Scientific Program

SPC-RESEARCH IN BRAZIL AS AN OPTION: 40 YEARS OF EXPERIENCE AND DEDICATION

Antoniana U. **Krettli**

Laboratory of Malaria, Centro de Pesquisas René Rachou / FIOCRUZ &
Universidade Federal de Minas Gerais, Belo Horizonte, MG
akrettli@cpqrr.fiocruz.br

Research entered my life by chance, as a student of Zigman Brener. Chloroquine treatment was no longer efficient against *P. falciparum* human malaria, and we tested drugs against murine malaria. Among hundreds of molecules, sulfadiazines (Hoffman la Roche) were active against a chloroquine resistant strain selected by drug pressure. My master thesis was on *P. juxtanuclearem* a chicken malaria parasite discovered in Belo Horizonte, MG. Later, I went to New York, working with Ruth Nussenzweig on malaria anti-sporozoite vaccine and on immunopathology. These 30 months at NYU were intense and exciting, especially learning about neutralizing antibodies after vaccination. Back home, decided to study this antibody mediated protection and not finding vectors to raise sporozoites - a research drawback up to now - I began with *Trypanosoma cruzi* antibody mediated immunity using mice chronically infected. Soon had a paper published (J. Immunol. 1976) and for two decades, in collaboration with several groups, in and outside Brazil, I focus on antibodies against live trypomastigotes describing the “lytic antibodies”.

My students worked with malaria models, less dangerous, rather amusing and important. We infected *Aedes fluviatilis* mosquitoes with *Plasmodium gallinaceum*, successfully, and produced sporozoites used to: vaccinate chickens; characterize antibodies during infections; infect macrophages; and raise monoclonal antibodies to the circum-sporozoite protein (CS). Our work in human malaria, driven to the anti-CS immune response, used recombinant *P. falciparum* (Pf) and *P. vivax* CS proteins and sera from individuals living in malaria endemic areas, or those briefly exposed to transmission. All sera had similar levels of antibodies. Later, as a visiting researcher at NIH (1990), I worked with *P. gallinaceum* (Pg) and aspects of sporozoite and vector interactions. We cloned and sequenced the PgCS protein, a data which confirmed previous hypothesis of Pg and *Pf* being closely related. Back home, we studied possible reactivity of Pg sporozoites with human malaria. Sera from subjects exposed to malaria in hyper-endemic areas of intense Pf transmission strongly reacted with PgCS, not sera from *P. vivax* infections. If Pg sporozoites protect humans against Pf infections is yet to be tested.

At present, our research aims the discovery and development of new antimalarials exploring the Brazilian biodiversity and ethnopharmacology. With phytochemists groups we select and test substances against *P. falciparum* blood stage (comparing traditional microscopy, hypoxanthine incorporation, enzymatic tests, green fluorescent parasites). Those selectively active (low toxicity) are tested in rodent malaria. Among many synthesized molecules, a hybrid between mefloquine and artesunate, more active and less toxic than the antimalarial combination, should undergo clinical trials. The financial support for this multidisciplinary project has allowed our groups to work with students and technicians, train specialists at various levels, from undergraduate to postdoctoral fellows, work on chemistry and malaria, important for the country development. Learning, teaching and doing science has been great fun, and hope to transmit my enthusiasm to future generations. To show them how pleasant research can be, in spite of the intense labor it requires, might be my best contribution to science. Supported by CNPQ, FIOCRUZ-MS.

CO.01 – INSIGHTS INTO THE PATHOGENESIS OF VIVAX MALARIA

Barral-Netto, Manoel

Centro de Pesquisas Gonçalo Moniz (FIOCRUZ), Bahia, Brazil, Faculdade de Medicina da Bahia, Universidade Federal da Bahia (UFBA), Brazil, Instituto de Investigação em Imunologia - Instituto Nacional de Ciência e Tecnologia (iii-INCT), São Paulo, Brazil

