MC001 - PROTEIN SUMOYLATION IN THE LIFE CYCLE OF *GIARDIA LAMBLIA*

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The post-translational modification SUMOylation is a major regulator of protein function playing an important role in a wide range of cellular processes such as chromatin organization, transcription, DNA repair, macromolecular assembly, protein homeostasis, cell cycle progression, trafficking, and signal transduction. SUMOylation involves the covalent attachment of a member of the SUMO (small ubiquitin-like modifier) family of proteins to lysine residues in specific target proteins via an enzymatic cascade analogous to, but distinct from, the ubiquitination pathway. SUMO proteins are ubiquitously expressed throughout the eukaryotic kingdom. In mammalian there are four SUMO paralogs, named SUMO-1 to SUMO-4, and an increasing number of proteins are being identified as SUMO substrates. While, new findings are improving the current knowledge on the SUMOylation process in mammals, little is known about the SUMO pathway and its targets in primitive eukaryotes like *Giardia lamblia*.
G. lamblia is one of the most common causes of human intestinal diarrhea and is of great biological interest because it derives from the earliest branch of the eukaryotic line of descent. In this work, some of the components and substrates of the SUMOylation pathway in *G. lamblia* is presented.
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MC002 - A 100 YEARS OF OMISSION: UNDERSTANDING TRIATOMINE SEXUAL COMMUNICATION THROUGH ANALYTICAL, MOLECULAR AND BEHAVIORAL STUDIES.

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Chagas disease is a health burden that affects more than 10 million people in Latin America and its control relies mostly in eliminating triatomines, the insects vectoring it. The behavior of triatomine bugs is usually triggered by odors, whose detection is mediated by olfactory receptor neurons (ORNs) that present olfactory receptors (ORs and IRs) in their membranes. Analytical chemistry studies allowed uncovering the identity of compounds produced by the metasternal glands (MGs) of three species of vector bugs. These secretions include ketones, alcohols and dioxolanes. Behavioral experiments revealed that these volatiles are responsible for mediating the activation of sheltered males in the presence of females. Moreover, males showed oriented responses towards airstreams laden with female odors and female MGs function was necessary for triggering this behavior. Besides, female MG secretions were responsible for promoting male aggregations around mating pairs. Bioinformatic searches combined with molecular biology analyses of gene expression in the antennae of *Rhodnius prolixus* allowed us identifying the olfactory co-receptors for ORs (OrCo) and IRs (IR8a, IR25a and IR 76b). qPCR results have shown that there is no significant difference in the expression profiles of OrCo and IR25a between males and females, suggesting that both sexes may expose similar amounts of odorant receptors to the environment. Transcriptomic analyses of *R. prolixus* antennae are being developed and will allow determining whether specific genes (either ORs or IRs) are more expressed in males than females. These sequences would represent potential candidates for evaluation as pheromone receptors. Olfactometer studies are being developed to identify the active compounds that mediate male orientation to females. The control of Chagas disease vectors would greatly benefit from alternative tools based in the manipulation of bug behavior, as insecticide resistance is already a serious problem with the main vector species.
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MC003 - FREE-LIVING AMEBAS, AN EMERGING DISEASE IN LATIN AMERICA

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Free-living amebas are ubiquitous protozoans distributed worldwide that have great capacity to survive and replicate in the environment without the need of a host or vector for transmission. The first case of human meningitis due to free-living amoeba was described by Fowler in 1965, and four genera can be human pathogens: *Naegleria*, *Acanthamoeba*, *Balamuthia* and *Sappinia*. These parasites can cause central nervous system (CNS) involvement in two clinical syndromes: primary amebic meningoencephalitis (PAM) and granulomatous amebic encephalitis (GAE). Both are uncommon and usually fatal.

PAM presents as an acute hemorrhagic meningoencephalitis caused by *N. fowleri* in immunocompetent hosts. *Naegleria* lives in fresh contaminated water and it infects humans through the nasal mucosa by inhalation and activities with close contact to contaminated water.

In contrast, GAE is a subacute or chronic infection, lasting from a few weeks to 2 years, associated with *Acanthamoeba spp.*, *B. mandrillaris* and *S. pedata* (only one case reported). It is acquired via inhalation of cysts through the respiratory mucosa or by direct skin inoculation with posterior dissemination to CNS, lungs, kidney, thyroid, and liver. *Acanthamoeba* usually affects immunocompromised patients while *B. mandrillaris* can produce disease in immunocompetent as well as in immunosuppressed hosts.

Acanthamoeba spp. can also cause keratitis, a threatening infection of the cornea in healthy users of contact lenses with inappropriate cleaning procedures or contaminated solutions; and nasopharyngeal infection in severely immunosuppressed HIV patients.

