HP1 - BIOLUMINESCENCE IMAGING OF CHRONIC TRYPANOSOMA CRUZI INFECTIONS REVEALS TISSUE-SPECIFIC PARASITE DYNAMICS AND HEART DISEASE IN THE ABSENCE OF LOCALLY PERSISTENT INFECTION

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Chronic Trypanosoma cruzi infections lead to cardiomyopathy in 20-30% of cases. A causal link between cardiac infection and pathology has been difficult to establish because of a lack of robust methods to detect scarce, focally distributed parasites within tissues. We developed a highly sensitive bioluminescence imaging system based on T. cruzi strain CL Brener expressing a novel luciferase that emits tissue-penetrating orange-red light. This enabled long-term serial evaluation of parasite burdens in individual mice with an in vivo limit of detection of significantly less than 1000 parasites. Parasite distributions during chronic infections in BALB/c mice were highly focal and spatiotemporally dynamic, but did not localize to the heart. End-point ex vivo bioluminescence imaging allowed tissue-specific quantification of parasite loads with minimal sampling bias. During chronic infections, the gastro-intestinal tract, specifically the colon and stomach, was the only site where T. cruzi infection was consistently observed. Quantitative PCR-inferred parasite loads correlated with ex vivo bioluminescence and confirmed the gut as the parasite reservoir. Chronically infected mice developed myocarditis and cardiac fibrosis, despite the absence of locally-persistent parasites. In summary, our data identify the gut as a permissive niche for long-term T. cruzi infection and show that canonical features of Chagas disease can occur without continual myocardium-specific infection. **Supported by:**Wellcome trust and CNPq

HP2 - MELATONIN CONTROLS THE SUBVERSION OF MIRNA PROFILE OF MACROPHAGES BY *LEISHMANIA (LEISHMANIA) AMAZONENSIS* INFECTION

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Melatonin is the dark hormone produced by pineal gland. However, it can acts as an anti-inflammatory and antioxidant and it can be produced by other cells/tissues. Melatonin modulates the expression of genes involved in inflammatory response. We showed that melatonin is able to reduce the L. (L.) amazonensis infection in vitro regulating the expression of genes involved in NO and polyamines production. The miRNAs are modulators of inflammatory mechanisms during immune response by post-transcriptional regulation of genes involved in the activation of macrophages. In this communication, we show the role of melatonin in regulating the miRNA profile of macrophages during L. (L.) amazonensis infection. We analyzed the miRNA prolife of total RNA from BALB/c mice Bone Marrow-Derived macrophages (BMDMs), treated or not with melatonin (30nM) for 4h before L. (L.) amazonensis inoculation. At 4h and 24h post-infection, we verified that melatonin promote a reduction in the infectivity (60%) compared to non-treated or vehicle-treated macrophages, but It did not modulate the pro- and anti-inflammatory cytokines production. We detected an up-regulation of 20% and 28% of the 84 miRNAs analyzed in non-treated and vehicle-treated BMDMs infected with L. amazonensis WT, respectively. The melatonin treatment promotes the reduction in 50% of the numbers of up-regulated miRNAs (11,9%) after 4 and 24h of infection being the period more pronounced. In silico analyzis showed that the mRNA targets for the miRNAs included pathways involved in phagolysosome maturation (LAMP-1), regulation of iNOS, cationic amino acidic transporter (CAT) and Tumor-necrose factor receptor (TNFR). We concluded that melatonin modulate the miRNAs expression in macrophages infected by L. amazonensis infection to reduce infectivity. **Supported by:**FAPESP and CNPq

HP3 - MULTIPLE INNATE IMMUNE RECEPTORS PARTICIPATE OF THE AUTOPHAGY-MEDIATED RESTRICTION OF *TRYPANOSOMA CRUZI* REPLICATION IN MACROPHAGES

