

HP-01 - Gasdermin-D activation in response to *Leishmania* infection induce a transient cell permeabilization to promote NLRP3 activation and host resistance to infection

DE SÁ, K.S.G.¹; AMARAL, L.A.²; RODRIGUES, T.S.²; ISHIMOTO, A.Y.²; DE ANDRADE, W.A.C.²; DE ALMEIDA, L.²; CASTRO, F.F.²; BATAH, S.S.²; PASTORELLO, M.T.²; FABRO, A.T.²; ZAMBONI, D.S.².

1. DEPARTAMENTO DE BIOLOGIA CELULAR E MOLECULAR E BIOAGENTES PATOGENICOS, FMRP, BRA; 2. USP, RIBEIRÃO PRETO - SP - BRA.

E-mail: keyla-sa@hotmail.com

Leishmania is an obligate intracellular parasite that causes Leishmaniasis, a disease that affects millions of people worldwide. *Leishmania* evades immune response by inhibiting specific processes on parasite-containing immune cells, yet the NLRP3 inflammasome activation is key for disease outcome. The molecular mechanisms upstream of the inflammasome activation are still unclear and there is no evident host cell death in *Leishmania*-infected cells. Here, we investigated the participation of Gasdermin-D (GSDMD, a pore-forming effector protein associated with pyroptosis) during *Leishmania* infection in macrophages and in vivo. We demonstrated that despite the absence of pyroptosis, GSDMD is active at the early stages of *L. amazonensis* infection in macrophages, allowing a transient cell permeabilization and potassium efflux, promoting NLRP3 inflammasome activation. *Gsdmd*^{-/-} macrophages exhibit less ASC puncta formation and IL-1 β production in response to infection, suggesting that the transient GSDMD-mediated permeabilization contributes for NLRP3 inflammasome activation. Mouse and macrophages deficient in GSDMD were highly susceptible to infection by several *Leishmania* species, including *L. amazonensis*, *L. major*, *L. braziliensis* and *L. mexicana*, confirming a key role of Gasdermin-D for inflammasome-mediated host resistance to infection. Finally, ASC/NLRP3 puncta and cleaved Gasdermin-D were present in skin biopsies of leishmaniasis patients, supporting the role of these molecules during active disease in humans. Altogether, our findings reveal that *Leishmania* infection triggers a transient activation of GSDMD and this molecule is critical for inflammasome activation and immunity in Leishmaniasis.

Supported by:FAPESP 2018/16777-8 **Keywords:**Leishmania;Inflammasome;GSDMD.

HP-02 - Technological development of a new vaccine candidate against canine visceral leishmaniasis caused by *Leishmania infantum*.

MELO JUNIOR, O.A.D.O.¹; DOS SANTOS, J.S.¹; GONDIM, F.C.¹; MACIEL, D.O.¹; BORSODI, M.P.G.¹; DE FARIAS, V.P.²; LIMA, E.M.³; MOREIRA, O.D.C.⁴; SOUSA-BATISTA, A.D.J.¹; GUEDES, H.L.D.M.¹; BERGMANN, B.R.¹.

1. UFRJ- RJ - BRA; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ- BRA; 3. UNIVERSIDADE FEDERAL DE GOIAS, GOIANIA - GO - BRA; 4. INSTITUTO OSWALDO CRUZ, RJ- BRA.

E-mail: otoni@biof.ufrj.br

Visceral leishmaniasis is a lethal neglected disease caused by *Leishmania infantum* and *L. donovani*. In Brazil, dogs are the main reservoir of *L. infantum*. We designed a novel vaccine formulation (LaAg RA-LipPEG) for intranasal administration to newborn puppies consisting of *L. amazonensis* antigens associated with retinoic acid nanostructured with pegylated liposomes as an adjuvant. Our pre-clinical tests in mice demonstrated the efficacy of intranasal LaAg RA-LipPEG in preventing parasite growth in the spleen, liver and bone marrow after infection. Also, a clinical trial with 30 *L. infantum*-free mongrel dogs, non-reactive by SNAP, ELISA and PCR, is under way. Dogs received two or three intranasal doses of LaAg RA-LipPEG at 15- day intervals, or 3 subcutaneous doses of the marketed Leish-Tec® as controls. LaAg RA-LipPEG showed to be immunogenic, increasing the serum levels of anti-promastigote IgG, IgA and IgM and anti-amastigote IgG. Of note, all dogs tested negative for the DPP rapid test 30 days after the last dose, indicative of vaccine non-interference with current CVL diagnostic test. Dogs were challenged with *L. infantum* 3 months after vaccination and tested for PCR 2 months after infection. Overall, LaAg RA-LipPEG is a promising candidate for the prevention of canine visceral leishmaniasis caused by *L. infantum*. **Supported by:**Global NTD Network **Keywords:**Canine Visceral Leishmaniasis ;Vaccine;Intranasal.

HP-03 - **TNFR1, the maestro of inflammatory response to infection with *Leishmania amazonensis***

VAZ, L.G.; DINIZ, C.; DE ANDRADE, A.C.; NOLASCO, A.E.; AMARAL, F.; VIEIRA, L.Q..
UFMG - MG - BRA.
E-mail: leoc_bio@yahoo.com.br

TNF is pleiotropic cytokine involved in inflammation, host defense, tissue degeneration and tissue regeneration, among other functions. TNF acts through two cognate receptors. Signaling through TNFR1 is paramount against intracellular parasites, including *Leishmania*. We investigated the impact of TNFR1 on the infection by *L. amazonensis* using wild-type (WT) and TNFR1 knockout (TNFR1ko) mice. Although expression of TNFR1 by WT mice is not enough to eliminate the parasite, this receptor mediates control of parasite replication and inflammatory response. At nine weeks, lesions were larger in TNFR1ko mice, and at 12 weeks both lesions and parasite number were larger in TNFR1 ko mice. WT mice presented more F4/80⁺MHCII⁺ cells (P3) than TNFR1ko mice at 9 and 12 weeks of infection. At 9 weeks WT had more iNOS⁺ and arginase 1 (Arg1) double positive iNOS⁺Arg1⁻ in P3 cells than TNFR1ko mice. At 12 weeks of infection, iNOS⁻Arg1⁺ P3 cells were higher in WT than in TNFR1ko mice. We could not detect differences in the number or percentage of infected myeloid cells or in the presence of NO in these cells *in vivo*. However, BMDM derived from TNFR1ko mice were more susceptible to infection with *L. amazonensis*. Moreover, these cells produced less NO when activated with IFN- γ and infected with *L. amazonensis*. We also found larger number of T lymphocytes in TNFR1 ko mice. Furthermore, most of the few lymphocytes found in WT mice at 12 weeks of infection were regulatory T cells. At this time point we also found higher levels of IL-10 in lesions of WT mice. In summary, at the last time point of infection we investigated, WT mice presented a regulatory response characterized by macrophages iNOS⁻Arg1⁺ (M2), Tregs at the site of infection and production of IL-10. We conclude that for successful clearance and wound healing during leishmaniasis, different phenotypes of macrophages need to appear coordinately in the appropriate time. TNF, through TNFR1, seems to act as this conductor. **Supported by:** CAPES, CNPq, FAPEMIG **Keywords:** TNFR1; Leishmania; macrophage polarization.

HP-05 - **GLYCOCONJUGATES (LPG/GIPLS) FROM DERMOTROPIC AMAZONIAN LEISHMANIA SPECIES DISPLAYS INTERSPECIES VARIATIONS IN THEIR BIOCHEMICAL AND FUNCTIONAL PROPERTIES IN C57BL/6 MACROPHAGES**

SOARES, R.P.P.¹; RÉGO, F.D.¹; LAURENTI, M.D.².

