RT.01 - Acidolcalcisomes: 20 years studying

RT.01.001 - TWENTY YEARS OF STUDIES ON THE STRUCTURE OF ACIDOCALCISOMES

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I entered in contact with the word acidocalcisome with a message from Roberto Docampo in 1994 before the publication of the classical work by Anibal Vercesi, Silvia Moreno and Roberto Docampo, in 1994. The first images were obtained by fluorescence microscopy of T. brucei incubated in the presence of Acridine Orange. I then realized that the structure described by them was an old known structure that we referred to as “electron dense organelle”, “volutin granules”, etc. Old studies carried out with bacteria in 1895 and 1904 revealed the presence of very small metachromatic structures that stained in red when the cells were incubated in the presence of basic blue dyes. Such structure was also seen by Carlos Chagas in 1909 and clearly depicted in his detailed illustrations of the various developmental stages of new protozoan designated as Trypanosoma cruzi. Following the Docampo message, we started a fruitful collaboration where several colleagues from our laboratory played an important role. These included Kildare Miranda, Marlene Benchimol, Helmut Plattner, Joachim Hentschel, Wendell Girard Dias, and Lia Carolina Soares Medeiros. Using several microscopy approaches we have obtained information on (a) the general shape, dimension, appearance and localization of acidocalcisomes of trypanosomatids and apicomplexans, (b) localization of different ions in the organelle as assayed using X-ray microanalysis, electron energy loss, and energy-filtered electron microscopy images, (c) immunocytochemical localization of acidocalcisome enzymes such as Ca2+-ATPase, V-H+-ATPase, and Pyrophosphatase, and (d) relationship of the acidocalcisomes with the mitochondrion and the contractile vacuole as seen using three-dimensional reconstruction techniques. Taken together these studies showed (a) that the general appearance of the acidocalcisome varies according to the procedure used to process the cells for EM studies, (b) that the electron density is due mainly to the polyphosphate accumulated within the organelle and that is easily dissolved when the section enter in contacts with water, (c) that the shape of the organelle varies according to the protozoan species and the growth conditions, (d) that there is a species specific ionic composition, as evaluated using X-ray microanalysis, (e) the presence of iron and zinc in the acidocalcisome of some trypanosomatids, (f) that in some cells the organelles display an organized array at some regions, (g) that the acidocalcisomes interact and fuse with the contractile vacuole, and (h) that the different ions do not co-localize within the organelle but are localized in distinct domains.
Taken together the morphological studies associated to those obtained using biochemistry, molecular biology and physiological analysis it is clear that we now have a good idea about the function of the acidocalcisomes. However, further studies are necessary to clarify some points. From a cell biology perspective, I would point out some topics that will require further studies. First, it is important to better understand the biogenesis of the acidocalcisomes. Recent studies point to a role of small GTPases, such as Rab32, that may play some role on the biogenesis of the organelle. Second, it is important to clarify the fact that the acidocalcisomes found in monoxenic trypanosomatids are not labeled when the cells are incubated in the presence of markers of acidic compartments such as acrydine orange, lysotracker or lysosensor thus suggesting the possibility of the existence of non-acidic calcisomes. **Supported by:**CNPq, Faperj and FINEP
Keywords: Acidocalcisome; trypanosomatidae; apicomplexa

RT.01.002 - POLYPHOSPHATE AND ACIDOCALCISOMES: ORGANELLES CONSERVED FROM BACTERIA TO HUMAN CELLS

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Acidocalcisomes are acidic calcium stores that have been found from bacteria to human cells (1). They are rich in phosphorus compounds in the form of orthophosphate, pyrophosphate, and polyphosphate (polyP) and their acidity are maintained by proton pumps such as the vacuolar proton pyrophosphatase, the vacuolar proton ATPase, or both (2). It has been claimed that acidocalcisomes could be present in all domains of life, including archaea, and may thus date back as far as to the last universal common ancestor (3). On the other hand, acidocalcisomes of trypanosomes share characteristics with organelles known as lysosome-related organelles (LROs), such as human platelets dense granules (4) and mast cell granules (5), which are also considered acidocalcisome-like organelles. This does not necessarily suggest a different origin of acidocalcisomes in eukaryotes but a potential further adaptation in these cells. Acidocalcisomes could have appeared either autogenously or by convergent evolution. Recent studies in trypanosomatids and in other species have revealed their role in phosphate metabolism, and cation and water homeostasis, as suggested by the presence of novel pumps, transporters, and channels (6). The presence of calcium uptake (calcium ATPase) and calcium release (inositol 1,4,5-trisphosphate receptor) mechanisms (7) suggests an active role of acidocalcisomes of Trypanosoma brucei in calcium signaling. The study of the biogenesis of acidocalcisomes as well as of the interactions of these LROs with other organelles (8), have uncovered important roles in calcium signaling and osmoregulation.

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Keywords: Acidocalcisome; polyphosphate; acidic calcium store

RT.01.003 - INITIAL CHARACTERIZATION OF THE ACIDOCALCISOMES IN TRYPANOSOMES

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In 1992, Eamonn K. Rooney and Julian D. Gross (1) reported the presence of a Ca2+-ATPase in organelles called acidosomes present in Dictyostelium discoideum. The name acidosome was given because they were acidic, as indicated by their sensitivity to nigericin (a K+/H+ ionophore). Dan Zilberstein and collaborators (2) as well as Larry Ruben and collaborators (3) had also described nigericin-sensitive calcium compartments in L. donovani and T. brucei, respectively. These data led us to look for a Ca2+-ATPase activity in the calcium-containing acidic compartment of trypanosomes. Indeed, we demonstrated the presence of proton uptake sensitive to vacuolar ATPase (V-H+-ATPase) inhibitors, and Ca2+ uptake sensitive to vanadate (Ca2+-ATPase) in permeabilized cells. We also found that there were organelles in these parasites that stained with acridine orange, presented a characteristic electron density, when observed by electron microscopy, and were responsible for these responses to the inhibitors and ionophores (4). We named these organelles the acidocalcisomes to indicate that they were acidic and contained calcium. Further work in T. cruzi (5) and T. brucei allowed the biochemical characterization of a vacuolar-type H+-ATPase for H+ uptake, a Ca2+/H+ countertransporting ATPase for Ca2+ uptake, a Ca2+/nH+ antiporter for Ca2+ release and in T. brucei procyclic trypomastigotes and Leishmania donovani promastigotes a Na+/H+ antiporter that may participate in Ca2+ release from these organelles.