In Latin America, the first report of a meningoencephalitis by free living amoeba was described in 1977. *B. mandrillaris* affects more frequently people of Hispanic origin, perhaps due genetic susceptibility or environmental exposure. It was isolated for the first time in 1986, from the brain of a mandrill baboon which died due a meningoencephalitis at the San Diego Zoo. Since 1991, more than 200 human cases have been described almost all over the world, except Africa. Most cases are present in South America, Mexico and United States. Many cases develop cutaneous lesions prior to CNS infection. Nonspecific symptoms and the lack of availability of a reliable diagnostic test will obstruct and delay the diagnosis, most of the time it is made only at autopsy. Diagnostic tools have been developed lately, but their availability is limited to reference centers, such as the CDC, and research sites, which delays the diagnosis.

Early suspicion and recognition of these entities during early stages of disease (before of CNS affection or initial affection) are crucial for the prognosis and possibility of survive to this otherwise fatal infection. Unfortunately, an effective therapy is not available. A few survivors were treated with a combination of antimicrobials including pentamidine isethionate, sulfadiazine, clarithromycin, fluconazole, and flucytosine; or albendazole, itraconazole, and lately miltefosine. Surgical excision has been used in association to these combinations of drugs in some of these survivors.

Similarly, *Naegleria* and *Acanthamoeba* infection with disseminated or CNS affection result in death in most cases. An *Acanthamoeba* infection survivor responded to the same combination of drugs used for B. mandrillaris, voriconazol was useful on a disseminated case. PAM survivors received combination of Amphotericin B (intravenous and/or intrathecal), rifampicin and fluconazole, with or without ventriculoperioneal shunt to manage obstructive hydrocephalous.

Although the prognosis of these infections is still ominous, the last reports provide hope for attaining clinical cure. More clinical studies are needed to determine which is the most effective and appropriate approach and treatment for these entities.

MC004 - SELECTIVE TRANSMISSION OF *TRYPANOSOMA CRUZI* AND *T. RANGELI* GENOTYPES: COEVOLUTION OR PARASITE-VECTOR ADAPTATION?

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There has been growing interest in understanding the factors determining *Trypanosoma cruzi* and *T. rangeli* genotypes' geographical distribution in Latin-America and their association with domestic or sylvatic transmission cycles. The nomenclature for six *T. cruzi* discrete taxonomic units (DTU) has been unified recently. However, few studies have dealt with the transmission dynamics of such DTUs in different triatomine species. We have observed that *Panstrongylus geniculatus* has had greater ability to transmit *T. cruzi* II than *T. cruzi* I in experimental infections, whereas *Rhodnius prolixus*, *R. colombiensis* and *R. pallescens* have had greater ability for transmitting *T. cruzi* I than *T. cruzi* II. Such results agree with greater natural *T. cruzi* I infection prevalence in domiciliated and sylvatic vectors from the *Rhodnius* genus in Colombia. Nevertheless, different molecular groups of *T. rangeli* have been described based on their kDNA minicircle organisation and called *T. rangeli* KP1(+) and *T. rangeli* KP1(-). *T. rangeli* KP1(+) strains have been isolated from *R. prolixus*, *R. robustus* and *R. neglectus* ("Robustus" group) and KP1(-) strains have been isolated from *R. pallescens*, *R. colombiensis* and *R. ecuadoriensis* ("Pictipes" group). These findings have supported the hypothesis that two main groups of *T. rangeli* circulate through two phylogenetic *Rhodnius* groups, denoted as "Robustus" and Pictipes". Several works have shown that the vector's immune response is determinant in the selective transmission of different *T. cruzi* and *T. rangeli* genotypes. We have shown that the presence of a trypanolytic factor in *R. prolixus* and *R. robustus* haemolymph selectively lyses *T. rangeli* KP1(-) populations but not KP1(+) populations so that KP1(+) strains become isolated in vectors and vertebrates but not KP1(-) strains in regions where *R. prolixus* or *R. robustus* are distributed. We have observed that both *T. rangeli* genotypes can be isolated from different vector species which are even in sympatry with loss of genetic flow between parasites. We have confirmed the presence of trypanolytic factors in *R. prolixus* haemolymph and intestine against *T. cruzi* DTUs, finding evidence of trypanolytic activity against *T. cruzi* II, V and VI, agglutination against *T. cruzi* IV and a lack of activity against *T. cruzi* I and III. We have observed that *R. prolixus* haemolymph has lytic activity against genotypes which in Colombia are absent in areas where this vector is distributed but lacks lytic activity against *T. cruzi* I, this being precisely the predominant genotype in areas of *R. prolixus* distribution. These observations strengthen the hypothesis that *T. cruzi* (I-VI) and *T. rangeli* (KP1+, KP1-) genotypes' geographical distribution is determined by local species' ability to transmit determined genotypes and impede the transmission of others. A preliminary study comparing *R. prolixus, R colombiensis*, *R. pallescens* and *T. maculata* proteomes revealed the presence of around 23 proteins exclusive for *R. prolixus* haemolymph which could be implicated in the differential lysis of *T. cruzi* and *T. rangeli* genotypes. Ten of the 35 haemolymph proteins have been identified by mass spectrometry. Protein overexpression has been observed in the haemolymph of *R. prolixus* infected by *T. cruzi* and *T. rangeli* genotypes. The differentially expressed proteins could be associated with parasites' selective transmission mechanisms. The results concerning *T. cruzi* and *T. rangeli* genotypes' interaction with different triatomine species has suggested a certain degree of evolutionary association that invites the exploration of new methodologies for strengthening the parasite-vector co-evolution hypothesis and for identifying genes from parasites or genes from vectors implicated in the transmission of determined trypanosome genotypes. **Supported by:**Colombian Science, Technology and Innovation Department (COLCIENCIAS) (grant No. 110551929038)