1. INSTITUTO RENÉ RACHOU/FIOCRUZ, BH- MG - BRA; 2. FACULDADE DE MEDICINA DA USP/FMUSP, SÃO PAULO - SP - BRA.

E-mail: rsoares@cpqrr.fiocruz.br

Lipophosphoglycans (LPGs) and Glycoinositolphospholipids (GIPLs) are *Leishmania* glycoconjugates involved in host-parasite interaction in the vertebrate host. Information on the glycobiology of dermatropic Amazonian *Leishmania* species is scarce. For this reason, the objectives of this project were to report the biochemical and functions properties of LPGs and GIPLs. The species included: *Leishmania braziliensis*, *L. guyanensis*, *L. shawi*, *L. lainsoni*, *L. lindenbergui* and *L. naiffi*. After extraction and purification of LPGs and GIPLs, the preliminary biochemical structures were obtained using fluorophore-assisted carbohydrate electrophoresis. Interesting polymorphisms were observed in the LPGs and GIPLs of the six species. Most of the LPGs were devoid of sidechains (type I) showing the typical Gal-Man-PO₄ repeat units. Those included *L. shawi*, *L. lainsoni*, *L. lindenbergui* and *L. naiffi*. LPGs of *L. braziliensis* and *L. guyanensis* showed galactose and glucose sidechains, respectively. GIPLs from dermatropic *Viannia* species also had variations in their monosaccharide content. GIPLs from *L. guyanensis* and *L. naiffi* possesses galactose and mannose (Type II/hybrid), whereas for the other species, galactose was the main sugar (Type I). Those glycoconjugates were assayed in mouse peritoneal macrophages and respective TLR2 ^{-/-} and TLR4 ^{-/-} knock-outs for NO and cytokine/chemokine production. Regardless the species, NO and cytokine/chemokine production were primarily via TLR4/TLR2. A higher pro-inflammatory activity was detected in *L. lainsoni* (type I LPG). We did not establish any correlation between biochemical structure and functional activity. In conclusion, there are several biochemical polymorphisms in the LPGs and GIPLs of Amazonian dermatropic *Viannia* species. Those glycoconjugates triggered variable innate immune responses in macrophages and may contribute to the spectrum of clinical manifestations in dermatropic *Viannia* species. **Supported by:** FAPESP (2021/01243-0); CNPq (302972/2019-6); FAPEMIG PPM-XII (00202-18) **Keywords:** LIPOPHOSPHOGLYCAN; GLYCOINOISTOLPHOSPHOLIPIDS; INNATE IMMUNITY.

HP-06 - Congenital *Toxoplasma gondii* infection affects retinal proliferation and differentiation in mice.

DE CAMPOS, V.S.¹; DA COSTA, K.C.¹; ADESSE, D.P.². 1. UNIVERSIDADE FEDERAL FLUMINENSE, NITERÓI - RJ - BRA; 2. FIOCRUZ, RJ- BRA.
E-mail: vvincampos@gmail.com

Toxoplasmosis affects one third of the world population and has the protozoan *Toxoplasma gondii* as its etiological agent. Congenital infection (CI) can cause severe damage to the fetus, such as abortions, intracranial calcification, hydrocephalus and retinochoroiditis. The severity of the impairment depends on the gestational period in which infection occurs. Even so, there are few data that clearly demonstrate the occurrence of alterations at the cellular level during the stages of retinal development, after congenital infection. The present work aims to investigate the impact of CI by *T. gondii* on the retina of mice. We proposed a model for CI, in which pregnant females of the C57bl6 strain are separated into two groups, control and infected, and the offspring are analyzed at embryonic day (E) 18 and E20. At E10, pregnant females are infected intragastrically with 2 cysts of the Me49 strain of *T. gondii*, while controls received saline solution. At E18 and E20, the infected pups had significantly smaller body size and weight than the controls, indicating that embryonic development was affected. A significant increase in the number of Ki67-positive cells (marker of proliferating cells) in the neuroblastic layer (NBL) and an augment in the number of cells in mitosis in the apical region of the NBL was detected in the retina of the infected mice compared to the control. In agreement, cell cycle proteins, such as cyclin D3, Cdk6 and pChk2, were also significantly altered in infected retinas. Interestingly, the immunohistochemical analysis showed a significant increase in the population of β -III-tubulin-positive cells, one of the earliest markers of neuronal differentiation. Together, the data suggest that CI alter cell cycle, increasing proliferation but inducing the arrest of these cells at G2/M phase. It is possible that these alterations influence the differentiation, anticipating/increasing neuronal maturation, and therefore leading to abnormal retinal formation. **Supported by:** INOVA no. 3231984391; CNPq 401772/2015-2 - 444478/2014-0; INCT/INNT 465346/2014-6 for KC, FAPERJ no. E-26/010-001199/2015; Projetos Temáticos no. E26/010.101037/201; Sediadas grant no. E-26/010.001493/2019 for KC). **Keywords:** Congenital toxoplasmosis; Retina; Development.

HP-09 - Modifications in lipid metabolism of swiss mice infected with *Toxoplasma gondii*.

ANDRADE, S.V.D.; CABRAL, S.S.; KLUCK, G.E.G.; MARTINS-DUARTE, É.D.S.; DE SOUZA, W.;
VOMMARO, R.C.; ATELLA, G..
UFRJ- RJ - BRA.
E-mail: savadean22@hotmail.com

Toxoplasmosis is a neglected tropical disease of which Brazil stands out as the third country with highest prevalence of IgG against this parasite. The disease, caused by an intracellular protozoan of the genus *Toxoplasma*, affects the felids and several other species, as humans. *Toxoplasma gondii* does not have complete degradation and synthesis lipid pathways and, thus, it depends on hosts for its development. Therefore, the aim of this work is to characterize the liver lipid metabolism of mice infected by *Toxoplasma gondii*. For this, 3 groups of Swiss mice, male and female, were submitted to an infection time course of 3,4,7 weeks or 4 months, at two different parasite loads, 50 and 250 parasites (CTR, n=32; INF 50, n=28; INF 250, n=27). After the infections, livers were submitted to proteins, cholesterol, glucose and triacylglycerols (TAG) dosage using enzymatic colorimetric method (DOLES). Also, lipids were extracted and submitted to thin layer chromatography (TLC) to assess whether the infection would alter the lipid metabolism. From the results obtained, it was observed a significant decrease in the amount of proteins in 7-week-old females and males, a significant reduction in TAG concentration in 3-week-old females and in 4-month-old females; a significant glucose decrease in females and 3-week-old males and a cholesterol decrease in 7-week-old females. About the lipids assess, it was observed a significant increase in TAG and phospholipids and, also, a decrease of monoacylglycerol (MAG) in 3-week-old females; a decrease of TAG in 7-week-old females; a decrease in esterified cholesterol, TAG, 1,3-diacylglycerol and MAG in 4-month-old females. Thus, *Toxoplasma gondii* modulates lipid metabolism in the liver of Swiss mice. These alterations may be involved as an attempt to acquire these macromolecules for its own benefit, in order to complete their life cycle. **Supported by:** FAPERJ, CNPq, INCT **Keywords:** Metabolism; Lipids; *Toxoplasma gondii*.

HP-10 - Modulation of the Host Nuclear Compartment by *Trypanosoma cruzi* Uncovers Effects on Host Transcription and Splicing Machinery

DE CASTRO, C.G.¹; CASTRO, F.F.²; CÓRDOVA, R.A.G.³; DA FONSECA, C.K.³; GOMES, M.D.³; ISHIKAWA-ANKERHOLD, H.C.⁴; BAQUI, M.M.A.³.

1. INSTITUTO BUTANTAN, SÃO PAULO - SP - BRA; 2. USP - SP - BRA; 3. FMRP-USP, RIBEIRÃO PRETO - SP - BRA; 4. UNIVERSIDADE DE MUNIQUE, MUNICH - ALEMANHA.

E-mail: camilagachet@gmail.com

Host manipulation is a common strategy for invading pathogens. *Trypanosoma cruzi*, the causative agent of Chagas Disease, lives intracellularly within host cells. During infection, parasite-associated modifications occur to the host cell metabolism and morphology. However, little is known about the effect of *T. cruzi* infection on the host cell nucleus and nuclear functionality. The methodology used in LLC-MK2, HeLa and THP-1 cells infected with *T. cruzi* compared to uninfected cells (control) was based on analysis of images obtained in a confocal and electron microscope, of gene and protein expression, and the use of a mini reporter gene for check splicing *in vivo*. The results showed that the parasite migrates close to the nucleus causing deformation in the nuclear envelope, and altering the chromatin conformation during the infection. Also, we showed that *T. cruzi* can modulate host transcription and splicing machinery in non-professional phagocytic cells during infection. We found that *T. cruzi* regulates host RNA polymerase II (RNAPII) in a time-dependent manner, resulting in a drastic decrease in RNAPII activity. Furthermore, host cell ribonucleoproteins associated with mRNA transcription (hnRNPA1 and AB2) are downregulated concurrently. We reasoned that *T. cruzi* may hijack the host U2AF35 auxiliary factor, a key regulator for RNA processing, as a strategy to affect the splicing machinery activities directly. In support of our hypothesis, we carried out *in vivo* splicing assays using an adenovirus E1A pre-mRNA splicing reporter, showing that intracellular *T. cruzi* directly modulates the host cells by appropriating U2AF35. For the first time, our published results provide evidence of a complex and intimate molecular relationship between *T. cruzi* and the host cell nucleus during infection. **Supported by:**FAPESP 2010/19547-1; 2018/03677-5; CAPES finance code 001; FAEPA-FMRP-USP **Keywords:**host-pathogen interaction;trypanosomatid;nucleus.