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The control of intracellular parasite replication is dependent upon an effective immune response that is initiated by pattern recognition receptors (PRRs). Some PRRs trigger autophagy, a process that target intracellular pathogens for degradation. The induction of autophagy in macrophages infected with *Trypanosoma cruzi* has never been reported. Here, we investigate the induction of autophagy in response to *T. cruzi* infection and assessed the signaling pathways involved in the autophagy induction. Bone marrow-derived macrophages obtained from C57BL/6 (WT) mice were transduced with lentivirus encoding GFP-LC3 and infected with T. cruzi. We found that infected macrophages presented higher number of LC3 puncta when compared with uninfected cells. In addition, we found that LC3 colocalizes with the anterior region of the amastigote forms of *T. cruzi*. To address the role of autophagy in *T. cruzi* infection we inhibited autophagy by using shRNA to mouse Atg5. We found that Atg5 silenced macrophages display a higher numbers of amastigotes as compared to macrophages transduced with control shRNA. By using immunofluorescence, we found that Atg5 facilitated the colocalization of *T. cruzi* with LAMP1 positive vesicles surrounding the parasites, thus suggesting an autophagy mediated targeting of the parasites for degradation. Finally, we investigated the intracellular signaling pathways required for autophagy induction in response to *T. cruzi*. By using macrophages from several gene deficient mice we found that macrophages from Nod1-/-, Rip2-/-, ASC-/-, Caspase-1-/-, MyD88-/- and TLR3/7/9-/-, but not Nod2 and NLRC4, were defective to trigger autophagy in response to infection. This was demonstrated by the reduced formation of LC3 puncta in macrophage cytoplasm and diminished colocalization of LC3 with amastigotes of *T. cruzi*. All together, our data indicate that the recognition of *T. cruzi* by PRRs lead to autophagy-mediated restriction of *T. cruzi* replication in macrophages. **Supported by:**FAPESP

HP4 - STUDY OF SPLENIC AND PERIPHERAL BLOOD LYMPHOCYTES FROM BALB/C MICE IMMUNIZED WITH IRRADIATED TACHYZOITES OF *TOXOPLASMA GONDII*

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Toxoplasmosis is a major public health problem, infecting one-third of the world human beings, and leads to abortion in domestic animals. A vaccine strategy would be an ideal tool for improving disease control. In this study, we evaluate the responsive immune cells from blood and spleen in immunized BALB/c mice (3 doses) by intraperitoneally (i.p.) and orally (v.o.) with irradiated 10^7 tachyzoites of RH strain of *T. gondii*. T and B cell populations were analyzed by flow cytometry and antibody detection in sera by ELISA. Protection was determined after challenge with 10 cysts of strain ME-49 or VEG orally by *T.gondii* DNA quantification by Real-time PCR in brains after 90 days. The i.p. or v.o immunized mice showed an increase in the population of B cells (CD19+) in the spleen and blood ($p < 0.05$), but expression CD69+ in B cells were increased only in the spleen of ip mice ($p<0.05$). Plasma cells were increased in spleen of ip mice ($p < 0.05$) and these cells were increased only in blood of vo mice ($p<0.05$). Specific IgG antibodies in serum was present only in i.p. mice, earlier and after 90 days of the last dose ($p < 0.05$). CD4+ and CD8+ cells were increased in spleen and blood in ip mice, but only CD8+ cells were increased in spleen of vo mice ($p<0.05$). Both i.p. and v.o. showed a decrease ($p < 0.05$) of expression of CD45RB and an increase of CD69 in splenic CD4+ T lymphocytes. The decreased of expression of CD45RB by CD8+ cells from blood ($p < 0.05$) while there was an increased expression of CD69 by splenic CD8+ T cells in i.p. mice. Both i.p. and v.o mice were protected against a challenge with ME-49 or VEG cysts, as detected in brains($p < 0.01$). We found that our model induces a cell mediated immune response by B and T cells and immune protection after challenge, which is similar to natural infection and a model for vaccine-induced immune response for testing new immunogens in the future. Mucosal protection was effective but needs more studies for its clarification. **Supported by:**CNPq

