions still to be answered.

CO4 - MALARIA PARASITE IMMUNE EVASION: FROM BASIC RESEARCH TO NEW INTERVENTION STRATEGIES

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The persistence of the human malaria parasite Plasmodium falciparum during blood stage proliferation in its host depends on the successive expression of variant molecules at the surface of infected erythrocytes. This variation is mediated by the differential control of a family of surface molecules termed PfEMP1 encoded by approximately 60 var genes. Each individual parasite expresses a single var gene at a time, maintaining all other members of the family in a silent state. PfEMP1/var enables parasitized erythrocytes to adhere within the microvasculature, resulting in severe disease. I will review key regulatory mechanisms thought to be critical for antigenic variation. Different epigenetic layers orchestrate this process including perinuclear spatial domains, differential histone marks on otherwise identical var genes and var silencing mediated by heterochromatin. Recently, we discovered a novel type of post-transcriptional mechanism that involves an RNase-mediated silencing process via the degradation of var gene mRNA (cryptic RNA). Insight into the var gene regulation process provided us with new insight into molecules essential for parasite proliferation. We investigated P. falciparum histone 'writers' and 'readers' as potential target class for the development of novel antimalarial. Our data position histone lysine methyltransferases as a previously unrecognized target class, and BIX-01294 as a promising lead compound, in a presently unexploited avenue for antimalarial drug discovery targeting multiple life-cycle stages. **Supported by:**ERC advanced grant

CLOSURE CONFERENCE

CC001 - ARGININE FATE IN *LEISHMANIA* - A GAME *OF MANY PLAYERS.*

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Amongst several enzymes that participate in the Krebs-Henseleit cycle, *Leishmania* presents L-arginine amidinohydrolase, E.C. 3.5.3.1 (ARG), a metalloenzyme that hydrolyses L-arginine to L-ornithine and urea.
The question initially addressed in our studies was why protozoa that live in an aqueous environment, need to eliminate nitrogen in urea form? Works of Camargo et al indicated that the physiological role of the enzyme could be assigned to the ornithine production.
As the enzyme inducible Nitric Oxide Synthase (iNOS) uses the same substrate to produce nitric oxide (NO), a potent microbicide molecule, new light came into the discussion pointing that arginase could also act facilitating parasite escape from macrophage attack.
Our lab characterized *L. amazonensis* ARG coding gene, and also established method to purify an active recombinant ARG expressed in *E.coli*. The purified enzyme presented a Km and a Vmax similar to the native enzyme. Antibodies produced against ARG localized the enzyme in the glycosomal compartment in both promastigote and amastigote. We obtained ARG knockout as well as add-back mutants, addressed or not to glycosome. The *L. amazonensis* null mutant and the non-glycosome addressed add-back, both lacking ARG enzymatic activity, presented a low infection rate in *in vitro* and *in vivo* infection. This allowed us to postulate that the correct compartmentalization of the enzyme is important for parasite growth in the host, since ornithine is the precursor of polyamine pathway, and contributes to the success of the infection, probably by allowing the escape from macrophages defenses.
We also explored arginine uptake regulation in *L. amazonensis.* The two copies of amino acid transporter AAP3-like gene, organized *in tandem* in the genome, showed similar ORFs, but 5' and 3' untranslated regions with significant differences. It is interesting that the transcript of one copy is more stable in response to amino acid starvation, increase of temperature and pH decrease.
We also determined the metabolomic polyamine pathway profile of the wild type *L. amazonensis* (WT) and the ARG knockout mutant submitted or not to arginine starvation. We found a decrease in arginine, ornithine and putrescine after starvation of WT parasites, but the levels of urea, glutamate, spermidine, spermidine or agmatine were not altered. However, the absence of ARG resulted in an increase of arginine and citrulline levels and a decrease in ornithine and putrescine.
We know that Th1 and Th2 cytokines induce the expression of macrophage CAT-2B transporter in macrophages. Besides, if iNOS is activated by a Th1 response, the NO production kills the parasite. But if Th2 response activates arginase I (ARG I) we have the production of polyamines and parasite proliferation. We don't understand yet the complete orchestration of host and parasite ARG and NOS enzymes, but we show that arginine is essential in determining the fate of *Leishmania* infection.
Recently, we observed that the dark hormone melatonin diminished the mammal host infection rate. Searching for mechanisms that lead to this protection, we observed that macrophages treated with melatonin reduced the transcripts of host ARG I and CAT-2B but not iNOS, with a possible involvement of miRNAs in this regulation. Therefore, melatonin modulates macrophage arginine uptake, inactivating host ARG I leading to the inhibition of *L. amazonensis* replication at the lesion. This may represent a protective mechanism in the initial steps of the infection.
The use of *Leishmania* ARG as a target for chemotherapy bumps on its glycosomal location. A potential drug has to cross all way through the macrophage membrane, the phagosome membrane, the amastigote membrane and the glycosomal membrane. So, we propose that a more rational way to control the infection is to prevent arginine to reach the parasite ARG disturbing this route somewhere inhibiting arginine uptake.
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