HP-11 - Mechanisms of FAZ assembly in *Trypanosoma cruzi*

MELO, N.S.; ALCANTARA, C.D.L.; VIDAL, J.C.; ROCHA, G.M.; DE SOUZA, W..
UFRJ, RJ- BRA.

E-mail: normandasouzamelo@gmail.com

T. cruzi has a single flagellum responsible for motility. The flagellum/cell body connection is established via the flagellar attachment zone (FAZ), an adherent network composed of a set of fibers, filaments, and junctional complexes that starts from the flagellum domain and is anchored to the cell cytoskeleton and remains attached along the body, except the distal portion of the flagellum. Assembly dynamics of FAZ are poorly characterized in *T. cruzi*, although in other trypanosomatids it is a key regulator of cellular processes. Despite the classic phenotype and depletion of TcGP72 well established little is known about interaction with other proteins and its structural features during the life cycle of *T. cruzi*. TcGP72 protein is a homolog to FLA1, 2 e 3 of *T. brucei*. In *T. brucei* it was described that TbFLA1 protein interacts with FLA1 Binding Protein (TbFLA1BP) promoting the membrane adhesion between the flagellum/cell body. This TbFLA1/TbFLA1BP association favors the assembly of FAZ and flagellum and is responsible for the regulation of morphogenesis. In this study we analyze the role of GP72 and FLA1BP proteins during the life cycle of *T. cruzi*, evaluating tagged parasites and knockout for both proteins using CRISPR/Cas9 system. We report that C-terminal is required for correct targeting of the TcGP72 and mutants labeled on the C-terminal showed flagellum detachment as well as a knockout. TcFLA1BP protein exhibits localization along the FAZ in the cell body in all phases of the cell cycle. Consistent with previous studies we observed that the TcGP72 *-/-* showed flagellum detachment from the cell body in all *T. cruzi* forms, in addition, to presenting drastic morphological changes in organelle positioning during metacyclogenesis and altered flagellum/cell length. TcFLA1BP *-/-* also showed morphological changes, flagellum partially detached flagellum and ratio flagellum/cell size diminished. Both knockouts exhibited inhibition of growth, cytokinesis, and infection. **Supported by:**FAPERJ **Keywords:**flagellar attachment zone;FAZ protein;GP72/FLA1 Binding Protein.

HP-13 - Physical exercise protects mice from muscular and neural pathology after *Toxoplasma gondii* infection

VIEIRA, P.D.C.¹; DE CASTRO, L.L.²; ESTRELA, L.P.¹; RODRIGUES, K.L.¹; PEREIRA, E.N.G.¹; DALIRY, A.¹; BARBOSA, H.S.¹; ADESSE, D.P.¹.

1. FIOCRUZ, RJ- BRA; 2. UFRJ, RJ- BRA.

E-mail: paloma.vieira.rj@gmail.com

Toxoplasmosis has a worldwide distribution and is caused by the intracellular parasite, *Toxoplasma gondii*. Acquired toxoplasmosis can lead to myositis, polymyositis and cardiac compromising. Although etiological treatments are available, none of them can fully prevent effects of the disease. Physical exercise (PE) has been described as a non-invasive treatment for many diseases such as diabetes, obesity, and has preventive action for others such as anxiety and cancer. We evaluated if PE could prevent the effects of acquired Toxoplasmosis. Swiss Webster male mice were trained for 8 weeks in an aerobic wheel for 5 days/week in 30-min sessions at 60% speed of exhaustion capacity. O₂ consumption (VO₂), maximum speed and time of exhaustion were evaluated during the protocol. After training period, mice were infected intragastrically with 10 cysts of ME49 *T. gondii* strain. Every 2 days after infection, grip strength was evaluated, and 10 days post infection animals were evaluated for aerobic performance and microcirculatory parameters of muscle and brain. As expected, PE increased maximum speed and time until exhaustion. *T. gondii* infection decreased the maximum speed (VO₂ at rest and maximum VO₂ during the exercise) in sedentary animals. Grip strength peak was also increased with exercise, and although it did not change with infection, the test time was reduced in sedentary-infected mice. Tibialis anterior muscle (TA) showed no weight change, but soleus muscle weight increased with training but not after infection. *T. gondii* infection decreased brown, but not epididymal and retroperitoneal white adipose tissue. Laser speckle imaging showed an extensive reduction on TA and brain blood flow in sedentary infected mice, which was prevented by PE. Rolling and adherent leukocytes was also drastically increased in sedentary infected mice, but not in exercised ones. Our results indicate that PE is a powerful tool for preventing muscle and cerebral damages of toxoplasmosis. **Supported by:** INOVA Fiocruz, IOC, CNPq, Edital PAPES VII, Faperj **Keywords:** Toxoplasmosis; Physical exercise; Muscle and cerebral damage.

HP-15 - PROFILING OF ANGIOGENIC FACTORS RELEASED BY TOXOPLASMA GONDII CYST-BEARING CORTICAL NEURONS

DE CASTRO, L.L.¹; DINIZ, L.²; SCHARFSTEIN, J.¹; ADESSE, D.P.³.

1. INSTITUTE OF BIOPHYSICS CARLOS CHAGAS FILHO | IBBCF - UFRJ, RJ- BRA; 2. INSTITUTO DE CIÊNCIAS BIOMÉDICAS | ICB - UFRJ, BRA; 3. INSTITUTO OSWALDO CRUZ | IOC - FIOCRUZ, RJ- BRA

E-mail: leonardoleal@biof.ufrj.br

Central Nervous System is a major target of *Toxoplasma gondii* infection *in vivo* and tissue cysts are found primarily in neurons. Recent evidence indicates that acquired toxoplasmosis in mice can lead to neuroinflammation and -degeneration as well as behavioral abnormalities. We have shown that *T. gondii* reduces cerebral blood flow and leads to a microvascular rarefaction, with increased Blood-Brain Barrier (BBB) permeability. Because *T. gondii* resides predominantly in neurons and the cellular interactions of the BBB with other neural cells are relevant for the maintenance of BBB integrity, we evaluated the pro- and anti-angiogenic factors released by *T. gondii* cyst-bearing neurons. Primary cultures of Swiss Webster mouse embryo cortical neurons were infected with tachyzoites of ME49 strain. We confirmed that our cultures were 100% neuron-enriched, as showed by β -III-tubulin and neurofilament immunostaining. Moreover, tissue cysts were found, as shown by CST-1 and DBA staining. At 7 dpi, culture supernatants were submitted to Proteome Profiler Angiogenesis Array Kit and we verified that VEGF, Fractalkine (CX3CL1), SDF-1 (CXCL12), NOV (CCN3), PDGF-AA and MMP-3 analytes were decreased in infected cultures. In order to further validate our findings in a relevant animal model of acquired toxoplasmosis, we analyzed the expression of genes of interest in the cortices of SW mice infected with *T. gondii* for 10 and 40 days, when neuroinflammation and BBB damage occur. ZO-1, a tight junction adaptor protein, was significantly increased in infected brains compared to controls, which corroborates our findings of microvascular dysfunction induced by infection. Fractalkine 3, a neuron-derived chemokine that signals to CX3CR1 in microglia, was reduced at 10 and 40 dpi by 0.71- and 0.68-fold, respectively. Our results point to a possible mechanism by which latent toxoplasmosis disturbs cerebral microvasculature, thus leading to neurological dysfunction. **Supported by:** Fiocruz, Programa INOVA Fiocruz, FAPERJ **Keywords:** cortical neurons; angiogenesis; toxoplasma gondii.

HP-16 - CHARACTERIZATION OF REGULATION MECHANISMS INVOLVED IN *LLCHIT1* EXPRESSION, A MIDGUT-SPECIFIC CHITINASE OF *LUZOMYIA LONGIPALPIS*, THE MAIN VECTOR OF AMERICAN VISCERAL LEISHMANIASIS.

RIBEIRO, A.C.P.S.¹; NEGREIRA, G.O.L.²; TRAUB-CSEKO, Y.M.¹; TEMPONE, A.J.¹.

