RT001A - The FML-vaccine Leishmune (R) and the Nucleoside hydrolase synthetic vaccine in vaccination and therapy of visceral leishmaniasis

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The FML-saponin vaccine (Leishmune®) composed of the Fucose–Mannose ligand antigen of L. donovani promastigotes and the QS21 and deacylated saponins of Quillaja saponaria was immunogenic, immunoprophylactic and immunotherapeutic in mice and hamsters and field trials for dogs. The vaccine was considered safe and was well tolerated. After two years of vaccination, only 1% of 550 vaccinated and exposed dogs died of ZVL and 1.2% was symptomatic while 39% of deaths and 20.6% of symptomatic cases were detected among untreated exposed controls. The vaccine reduced the parasite burden accessible for sand flies, as disclosed by the negative results of PCR, immunohistochemistry and xenodiagnosis. The generated antibodies block the transmission of the disease by sand flies. When formulated with double saponin concentration it promoted parasitological and clinical cure while sterile cure required immunochemotherapy. Leishmune® induced enhanced levels of IFN-γ, NO and anti-L. chagasi IgG2, the early and persistent activation of neutrophils and monocytes, and increased the CD8+ T-cells expressing IFN-γ. Dogs vaccinated with Leishmune® did not become seroreactive in the official test used by the control enquire. Only 1.3% of positivity (76 among 5,860) was detected in a 110.000 dogs enquire. Leishmune ® vaccination displayed an additive effect over dog culling, on the decrease of the incidence of canine and human ZVL. The districts of greater vaccine coverage exhibited declined or sustained levels of canine and human cases of ZVL, while those with less vaccine coverage, showed rising curves of canine and human cases of the disease. Our data confirmed the relevance of dog vaccination as predicted by Dye (1996).

The main antigen of the FML complex is the Nucleoside hydrolase of Leishmania donovani (NH36). Protection against L. chagasi in mice is related to its C-terminal domain (F3= amino-acids 199-314) and is mediated mainly by a CD4+ T cell driven response with a lower contribution of CD8 + T cells. Immunization with this peptide exceeds in 36.73 ± 12. 33% the protective response induced by the cognate NH36 protein. Increases in IgM, IgG2a, IgG1 and IgG2b antibodies, CD4+ T cell proportions, IFN-γ secretion, ratios of IFN-γ/IL-10 producing CD4+ and CD8+ T cells and percents of antibody binding inhibition by synthetic predicted epitopes were detected in F3 vaccinated mice. The increases in DTH and in ratios of TNFα/IL-10 CD4+ producing cells were strong correlates of protection which was confirmed by in vivo depletion with monoclonal antibodies, algorithm predicted CD4 and CD8 epitopes and a pronounced decrease in parasite load that was long-lasting. No decrease in parasite load was detected after vaccination with the N-domain of NH36 (Nico et al., 2010). Both peptides reduced the size of footpad lesions, the parasite load and PCR evidence of Leishmania DNA in prophylactic and therapeutic vaccination against L. amazonensis and in immunotherapy against L. chagasi infection.

Nucleoside hydrolases are involved in the purine salvage pathway, are targets for the development of anti-leishmanial drugs and are not found in mammal cells. The L. donovani nucleoside hydrolase accepts inosine, guanosine, adenosine, uridine and cytidine as substrates with a slight preference for adenosine and inosine. Guanosine is not a good substrate. The catalytic specificities showed relative values of Kcat with inosine, adenosine, guanosine, uridine and cytidine of 32, 53, 1.2, 16 and 9, respectively. In collaboration with Prof. VL Schramm (A Einstein College of Medicine NY, USA) we have analyzed potential nucleoside hydrolase inhibitors, as an approach to block purine salvage. Immucillin-H and Immucillin-A gave Ki values of 19 and 80 nanomolar respectively. Immucillin-H (Forodesine) is currently in human clinical trials against lymphatic cancers. Our results may be useful as leads for anti-leishmania agents. **Supported by:**CNPQ e FAPERJ

RT001B - A2 antigen based vaccine formulations: rational, update and perspectives towards an anti-amastigote vaccine for visceral leishmaniasis

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Aiming to develop a vaccine against visceral leishmaniasis (VL) targeting the intracellular amastigotes, we have identified an amastigote specific antigen (A2) that contains an immunogenic epitope for CD4+ T helper (Th) cells and multiple repetitive units encoding CD8+ cytotoxic T lymphocyte (CTL) epitopes. A2 proteins are amastigote virulence factors, and are associated to the ability of leishmania viscetropic species to survive at high temperatures and migrate to visceral organs. Several vaccine formulations containing the recombinant antigen A2 have been tested and were shown to be protective in mice, dogs and nonhuman-primates. Among these vaccine formulations, Leish-Tec®, an A2 recombinant protein plus saponin formulation, is the third prophylactic vaccine against canine visceral leishmaniasis (CVL) and the first recombinant one, to be licensed in the world. The ability to induce type I immune responses and specific humoral responses that do not cross react with antigens used in routine tests for serodiagnodiagnosis of CVL, are main immunogenic properties of Leish-Tec®, though evaluated in a small, single breed (beagle dogs) sample of a phase II trial. In the current presentation are presented the steps forward better characterize the humoral responses induced by Leish-Tec® in larger and heterogeneous dog population and its efficacy and ability to reduce transmission rates. through a randomized, masked, placebo controlled phase III trial. In addition, results of pre-clinical trial in Rhesus monkeys aiming to test, through homologous or heterologous prime-boost protocols, the protective responses induced by adenovirus or the recombinant A2 will be also discussed. Altogether, the results obtained so far constitute a solid base for the development of an A2 based vaccine for human VL. **Supported by:**National Institute for Science and Technology of Vaccines (CNPq) and The Network Research in Bio-mol