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Keywords: Acidocalcisome; calcium homeostasis; proton atpase

RT.02 - Latest News on Control of Gene Expression in Trypanosomatids

RT.02.001 - EPIGENETIC REGULATION OF RNA POL II TRANSCRIPTION TERMINATION AND GENE EXPRESSION BY DNA MODIFICATION AND HISTONE VARIANTS IN TRYPANOSOMATIDS

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Kinetoplastids are a group of early-diverged eukaryotes that includes the human parasites Trypanosoma brucei, Trypanosoma cruzi, and Leishmania major. Unique for a eukaryote, the genomes of kinetoplastids are organized into polycistronic gene clusters that contain multiple genes that are co-transcribed from a single promoter. Because of this genome arrangement, it is thought that all gene regulation in kinetoplastids occurs after transcription at the level of RNA (processing, stability, and translation). However, multiple epigenetic modifications have been found enriched at transcription initiation and termination sites in kinetoplastids, including histone modifications, histone variants, and the modified DNA base J (beta-D-glucosyl-hydroxymethyluracil), suggesting mechanisms of transcriptional control of gene expression. We have shown that base J regulates chromatin structure at RNA Polymerase (RNAP) II initiation sites in T. cruzi, where the loss of J results in increased RNAP II recruitment at promoters and transcription of gene clusters, and global changes in gene expression. In L. major the loss of J leads to a defect in RNAP II termination resulting in transcription into opposing (convergent) gene clusters and production of antisense RNAs. Readthrough transcription at convergent gene

arrays leads to decreased levels of mRNA from genes at the end of the cluster and cell death. Whether this downregulation is due to polymerase collision restricting transcription of the sense strand or mRNA degradation via antisense RNA production is currently being addressed. The current model is that the glucose moiety of base J sticking out in the major groove of DNA provides a steric block for transcription elongation stimulating termination. In Leishmania J is important to prevent readthrough transcription at the end of polycistronic gene arrays.

We have recently demonstrated that J also regulates RNAP II termination in T. brucei, but at sites within specific gene clusters, such that the loss of J results in the increased expression of downstream genes. The kinetoplastid-specific histone variant H3.V co-localizes with base J at RNAP II termination sites, however its role in regulating termination is unknown. We now demonstrate that the loss of histone variant H3.V from RNAP II termination sites in T. brucei also leads to defects in RNAP II termination within gene clusters and increased expression of downstream genes. Gene derepression is intensified upon the combined loss of J and H3.V. Additionally, H3.V inhibits transcription of siRNA producing loci. Our results indicate base J and H3.V can act independently as well as synergistically to regulate transcription termination and expression of coding and non-coding RNAs in T. brucei, depending on chromatin context. As such these studies significantly expand our understanding of epigenetic regulatory mechanisms underlying transcription termination in eukaryotes, including providing the first direct evidence of a histone variant negatively influencing transcription elongation to promote termination.
Furthermore, these findings highlight the importance of chromatin modifications in the regulation of transcription, particularly in early-diverged eukaryotes with unique polycistronic transcription and have direct implications for a strictly post-transcriptional model of gene expression in kinetoplastids.**Supported by:**NIH
Keywords: Gene expression; epigenetics; transcription

RT.02.002 - A DEAD-BOX RNA HELICASE OF THE DDE1/DDX3 SUBFAMILY PLAYS A CENTRAL ROLE IN MITOCHONDRIAL QUALITY CONTROL AND THE RESPONSE TO STRESS IN LEISHMANIA

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Members of the Ded1/DDX3 subfamily belong to the DEAD-box proteins, the largest family of ATP-dependent RNA helicases that participate in most aspects of RNA metabolism. We have previously characterized a DEAD-box RNA helicase of 67 kDa, the Ded1/DDX3 ortholog of *Leishmania*, and showed that this helicase binds ribosomal RNA and prevents rRNA degradation and translational arrest upon stress and drug-induced apoptosis by a novel mechanism involving antisense rRNA processing. Here, we provide novel insights into the function of this Ded1/DDX3 RNA helicase as a major regulator of the mitochondrial stress response. We show that the *Leishmania* Ded1/DDX3 ortholog plays a central role in preventing mitochondrial damage from the accumulation of reactive oxygen species (ROS) under normal conditions and most importantly upon heat and oxidative stress. Leishmania genetically depleted for Ded1/DDX3 are indeed unable to scavenge ROS induced under various stresses, which leads to loss in mitochondrial membrane potential, mitochondrial fragmentation, and cell death. The high susceptibility of the Ded1/DDX3(-/-) knockout strain to various stresses encountered in the host macrophage impedes amastigote differentiation and blocks intracellular survival and disease progression in an experimental mouse model. In line with its protective role against mitochondrial stress, Ded1/DDX3 was shown to associate with key components of the cellular antioxidant defense mechanisms and also to the ubiquitin-selective chaperone VCP/p97/Cdc48, a component of the ubiquitin-proteasome system that regulates mitochondrial protein quality control. Accordingly, in the absence of Ded1/DDX3, parasites accumulate higher levels of ROS and polyubiquitinated proteins. Collectively, these observations support a model where Ded1/DDX3, through intermolecular interactions with components of the cellular stress responses, protects mitochondria from ROS damage, hence contributing to the maintenance of mitochondrial health. These studies provide new insights into a central role of Ded1/DDX3 RNA helicase in monitoring the response to mitochondrial stress and opens new avenues for the development of strategies to therapeutically target DDX3 as a novel anti-parasitic drug.
Supported by: Canadian Institutes of Health Research
Keywords: Leishmania; dead-box rna helicases; mitochondrial quality control