1. FIOCRUZ, RJ- BRA; 2. INSTITUTE OF TROPICAL MEDICINE ANTWERP, ANTWERPEN - BEL
E-mail: ac_arolina@outlook.com

Our group identified a midgut-specific chitinase, named *Llchit1*, in *Lutzomyia longipalpis*, the main vector of American Visceral Leishmaniasis. *Llchit1* expression is induced upon blood feeding and the level of *Llchit1* mRNA appears to peak at 72h PBM, when occurs the degradation of the peritrophic matrix (PM), an important event in the establishment of *Leishmania* infection in the vector. Aiming to understand the mechanisms involved in this gene regulation, we previously started the characterization of its promoter. The *Llchit1* promoter sequencing analysis revealed the presence of putative transcriptional factors binding sites including GATA, Ecdysone receptor (ECR), and Ecdysone-induced proteins (EIP). Through luciferase assays, we observed that, in this promoter, the exonuclease deletion of GATA factor binding sites interrupts the reporter induction. Furthermore, a fragment of this promoter encompassing the region between -1200 e +68 (P1268) containing a corresponding sequence to the ECR and binding sites to EIP strongly induced the luciferase expression upon ecdysone stimulus. In this way, the focus of the present work was to characterize the role of GATA and ecdysone on *Llchit1* expression. In *L. longipalpis* LL-5 embryonic cells we silenced the GATA factor and, through qPCR, we observed a decrease in *Llchit1* expression. Furthermore, incubating these cells with ecdysone we observed an increase in *Llchit1* expression. Considering that the blood meal may raise the production of both ecdysone and GATA through the TOR pathway, we evaluated the role of the mTOR kinase on *Llchit1* expression. Although GATA and ecdysone act like transcriptional activators of *Llchit1*, mTOR knockdown did not affect *Llchit1* expression. Thereby, the present work characterizes a sandfly promoter that, due to tissue and stimulus-specific expression becomes a possible tool for the development of transgenic sandflies refractory to *Leishmania* infection. **Supported by:**FAPERJ. 26/200.578/2020 **Keywords:**Lutzomyia longipalpis; Chitinase gene promoter; gene regulation.

HP-17 - High-dimensional flow cytometry to evaluate CD4⁺ T cell heterogeneity during Chagas cardiomyopathy .

ALMEIDA, G.G.¹; RIMKUTE, I.²; DO VALE, I.N.P.C.¹; LIECHTI, T.²; HENRIQUES, P.M.¹; ROFFE, E.D.S.²; DE ARAUJO, F.F.¹; ROCHA, M.O.D.C.³; SANTOS, S.M.E.³; MARTINS-FILHO, O.A.¹; JANKOVIC, D.²; SHER, A.²; DE CARVALHO, A.T.¹; ROEDERER, M.²; ANTONELLI, L.R.D.V.¹.

1. INSTITUTO RENÉ RACHOU, MG - BRA; 2. NIH, WASHINGTON - USA; 3. UFMG - BRA
E-mail: vet.greg@gmail.com

Chagas' disease is a neglected tropical disease caused by the infection of *Trypanosoma cruzi* protozoa. Chronic chagasic cardiomyopathy (CCC) is a result of a persistent myocarditis during Chagas' disease, leading to heart failure and death in several cases. Although much of the immune response during Chagas' disease is known to rely on cytotoxic T cells, the role of CD4⁺ T cells is less understood. Also, a comprehensive analysis of activation markers on CD4⁺ T cells during different clinical forms was not performed so far. To explore a broad phenotyping of CD4⁺ T cell in different stages of Chagas' disease, we employed a high-dimensional flow cytometry panel combining 27 markers. Results were analyzed by both supervised and unsupervised strategies. Unsupervised strategy revealed several phenotypes of CD4⁺ T cells that are differentially represented in the different stages of the disease. CD69⁺CD4⁺ T cells are expanded during Chagas' disease in all memory compartments and among naïve T cells, although decreased in mild CCC compared to both patients without cardiac disease and established CCC. In addition, regulatory T cells expressing CD39 display a lower frequency in mild CCC compared to controls. The frequency of CD4⁺ T cells co-expressing granzyme B and perforin is increased in chagasic patients compared to controls and is more pronounced among patients with mild CCC compared to controls. Patients with mild CCC produce cytokines upon antigen stimulation and display higher frequencies of effector memory CD4⁺ T cells with multifunctional phenotype. Altogether, our results showed an imbalance of proinflammatory and regulatory responses of CD4⁺ T cells in the establishment of mild Chagas cardiomyopathy. Our results showed the heterogeneity of the phenotypes associated with disease worsening, providing insights on the pathology of the disease and potential markers to guide clinical decisions. **Supported by:**INCTV, Fiocruz, NIH **Keywords:**Chagas' disease;T cell;immunoresponse.

HP-18 - **ROLE OF P2X7 PURINERGIC MOLECULE IN RESPONSE TO *Trypanosoma cruzi* IN THE ACUTE PHASE OF CARDIAC INFECTION.**

XAVIER JUNIOR, J.T.¹; SANTOS, G.D.A.²; DO NASCIMENTO, R.S.²; FEVEREIRO, M.R.²; DE SÁ, K.S.G.¹; ZAMBONI, D.S.¹; DE CHAVES, M.L.M.B.²; LIMA, M.R.D.I.²; MOSIG, J.M.A.².

1. USP, RP - SP - BRA; 2. USP - SP - BRA

E-mail: txjoaquim@outlook.com

Chagas disease, caused by *Trypanosoma cruzi*, is an important cause of acute and chronic cardiomyopathy worldwide. *T. cruzi* is capable of infecting and replicating in several cell types, which can lead to cell death by necrosis, releasing danger signals such as ATP. The extracellular ATP can be recognized by the P2X7 ion channel, thus being able to act in cell activation. However, overstimulation of this receptor can lead to cell death by pyroptosis or necrosis. Considering that, in the heart of *T. cruzi*-infected C57BL/6 mice we observed an increase in the number of cells expressing P2X7, as well as in the level of *P2rx7* transcripts. In this work we evaluated the role of P2X7 in the heart during an acute phase of infection by *T. cruzi*. *P2rx7*^{-/-} infected mice showed no differences in body weight, blood parasitemia, heart weight and cellular infiltrate in the cardiac tissue when compared to C57BL/6 equally infected mice. However, an increase in *T.c. 18s* satellite DNA of *T. cruzi*, *IL-10* and *Arg1* mRNA transcripts, as well as a decrease in *IL-6*, *Nos2* and *IFN-γ* transcripts, was observed in the heart of the infected *P2rx7*^{-/-} mice. Furthermore, in the protein analysis, the heart of *T. cruzi*-infected *P2rx7*^{-/-} mice presented a reduction in the levels of IFN-γ and IL-1β and an increase in IL-10 and IL-12p70 when compared to C57BL/6 equally infected, indicating that in the cardiac tissue of *P2rx7*^{-/-} mice, the effector cells of the immune system, such as macrophages, monocytes and lymphocytes, are less responsive to *T. cruzi* infection, maybe by the less signaling via NLRP3 inflammasome. However, no significant differences were observed in lymphocyte activation or macrophage polarization to M1 and M2. The data obtained suggest that the P2X7 molecule plays a role in macrophage activation, and its absence may culminate in the possible worsening of the disease. **Supported by:**FAPESP 2018/25984-7 **Keywords:**P2X7;Trypanosoma cruzi;Macrophages.

PV-01 - **Identification and testing of candidates for the development of leishmaniasis Transmission Blocking Vaccines**

LEMOS-SILVA, T.¹; COELHO, F.S.¹; TELLERIA, E.L.¹; TEMPONE, A.J.¹; SACKS, D.L.²; TRAUB-CSEKO, Y.M.¹.

1. IOC, FIOCRUZ, LABORATÓRIO DE BIOLOGIA MOLECULAR DE PARASITAS E VETORES, RJ-BRA; 2. NIH, DEPARTMENT OF LABORATORY MEDICINE, USA

E-mail: ytraub@ioc.fiocruz.br

There has been a significant increase in the number of human cases of visceral leishmaniasis in recent years, which in Brazil is caused by *Leishmania infantum chagasi*, transmitted by *Lutzomyia longipalpi*. There is a demand for new approaches in the control of leishmaniasis due to limitations in the use of traditional methods, such as insecticide resistance, costs, etc. Among these alternatives is the use of transmission blocking vaccines (VBTs). The targets of VBTs are pathogen or insect proteins responsible for establishing the pathogen in the vector. We are working with several promising candidates in the parasite, both testing insect infection with mutant parasites (either obtained through collaborations or by creating mutants through CRISPR) and performing artificial infections in the presence of antibodies against proteins of interest. Among the targets with promising results are genes involved in sugar and amino acid metabolism which are upregulated at times of high parasite proliferation inside the insect. As vector targets, we are investing in the identification of molecules involved in vectorial capacity. We are currently performing experiments where these target proteins of the vector or parasite are being inoculated into mice and/or hamsters, which will then be infected with *Leishmania* and exposed to *L. longipalpis* bites. Levels of vector infection are being determined in relation to insects fed on mock-immunized control animals.

-TLS is developing part of this work at the NIH during a Graduate Sandwich Fellowship.

-ELT is presently at the Charles University, Czech Republic. **Supported by:** INCTEM, IOC-Fiocruz, INOVA-Fiocruz, FAPERJ **Keywords:**Leishmaniasis;transmission blocking vaccines;vector-parasite interaction.