RT001C - Use of a recombinant cysteine proteinase from Leishmania (Leishmania) chagasi for immunochemotherapy of canine visceral leishmaniasis

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A recombinant cysteine proteinase from *Leishmania (Leishmania) chagasi*, rLdccys1, was previously shown to be a useful immunological marker for stages of visceral leishmaniasis (VL) in humans and dogs, as well as a potential tool for the diagnosis of human and canine VL (Pinheiro et al., 2005; Dias et al., 2005; Pinheiro et al., 2009). Furthermore, immunization with rLdccys1, as well as with the gene encoding the cysteine proteinase Ldccys1, induced significant protective immune responses in a mouse model of VL (Ferreira et al., 2008). Preliminary results also revealed that immunization with rLdccys1 resulted in a significant protection in dogs after challenge with *L. (L.) chagasi* (Pinheiro, 2009). These results encouraged us to ask if rLdccys1 could be used in immunotherapy of naturally infected dogs from an endemic region of VL, Teresina, Piauí state of Brazil. Thirty symptomatic naturally infected mongrel dogs were divided in three groups. One group received three doses, with one month interval, of rLdccys1 plus *Propionibacterium acnes* as adjuvant, a second group received three doses of *P. acnes* only and a third group received saline. Improvement of clinical parameters (alopecia, onychogryphosis, cachexia, anorexia, apathy, skin lesions, hyperkeratosis, ocular secretion, increasing of lymph nodes and weight loss), besides a significant reduction of spleen parasite load were observed within three months in dogs that received rLdccys1 + *P. acnes*, in comparison to controls which received either *P. acnes* only or saline. Evaluation of humoral and cellular immune responses was performed one month after the end of treatment. A more intense delayed type hypersensitivity reactions against *L. (L.) chagasi* lysate were observed in dogs treated with rLdccys1 when compared to controls. Furthermore, a higher IgG2 and a decreased IgG1 response followed by a high IFN-y and a low IL-10 concentration were detected in serum from rLdccys1-treated dogs, pointing to a Th1 cell

mediated response. In contrast, dogs which received *P. acnes* only or saline exhibited low serum levels of IgG2 and IFN-y and high concentrations of IgG1 and IL-10. All of the dogs treated with rLdccys1 until 12 months after treatment survived, whereas control dogs which received either saline or *P. acnes* only died between 3 and 6 months. These findings support the potential use of recombinant *L. (L.) chagasi* cysteine proteinase for immunotherapy of canine visceral leishmaniasis. **Supported by:** FAPESP.

RT002A - Insect vectors and trypanosomatids: a neglected interface

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The interaction between insect hosts and trypanosomatids is mediated by the surface parasite molecules. The study of such molecules in the context of the insect vector has largely been overlooked. Here, we presented for the first time, the possible involvement of Trypanosoma cruzi cruzipain in the interaction process with the invertebrate host. Cruzipain is a lysosomal cysteine peptidase, which plays an important role in parasite infectivity, intracellular growth and differentiation, and is abundantly expressed on the surface of epimastigotes. Since these forms face the insect vector environment during the life cycle, it is conceivable that cruzipain may participate in the interaction process with the invertebrate host. In this sense, we showed that adhesion of *T. cruzi* to the insect midgut cells was inhibited by the blockage of cruzipain function. Cysteine peptidase inhibitors, in a dose-dependent manner, and anti-cruzipain antibodies were able to reduce the binding of epimastigote forms to the *Rhodnius prolixus* midgut. Similarly, *T. cruzi* transfectants that overexpress chagasin, the endogenous cruzipain inhibitor displayed low levels of adhesion. Accordingly, the supplementation of exogenous cruzipain partially restored the adherence of the transfected line. Additionally, the ability of the chagasin overexpressing transfectants to colonize the insect *in vivo* was drastically reduced, and the levels of cruzipain expression by wild-type parasites were enhanced after *in vivo* passage in *R. prolixus.* Collectively, our results strongly suggest that cruzipain is required for successful colonization of *R. prolixus* by *T. cruzi*. The possible participation of other molecules, such as Tcgp63 was also discussed.