RT.02.003 - THE ROLE OF HEME IN PROTEIN EXPRESSION IN TRYPANOSOMA CRUZI

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In eukaryotes, translation is regulated by the availability of protein initiation factors that promote ribosome assembly in the presence of mRNA and tRNAMet. One of the required components is the complex formed by the translation initiation factor 2 (eIF2), a trimeric protein associated with one molecule of GTP and tRNAMet. Once the AUG codon is found, the elongation phase of the protein synthesis starts and eIF2 is released associated with GDP. For a new round of translation initiation, the GDP should be exchanged through the action of a guanine exchange factor (eIF2B). However, in the presence of unfavorable conditions, the α-subunit of eIF2 (eIF2α) is phosphorylated, inhibiting the action of eIF2B. This reduces the levels of available eIF2-GTP-tRNAMet and consequently decreases translation. Paradoxically, some mRNA, coding proteins involved in the stress remediation are specifically translated. In mammals, four different protein kinases have been shown to phosphorylate eIF2α. GCN2, a highly conserved protein kinase, which is activated by uncharged tRNAs. PERK, a protein kinase located in the membrane of the endoplasmic reticulum (ER), which is activated by an excess of unfolded proteins in this compartment. PKR, which is activated by double strand RNA, usually present during virus infection. HRI, which is activated when heme availability is low. In Trypanosomes, three eIF2α kinases can be recognized by sequence comparison. One is homologous to GCN2 (K1), another with the same topology of PERK (K2) and a third one with minimal resemblance with the mammalian kinases (K3). We have shown that K2 is able to phosphorylate eIF2α in Trypanosoma brucei but is located in the membrane of the flagellar pocket of T. brucei [1]. The Trypanosoma cruzi K2 (TcK2) is not present in the ER and is found in endosomal membranes [2]. In this work we also demonstrated that TcK2 is activated when heme levels decrease in the medium. Heme binds specifically to the catalytic domain of the kinase, inhibiting its activity. Parasites knocked-out for the TcK2 lose the parasite differentiation capacity and heme is not stored in endosome, remaining in the cytosol. TcK2 null cells display growth deficiencies, accumulating hydrogen peroxide that drives the generation of reactive oxygen species. The absence of TcK2 also impaired intracellular growth of amastigotes in cultured mammalian cells. We have also shown that eIF2α phosphorylation is involved in protein synthesis arrest and differentiation of proliferative T. cruzi into non-proliferative forms [3]. Therefore, it is possible that by regulating eIF2α phosphorylation and protein synthesis, heme through eIF2α phosphorylation could direct the specific translation of a certain group of proteins. We will present data of a comparative analysis of protein and mRNA levels of key proteins involved in the oxidative stress control that suggest a direct involvement of eIF2α in the control of protein synthesis that could lead to parasite differentiation.

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Keywords: síntese proteica; diferenciação; heme

RT.02.004 - NETWORKS OF GENE EXPRESSION CONTROL IN TRYPANOSOMES.

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African trypanosomes have almost no regulation of polymerase II transcription. Bloodstream forms multiply in mammals, procyclic forms in Tsetse flies. Transcriptome-wide measurements revealed a huge range of mRNA half-lives (1) and extensive translation regulation. Using results of high-throughput RNAi (2) and tethering (3) screens, and the mRNP proteome, we identified the proteins that are bound to mRNA, affect mRNA abundance or translation, and are essential for cell survival. This subset is highly enriched in proteins with RNA-binding domains, but also

includes proteins of unknown function. Several proteins that activate expression act via trypanosome MKT1 (4), while results for others suggest a link between translational suppression and decreased mRNA abundance. One of the 6 T. brucei eIF4E variants, eIF4E1, is a suppressor in the tethering assay. eIF4E1 does not interact with any eIF4G, but instead with 4E-IP (5), which is also a suppressor when tethered. When tethered-4E-IP expression is induced, the target RNA amount decreases, and the remainder loses association with polysomes. We speculate that 4E-IP can be recruited to mRNAs either by specific mRNA-binding proteins. For example, expression of RBP10 correlates with for expression of mRNAs encoding enzymes of bloodstream-type energy metabolism. Inappropriate expression of RBP10 in procyclics causes expression of bloodstream-form mRNAs and loss of procyclic ones (6). RBP10 suppresses gene expression in the tethering assay. It interacts with 4E-IP, and evidence from tethering, yeast two hybrid, and co-immunoprecipitations suggests that the interaction is required for RBP10 suppressive action. Since both proteins have low-complexity domains, we speculate that protein aggregation may play a role in their activity.

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Supported by:DFG
Keywords: Trypanosoma; mrna; translation

RT.03 - Molecular Interactions Between Parasites and Vectors

RT.03.001 - GENOME-WIDE ANALYSIS OF RHODNIUS PROLIXUS GUT GENE EXPRESSION: INFLUENCES OF THE BLOOD MEAL AND TRYPANOSOME INFECTION

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The annotated genome of Rhodnius prolixus recently became available, allowing us to provide a genome-wide description of the physiology of blood digestion in this triatomine insect and to study the impact of infection by T. cruzi on the insect midgut. During annotation of the genome all immune genes were identified, which revealed that although several genes of the IMD pathway were lacking – similar to what had been reported for the pea aphid genome – the pathway was functional, controlling the proliferation of intestinal microbiota. However, surprisingly, silencing of either toll pathway or IMD pathway did not change T. cruzi levels in any of the intestinal segments of adult R. prolixus insects, suggesting the existence of immune evasion mechanisms by the parasite. A more extensive analysis of the infection of the insect with this parasite was provided by a gut transcriptome using first stage nymphs (N1). The N1 stage was used as an experimental model instead of older insects because it allows a much more simple logistics for robust experimental design (larger experimental replicas and less expensive setups). N1 nymphs also were at least as susceptible to parasite infection as older stages, and field data revealed that N1 infection did occur under natural conditions. Insects were infected with T. cruzi using both epimastigote or trypomastigote parasite forms, and gene expression changes were followed in the gut at several different time points after the ingestion of the infectious blood meals. Data obtained revealed that the presence of the parasite induced significant global changes in the insect gene expression, with distinct responses being obtained according to time post-infection, parasite developmental form or parasites levels in the blood meal. Altogether, these data presents the first comprehensive molecular analysis of the impact of T. cruzi infection on the physiology of its insect vector.**Supported by:**CNPq;FAPERJ;CAPES;INCTEM
Keywords: Rhodnius prolixus; trypanosoma cruzi; host-parasite relationship