PV-02 - Glucose Metabolism is Essential to Support Heme-Induced Epimastigotes Proliferation of *Trypanosoma cruzi*

VIEIRA, C.S.D.¹; AGUIAR, R.P.²; NOGUEIRA, N.P.D.A.¹; DOS SANTOS JUNIOR, G.C.¹; PAES, M.C.¹.

1. UERJ, RJ- BRA; 2. UFRJ, RJ - BRA.

E-mail: carolina.sd.vieira@gmail.com

Trypanosoma cruzi is the causative agent of Chagas disease, a neglected illness transmitted by triatomine insects during its blood meal. Heme, an abundant product of blood digestion, is a physiological oxidant molecule that triggers epimastigotes proliferation and transcriptionally regulates the expression of genes related to energy metabolism, mostly upregulating genes involved to glycolysis and aerobic fermentation process in epimastigotes. Once these metabolic pathways are essential to energy demand in this proliferative stage, this work aimed to investigate the glucose metabolism of epimastigotes cultured with heme through metabolomic and bioenergetic approaches. In response to heme, *T. cruzi* epimastigotes enhanced the D-glucose consumption after 24h and over the days, producing and secreting to its culture medium increased succinate levels, the main product of succinic fermentation of *T. cruzi*. High levels of succinate in supernatant promoted acidification of extracellular pH, being this effect inhibited by addition of glycolysis inhibitor, 2-deoxy-D-glucose (2-DG). Also, was shown that D-glucose supplementation increased epimastigotes proliferation exclusively in the presence of heme, and glycolysis inhibition impaired significantly the heme-induced proliferation. Regarding the mitochondrial physiology, heme only increased epimastigotes electron transport system-related O₂ consumption rate (OCR) compared to control. But, immediately after D-glucose addition, this OCR was impaired, similar a Crabtree effect (the glucose-mediated inhibition of mitochondrial respiration). Taken together, our data substantiate the idea previously pointed by transcriptional analysis, that heme signaling modulate the energy metabolism of *T. cruzi* epimastigotes promoting a metabolic adaptation towards aerobic fermentative of glucose, negatively regulating the oxidative metabolism in the presence of this sugar to sustain its fast proliferation.

Supported by:FAPERJ (E-26/203.213/2015, E-26/010.001706/2019), ARC (26/010.100.623/2018), CNPq (421676/2017), CAPES (Finance Code 001) **Keywords:**Trypanosoma cruzi;Heme;Energy metabolism.

PV-05 - Development of a computer visualization tool for the morphological classification of Free-Living Amoebas (FLAs)

ISSA, M.¹; BELLINI, N.K.²; OLIVEIRA, D.C.³; THIEMANN, O.H.¹.

1. INSTITUTO DE FÍSICA DE SÃO CARLOS, USP (IFSC/USP), BRA; 2. INSTITUTO BUTANTAN, BRA; 3. INSTITUTO FEDERAL GOIANO, RIO VERDE - BRA

E-mail: matheus.issa@usp.br

Free-Living Amoebas (FLAs) are unicellular eukaryotic microorganisms widely found throughout nature that belong to the Kingdom Protista. Although the majority of them lives freely in the environment, some genera are facultative opportunistic parasites, such as *Naegleria* spp. and *Acanthamoeba* spp., which normally cause infections associated with the Central Nervous System (CNS). Due to its high pathogenicity, these infirmities progress so fast that death ensues rapidly, such is the case of Primary Amebic Meningoencephalitis (PAM), caused by *N. fowleri*, as well as Granulomatous Amebic Encephalitis (GAE), caused by some *Acanthamoeba* spp.. Studies related to these microorganisms in Brazil are very scarce, without a precise mapping of its environmental distributions and an optimized method for morphological analysis, increasing the difficulty of efficiently processing samples. Therefore, we are currently elaborating a computer visualization program based on taxonomic keys from the classification guide Page (1988) to be available to the community for future taxonomic identification of FLA. We are collecting the morphological characteristics described in the guide and organizing them into a tabular dataset. This dataset is the input for the software development called Page's Visualization Tool (PVT), which is based on high-dimensional data visualization. Thus, we are proposing a computational tool able to contribute to the optimization of the analysis and improve future research on the morphological characterization of FLAs. **Supported by:**Agência financiadora: FAPESP; N° do processo: 2021/04364-3 **Keywords:**Free-Living Amoeba;PVT;Page.

PV-06 - **Updated phylogenomic reconstruction of amoebozoan testate amoebae**

LAHR, D.J.G.¹; DE SOUSA, A.L.P.¹; TICE, A.²; BLANDENIER, Q.²; RIBEIRO, G.M.¹; DUMACK, K.³; SINGER, D.⁴; LARA, E.⁵; SOUZA, M.B.G.E.¹; KLEITZ-SINGLETON, F.²; FRY, N.²; HENDERSON, T.²; BROWN, M.W.².

1. USP, BRA; 2. MSU, JACKSONVILLE - USA; 3. KOLN UNIVERSITY - GER; 4. ANGIERS UNIVERSITY-FRA; 5. REAL JARDIN BOTÁNICO DE MADRID, ESP

E-mail: dlahr@ib.usp.br

Shelled (testate) amoebae, unicellular eukaryotes capable of building a shell, appeared multiple times throughout the evolutionary history of life. The eukaryotic 'supergroup' Amoebozoa alone is home to two diverse testate amoebae groups: Arcellinida and Corycidia. Arcellinida is a diverse order of testate amoebae represented by lineages able to build hard shells and have left exceptionally preserved microfossils in the Neoproterozoic, making it a pivotal group to illuminate the early evolution of Eukaryotes. Corycidia is a recently established subclade of Amoebozoa represented by the lineages of testate amoebae that produce flexible shells and are distantly related to Arcellinida. Recent efforts of sampling diverse arcellinids and corycids in a phylogenomic framework have resolved the deep phylogenetic relationships within these groups and demonstrate that most major lineages of Arcellinida have already diversified as early as 730 million years ago. Also, a genus of flexible shell testate amoebae (*Microchlamys*) has found a home within Arcellinida. Despite the recent advance, most genera of Arcellinida and Corycidia remain unsampled, impairing further insights on testate amoebae evolution and classification. Here, we report the new phylogenomic reconstruction of the amoebozoan testate amoebae. We constructed a new phylogenomic dataset (224 genes) of arcellinids and corycids using PhyloFisher, a pipeline that enables the construction of datasets easy to share and update. We included taxa with available phylogenomic data and ten newly sampled taxa, including *Microcorycia* and *Spumochlamys*, which produce flexible shells and lack precise placement. Our reconstruction demonstrates that *Microcorycia* and *Spumochlamys* are members of Arcellinida, forming a monophyletic clade with *Microchlamys*. These results show that the diversity within Arcellinida is higher than previously thought, also implying a high diversity of eukaryotic life already in the Neoproterozoic. **Supported by:** 2019/22815-2 FAPESP **Keywords:** amoebae; phylogenetics; evolution.

PV-07 - **Is there a linker histone in *Toxoplasma gondii*?**

SEVERO, V.R.¹; SOUZA, R.O.O.²; VITORINO, F.N.D.L.³; DA CUNHA, J.P.C.³; ARRIZABALAGA, G.A.²; AVILA, A.R.¹; NARDELLI, S.C.¹.

1. INSTITUTO CARLOS CHAGAS - FIOCRUZ, PR - BRA; 2. INDIANA UNIVERSITY SCHOOL OF MEDICINE, USA; 3. INSTITUTO BUTANTAN, SP BRA.

E-mail: vanessarsevero@gmail.com

The chromatin is a natural barrier to all DNA-dependent processes such as transcription. The chromatin compaction levels are regulated mainly by histones and their post-translational modifications (PTMs) that may act by facilitating or preventing access to DNA. *Toxoplasma gondii* has the four canonical histones (H2A, H2B, H3 and H4), but the fifth histone (H1 or linker histone) has not been identified. In other eukaryotes, H1 links nucleosomes, and its absence could interfere with chromatin condensation. We have identified a small and basic protein in *Toxoplasma*, similar to H1-like of bacteria, which we have named TgH1-like. By immunofluorescence, we were able to locate TgH1-like exclusively in the nucleus of tachyzoite. In addition, using Ultrastructural expansion microscopy (U-ExM) it was possible to observe that TgH1-like accumulates in the nucleolus and nuclear periphery. Performing standard histone extraction protocols, we observed TgH1-like in the same fraction as the histone H4, confirmed by immunoprecipitation assays that also detected histones H3, H2A1, H2Bb and H2B.Z. Knockout parasites showed 22% of vacuoles in asynchronous replication and endopolygeny division. TgH1-like has two post-translational modifications (PTM) sites already described. Parasites mutated at the phosphorylation site (S43A) showed asynchronous division and early karyokinesis events. Mutants for both phosphorylation and ubiquitylation sites (S43A_K45R) showed the same phenotype pattern related to cell division, analyzed using U-ExM. Next, we investigate the nuclei architecture by transmission electron microscopy, where *Δtgh1-like* showed a decrease in peripheral chromatin compaction. Our results demonstrate that TgH1-like has histone characteristics, playing a role in chromatin compaction and coordination of cell division. To our knowledge, this would be the first linker histone identified in Apicomplexan parasites and will provide new insights into the chromatin dynamics in *Toxoplasma*. **Supported by:** CAPES, CNPq, Fundação Araucária, Inova-FIOCRUZ **Keywords:** *Toxoplasma gondii*; Chromatin; Linker Histone.