RT002B - Regulation of 26S proteasome and proteasome related HslV/U in Trypanosoma cruzi

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Trypanosoma cruzi is a rare example of an eukaryote that has genes for two threonine proteases: HslVU complex and 20S proteasome. HslVU is an ATP-dependent protease consisting of two multimeric components: the HslU ATPase and the HslV peptidase. In this study, we expressed and obtained specific antibodies to HslU and HslV recombinant proteins and demonstrated the interaction between HslU/HslV by coimmunoprecipitation. To evaluate the intracellular distribution of HslV in *T. cruzi* we used an immunofluorescence assay and ultrastructural localization by transmission electron microscopy. Both techniques demonstrated that HslV was localized in the kinetoplast of epimastigotes. We also analyzed the HslV/20S proteasome co-expression in Y, Berenice 62 (Be-62) and Berenice 78 (Be-78) *T. cruzi* strains. Our results showed that HslV and 20S proteasome are differently expressed in these strains. We also analyze the proteasome activities and observed a positive correlation between proteasome levels and activity between these strains. Curiously, the ubiquitin-proteasome activity is markedly different in the Be78 strain when compared to Y and 62. To investigate whether a proteasome inhibitor could modulate HslV and proteasome expressions, epimastigotes from *T. cruzi* were grown in the presence of PSI, a classical proteasome inhibitor. This result showed that while the level of expression of HslV/20S proteasome is not affected in Be-78 strain, in Y and Be-62 strains the presence of PSI induced a significantly increase in Hslv/20S proteasome expression. Together, these results suggest the coexistence of the protease HslVU and 20S proteasome in *T. cruzi* regulated by strain specific mechanisms, reinforcing the hypothesis that non-lysosomal degradation pathways have an important role in *T. cruzi* biology. **Supported by:**FAPEMIG, CNPq

RT003A - Toxoplasma gondii microneme proteins: new roles in host-parasite relationship

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Toxoplasma gondii, like other parasites of the phylum apicomplexa, actively invade host cells, in a process that critically depends on microneme proteins (MICs), which are released from intracellular organelles called micronemes. Microneme proteins 1 (TgMIC1), 4 (TgMIC4) and 6 (TgMIC6) forms a complex on the parasite surface, which promotes *T. gondii* adhesion to host cells. TgMIC1 and TgMIC4 are both glycan-binding proteins (GBPs), with specificity to sialic acid- and galactose-terminating glycans, respectively. These GBPs constitute the subcomplex Lac+, obtained through adsorption of soluble *Toxoplasma* antigens to immobilized lactose. When used to immunize C57BL/6 mice, Lac+ conferred resistance against *T. gondii* infection. Protection was also provided by mice vaccination with recombinant forms of microneme GBPs, as demonstrated by increased survival to infection and reduced parasitism, associated with Th1 immunity. We also examined the activities exerted by microneme GBPs on innate immune cells. Both GBPs activated macrophages to produce inflammatory mediators through a TLR2- and TLR4-dependent mechanism, since the response was significantly inhibited when cells from MyD88, TLR2, or TLR4-KO mice were assayed. By using HEK293 cells transfected with TLR-4, TLR-2/1, and TLR-2/6, we demonstrated that direct interactions of rTgMIC1 and rTgMIC4 with TLRs were responsible for cell activation, manifested by NF-kB activation and IL-8 production. Finally, HEK293 cells transfected with mutated TLR2 molecules for their N-glycosylation sites allowed to localize the N-glycan targeted by rTgMIC1, but not by rTgMIC4. Therefore, our study attributes new roles to microneme GBPs in host-parasite relationship exerted through their interaction with TLRs, which results in activation of innate immune cells. **Supported by:**FAPESP

RT003B - Dissection of essential invasion factors in Toxoplasma gondii

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Apicomplexan parasites invade their host cell in an active process that is independent of the hosts endo/phagocytic system. It is generally believed that gliding motility is required for active invasion. The current model prdeicts that the MyoA-motor complex, consisting of myosin A (MyoA), the myosin light chain (MLC1) and the gliding associated proteins (GAP40,45 and GAP50) interact with short actin filaments to generate the force that is transmitted to the parasite-host cell junction via micronemal proteins, such as MIC2 or AMA1 resulting in the invasion of the host cell. Here we provide data that challenge this model. We generatedknockout mutants using a novel conditional recombinase system in Toxoplasma gondii for all of these "essential" factors. Interestingly we were able to keep clonal knockouts for several of these genes continously in culture and show that host cell penetration occurs with the same kinetic as in control parasites. We conclude that gliding motility and host cell penetration are two mechanistically distinct steps during invasion of the host cell. We will discuss a novel model for host cell invasion by apicomplexan parasites.