Malaria is considered one of the most important infectious diseases that ever threaten the world. This disease is caused mainly by the infection with *Plasmodium falciparum* or *Plasmodium vivax* transmitted by *Anopheles* mosquitoes. Despite governmental and private efforts for the development of key strategies for the disease control, the actual panorama of the *Plasmodium* infection is getting worse due to the emergence of drug resistant parasite strains. The lethal cases are reported mostly in Africa and are caused by *Plasmodium falciparum*. Albeit being less lethal, *Plasmodium vivax* infections are more widely distributed and can cause high morbidity and eventually death. In most endemic areas, studies have indentified a number of factors related to clinical immunity or susceptibility to the parasites. Thus, at least regarding the falciparum malaria, age, genetic polymorphisms and repeated exposure to *Plasmodium* are considered most important determinants of the disease outcome. Unfortunately, little has been made in the screening of reliable predicting factors that could be ultimately used for clinical evaluations. This landscape is even worse for vivax malaria, probably because many researches consider it as a benign disease. Moreover, as most of the current knowledge about the malaria pathogenesis did not truly help to relieve the disease burden, new insights are necessary to overcame unfavorable scenario. The lecture will present data that aim to identify potential determinants of the vivax malaria severity linked to the immunopathogenesis in an endemic area from the western Brazilian Amazon. First, a precise and effective method for malaria diagnosis was screening by comparing multiple tests, including a software based of artificial neural networks. The molecular assay showed to be the most efficient for the diagnosis of symptomatic and asymptomatic malaria. In addition, the rational use of a rapid test for the diagnosis of malaria may be promising in areas where there is difficulty in continued training of technical human resources. The artificial neural network indicated that the cytokine balance is a strong determinant of the clinical presentation. In another study, the use of serology for measuring IgG antibodies against the sonicate salivary gland of the *Anopheles darlingi* vector showed to be a powerful tool for the estimation of exposure to *Plasmodium vivax* and also to predict clinical immunity. Intriguingly, the natural exposure to the hepatitis B virus appeared as an important factor associated with reduced clinical severity for both vivax and falciparum malaria. Concerning solely the vivax malaria, severe cases have an intense and unregulated inflammatory response. In these patients, the antioxidant enzyme superoxide dismutase-1 has emerged as an excellent marker of severity and was involved in the pathogenesis of the severe disease in which there is a release of large amounts of free heme. Together, these data add important information in understanding the mechanisms that determine the severity of vivax malaria.

CO.02 – TELOMERE DYNAMICS AND ANTIGENIC VARIATION IN *Trypanosoma brucei*

CROSS, G. A. M.
The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

Telomeres are specialized DNA-protein complexes that stabilize chromosome ends, protecting them from nucleolytic degradation and illegitimate recombination. Telomeres form a heterochromatic structure that can suppress the transcription of adjacent genes. Telomeres may have additional roles in *Trypanosoma brucei,* whose variant surface glycoproteins, the mediators of antigenic variation and the primary cause of persistent host infections, are expressed from one subtelomeric locus at any given time. Many silent VSG genes are stored at subtelomeric loci, both at the 44 ends of the diploid chromosomes and in minichromosomes, whose abundance is unique to *T. brucei*. Telomere-induced silencing is probably partially but not entirely responsible for VSG silencing. I will summarize our principal findings about telomere structure and dynamics in *T. brucei*. Based on observations of the structure and dynamics of telomeres in the absence of telomerase and after its restoration, we have proposed that growth and breakage of telomeric repeats plays an important role in regulating the rate of antigenic switching, for which I will present new evidence.

CO.03 – RNA-BINDING PROTEINS AND EXORIBONUCLEASES INVOLVED IN mRNA DEGRADATION IN *Trypanosoma brucei*

Clayton, CE

Zentrum für Molekularbiologie der Universität Heidelberg

Kinetoplastid protists control their gene expression primarily at the levels of mRNA processing, degradation and translation. We have shown that in *Trypanosoma brucei,* the 5'-3' exoribonuclease XRNA is important in the rapid degradation of developmentally regulated mRNAs, whereas the deadenylase CAF1 is required for general, constitutive mRNA degradation.
The rates of mRNA degradation and translation can be influenced by association with specific RNA-binding proteins. We have discovered that a pumilio domain protein, PUF9, specifically stabilises a few mRNAs during S-phase, while a zinc-finger-domain protein is involved in the stress response. Examples of this control will be described.
To investigate the importance of mRNA degradation in more detail, we have used deep RNA sequencing to analyze the transcriptomes of trypanosomes that have reduced expression of XRNA, and to correlate the half-lives of mRNAs with their abundances. Some preliminary results of these experiments will be presented.