PV-08 - The genetic knockout of pyrroline-5-carboxylate synthetase and pyrroline-5-carboxylate reductase reveals a shortcut in the glutamate-proline pathway in *Leishmania braziliensis*

CAMPAGNARO, G.D.; CRUZ, A.K..
FACULDADE DE MEDICINA RIBEIRÃO PRETO, USP, RIBEIRÃO PRETO - SP - BRA.
E-mail: campagnarogd@gmail.com

Proline (Pro) is a valuable energy source as its catabolism originates substrates for the tricarboxylic acid cycle. In trypanosomatids, Pro is obtained by uptake from the environment or by *de novo* biosynthesis from glutamate (Glu) via the activity of pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR). *Trypanosoma brucei* is auxotrophic for Pro, whereas *T. cruzi* is able of both Pro uptake and biosynthesis, which unables it to use Glu as sole Carbon source. Starved (4h in PBS) procyclic promastigotes (PRO) of *Leishmania braziliensis*, *L. amazonensis* and *L. major* recovered mitochondrial function with various Carbon sources, including Glu, which suggested a functional *de novo* Pro biosynthesis pathway. Thus, we investigated the functionality of P5CS and P5CR in Pro biosynthesis in *L. braziliensis*. The knockout (KO) of *LbrP5CS* and *LbrP5CR*, however, did not affect their ability to efficiently use Glu as energy source. Parasites KO for Pro dehydrogenase, the first catabolic enzyme of Pro, were able to utilize Glu, but not Pro, as sole energy source, indicating that energy generation from Glu follows a separate route from the Pro pathway. Also, parasites starved in PBS and recovered in Glu did not restore their intracellular free Pro levels, indicating that Pro biosynthesis is actually not functional in *L. braziliensis*. Yet, mRNAs encoding for *LbrP5CS* and *LbrP5CR* are found in PRO, metacyclic (META) and axenic amastigotes (AMA) forms, but displays highest levels in META. Moreover, myc-tagged *LbrP5CS* and *LbrP5CR* showed a variable pattern of expression across different biological forms: whereas *LbrP5CS* expression is reduced in META and absent in AMA, *LbrP5CR* expression was detected in all three life cycle stages with highest levels in META. Our results indicate that Pro biosynthesis is not functional in *L. braziliensis* and may suggest that *LbrP5CS* and *LbrP5CR* have moonlight functions.

Supported by:FAPESP 2020/02372-6 **Keywords:**Leishmania braziliensis;Glutamate;Proline.

PV-09 - IDENTIFICATION OF GENES INVOLVED IN SYNTHESIS OF BIOACTIVE PHOSPHOLIPIDS AND MOLECULAR ANALYSIS OF A PLATELET ACTIVATING FACTOR ACETYLHYDROLASE (PAF-AH) OF TRYPANOSOMA CRUZI

COELHO, F.S.; MOREIRA, I.C.D.F.; TORRES, P.M.; LAZOSKI, C.V.D.S.; VIEIRA, D.P.; LOPES, A.H.D.C.S..
UFRJ, RJ- BRA.
E-mail: lipesc1@micro.ufrj.br

Lysophosphatidylcholine (LPC) is a phospholipid that presents ubiquitous distribution among eukaryotes, including protozoan parasites, such as *Trypanosoma cruzi*. LPC is obtained from phosphatidylcholine (PC) by the action of a cholesterol acetyltransferase or one of the enzymes of the phospholipase A₂ (PLA₂) family, which includes the platelet-activating factor acetylhydrolase (PAF-AH). LPC acts in various biological and pathophysiological processes, such as inflammatory diseases, especially in atherosclerosis, as well as in innate immunity. Also, *T. cruzi* synthesizes several LPC species, one of which (C-18:1 LPC) behaves similarly to PAF, aggregating platelets and triggering cell differentiation and infectivity of *T. cruzi* towards mouse macrophages. The present study aimed to identify the genes involved in the synthesis of LPC in *T. cruzi* and characterize proteins that participate in the biosynthesis. Enzymes related to the synthesis of LPC were selected from the KEGG pathways database, and a search for their gene sequences was performed on genomic data bank TritypDB. To confirm the existence of these genes, polymerase chain reactions (PCR) were performed, and the amplified regions were sequenced. Ten genes that code for enzymes of the LPC biosynthetic pathways in *T. cruzi* were amplified using PCR, including a phospholipase A₂ (PLA₂), related to cell invasion events in several parasitological protozoa. Then, we constructed a three-dimensional model of that PLA₂, which in fact is a PAF-AH. The PAF-AH activity was detected in the cell extract of *T. cruzi* and a specific PAF-AH inhibitor abolished the activity. In summary, this is the first study to shed light to the molecular structure and function of a PAF-AH in *T. cruzi*. Additional studies will be carried out to determine the role of PAF-AH from *T. cruzi*, as well as its inhibitors, in the invasion of the parasite in mammalian cells. **Supported by:**CNPq, CAPES, FAPERJ and INCT-EM **Keywords:**phospholipids;LPC biosynthesis;phospholipase.

PV-10- Does *Leishmania* ATM play a role in DNA repair and genome plasticity?

BLACK, J.A.; DA SILVA, G.L.A.; TOSI, L.R.O..
USP, RIBEIRAO PRETO - SP - BRA.
E-mail: jennifer.stortz@glasgow.ac.uk

DNA damage arises from a myriad of sources leading to a broad spectrum of lesion types including Double Strand Breaks (DSBs). DSBs are considered the most deleterious, and if persistent, can lead to chromosome breakage, genome instability, tumorigenesis, and cell death. In response, eukaryotic cells have evolved a network of pathways known as the DNA Damage Response (DDR). At the forefront of the cellular response to DSBs, is the kinase ATM (Ataxia-telangiectasia Mutated). ATM is recruited to DSBs via the actions of the MRN complex (Mre11-Rad50-Nbs1). Together, these events lead to a phosphorylation cascade promoting the recruitment of repair factors and the establishment of a repair permissive environment. Paradoxically, DSBs can also drive genome diversity, particularly if the DSB is 'controlled', for instance during V(D)J recombination, which underpins immune system diversity. In *Leishmania*, the DDR is less understood. *Leishmania* ATM has been shown to act in response to oxidative stress, but the wider functionalities of this kinase are unknown. Nor is it clear if ATM has functions pertaining to the remarkable plasticity of the *Leishmania* genome; studies implicate DNA damage and DDR activities as drivers of this process. Here, using CRISPR/Cas9 we deleted ATM in *L. major* promastigotes. We found loss of ATM moderately affects growth *in vitro*, but significantly sensitises cells to DSB-inducing genotoxins. Additionally, by sequence analysis, we found evidence of wider genomic alterations (ploidy) and increased instability in ATM's absence suggesting this kinase plays a key role in maintaining *Leishmania* genome stability.

Supported by:FAPESP 2020/01883-7

Keywords:Leishmania;ATM;DNA repair.