RT.03.002 - GP63 IS ONE OF THE MOLECULES RESPONSIBLE FOR TRYPANOSOMATID-INSECT INTERACTION

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The GP63 of the protozoan parasite Leishmania is a highly abundant zinc metallopeptidase, mainly glycosylphosphatidylinositol (GPI)-anchored to the parasite surface, which contributes to a myriad of well-established functions for Leishmania in the interaction with the mammalian host. However, the role of GP63 in the Leishmania-insect vector interplay is still a matter of controversy. GP63 homologues have been observed in all monoxenic trypanosomatids examined to date, as well as in the phytomonads and heteroxenic mammalian parasites - Trypanosoma cruzi, Trypanosoma brucei and Trypanosoma rangeli. The identification of GP63 homologues among trypanosomatids with different life cycles may help to improve the knowledge on GP63 function and evolution. A critical revisiting of the data available reveals an indirect correlation between gene expansion and proteolytic activity, as seen in T. cruzi and Leishmania tarentolae. Also, does the ubiquitous presence of GP63 on the surface of the trypanosomatids developmental forms that face the invertebrate host an indirect evidence of GP63 role in the insect interaction or shall it simply be an evolutionary vestige? There is substantial data suggesting that GP63 homologues found in monoxenic trypanosomatids and phytomonads play essential roles in the parasite nutrition through acquisition through degradation of gut content, as well as in the binding to the insect gut. In Leishmania, the actual role of GP63 on the interaction with the vector is still a matter of controversy, while in T. cruzi only preliminary data is available suggesting the participation of GP63 in T. cruzi binding to R. prolixus gut. In T. brucei and T. rangeli, GP63 possible role in the insect interaction has never been explored. Also, to which extent does GP63 mediate the interaction process in comparison to other molecules? An interesting model to help to address this last question is Strigomonas culicis, which harbors an endosymbiont bacterium that can be eliminated through antibiotic treatment, originating aposymbiotic strains that are unable to colonize the insect gut. This model was employed in a comparative shotgun proteomic approach of wild type and aposymbiotic strains aiming to identify proteins that shift abundance between strains that could be related to the differences observed in the colonization process. Certainly, in the forthcoming years, more data generated by distinct research groups will help to fulfill all these gaps on GP63 knowledge.
Supported by:CNPq, CAPES, FAPERJ and Fiocruz.
Keywords: Leishmanolysin; trypanosomatids; insect

RT.03.003 - SAND FLY-LEISHMANIA INTERACTION AND VACCINE DEVELOPMENT

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Leishmaniasis are important vector-borne diseases caused by Leishmania parasites and transmitted by phlebotomine sand flies. In the sand fly midgut, leishmania is taken up as amatigotes and subsequently differentiates into the free-swimming promastigote forms. Among the later, the metacyclic promastigotes are the infective forms transmitted to the mammalian hosts. In order for leishmania to successfully develop in the sand fly midgut, this parasite overtakes some challenging steps that could otherwise completely abrogate its development. Among such midgut barriers, the digestive enzymes, the peritrophic matrix, the immune system's Imd pathway, and epithelium docking are the best well studied. In the sand fly-leishmania interplay, such barriers alone or in combination can define the sand fly vector competence to harbor a specific species of leishmania. In that regard, there are two sorts of sand fly vectors, the restrictive and the permissive ones that can harbor one or multiple species of leishmania, respectively. As vector competence is the exponential component of the McDonald's vectorial capacity equation, the ability to harbor leishmania can define the sand fly's ability to transmit it. By the same token, strengthening or weakening such midgut barriers can halt leishmania transmission to the vertebrate hosts. One such away to accomplish that is through the development of transmission blocking vaccines (TBVs). Such vaccines aim to block parasite development in the sand fly midgut through the neutralizing activity of specific antibodies. Previously, a proof-of-concept was published whereby antibodies targeting the

receptor for binding *Leishmania major* to the midgut of its natural sand fly vector, Phlebotomus papatasi, dramatically reduced the Leishmania parasite load and completely eliminated metacyclics. Those findings prompted us to search the midgut of the sand fly *Lutzomyia longipalpis* for the receptor that binds Leishmania infantum. In order to accomplish this goal, we developed a leishmania-binding assay and performed protein purification experiments with *L. longipalpis* midgut extracts. Various midgut receptor candidates were identified using mass spectrometry analysis from HPLC purified fractions, and further experiments will be performed in order to verify their ability of bind to leishmania *in vitro* and *in vivo*. The identification of new targets in the midgut of the vectors of visceral leishmaniasis will be ideal for TBV development because it will allow for the vaccination of dogs, preventing the spread of the disease.
Keywords: Sandfly-leishmania interaction; transmission-blocking vaccines; proteomics

RT.03.004 - SAND FLY SALIVARY ANTIGENS AND ANTIBODY RESPONSE IN BITTEN HOSTS: SCREENING OF DOMESTIC ANIMALS EXPOSED TO OLD-WORLD VECTORS OF VISCERAL LEISHMANIASES.