RT003C - *Trypanosoma rangeli:* A model to assess virulence, pathogenicity and host/vector-parasite interactions

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Trypanosoma rangeli is a non-virulent hemoflagellate protozoan parasite of mammals occurring in sympatry with *T. cruzi* in a wide geographical area in Central and South Americas. Sharing reservoirs, vectors and a variety of soluble antigens, co-existence of these species represents a major problem for serological diagnosis of Chagas disease. Little is know about the *T. rangeli* biology, especially on the mammalian hosts. Thus, the needs for information on this taxon lead

us to sequence the parasite genome, whose preliminary findings including some of the initial comparative analysis with pathogenic trypanosomatids are presented. The *T. rangeli* genome seems to be as large as the *T. cruzi* genome as revealed by flow cytometry, with an average of ~154Mb in size, showing a nuclear genome of ~123Mb. Karyotyping of *T. rangeli* Choachí KP1(+) and SC58 KP1(-) strains by PFGE revealed a variable intra-specific profile, both formed by 16 chromosomal bands (0.40-3.44Mb). Sequencing of the SC58 strain genome was carried out in a Roche 454 FLX platform and consisted in a single paired-end plus a single shotgun run. With an average G+C content of 49.91%, the assembled genome comprises, so far, 237 contigs that were assembled with the support of formerly generated EST/ORESTES. Automated annotation via SABIÁ system revealed 7,723 ORFs, being 2,413 valid, 5,157 hypothetical and 153 partial/truncated ORFs. These ORFs showed 93% matches on KEGG (7,238) and 76% on InterPro (5,912) databases. Analysis of the *T. rangeli* ORFs by Gene Onthology showed 273 descriptions related to biological processes (2,227 genes), 97 descriptions related to cellular components (1,159 genes) and 354 descriptions related to molecular function (3,259 genes). Based on their distinct pathogenic/non-pathogenic behavior in mammals, comparison of *T. rangeli* to *T. cruzi* genome reveals that *T. rangeli* possess several homologous genes that are implicated in cell recognition, virulence or pathogenicity. Among these, genes coding for surface proteins are of special interest. Also, expression by *T. rangeli* of *T. cruzi* genes related to pathogenesis, virulence or interaction with hosts/vectors that are absent or inactive on *T. rangeli* proved to be a reproducible and reliable model to assess distinct biological aspects of both species. **Supported by:** CNPq, CAPES, FINEP and UFSC

RT004A - FROM DIGESTION TO IMMUNITY: NEW PERSPECTIVES FOR THE CONTROL OF INSECT-BORNE DISEASES

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Sequencing the transcriptome of the midgut and analysis of digestive enzymes of Rhodnius prolixus adults, Lutozmyia. longipalpis larvae and Aedes aegypti larvae showed new aspects of the digestive habits of these vectors, revealing several enzymes related to digestion of microorganisms, which are promising targets for inhibiton and control.

Purification, characterization and cloning of beta-1,3-glucanases from Periplaneta Americana (Dictyoptera), Abracris flavolineata (Orthoptera), Tenebrio molitor (Coleoptera), Spodoptera frugiperda (Lepidoptera), several termite species (Isoptera), L. longipalpis (Diptera) and A. aegypti (Diptera) revealed that these enzymes could fulfil distinct physiological roles. These enzymes are related to beta-glucan recognition factors and gram-negative binding proteins from glycoside hydrolase family 16. Recently, some of these enzymes were proposed as a part of a midgut recognition cascade for pathogenic microorganisms. Nevertheless, studies on Aedes and Lutzomyia members of this family showed that hydrolytic and immune functions are related to the expression of different genes, and phylogenetic analysis suggest that immune factors resulted from a duplication of an ancestral digestive enzyme.

Insect chitinases are members of family 18 of glycoside hydrolases. The purification and cloning of T. molitor midgut chitinase showed the presence of digestive chitinases lacking the chitin binding domain, which were shown later to constitute an important subfamily of insect chitinases. R. prolixus genome contain nine chitinase genes, coding for 10 transcripts. In spite of the absence of a perithrophic membrane, R. prolixus express a midgut chitinase, related to the digestive chitinases found in other insects. This chitinase is putatively involved in controlling the gut microbiota and seems to be an inherited trait from detritivore ancestors of the Hexapoda lineage. **Supported by:**FIOCRUZ, CNPq, FAPERJ, CAPES, FAPESP and PETROBRAS/CENPES

RT004B - A bacterium against dengue: our challenge

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Dengue has reemerged as a major public health problem in Brazil, with more than 3 million reported cases between 2000-2005, representing more than 70% of all cases reported in the Americas, and 61% of all cases reported to the WHO globally. Current control methods rely on insecticides for mosquito control and because of that, resistance against commonly used chemicals is increasingly widespread. Our project involves the use of a naturally occurring bacterium called *Wolbachia* as a novel biological control agent. *Wolbachia* manipulates the reproduction of their host in order to be vertically transmitted from the mother to offspring. This bacterium is believed to be present in up to 70% of all insect species worldwide but it has never been found in the *Aedes aegypti* mosquito (dengue vector). When stably introduced into *Aedes aegypti*, *Wolbachia* was able to block dengue virus transmission by these mosquitoes, constituting a great potential for control of dengue disease. Currently field tests are been carried out in Australia, where *Wolbachia* infected mosquitoes were able to invade local populations of *A. aegypti*. Next, the strategy will be applied in dengue endemic countries, like Brazil, to test whether it might be used as a sustainable dengue control strategy.

Eliminate Dengue Brasil is funded in part by the Ministry of Health in Brazil (DECIT/ SVS/ CNPq) and by a grant from the Foundation of the National Institutes of Health through the Vector-based Transmission of Control: Discovery Research (VCTR) programs of the Grand Challenges in Global Health initiative of the Bill & Melinda Gates Foundation (www.eliminatedengue.com).