PV-11 - Dynamics of TcRab5 isoforms in *Trypanosoma cruzi* epimastigotes

AMARAL, A.A.; ALCANTARA, C.D.L.; DA CUNHA-E-SILVA, N.L..
UFRJ, RJ- BRA.
E-mail: alexia.achilles@gmail.com

Rab5 is a small GTPase that regulates the budding of vesicles and directs the endocytosed cargo from the plasma membrane to the early endosomes. Its function and dynamics are well established in mammalian cells, but in *T. cruzi*, which has a peculiar and poorly elucidated endocytic pathway, there is no information but the annotation of two isoforms, TcRab5a and TcRab5b, at the TriTrypDB database. Thus, our goal is to characterize their dynamics and functions. For this, epimastigotes of the Dm28c strain were genetically modified using the CRISPR-cas9 technique. Two populations of mutants were generated by the insertion of the mNeonGreen (mNG) fluorescent protein and the myc peptide genes into the N-terminal region of TcRab5a and TcRab5b. After selection of the transfected parasites, observation of live cells expressing mNG:TcRab5a showed its localization concentrated close to the bottom of the flagellar pocket. In mNG:TcRab5b mutants, signal was found in compartments at the perinuclear region. Endocytosis assays using fluorophore labelled transferrin (Tf), albumin (BSA), ferritin (Ferr) and hemoglobin (Hb), showed that TcRab5a and TcRab5b participate in endocytosis of Tf and BSA but not Ferr and Hb. More than one attempt to obtain knockout mutants for TcRab5a failed, suggesting that these proteins are essential for epimastigotes. On the other hand, we managed to generate TcRab5b KO mutants, and they were unable to endocytose transferrin. Transmission electron microscopy of these cells showed increased number of lipid droplets and lipid inclusions in the lysosome-like organelles and mitochondrial alterations. To evaluate the non-redundant functions of the TcRab5 isoforms, endogenous labeling of the two isoforms in the same cell are under analyses. **Keywords:**Trypanosoma cruzi;TcRab5;Early endosomes.

TB-01 - EXPANDING THE ARSENAL AGAINST LEISHMANIASIS: NOVEL TAMOXIFEN/CLEMASTINE CHIMERA AS POTENTIAL ANTILEISHMANIALS

AGOSTINO, V.D.S.¹; BUERDSELL, M.L.¹; ULIANA, S.R.B.²; DENNY, P.W.¹; COELHO, A.C.³; STEEL, P.¹.
1. DURHAM UNIVERSITY, GBR; 2. ICB-USP, SP - BRA; 3. UNICAMP, SP - BRA.
E-mail: victor.agostino@gmail.com

Collectively, the shortcomings of current treatments make the discovery of new alternative treatments an urgent matter. In this context, tamoxifen, a selective oestrogen receptor modulator (SERM) and known anti-breast cancer drug, has been identified as a potent anti-leishmanial, displaying significant activity against both *in vitro* and *in vivo* infection models. Similarly, clemastine fumarate, an over-the-counter first-generation antihistamine drug, has submicromolar activity against intramacrophage amastigotes of *Leishmania amazonensis* as well as equivalent activity to glucantime in a mouse model infection. Interestingly, both molecules display similar chemical features and have also been proposed to target the same enzyme: the inositol phosphorylceramide synthase (IPCS), an essential enzyme to the parasite which is part of the sphingolipids biosynthetic pathway. However, previous studies have shown that clemastine and tamoxifen have multiple intracellular targets. These could lead to toxicity and lack of selectivity, and are yet to be explored. To investigate this in greater detail – and develop more effective selective compounds –, we have built a library of tamoxifen/clemastine hybrids based on the common chemical features shared by these molecules. Following initial screening against *L. major* and *L. amazonensis* promastigotes, as well as cytotoxicity assays using HepG2 cells, several hybrids have shown submicromolar activity and no toxicity against human cells. This showed an improvement from parental molecules, which are toxic against HepG2 cells. The most active compounds ($EC_{50} < 2 \mu\text{M}$ against both species of promastigotes together with $SI > 10$ versus HepG2) are currently being tested against intracellular amastigotes. This presentation will describe these studies together with the ongoing experiments designed to explore the mode of action and molecular target(s) of these chimeric compounds. **Supported by:**GCRF-CDT (UK)
Keywords:Drug discovery;Treatment;Medicinal chemistry.

TB-02 - Effect of apigenin *in vitro* and *in vivo* in *Leishmania infantum*.

EMILIANO, Y.S.D.S.; AMARAL, E.E.A.. FIOCRUZ, RJ- BRA.
E-mail: yagoemiliano92@gmail.com

Leishmaniasis, a neglected tropical disease, has been reported in 98 countries and affects 12 million people around the world. *Leishmania infantum* is responsible for the most severe clinical manifestation, visceral leishmaniasis (VL). Current treatment for leishmaniasis is based on pentavalent antimony, amphotericin B and miltefosine, but these treatments have several collateral effects, resistance and therapeutic failure. Apigenin is a flavonoid present in common fruits and vegetables, and it is believed to have several biological functions. In the present study, apigenin demonstrated concentration-dependent inhibition for 72 hours on the *L. infantum* promastigote, reaching an inhibition of 94.6% at its highest concentration used (96 μM), demonstrating an IC_{50} value of 29.9 μM . Its effect was also evaluated in *L. infantum*-infected murine peritoneal macrophages, which were incubated for 72 hours with different concentrations of apigenin (0-24 μM). A concentration-dependent inhibition was observed, reaching 85% and 88% inhibition at 12 μM and 24 μM , respectively. The IC_{50} value of intracellular amastigotes was 2.35 μM . Concerning the murine model of visceral leishmaniasis, *in vivo*, two types of treatments were used, long-term and short-term. BALB/c mice were infected via the peritoneum with *L. infantum* promastigotes (1×10^9 cells/ml) and treated with vehicle (control group), apigenin (2 mg/kg/day) or glucantime (100 mg and 200 mg/kg/day). Apigenin demonstrated a reduction of 94% and 99.7% in the parasite load in the liver in the long-term treatment model and in the short-term treatment model, respectively. Furthermore, toxicity was not demonstrated in the Balb/c mice. Taken together, these results suggest that apigenin may be a more efficient and less toxic molecule for the treatment of leishmaniasis.

Supported by:CAPES, CNPq e FAPERJ **Keywords:**Apigenin;Treatment;Visceral Leishmaniasis.

TB-03 - Effect of MMS as an alternative therapy for the treatment of leishmaniasis

OZÓRIO, G.G.; EMILIANO, Y.S.D.S.; AMARAL, E.E.A..
FIOCRUZ, RJ- BRA.
E-mail: gabriella.ozorio@gmail.com

Leishmaniasis is a severely neglected tropical disease caused by different species of *Leishmania*. The treatment of leishmaniasis involves a limited drug arsenal and is associated with problems such as therapeutic failure, high toxicity, high costs and the emergence of resistant cases in different parts of the world. Among the search for new alternatives to combat these diseases, drug repurposing stands out. In this scenario, we highlight MMS, a drug currently used in the clinic for the treatment of another disease whose activity was described in *Trypanosoma cruzi*. This study evaluated the effect of MMS *in vitro* and *in vivo* and its possible mechanism of action. Promastigotes of *L. infantum* and *L. amazonensis* were treated with different concentrations of MMS (18,75 μ M – 1200 μ M) for 24 and 72 hours and demonstrated an inhibition of cellular viability in a concentration-dependent manner. To investigate a possible mechanism of action, promastigotes treated with MMS showed an increase in ROS levels in both species at 24 and 72 hours, and the preincubation with antioxidant molecules were not capable of protecting cells from the inhibition promoted by MMS. Against the intracellular amastigote, MMS (4 – 280 μ M) demonstrated an inhibition of the infection index in a concentration-dependent manner after 72 hours of treatment and proved to be not toxic in the macrophage toxicity assay. In the *in vivo* study, BALB/c mice were infected with *L. infantum* promastigotes for 7 days and treated with 1, 5, 3 or 6 mg/kg/day of MMS, 100 mg/kg/day of glucantime or the vehicle. MMS was able to decrease the parasite load in the liver of infected mice compared to the control and Glucantime groups. Serological toxicology markers were evaluated, and no significant changes were observed, suggesting the absence of liver and kidney toxicity. Taken together, these results suggest that MMS is a good possibility for leishmaniasis chemotherapy. **Supported by:** CNPq; CAPES; Faperj; IOC/Fiocruz **Keywords:** Leishmaniasis; drug repurposing; Mechanism of action.