MC005 - TARGETING A *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN 1 FRAGMENT TO THE DEC205+ DENDRITIC CELL POPULATION ELICITS STRONG ANTIBODY AND T CELL RESPONSES.

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Antigen targeting to different dendritic cell (DCs) populations using fusion monoclonal antibodies (mAbs) has been successfully used to elicit T and B cell responses in recent years. This is accomplished by the administration of small doses of the fusion mAb in the presence of a DC maturation stimulus that can be a toll-like receptor (TLR) ligand, for example. When different antigens were targeted to a DC population that expresses the DEC205 receptor and the CD8α marker, strong immunity was elicited and even protection was observed in some cases. In this work, we genetically fused the αDEC205 mAb with the 42 kDa fragment derived from the *Plasmodium vivax* merozoite surface protein 1 (MSP1), an antigen candidate for the development of a malaria vaccine. During *Plasmodium* invasion into the red blood cell, the 42 kDa fragment is further cleaved into 33 and 19 kDa fragments. The 19 kDa fragment is normally target for antibody responses while the T cell epitopes are restricted to the 33 kDa portion of the molecule. We administered two doses of the αDEC-MSP1$_{42}$ fusion mAb in the presence of the TLR3 agonist poly I:C to either C57BL/6 or C57B10.A mice. We will report on the induction of humoral and cellular immune responses against MSP1$_{42}$ using this strategy.
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MC006 - THE LIPID FLOW BETWEEN PARASITES AND HOSTS: AN UNEXPLORED CASCADE OF BIOCHEMICAL MECHANISMS

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Parasites lack *de novo* synthesis of lipids classes and therefore must scavenge them from their hosts or the environment. We have evaluated such mechanism in two different models in the lab. We have shown that the malaria parasite *Plasmodium gallinaceum* and *Leishmania amazonensis* take up and metabolize lipoprotein particles from their invertebrate and vertebrate hosts in both a time and dose-dependent manner. In both parasites these mechanisms rely on the presence of a true lipoprotein receptor. Lipiod uptake is blocked by both low temperature and by the excess of non-labelled lipoprotein. The major hemolymphatic mosquito lipoprotein, lipophorin, labelled with FITC was found inside the oocyst structure suggesting that the particle is not only binding to the membrane but it its also being internalized. Lp uptake seems to be a demand of growing oocysts. In order to a single ookinete to give raise to thousands of sporozoites the oocysts must undergo several rounds of cell duplication. Such duplicative effort requires the synthesis and assembly of a huge amount of cell membrane units. Therefore, fatty acids to be incorporated in such phospholipid membranes are derived from circulating insect lipophorin.
In *L. amazonensis* the putative lipoprotein receptor was found associated with specific detergent-resistant lipid microdomains (DRMs) in the membrane of the parasite. Its molecular weigth was estimated as 69 kDa by *western-ligand blotting technique*. Lipid uptake in this model is blocked by methyl-β-cyclodextrin (MCBD), a DRM disruptor. In *L. amazonensis* fluorescently-labeled LDL was used to follow the intracellular distribution of this marker after uptake in both MBCD-treated and non-treated parasites. The accumulation of LDL was analyzed by flow cytometry using FITC-labeled LDL particles. After incubation with ^{3}H-Cholesterol-LDL we observed the appearance of radioactive cholesteryl ester in the parasites. These observations indicate that *L. amazonensis* can esterify free cholesterol from LDL particles. Evidence that cholesterol can be esterified by fatty acids, such as oleate or palmitate, suggests the existence of the enzyme acyl-coenzyme A: cholesterol *O*-acyltransferase (ACAT).
Altogether these data show that *L. amazonensis* and *Plasmodium* are able to compensate for their lack of lipid synthesis through the use of a lipid importing machinery largely based on the uptake of LDL and lipophorin particles from their hosts. Understanding the details of the molecular events involved in these mechanisms may ultimately lead to the identification of novel targets to block parasite infection in vertebrate and invertebrate hosts. **Supported by:**CNPQ, FAPERJ, INCT