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During blood feeding sand flies deposit into the host skin immunogenic salivary proteins which elicit a specific antibody response. These anti-saliva antibodies enable to estimate the host exposure to sand flies and also the risk of Leishmania infection. As screening of specific anti-saliva antibodies is limited by the availability of salivary gland homogenates (SGH), utilization of recombinant salivary proteins is a promising alternative.
We tested this approach in serum samples of various domestic and wild animals bitten by Phlebotomus perniciosus and P. orientalis, important vectors of visceral leishmaniases (VL).
In the Mediterranean area, P. perniciosus is the main vector of Leishmania infantum, the causative agent of zoonotic visceral leishmaniasis. The main reservoir of the disease is dog and therefore measuring the canine exposure to sand fly bites is important for estimating the risk of L. infantum transmission. The reactivity of six bacterially-expressed P. perniciosus salivary proteins, yellow-related protein rSP03B, apyrases rSP01B and rSP01, antigen 5-related rSP07, ParSP25-like protein rSP08 and D7-related protein rSP04, was tested with sera of mice and dogs experimentally bitten by this sand fly using immunoblots and ELISA. In immunoblots, both mice and canine sera positively react with yellow-related protein, both apyrases and ParSP25-like protein. A similar reaction was observed by ELISA, where the reactivity of yellow-related protein and apyrases significantly correlated with the canine antibody response against SGH. Recombinant yellow-related protein rSP03B and the apyrases rSP01B and rSP01, were identified as the best candidates for evaluating the exposure of mice and dogs to P. perniciosus bites.
Same recombinant salivary proteins of P. perniciosus were subsequently used to determine the exposure of wild reservoir hosts to sand fly bites in L. infantum focus in the south-west of the Madrid region. Sera from hares and wild rabbits captured in the study area presented higher anti-saliva antibody response in comparison to negative control sera and showed very high positive correlation between SGH and yellow-related protein rSP03B. Data confirmed the exposure of hares and rabbits to P. perniciosus bites in the context of an outbreak of human leishmaniosis in Spain, highlighting their involvement in Leishmania transmission by supporting their role as potential reservoirs.
In East Africa, P. orientalis is the main vector of VL caused by L. donovani. The epidemiology of the disease is not clear but domestic dogs and other domestic animals were repeatedly found PCR-positive for L. donovani. Here, we tested sera of domestic animals (dogs, sheep and goats) from Ethiopia. We proved that animals are bitten by P. orientalis and found good correlations of whole salivary lysate with P. orientalis recombinant ParSP25 and apyrase.
In conclusion, these results suggest that recombinant sand fly salivary proteins represent a valid alternative to whole salivary lysates and could be used in large-scale of serological studies on domestic as well as wild mammals. This novel methodology enables to detect host exposure to sand fly and represents a promising tool for further epidemiological studies that would help to design better strategies for the control of leishmaniases.
Keywords: Visceral leishmanises; sand fly saliva; recombinant antigens

RT.04 - Immunology of Protozoan Infections

RT.04.001 - HOST RESPONSES IN THE CLINICAL AND THERAPEUTIC OUTCOMES OF L. V. PANAMENSIS INFECTION

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The Leishmania-host interaction and the balance of this relationship determine the outcome of infection, which can extend from asymptomatic to severe clinical manifestations precipitating significant host morbidity and mortality. Although infection by species of the L. Viannia subgenus typically presents with localized, sometimes spontaenously self-resolving lesions, it can also result in chronic and recurrent clinical manifestations as a consequence of exacerbated immuno-inflammatory responses. The mechanisms and molecules that modulate these uncontrolled responses and their role in the therapeutic outcome, however, have yet to be determined.

Recent findings from our group showed a dichotomy in the chemokine response induced by infection of human macrophages with L.V. panamensis strains isolated from patients with self-healing cutaneous leishmaniasis (CL), n=4, and patients with chronic CL (n=5) defined as lesions of > 6 months of evolution. A consistent pattern of induction of CCL (ccl2, ccl8) and CXCL (cxcl1, cxcl2, cxcl3 y cxcl5) chemokines was observed upon infection with strains derived from patients with chronic CL. In contrast, induction of these chemokines was not detected in primary human macrophages infected with strains isolated from patients with self-healing disease, substantiating the role of the Leishmania strain in the clinical outcome and pathogenesis of infection (Navas et.al, 2014).

To explore the contribution of host cell responses in the therapeutic outcome, we profiled the transcriptome of infected and drug-exposed macrophages from CL patients who failed or responded to treatment with Glucantime® (n=2/group), defined at 13 weeks after beginning of treatment. Five times more transcripts were modulated in macrophages from patients who responded to treatment (631 vs. 128 in non-responders). Gene grouping by Gene Ontology and network sub-analyses showed that biological processes predominantly related to immunological functions, and the chemokine network were significantly up-regulated and enriched in macrophages from responders, potentially contributing to early control of intracellular parasite replication. Conversely, oxidative-stress response genes were enriched in macrophages from patients who failed treatment, likely impacting on the redox and detoxification of antimonials. Induced expression of cxcl3 and ccl2 was corroborated in macrophages from patients who cured (n=9). In vivo responses were evaluated by gene expression profiling of 84 genes of the inflammatory response in lesion biopsies from CL patients obtained pre- and post-treatment with Glucantime®. The immune response at the end of treatment in patients who cured (n=6) was characterized by downregulation of CCL and CXCL chemokines. Although inhibition of these mediators was also observed in biopsies from patients who failed to respond to treatment (n=8), the magnitude of the inhibition was lower ($p<0.05$) than that of patients who cured.

Our findings suggest that modulation of the chemokine network, mediated by both Leishmania and the anti-leishmanial therapy, and the dynamics of chemokine gene expression in host cells and cutaneous lesions are critical determinants of pathogenesis and of the outcome of treatment with Glucantime® of cutaneous leishmaniasis caused by L. (Viannia) panamensis.

Supported by:COLCIENCIAS 222956933302, NIH/NIAID 1R01AI104823, NIH/NIAID R01 AI093775
Keywords: Leishmania; immune response; treatment outcome

RT.04.002 - CONTRIBUTION OF MONONUCLEAR PHAGOCYTES TO INFLAMMATORY RESPONSE IN CUTANEOUS LEISHMANIASIS

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Skin ulcer development in cutaneous leishmaniasis (CL) patients is associated with exaggerated inflammatory response with high levels of TNF. Leishmania braziliensis is the most prevalent species causing CL in Brazil and also the one responsible for the most severe skin forms of the disease. Lesion infiltrate is mainly composed by lymphocytes and mononuclear phagocytes, and few parasites are observed. To investigate the presence of inflammatory mediators in ulcer from CL patients we first conducted an unbiased microarray analysis of lesion biopsies and compared with healthy skin transcripts. Among the upregulated genes in CL lesions we found the inflammatory genes Metalloproteinase-9, CXCL9, CXCL10, TNF and IL-1β. We than confirmed these findings by looking at protein of these mediators in supernatants of peripheral blood mononuclear cells culture and also in supernatants of lesion biopsies, in response to soluble Leishmania antigen. Interestingly, these chemokines and cytokines were present in groups of patients with ulcerated lesions and those with pre-ulcerative lesions. Circulating monocytes in human are known to be heterogeneous and based on CD14 and CD16 expression they are subdivided in classical, intermediate and non-classical monocytes. We found that while all monocyte subsets produce Metalloproteinase-9 and CXCL9, the intermediate monocyte population is the main source of CXCL10, TNF and IL-1β. The production of IL-1β can happen upon activation of inflammasomes. To test the pathway by which L. braziliensis triggers IL-1β production we infected C57BL/6 mouse macrophages lacking NLRP3, AIM2, Caspase1, ASC and IL-1R. We found that L. braziliensis-induced IL-1β production is dependent on NLRP3, Caspase1 and ASC. Altogether our data show that intermediate monocytes are the main population producing inflammatory cytokines and hence may be a potential therapeutic target candidate.
Keywords: Cutaneous leishmaniasis; inflammation; immune response