RT004C - Fighting malaria with engineered bacteria

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The unbearable burden of malaria is increasing worldwide and devising novel approaches to fight this deadly disease is urgently needed.

Recent technical advances in vector biology, made possible a new strategy to combat malaria namely, genetically modifying the mosquito to reduce its vectorial competence. However, one crucial unresolved aspect of this approach is how to introduce transgenes into wild mosquito populations in the field (“genetic drive”). Several strategies have been proposed but there are concerns about the feasibility of their implementation.

We are exploring an alternative approach which is based on the fact that the mosquito, as all higher organisms, carries a microbiota in its midgut lumen. Rather than genetically modifying mosquitoes, the strategy is to genetically modify the bacteria that inhabit the mosquito midgut (paratransgenesis). We have shown that bacteria engineered to express and secrete molecules that interfere with Plasmodium development (i.e., effector molecules) strongly inhibit parasite development (up to 98%) and srongly decrease (up to 84%) vectorial competence of mosquitoes.

The recent finding that Asaia sp. bacteria may be vertically transmitted greatly increases the promise of this approach because it suggests means for introducing engineered bacteria into mosquito populations in the field. We note that the paratransgenesis strategy is compatible with existing control measures, including insecticides and insecticide-treated bed nets, and is not affected by mosquito population structure. The prospects for implementation of this strategy in a relatively near future are more favorable than for transgenic mosquitoes while being complementary to this approach (both approaches can be used simultaneously). Moreover, the entire approach is low-tech, since growing large numbers of bacteria is simple, in contrast to rearing millions of exclusively male transgenic mosquitoes for release in nature. **Supported by:**National Institutes of Health (USA)

RT005A - Monocytes versus Macrophages: Effector or Suppressor Cells in Leishmania major Infection?

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CD4 T cells play a key role in adaptive immunity to *Leishmania major,* by inducing nitric oxide-mediated parasite killing within macrophages (1). We hypothesized that NO-producing immature monocytes could also help immunity to *L. major* infection. By contrast myeloid-derived suppressor cells (MDSCs), including myeloid precursors and immature monocytes, play a deleterious role in tumor immunity, by producing NO and suppressing T cell responses (2). To investigate the role of MDSCs in *L. major* infection, we injected parasites in the peritoneum of B6 mice to elicit immature monocytes. Here we show that Gr1hi(Ly6Chi)CD11bhi MDSCs elicited by *L. major* infection suppressed both polyclonal and antigen-specific T-cell proliferation. On the other hand, MDSCs, bearing monocyte markers, killed intracellular *L. major* parasites in an NO-dependent fashion in vitro and reduced parasite burden in vivo (3). Furthermore, treatment with all-trans retinoic acid (ATRA), which induces immature monocytes to differentiate into mature macrophages (4), increased development of lesions, parasite load, and T cell proliferation in draining lymph nodes upon *L. major* infection (3). Next, we will address the functional phenotype of macrophages (5) induced by treatment with ATRA. Our results indicate that whereas NO-producing MDSCs help protective immunity to *L. major* infection, the macrophages differentiated from immature monocytes promote parasite infection. **Supported by:**FAPERJ, CNPq

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RT005B - Hemozoin and Inflammatory Biomarkers: Impact on Innate Immunity and Malaria Progression.

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Individuals infected with the protozoan parasite Plasmodium spp. are known to develop severe symptoms that are the consequence of intense inflammatory response triggered by the release of the merozoite form of the parasite into blood circulation. Of interest, hemozoin (HZ), a crystalline and brown pigment formed in the digestive vacuole of Plasmodium as a catabolism product of hemoglobin (Hb), is also simultaneously released. In the past, HZ was considered as a metabolic waste of the parasite, solely the result of heme detoxification. However, the fact that this molecule has been shown to be actively engulfed by phagocytes and to modulate MØ functions, as well as to be trapped in various organs, suggests that HZ can potentially contribute to the development of malaria immunopathogenesis. Following its release from ruptured Plasmodium-infected RBC, monocytes/macrophages rapidly engulf HZ. Furthermore, in human and murine malaria, a large number of circulating phagocytes are loaded with HZ, as well as those in lymphoid organs and the brain, where their presence seems to correlate with disease severity.

It has been demonstrated that human monocytes and murine MØ stimulated with HZ purified from various species of Plasmodium or synthetically generated as β-hematin, produce large amounts of cytokines, inflammatory molecules, MIF erythropoietic inhibitor and adhesion molecules. In accordance with these observations, we published the first report that in vivo inoculation of synthetic HZ rapidly induces the generation of various pro-inflammatory mediators including myeloid-related proteins, chemokines and cytokines, strongly suggesting that HZ per se may have an important role to play in the development of malaria-related pathologies. Additionally, we revealed that HZ significantly enhanced IFNγ-induced MØ NO generation, an important inflammatory event that could favor cerebral malaria development. Thereafter, we found that P. falciparum HZ (PfHZ)- and synthetic HZ (sHZ)-induced MØ chemokine expression were regulated by oxidative stress-dependent and –independent mechanisms involving PTP inhibition, MAP kinases and NF-κB activation. More recently, whereas previous report proposed that malarial DNA attaching to HZ was responsible for induction of “dendritic cells” activation is TLR9, we, and 2 other groups, have clearly demonstrated that induction of MØ pro-inflammatory cytokines (e.g. IL-1β) by HZ is TLR-independent but fully dependent on the activation of the NLRP3/Inflammasome complex, and that the up-stream Src kinase Lyn is pivotal for IL-1β secretion. Furthermore, we reported that HZ size influences MØ activation in vitro and provided in vivo demonstration that malarial DNA never interacts with HZ within Plasmodium-infected erythrocytes.