CO.04 – TOXOPLASMA PERFORIN-LIKE PROTEIN 1 DICTATES PARASITE EGRESS AND ACUTE VIRULENCE

Kafsack, B.F.C.[1], Commodaro, A.G.[2], Nepomuceno De Oliveira, N.[2], Hugunin, K.[3], Schultz, T.[1], Roiko, M[1], Eaton, K.[3], Pena, J.[4], Rizzo, L.V.[5], Carruthers, V.B.[1]

[1]Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, USA; [2]Vision Institute, Federal University of São Paulo, São Paulo, Brazil; [3]Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, USA; [4]Department of Immunology, Universidade Federal de Uberlandia, Uberlandia, Brazil; [5]Instituto Israelita de Ensino e Pesquisa Albert Einstein, São Paulo, Brazil

Pore-forming proteins are important virulence factors for many pathogens across the tree of life. With the exception of *Cryptosporidium spp.*, all of the sequenced apicomplexan genomes encode for multiple members of the Membrane Attack Complex/Perforin family of pore-forming proteins. Recent work has illustrated the importance of these so-called Perforin-Like Proteins (PLPs) in the lifecycles of both *Plasmodium spp*. and *Toxoplasma gondii*. In *Toxoplasma* PLP1 plays an important role in egress from host-cells by facilitating the breakdown of the parasitophorous vacuolar membrane. Virulence in Swiss Webster and C57BL/6 mice was attenuated for a PLP1-deficient strain with an LD_{50} of greater than 100,000 parasites, compared to less than 10 parasites for the wild-type RH strain. Using parasites expressing firefly luciferase, we monitored the infection of mice over-time by bioluminescence imaging. PLP1-defficient parasites replicated similar to WT at high doses of intra-peritoneal inoculation (100,000 and 10,000 tachyzoites) but lagged by approximately 2 days at a lower dose (1,000 tachyzoites). At a dose of 10,000 tachyzoites there was no apparent difference in time of dissemination from the peritoneal cavity to other organs. We also showed that the host mounts a milder cellular and inflammatory cytokine response against PLP1-deficient parasites compared to WT. Finally, we demonstrated that MyD88, IL12, and IFNgamma are not necessary for controlling infection with PLP1-deficient parasites, further underscoring the markedly attenuated virulence of this strain. Collectively, our findings suggest that the egress defect in PLP-deficient parasites leads to a moderately slower expansion of infection in mice, which survive by avoiding the severe immune pathology that normally accompanies infection with WT parasites.

CC – TALES OF EVOLUTIONARY DIVERSITY: HOW BLOOD-SUCKING INSECTS LEARNED TO USE OUR BLOOD.

Pedro L Oliveira

Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, RJ, Brazil and
Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular – INCT-EM

Infectious diseases that are prevalent in developing countries are either transmitted by blood feeding-arthropod vectors as in malaria, Chagas' disease and dengue, or have a blood-feeding organism as the etiologic agent, as in schistosomiasis and malaria. Half of the protein content of vertebrate blood is hemoglobin, and huge amounts of free heme (hemoglobin prosthetic group) are released during blood digestion. Heme is potentially cytotoxic, promoting oxidative damage to lipids, proteins and DNA. Hence, the avoidance of deleterious effects of free heme should be mandatory for blood-feeding organisms and to built protective mechanisms is a major evolutionary trend in the adaptation of these animals to hematophagy. This hypothesis is supported by the description of several protective mechanisms against heme toxicity in different species of hemoglobin-eating animals such as the kissing-bug (*R prolixus*), ticks (*R microplus*), mosquitoes (*A aegypti*) and the blood fluke (*S mansoni)*. During the course of evolution, these phylogenetically distinct groups of blood-feeding animals developed protective mechanisms that may be classified in two main groups: a) mechanisms that specifically deal with the heme molecule; such as heme aggregation, heme degradation by heme oxygenases and heme-binding proteins; b) strategies to control the redox balance, including both reactive oxygen species (ROS) depleting devices such as antioxidant enzymes and radical scavengers, together with regulation of radical production by cell metabolism. Besides the toxic effects of reactive oxygen species (ROS), it has been shown that heme have important roles in a wide range of physiological processes, from signal transduction, modulation of cellular response to stress and infection and microbial killing. This led us to study the regulatory effects of heme on redox metabolism of the midgut of blood feeding organisms. We have found that the production of ROS in the midgut of several blood sucking animals is markedly lowered after a blood meal, through heme-mediated cell signaling, affecting also the interaction with the midgut microbiota. We suggest that down-regulating production of ROS is a novel antioxidant mechanism to compensate for the ingestion of heme. Taken together, these data shows that the heme is a major component of the midgut scenario, profoundly affecting both the insect physiology and the midgut microbial ecology.
Supported by CNPq, FAPERJ and HHMI.