RT.04.003 - CHALLENGES IN THE APPROACH TO TREATING INFECTION TRYPANOSOMA CRUZI

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From the time of the discovery of Trypanosoma cruzi as the agent of Chagas disease, all the way to this very day, physicians and scientists have tried to develop safe, effective and inexpensive drugs to treat infection by this parasite. There are several challenges, including (1) the ability of the parasite to persist in the host, even in the face of effective immunity that keeps parasitemia and tissue parasitosis at low levels during chronic infection, (2) the existence of multiple mechanisms of pathogenesis, (3) the genetic heterogeneity of the human population, coupled with the pathogenetic heterogeneity of individual T. cruzi strains and clones, such that any given infection can have a wide variety of outcomes, and (4) the genomic plasticity of T. cruzi, which facilitates development of resistance to chemotherapy. Another curious aspect of this infection that was noted by the very earliest investigators is the frequent absence of parasites from sites of tissue inflammation. The mechanism underlying this finding was hypothesized to be “allergic” or “hyperergic” and, although presumably initiated by infection somewhere in the body, was thought to be ultimately independent of the parasite. This evolved into the autoimmune hypothesis of Chagas disease pathogenesis and the possible presence of autoimmunity in infected individuals. If present, autoimmunity poses an additional challenge to the development of therapies for both acute and chronic infection. In this presentation I will review the historical approaches to treatment of T. cruzi infection and discuss some of the modern approaches scientists are taking to prevent and cure this very important and emerging infection that is present throughout the Americas.**Supported by:**US National Institutes of Health
Keywords: Chagas; pathogenesis; treatment

RT.04.004 - MIRNA PROFILE IN *TRYPANOSOMA CRUZI* INFECTION: ASSOCIATION WITH PATHOLOGY DEVELOPMENT AND IMMUNE RESPONSE

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Chronic Chagas Cardiomyopathy (CCC) is the severe manifestation of Chagas disease, caused by infection with the protozoan parasite *Trypanosoma cruzi*. CCC is characterized by myocardial inflammation, arrhythmias, heart hypertrophy and fibrosis, affecting 30% of chronic infected patients. The molecular mechanisms associated with differential CCC progression is still poorly understood. CCC is a complex multifaceted disease with the involvement of multiple genes regulated by microRNAs (miRNAs) that together might play important roles in the disease. We simultaneously investigated miRNA and gene expression in myocardial tissue samples of CCC patients in comparison to samples from heart transplant donors as well as in heart of mice acutely infected with *T. cruzi*. We identified 20 differentially expressed miRNA in CCC myocardial tissue samples. Many of these have been related to cardiovascular diseases. MiRNA changes were integrated with genome wide gene expression profiles performed in the same samples to investigate predicted and experimentally validated miRNA targets. With this approach, we constructed CCC specific miRNA-Targets co-regulatory networks and found several hubs which dysregulated expression was validated by qRT-PCR. The identified networks may be potential regulatory mechanisms and also provide details of how miRNAs regulate gene expression during the establishment of each CCC clinical parameter: myocardial inflammation, arrhythmias, heart hypertrophy and fibrosis.

Keywords: Microrna; t.cruzi; chagas cardiomyopathy

RT.05 - Biology of Apicomplexa Parasites

RT.05.001 - TOXOPLASMA EFFECTOR GRA26 ACTIVATES THE CHROMATIN REPRESSOR EZH2 TO DAMPEN THE HOST IL-1β-MEDIATED INFLAMMATORY RESPONSE

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The intracellular parasite Toxoplasma strikes a subtle balance with the host immune system that not only prevents host death but also promotes parasite persistence. Although being enclosed within a parasitophorous vacuole, the parasite actively interfaces with host cell signaling pathways, thereby directing host cell responses. Dense granules are now recognized as critical in delivering products that occasionally traffic beyond the vacuole membrane (PVM) to the host cell and contribute to rewire host gene expression. In the same fashion to GRA16 (Bougdour et al. CHM, 2013) and GRA24 (Braun et al. JEM, 2013), GRA26 is a new parasite effector delivered in host cell nuclei where it interacts with host E2F transcription factors. The GRA26/E2F complex modulates positively the expression of the histone methyltransferase EZH2 which is suspected to restrain expression of host inflammatory genes, e.g. those encoding the neutrophil chemokine IL-8 or IL1β. Mice infected with type II gra26 KO strain are able to survive an otherwise lethal dose (up to 10e6) of tachyzoites. In the context of gra26 KO, we monitored a significant increase of neutrophils recruitment early during infection and a consecutive reduction of the pathogen loads that should contribute to extend the survival of mice. The proposed model suggests that GRA26 diverts EZH2 chromatin function to negatively regulate a network of genes involved in IL-1β/IFNγ-mediated inflammatory response. In this fashion, GRA26 is acting as a type II virulence factor that prevents a complete neutrophil-mediated parasite clearance at the site of infection and ultimately promotes parasite persistence. **Supported by:**European Research Council, ERC grant HostingToxo