Finally, having recently identified human inflammatory biomarkers (i.e. ApoE, SAA, LBP) from malaria patients that interact with the malarial HZ. We became interested to determine the impact of these sera biomarkers on the macrophage’s innate immune response triggered by HZ. Interestingly, we found that those adhering biomarkers differently but effectively influence HZ recognition by MØ, modifying not only IL-1β production, but in addition ROS generation and phagocytosis. Using ko mice for some of those host biomarkers, we found that their absence can strongly influence the development of cerebral malaria caused by P. berghei ANKA.

Collectively, our previous and present studies provide important clues about the impact of inflammatory mediators induced during malaria to modify, on one hand, the impact of HZ in its interaction with MØ, and on the other hand, to show that systemic release of those inflammatory biomarkers could play a critical role in the development of malaria-related pathologies. Findings stemming from our studies could lead to the development of new therapy to tame-down innate inflammatory response and potentially reduce the death rate cause by cerebral malaria.**Supported by:**Canadian Institute of Health Research

RT005C - Leukotriene B4 is essential for the development of experimental cerebral malaria

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Malaria is among mankind's worst diseases, affecting hundred thousands of people, particularly in the tropics, and having a 20 - 30% mortality rate despite adequate antimalarial treatment. Plasmodium berghei ANKA (PbA) infection in susceptible strains of mice induces experimental cerebral malaria, which reproduces, to a large extent, the pathological features of human cerebral malaria. Previous studies indicate that inflammatory mediators derived from arachidonic acid metabolism, collectively known as eicosanoids, participate in the pathogenesis of cerebral malaria. These studies indicate that prostaglandins are protective in cerebral malaria, while the role for leukotrienes in the disease pathogenesis has not been defined. In the present work, we directly characterize whether and which leukotrienes are involved in cerebral malaria in a mouse model. Here we show that the blockage of total leukotriene production by knocking out the 5-LO enzyme or using its pharmacologic inhibitor zileuton prevented the signs of cerebral malaria, increased the survival and prevented the inflammatory response without

affecting the parasitemia. Mice treated with a nonspecific inhibitor of the LTB4 receptors BLT1 and BLT2 (LY2552833) had increased survival and were resistant to cerebral malaria. This data was confirmed in mice genetically deficient for Blt1 receptors, which also present resistance to cerebral malaria development. In contrast, the pharmacologic inhibition of the cysteinyl leukotriene (CysLT) receptor CysLT-1 with montelukast did not change the survival curve and ECM incidence in PbA infected mice. Together these results indicate that LTB4 is essential for the development of cerebral malaria, affecting the inflammatory response and the pathogenesis despite not interfering with the parasite control. **Supported by:**FAPERJ, CNPq, Capes, NIH

RT006A - Novel approaches towards drug discovery against the human malaria parasite Plasmodium falciparum

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The malaria parasite *Plasmodium falciparum* is able to synthesize *de novo* pyridoxal-5-phosphate (PLP), the active form of vitamin B6. We show here that the de novo synthesized PLP is used by the parasite to detoxify singlet molecular oxygen (1O2), a highly destructive reactive oxygen species arising from haemoglobin digestion. The formation of singlet oxygen and the response of the parasite were monitored by life cell fluorescence microscopy, by transcription analysis and by determination of PLP levels in the parasite, respectively. Pull-down experiments of transgenic parasites overexpressing the vitamin B6 biosynthetic enzymes PfPdx1 and PfPdx2 clearly demonstrated an interaction of the two proteins in vivo which resulted in elevated PLP levels in the Pdx1/Pdx2-overexpressing cells and thus to a higher tolerance towards 1O2. In contrast, by applying cellular protein interference using inactive Pdx1 and Pdx2 mutants P. falciparum became susceptible to singlet oxygen. Our results clearly demonstrate the crucial role of vitamin B6 biosynthesis in the detoxification of singlet oxygen in P. falciparum. Besides the known role of PLP as a cofactor of many essential enzymes this second important task of the vitamin B6 de novo synthesis as antioxidant emphasizes the high potential of this pathway as a target of new anti-malarial drugs. **Supported by:**DFG, FAPESP

RT006B - Systems Immunology: Understanding Inflammatory Responses to Malaria

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P. falciparum-induced inflammation results in fever and other manifestations of malaria. Little is known about the immunological effects that persist after resolution of febrile malaria and to what extent these changes modulate the immune response and clinical course upon re-infection. A systems analysis of peripheral blood leukocytes collected in a longitudinal study of Malian children revealed that mediators of *P. falciparum*-induced inflammation are down-regulated after febrile malaria. This altered set-point relative to the healthy baseline was associated with dampened expression of pro-inflammatory molecules and upregulation of IL-10 producing FOXP3-CD4+T cells following *ex vivo* stimulation with *P. falciparum* lysate. This pattern of inflammation attenuation and tolerance after febrile malaria was lost in the absence of ongoing *P. falciparum* exposure, potentially explaining the observation that maintenance of malaria immunity requires ongoing *P. falciparum* exposure.