MC007 - ANTIMALARIAL DRUG DISCOVERY FROM NATURAL AND COMMERCIAL SOURCES

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Malaria control and eradication programs rely mainly on vector control and the efficacy of chemotherapy since there is no approved vaccine available. Artemisinin-based combination therapies (ACTs) are currently the preferred first-line antimalarial against Plasmodium falciparum (the deadliest of the five species that infect humans). Due to toxicity concerns, though, the World Health Organization (WHO) does not recommend the use of ACTs for pregnant women during their first trimester and for children weighing less than 5 kg. Moreover, the rapid development of resistance to the partner drug and the recent artemisinin resistance demonstrated in the Cambodia-Thailand border area vindicates the constant need for development of new drugs against different targets to expand our repertoire of antimalarials that can be used in combination therapies and has highlighted the relevance of more effective transmission-blocking drugs. The continuing search for new antimalarial compounds from plants and other organisms has indeed yielded many significant discoveries, but these have not, as yet, yielded effective new drugs. Recently, we isolated two new bioactive dimeric phloroglucinols from an ethanol extract of Mallotus oppositifolius collected in Madagascar, which have antimalarial activity against the asexual and sexual stages of P. falciparum. In addition, we interrogated the “malaria box,” which is available to the scientific community through Medicine for Malaria Venture (MMV), using phenotypic-based screens to identify gametocytocidal or apicoplast-targeting antimalarials. We identified one compound specifically targeting the apicoplast in the malaria parasites and twelve compounds that were active against late-stage gametocytes with half-minimum inhibitory concentration (IC50) values below 1 µM. During our screening, we stressed the identification of compounds showing activity against both asexual and sexual intraerythrocytic stages, which allowed us to identify promising lead compounds. Our efforts to identify their molecular target(s) will reveal cellular functions that are essential in both asexual and gametocyte stages; thus, more effective drugs can be developed to both cure malaria and stop its transmission.

MC008 - "THE ROLE OF MONOCYTES IN ANIMAL MODELS OF LEISHMANIA INFECTION"

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Monocytes are important cells from innate immunity that give rise to macrophages and dendritic cells. Recently, two subpopulations of monocytes were described based on the expression of the surface marker, GR1 (Ly6C). GR1+ monocytes are inflammatory cells that migrate to the site of inflamed tissues, producing cytokines as TNF-a and controlling some diseases such as toxoplasmosis and brucelosis. Leishmania species trigger a brisk inflammatory response and efficiently induce cell-mediated immunity. We examined the mechanisms whereby leukocytes were recruited into lesions after *Leishmania major* infection of mice. We found that a subpopulation of effector monocytes expressing the granulocyte marker GR1 (Ly6C) is rapidly recruited into lesions, and these monocytes efficiently kill *L. major* parasites. The recruitment of this subpopulation of monocytes depends on the chemokine receptor CCR2 and the activation of platelets. In addition, we have analyzed monocytes in mice treated with Anfotericin B for 4 weeks. Our results show that GR1+ monocytes increase in frequency in the blood during *L. major* infection and this increase is related to lesion size. C57BL/6 mice present decrease of GR1+ monocytes that are related to the decrease of lesion size. Similar result was observed in the BALB/c mice treated with Anfotericin B for 4 weeks. Thus, in mice, the analysis of monocyte frequency in the blood can be used as a predictor of prognosis in treated animals, where the decrease in the number of blood monocytes during treatment, represents an improvement in the animal's clinical status. Besides that we have analyzed the role of monocytes in canine visceral leishmaniasis. The dog (*Canis familiaris*) represents the major reservoir of the parasite, having a central role in the transmission to humans. Monocytes are always considered as a single population in dogs, that gives rise mainly to macrophages, which are the main host cell to the parasites. Our preliminary results show that dogs exhibit three distinct subpopulations of peripheral blood monocytes. One of these populations corresponds to 90% of the total monocytes, presenting high expression of CD14 molecule. The second and third populations represent about 10% of the total blood monocytes. Infected dogs show increased frequency of monocytes both in asymptomatic and symptomatic animals, when compared to uninfected animals. We are looking forward to see the role of these subpopulations during canine leishmaniasis.