Keywords: Toxoplasma gondii; host-parasite interaction; epigenetic

RT.05.002 - TOXOPLASMA MODULATION OF THE HOST CELL

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Toxoplasma gondii is an intracellular parasite, capable of infecting all warm-blooded animals. Upon infection of a host, Toxoplasma converts into cysts in brain and muscle tissue and establishes an infection that remains for the duration of the host's life. Host genetic differences determine the outcome of infection with Toxoplasma. Using rat strains that are resistant or susceptible to Toxoplasma we found that macrophages from resistant rats rapidly die following Toxoplasma infection, which prevents parasite replication. This rapid macrophage cell death is mediated by a cytosolic innate immune receptor, NLRP1, which activates caspase-1/11 (inflammasome activation). In mouse macrophages, Toxoplasma also activates the inflammasome but this does not lead to macrophage death and therefore Toxoplasma can replicate freely. Using a chemical mutagenesis strategy we have identified novel Toxoplasma dense granule proteins that are involved in inflammasome activation.
Keywords: Toxoplasma; inflammasome; nlrp1

RT.05.003 - GENETIC EXCHANGE, SURFACE ANTIGENS AND INFLAMMASOME SENSORS ACTIVATED BY PROTOZOAN PARASITES

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Using population genetic and WGS phylogenomic methods, our work has identified extant genetic exchange among circulating populations of natural isolates of Giardia, Leishmania, and *Toxoplasma*. To understand the biological consequences of such genetic admixture, we utilize *Toxoplasma* as our genetic model. Acute virulence during murine *Toxoplasma* infection is highly dependent on expression of polymorphic secreted pathogenesis determinants (SPDs) that are inherited in discrete haploblocks by genetic exchange. SPDs discharged from parasite secretory organelles target host immune signaling pathways and facilitate infection competency. Employing forward, reverse genetic, and genome-wide association (GWAS) techniques, we have identified novel SPDs activating inflammasome pathways, dysregulating immune homeostasis, or altering parasite pathogenesis. eQTL screening of progeny from a collection of T. gondii crosses that differentially modulate activation of the host inflammasome has identified three new parasite loci, in addition to GRA15, that upregulate IL-1β. Utilizing GWAS on WGS data from 56 T. gondii strains, we have identified four genomic regions (Chromosome VIIa, VIIb, VIII and IX) encoding novel SPDs associated with murine virulence. Finally, utilizing reverse genetics, we show that SRS29C expression negatively regulates murine virulence. The 1.8Å structure of SRS29C possesses a positively charged basic groove mediated principally by three ARG residues (K62, K68, K69) that specifically interact with heparan sulfate, a negatively-charged sulfated glycosaminoglycan (GAG). Mutation of active site residues K62 or K68/69 abrogates GAG binding and restores acute virulence. Our data suggest that the SRS29C-dependent GAG interaction is an important regulator of host immune responses required to maximize parasite transmissibility.
Keywords: Genetic exchange; toxoplasma; pathogenesis

RT.06 - Translational Research of Protozoan Parasites

RT.06.001 - TRANSLATIONAL RESEARCH, FROM MODELS TO HUMANS

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In the recent years, clinical trials in the field of parasitic disease are coming to light in order to bring strong evidence against neglected diseases.
Among others, this is the case of Chagas disease. After many years from its discovery, new clinical trials, testing novel compounds against Trypanosoma cruzi have been performed. The results obtained in the most recent clinical trials surprised both investigators and scientific community in general. According to the results obtained in the in vitro or the in vivo models, drugs evaluated had to cure the infection from the patients. Unfortunately, published data reveals disappointing and contrary results. Clinicians blame to basic researchers and doubt of the accuracy of the experiment design. Basic researchers complain to clinicians because of the wrong extrapolation from their results. At the end, animal models appears as the scapegoat.
New clinical trials will appear in the next coming years, evaluating new candidates, drug combinations or even new posology of old drugs.
It is mandatory to interpret correctly, data from animal model and mainly to be aware of its inherent limitations. Experimental models are essential prior to reach a clinical trial with patients but must be properly evaluated. We pretend to analyze the current available data from the experimental models and try to understand why clinical trials have failed to show the results previously obtained.
Bridges of fluent communication are needed between clinicians and basic investigators with the aim to complement the research and to reach a common goal. **Supported by:**CAPES/CSF
Keywords: Chagas disease; animal models; translational

RT.06.002 - RE-EDUCATION OF THE IMMUNE SYSTEM HAS A BENEFICIAL EFFECT ON EXPERIMENTAL CHAGAS' HEART DISEASE: UNVEILING TARGETS FOR THERAPY

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Chagas disease (CD) is caused by the protozoan Trypanosoma cruzi and affects millions of people mainly in Latin America. In the acute phase, immune response leads to parasite control. Ten to thirty years later, ~30% of the patients progress to the cardiac form of CD. The chronic chagasic cardiomyopathy (CCC) progresses with persistent parasitism, inflammatory processes, fibrosis and electrical and functional abnormalities. The severity of Chagas' heart disease, ranging from mild to severe, is directly associated with the intensity of the unbalanced immune response enriched in inflammatory mediators as tumor necrosis factor (TNF), interferon (IFN)γ and nitric oxide (NO). In this work, we challenge the hypothesis that immunological unbalance fuels heart abnormalities in CD. As consequence, regulation of the immune response in the chronic phase of T. cruzi infection was tested as an alternative to stop progression and even reverse CCC. For that, experimental model that reproduces aspects of Chagas' heart disease, including prolonged QRS and QTc and heart dysfunction, was treated with immunological tools as vaccine and target-based immunoregulator, associated or not with trypanocidal drug. Initially, aiming to re-educate the immune response we vaccinated mice with signs of CCC with a recombinant adenovirus-based vaccine (rAdVax) carrying the coding sequences of the amastigote surface protein-2 (ASP2) and trans-sialidase. In comparison with mice pre-therapy, vaccinated mice had less heart tissue damage and electrical abnormalities. rAdVax favored ASP2-specific immune CD8+IFNγ+ T-cells and reduced cytotoxic CD8+ T-cells. Further, rAdVax reduced the number of perforin+ cells, but preserved the number of IFNγ+ cells, in the heart tissue. Moreover, the rAdVax immunotherapy reduced the serum levels of NO and inducible (iNOS/NOS2) expression in the heart tissue. This study taught us that repositioning of key features of the unbalanced immune response was feasible and had as consequence the reversion of heart tissue injury. Thus, considering the association between the severity of CCC