RT006C - Distinct roles of splenic phagocytes in the different phases of blood-stage Plasmodium chabaudi AS malaria

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Malaria remains a major health issue, especially in the tropical areas of the globe. As an effort to develop new strategies to combat the disease, mouse models have been widely used to reveal the mechanisms of malaria immunity. These mechanisms generally comprise innate responses, such as macrophage activity and pro-inflammatory cytokines. It also includes effector CD4+ and CD8+ T cell activity during the early infection, and posterior generation of memory CD4+ T cells – similarly to humans exposed to repeated infections. The humoral immunity contributes to parasite clearance through neutralization and opsonization of infected red blood cells (iRBC) and merozoites.

By using in vivo and ex vivo approaches, herein we described some relevant aspects of the iRBC phagocytosis, as well as their implications in the immune response to P. chabaudi malaria. The phagocytosis of fluorescence-labeled iRBC was assessed in C57BL/6 mice at the different phases of infection by flow cytometry, fluorescence microscopy and intravital imaging. In some experiments, mice were depleted of phagocytes or GR1+ cells by treatment with clodronate liposomes or anti-Gr1 monoclonal antibodies, respectively. In naïve mice, F4/80+ red pulp (RP) macrophages and CD11c+ dendritic cells (DCs) actively internalized iRBC and subsequently migrated toward B-cell and T-cell areas, respectively. During the acute infection, alongside with RP macrophages and DCs, the CD11b+Gr1+ cell population increased and contributed to iRBC clearance. At that time, splenic phagocytes were primed by interferon (IFN)-γ and showed increased response to iRBC and toll-like receptor (TLR) agonists (lipopolysaccharides and CpG oligonucleotides). In vivo depletion showed that RP macrophages are fundamental for the development of both T- and B-cell responses to infection; DCs are particularly important for the T-cell proliferation stimulated by iRBC; whereas the absence of Gr1+ cells led to T-cell hyperactivation. In fact, enhanced parasitemias and mortality occurred only in mice depleted of RP macrophages and DCs. Moreover, CD11b+Gr1+ cells suppressed T-cell proliferation when co-cultured ex vivo with splenocytes stimulated with anti-CD3 monoclonal antibodies. These results suggest that splenic phagocytes contribute in different ways to induce parasite control during the acute infection with no excessive activation of the immune system.

On the other hand, an increased activation of the innate immune system appears to be necessary in conjunct with the acquired immunity to ensure the full protective immunity against reinfection. Chronic mice with residual parasitemia were fully protected against homologous (AS strain) and heterologous (AJ strain) parasite challenge, similarly to humans leaving in endemic areas of malaria. The full protection was associated with high proportions of effector-memory CD4+ T cells and hyperresponsiveness to TLR agonists. After parasite clearance, the size and phenotype of splenic phagocyte populations were again similar to those of non-infected controls and the priming effect of IFN-γ was no longer observed. In addition, parasite elimination led to a shift of experienced CD4+ T cells to central memory. Despite the presence of humoral immunity, these mice were not fully protected against reinfection, notably against the heterologous parasite challenge. However, when pre-treated in vivo with a low dose of recombinant IFN-γ, these mice recovered the ability to control homologous and heterologous parasite challenge. The acquisition of full protective immunity against both parasite strains occurred concomitantly with an increase in the response of splenocytes to TLR agonists. These results indicate that IFN-γ-induced priming is required to maintain the full protective immunity against P. chabaudi infection, given a molecular basis for the strain-transcending immunity hypothesis in human malaria. **Supported by:**CNPq (Brazil), FAPESP (Brazil), and FCT (Portugal)