TB-04 - 2'-hydroxyflavanone effects against wild type and antimony-resistant *L. infantum*, toxicity and pharmacokinetics

DE OLIVEIRA, L.G.F.; OZÓRIO, G.G.; PAES, T.F.; DOS SANTOS, J.P.R.; DA SILVA, J.D.P.A.;
MACHADO, M.P.; SILVA, A.C.A.; DA SILVEIRA, G.P.E.; PEREIRA, H.M.; PINTO, D.P.; DA CUNHA
JUNIOR, E.F.; AMARAL, E.E.A..
FUNDAÇÃO OSWALDO CRUZ, RJ- BRA.
E-mail: luizagervazoni@ig.com.br

Leishmaniasis deserves attention due to the wide variety of associated clinical manifestations and their high annual incidence. Although there are many drugs available as alternatives for leishmaniasis treatment, they remain mostly ineffective, toxic, expensive and longstanding, in addition to the resistance cases reported over the years. Among the search for new alternatives to treat this disease is the search for new medicines from natural sources. Studies have demonstrated the leishmanicidal effect of flavonoids, a class of plants secondary metabolites that have antioxidant and anti-inflammatory activity described in several diseases. 2'-Hydroxyflavanone (2HF) is a flavanone currently known for its activity in tumor cells. Previous results demonstrated 2HF *in vitro* and *in vivo* activity against wild-type and antimony-resistant *L. amazonensis*. Due to the promising effects of 2HF and the drug development process, this study evaluated 2HF against wild-type and antimony-resistant *L. infantum* and its toxicological and pharmacokinetic parameters. 2HF was able to inhibit *L. infantum* wild-type promastigotes and amastigotes in a concentration-dependent manner after 72 h as well as antimony-resistant promastigotes. In acute and subacute preclinical toxicity, 2HF proved to be safe, showing no mortality or changes in weight gain and water/food consumption. An LC–MS/MS selective and sensitive analytical method was developed for 2HF detection and quantification. This method was validated according to ANVISA guidelines. To determine the pharmacokinetic profile, BALB/c mice received a single oral dose of 2HF, and blood was collected at different times. 2HF pharmacokinetic parameters were calculated using a noncompartmental mathematical model. 2HF demonstrated a T_{max} of 5 min and a T_{1/2} of 97.52 min with a C_{max} of 2HF of 185.86 ng/mL. Taken together these results indicate 2HF promising effects against *L. infantum* and point out further *in vivo* studies with different formulations. **Supported by:** FAPERJ; CNPq; CAPES; PAPES; IOC/FIOCRUZ **Keywords:** Leishmania; Flavonoid; Pharmacokinetics.

TB-05 - Innovative microfluidic device to synthesize amphotericin B-loaded polymeric nanoparticles for cutaneous leishmaniasis treatment

MOTA, G.C.N.¹; SUZUKI, É.Y.¹; BERGMANN, B.R.¹; BALBINO, T.A.²; SOUSA-BATISTA, A.D.J.².

1. INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO – UFRJ, BRA; 2. PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA DA NANOTECNOLOGIA – PENT / COPPE – UFRJ, RJ- BRA

E-mail: giovanna@biof.ufrj.br

Cutaneous leishmaniasis (CL) treatment is based on multiple injections with drugs that produce inadmissible systemic toxicity. Therefore, effective local treatment is highly desirable. Previously, we demonstrated that polymeric nanoparticles loading amphotericin B (NP-AmB) as produced by conventional nanoprecipitation have shown promising efficacy by intralesional treatment. However, that is not a reproducible and industrially scalable method. Thus, we proposed to develop NP-AmB by microfluidic-assisted nanoprecipitation that employs precise mixing conditions, and is of easy scale-up. To reduce costs, we manufactured clean-room free and reproducible 3D devices, unlike the commercially available devices. First, NPs were produced by varying their TFR (Total Flow Rate) and FRR (Flow Rate Ratio). The optimized condition for lower size distribution (< 0.3) was FRR= 0.225 and TFR= 2000 $\mu\text{L}/\text{min}$. That yielded blank NPs with 247 nm and PDI = 0.076, and NPs-AmB with 148 nm and PDI= 0.253. Mouse macrophages were treated with NP, NP-AmB or free AmB for cytotoxicity studies. Blank NP CC50 was $> 100 \mu\text{g}/\text{mL}$, whereas NP-AmB and free AmB CC50 were 30 $\mu\text{g}/\text{mL}$ and 2 $\mu\text{g}/\text{mL}$, respectively, demonstrating a 15-fold reduced toxicity of AmB after nanoencapsulation. These results indicate the potential use of NP-AmB for a safer local treatment against CL with AmB employing an industrially scalable manufacturing process. **Supported by:**Vale **Keywords:**AmphotericinB;Leishmaniasis;Nanoparticles.

TB-06 - CHALCONE NANOCRYSTALS FOR ORAL TREATMENT OF CUTANEOUS LEISHMANIASIS

BORSODI, M.P.G.¹; LIMA, W.P.²; MENEZES, J.C.V.¹; DE OLIVEIRA, D.E.¹; COSTA, N.D.A.¹; CABRAL, L.M.¹; DA SILVA, A.J.M.¹; STEEL, P.³; SOUSA-BATISTA, A.D.J.¹; BERGMANN, B.R.¹.

1. UFRJ, RJ- BRA; 2. UNIGRANRIO, RJ- BRA; 3. DURHAM UNIVERSITY, DURHAM – ENG

E-mail: mariapaula@biof.ufrj.br

Treatment of cutaneous leishmaniasis (CL) needs safer and more active drugs especially for non-invasive use. We have synthesized a synthetic trimethoxylated chalcone (NAT22) showing high activity ($\text{IC}_{50} = 0.5 \mu\text{M}$, $\text{SI} = 13$) against *Leishmania amazonensis*. However, NAT22 is poorly soluble in water due to its large crystals ($\sim 200 \mu\text{m}$), hampering its oral use. Here, we aimed to reduce the size of NAT22 crystals by nanomilling in order to improve activity and oral efficacy. For that, NAT22 crystals were submitted to dry milling followed by wet milling yielding nanocrystals (nanoNAT22). Promastigotes were incubated with different concentrations of NAT22 or nanoNAT22 for 72 h, when cell viability evaluated by resazurin. For anti-amastigote activity, bone marrow-derived macrophages were infected with promastigotes (1:10) for 24 h at 37 °C and then treated for further 48 h with nanoNAT22 or NAT22 and cytotoxicity was assessed by LDH release in supernatants. NanoNAT22 in aqueous medium proved to be 18-times more active than NAT22 in the same medium. As expected, they were equally active when pre-solubilized in DMSO for both promastigotes ($\text{IC}_{50} = 0.7 \mu\text{M}$) and intracellular amastigotes ($\text{IC}_{50} = 0.5 \mu\text{M}$). The selectivity index was higher for nanoNAT22 ($\text{SI} = 20$) than NAT22 ($\text{SI} = 13$). For *in vivo* studies, BALB/c mice were infected in the ear with *L. amazonensis*-GFP. Seven days later, were orally treated daily (40 mg/kg) with nanoNAT22 or NAT22 for 5 weeks in propylene glycol. Controls received intralesional Glucantime in PBS (1.5 mg/kg, 1x/week). Lesion sizes were measured 2x/week. On day 52 of infection, ear parasites were measured by fluorimetric and limiting dilution assays. Oral nanoNAT22 was more effective than NAT22, reducing parasitic load by 52% and 30%, respectively, comparable to injectable Glucantime. In conclusion, nanomilling significantly improves solubility, anti-parasitic selectivity, oral bioavailability and efficacy of NAT22 chalcone against CL. **Supported by:** VALE DO RIO DOCE **Keywords:** CHALCONE; NANOCRYSTALS; LEISHMANIA.

TB-07 - MMV Pandemic box in vitro screening identifies new compounds highly active against the tachyzoite stage of *Toxoplasma gondii*

DOS SANTOS, M.; VAZ, G.H.D.S.; DE SOUZA, G.C.A.; VITOR, R.W.D.A.; MARTINS-DUARTE, É.D.S..
UNIVERSIDADE FEDERAL DE MINAS GERAIS (UFMG), BH- MG - BRA.
E-mail: mikesnts@outlook.com

Toxoplasmosis is an asymptomatic infection for most of the population, however, immunocompromised patients may evolve with ocular disorders and/or Central Nervous System involvement. Despite its importance, current chemotherapies are exclusively for the acute phase of the disease. In addition, the treatment presents therapeutic failures, teratogenicity, and frequent side effects, leading to low adherence. Thus, the development of new therapies that circumvent these problems has been a challenge. The Medicines for Malaria Venture (MMV), aiming to foster the development of new therapies for diseases with pandemic potential, has created the Pandemic Response Box. The box contains 400 compounds, which are either already in clinical use or development. To evaluate their activity against *T. gondii*, the 400 compounds were screened by treating tachyzoite infected cells for 7 days with 1 μ M. Of the 400 compounds, 24 were able to inhibit proliferation by more than 80%. IC₅₀ determination showed that 8 drugs/compounds inhibit *T. gondii* tachyzoite proliferation in concentrations lower than 100 nM and with selective index against the host cell. Further analysis showed that the different compounds have different modes of action, including both an immediate effect at the first replication cycle (48h of treatment) and a delayed effect (> 48h of treatment). The compounds that showed an immediate effect against the parasite replication had its mode of action studied by transmission electron microscopy (TEM) after 48h of treatment. TEM analysis confirmed that treatment with these compounds drastically affected tachyzoite morphology, inhibiting both its proliferation and leading to cell death. Thus in this study, we show new active drugs with potential for the future treatment of the acute stage of toxoplasmosis. **Supported by:**FAPEMIG, CNPq e PRPq-UFMG **Keywords:**Toxoplasmosis;Drug repoditioning;New therapies.