and increased cytokine concentrations in the serum of patients and mice, as proof of concept mice with signs of CCC were treated with immunomodulators. As common findings there was no change in parasite load, but the immune response was repositioned with reduction of inflammatory mediators and its receptors. Further, treatment of chronically infected mice with immunomodulators induced a more regulated profile with increased expression of IL-10 and reduced CD8+ T-cell cytotoxic activity. Additionally, there was a remarkable reduction of iNOS/NOS2 expression in the heart tissue and NO levels in the serum. In parallel to immunological repositioning, we observed improved heart function with reduction of the prolonged QRS and QTc. Altogether, our data support that TNF fuels immunological unbalance, which contributes to cardiac abnormalities. Lastly, immunomodulator associated with the trypanocidal drug benznidazole (Bz) diminished parasite load, reduced the expression of molecules of the TNF/TNFR1 and iNOS/NO pathways and ameliorated heart electrical function. Moreover, as the available treatments only mitigate CCC symptoms, the combined therapy appears to be feasible to control parasite and modulate the immune response aiming to improve the prognosis for Chagas' heart disease and the quality of life of the patients.
Keywords: Chagas disease; vaccine; immunotherapy

RT.06.003 - THE UNFOLDING STORY OF TAMOXIFEN IN LEISHMANIASIS CHEMOTHERAPY

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Leishmaniasis are highly prevalent diseases in Brazil. In recent years, the classical, predominantly rural, geographical distribution of the disease has changed and we have witnessed a considerable increase in the number of urban cases. Only a few drugs are available for leishmaniasis treatment and these display several disadvantages such as high cost and toxicity, parenteral use and, in some instances, low efficacy due to selection of resistant parasites. We have shown that tamoxifen, a selective estrogen receptor modulator, has antileishmanial activity in vitro against all *Leishmania* species tested so far. *In vivo* efficacy was demonstrated in experimental models of cutaneous and visceral leishmaniasis, after administration of tamoxifen by the parenteral, oral or topical routes. The mechanism of action of tamoxifen against the parasite involves damage to the plasma and mitochondrial membranes as well as disturbances of sphingolipid metabolism. A multi-targeted mode of action was also suggested by a complete lack of success in rescuing tamoxifen resistant *Leishmania* after *in vitro* or *in vivo* drug selection protocols. Tamoxifen has additive effects when combined with antimonials, amphotericin B or miltefosine. The potential for an off-label use of tamoxifen in leishmaniasis chemotherapy will be discussed. **Supported by:**FAPESP, CNPq, CAPES
Keywords: Leishmaniasis; chemotherapy; tamoxifen

RT.06.004 - RESEARCHING AND DEVELOPING NEW ANTIMALARIALS: WHERE ARE WE GOING?

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There is high resistance of Plasmodium falciparum to most traditional antimalarials like chloroquine (CQ), amodiaquine, mefloquine, and, to the artesunate derivatives, used in drug combinations. The discovery and development of new drugs and remedies are urgent, being the aim of our investigation, in collaboration with chemist and other research groups from all over Brazil, some collaborations abroad as well. The animal malaria models (mice) with experimental infection with blood-stage parasites allow for the selection of promising antimalarial drugs. But only best compounds, able to significantly reduce growth of P. falciparum (IC50 <5ug/ml) are submitted to tests in mice. Due to increasing constraints and restrictions on the use of experimentally infected animals, we try to adapt to this present rules, that have already affected our project, partly interrupted by our collaborators (Instituto Royal, S. Roque, SP), in charge of some pharmacological and toxicological tests of promising compounds was halted, under pressure of animal rights activists; such studies are critical before Phase I human clinical trials and to selected the less toxic drugs. Continuous in vitro cultures of P. falciparum are complex,

laborious and expensive, but they allow for a large scale drug testing; hundreds synthetic compounds are shown to be highly active to P. falciparum, like CQ analogues (nanomolar activity), and curative to mice with CQ-resistant malariain mice (DeSouza et al, Mal J, 2014). Various in vitro tests were used in parallel (assays with radioactive or colorimetric DNA intercalants; tests with monoclonal antibodies; or fluorescent SYBR green dye), and provided similar results. Cytotoxic molecules are discarded based on in vitro tests; and, those with the highest therapeutic (selectivity) indices, are prioritized for other tests. Hundreds extracts and fractions from medicinal plants anti-fever were also active in vitro, but the compounds responsible for activity are not always identified; as for instance from Aspidospermum nitidum (carapanauba), largely used in the Amazon to treat malaria (Pena-Coutinho et al MIOC, 2014), not provided in sufficient amounts for such specific studies. Other pharmacological tests in vivo including of activity, are required to develop antimalarials or phytotherapies. Complementary studies with fresh human isolates of P. falciparum and P. vivax, in ex-vivo tests, required the tedious thick blood-smears in traditional microcopy, but are valuable, and useful to define the profile of drug susceptibility (Aguiar et al Mal. J. 2014). But, most of our new antimalarial drugs, as well as traditional antimalarials, target only asexual blood stage parasites. An ideal drug should also target gametocytes, the sexual forms as well, and hamper parasite development in mosquito vectors. We try to study drugs that block malaria transmission using malaria in chicks and Aedes mosquitos; or gametocyte cultures, in phase of testing. But only one of our tested compounds shows this dual activity and was not toxic: MEFAS, a hybrid drug derived from mefloquine and artesunate (developed and produced at Farmanguinhos-RJ, by Nubia Boechat), previously shown to be active as a blood schizonticide. MEFAS´s ability to block the infectivity of Plasmodium falciparum gametocytes was accessed in preliminary studies, being more effective than mefloquine and artesunate alone (submitted). Our work, performed mostly by graduate students and young Posdoctors, has allowed training of highly specialized personnel, but, in spite of the hundreds active compounds -synthetic or from natural compounds- we described so far, only one drug that may undergo human trials, MEFAS. Produced on a large scale it has a chance to become a new human antimalarial with a dual activity and help in the control programs. **Supported by:**CNPq, FAPEMIG, CAPES and PAPES-FIOCRUZ
Keywords: Extratos de plantas medicinais; plasmodium falciparum; antimaláricos