RT007A - DETECTION OF LEISHMANIA (LEISHMANIA) INFANTUM RNA IN FLEAS AND TICKS COLLECTED FROM NATURALLY INFECTED DOGS

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The occurrence of the insect vector with low rates of *Leishmania* infection as well as autochthonous transmission in the absence of the natural vector in dogs has been reported. These unexpected data suggest a hypothesis of other arthropods as a possible way of *Leishmania* transmission. The prevalence of *Leishmania (L.) infantum* in fleas and ticks collected from dogs with canine visceral leishmaniasis (CVL), as well as parasite viability were evaluated herein. The presence of *L. (L.) infantum* was assayed by PCR in ectoparasites and PCR and ELISA in biological samples from 73 dogs living in a Brazilian endemic area. As the occurrence of *Leishmania* DNA in ticks and fleas is expected, given their blood-feeding habits, next we investigated whether parasites can remain viable inside ticks. PCR and ELISA confirmed that 83% of the dogs had CVL. Fleas and ticks (nymphs, male and female adults) were collected, respectively, in 55% and 63% of the 73 dogs. Out of the 60 dogs with CVL, 80% harbored ectoparasites infected with *L. (L.) infantum.* The infection rates of the ectoparasites were 23% and 50%, respectively, for fleas and ticks. The RNA analysis, extract from ticks left in laboratorial conditions during 7 to 10 days after removal from CVL dogs showed that parasites were alive. In addition, live parasites were also detected inside adult ticks recently moulted in laboratorial conditions. These findings indicate a higher infection rate of *L. (L.) infantum* in ticks and fleas, but they do not conclusively demonstrate whether these ticks can act as vectors of CVL, despite that their rates were higher than those previously described in Lutzomyia longipalpis. The presence of viable *L. (L.) infantum* in ticks suggests the possible importance of dog ectoparasites in CVL dissemination. **Supported by:** FAC was supported by fellowships from FAPESP. Proc-08/57245-7.

RT007B20 - Vertical transmission as a means of continuing a population of L. infantum-infected dogs

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Dogs are the predominant domestic reservoir for human L. infantum infection. Zoonotic visceral leishmaniasis (ZVL) is an emerging problem in some U.S. dog breeds, with an annual quantitative PCR prevalence of greater than 20% within an at-risk canine population. Classically Leishmania is transmitted by infected sand flies and phlebotomine sand flies exist in the United States, means of ongoing L. infantum transmission in U.S. dogs is previously unknown. Possibilities include vertical (transplacental/transmammary) and horizontal/venereal transmission. Several reports have indicated that endemic ZVL may be transmitted vertically. Our aims for this present study were to establish whether vertical/transplacental transmission was occurring in this population of Leishmania-infected US dogs and determine the effect that this means of transmission had on immune recognition of Leishmania. A pregnant *L. infantum*-infected dam donated to Iowa State University gave birth in-house to 12 pups. Eight pups humanely euthanized at the time of birth and four pups and the dam humanely euthanized three months post-partum were studied via *L. infantum*-kinetoplast specific quantitative PCR (kqPCR), gross and histopathological assessment and CD4+ T cell proliferation assay. This novel report describes disseminated *L. infantum* parasites as identified by kqPCR in 8 one day old pups born to a naturally-infected, seropositive US dog with no travel history. Despite presence of disseminated parasites, pups had a productive T cell proliferative response to parasite antigen at a day of age, also present at 12 weeks old, indicating absence of immunologic tolerance despite in utero infection. This is the first report of vertical transmission of *L. infantum* in naturally-infected dogs in North America, emphasizing that this novel means of transmission would possibly sustain infection within populations. Evidence that vertical transmission of SVL may be a driving force for ongoing disease in an otherwise non-endemic region has significant implication on current control strategies for ZVL, as at present parasite elimination efforts in endemic areas are largely focused on vector-borne transmission between canines and people. Determining frequency of vertical transmission and incorporating canine sterilization with vector control may have a more significant impact on SVL transmission to people in endemic areas than current control efforts.

RT007C - What makes Trypanosoma cruzi metacyclic forms highly fittet to infect by the oral route?

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Oral infection by *T. cruzi* has been responsible for frequent outbreaks of acute Chagas' disease in Brazil, Venezuela and Colombia. In the murine model of oral *T. cruzi* infection, it was found that metacyclic trypomastigotes invade the gastric mucosal epithelium, where the parasites replicate intracellularly. Metacyclic forms survive in the harsch conditions of the stomach because they express mucin-like surface molecules, extremely resistant to proteolysis. High efficiency of metacyclic forms in establishing infection by oral route is associated with the expression of gp82, a stage-specific surface molecule that selectively binds to gastric mucin, the main component of the mucus layer. *In vitro*, the parasites efficiently migrate through a transwell filter coated with gastric mucin. The gastric mucin-binding site of gp82 has been identified and the synthetic peptide (p7) based on that sequence was tested *in vitro* and *in vivo*. Transwell filters were coated with gastric mucin mixed with peptide p7 or with peptide p7* with the same composition as p7 bu with a scrambled sequence. Parasite migration through the filter coated with gastric mucin mixed with peptide p7 was almost completely abolished. Gastric mucin alone or mixed with peptide p7* had no inhibitory effect. *In vivo* assays consisted in giving Balb/c mice the peptide p7 or p7* before oral administration of metacyclic forms, followed by histological preparations of the stomach for parasite detection in the gastric epithelium. Mice that received peptide p7 exhibited significantly lower number of amastigote nests, as compared to mice inoculated with peptide p7*. As p7 sequence is located near p4, the main gp82 cell lbinding site, we envisage the possibility that, upon reaching the target cells, the recognition of the metacyclic trypomastigote gp82 sequence p4 by its receptor could facilitate the release of p7 sequence from the gastric mucin, enabling the parasites to initiate invasion. **Supported by:**FAPESP e CNPq