HP5 - IMMUNOMODULATORY ROLE OF SOCS2 IN MACROPHAGES STIMULATED WITH *TRYPANOSOMA CRUZI* ANTIGENS

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The immunopathogenesis of Chagas disease is extremely complex. In an immunocompetent individual, innate immune cells like dendritic cells and macrophages express high levels of inflammatory cytokines (IL-12, TNFα), chemokines, as well as cytotoxic molecules like nitric oxide and reactive oxygen species). This innate immune mechanism drives the typical Th1 adaptive immune response, in which CD4+ and CD8+ T cells controls the infection. Previously, we demonstrated that the Suppressor of cytokine signaling 2, SOCS2, is involved in modulating heart damage during experimental Chagas disease. Absence of SOCS2 was correlated with a reduced cell infiltrate in the heart and the decrease of pro inflammatory cytokines. To gain insight on the molecular function of SOCS2 during the activation of anti-*Trypanosoma cruzi* innate immune response, here we have studied several signaling pathways in macrophages from SOCS2 knockout (-/-) mice stimulated *in vitro* with *T. cruzi* antigens (TcAg). TcAg induced the rapid activation of the NFκB and Mitogen activated protein kinases (MAPK) signaling pathways (ERK, P38 and JNK) in WT cells. We found that the absence of SOCS2 leads to profound alterations in the intensity and kinetics of the signal-triggered through these three MAPK kinases. Remarkably, we found that the activation of P38 was fully dependent on SOCS2. IL-6 production was found reduced in SOCS2 -/- when compared with WT cells. This cytokine plays an important role in the survival of cardiomyocytes infected by *T. cruzi*. The reduction of IL-6 in the absence of SOCS2 might be related to the incapacity of these cells to signal through P38 and activate AP1, a transcription factor involved in IL-6 synthesis. Also, we demonstrated that TcAg abolished the induction of SOCS3 in response to IFN-γ and that this event was SOCS2-dependent. Our results shows that SOCS2 is involved in macrophages response to *T. cruzi* by regulating several signaling pathways, including MAPK kinases. **Supported by:**CNPq/FAPEMIG

HP6 - THE V0D2 SUBUNIT OF RAW 264.7 VACUOLAR ATPASE IS REQUIRED FOR THE BIOGENESIS OF LARGE LEISHMANIA AMAZONENSIS PARASITOPHOROUS VACUOLES

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Vacuolar ATPases (V-ATPase) are ATP-driven proton pumps distributed in the membrane of intracellular organelles, responsible for their acidification. V-ATPases are composed of a V1 domain (with eight different subunits, A-H) involved in ATP hydrolysis; and a V0 domain (with five subunits (a,c,c'',d and e) involved in the proton transport across the membrane. The subunit V0d2, also involved in membrane fusion, is up-regulated in BALB/c macrophages infected with Leishmania amazonensis, suggesting that this subunit could participate in the biogenesis of L. amazonensis large parasitophorous vacuoles (PVs). To investigate the involvement of V-ATPase V0d2 subunit (ATP6V0d2) in the intracellular establishment of L. amazonensis, we observed infected RAW 264.7 macrophages stably expressing shRNAi for this subunit by multidimensional live imaging. In ATP6V0d2 knocked-down macrophages, L. amazonensis PVs present a restriction in volumetric growth when compared to non-silenced macrophage controls. Although inducing the formation of smaller PVs potentially defective in protease activity (assessed by the absence of mature forms of cathepsin D in cell extracts), the absence of ATP6V0d2 did not impair PV acidification, inferred by the retention of lysosomotropic probes within vacuoles, nor parasite multiplication. To investigate the role of large PVs in the classically described resistance of L. amazonensis to nitric oxide (NO)-producing macrophages, ATP6V0d2 knocked-down macrophages were treated with Interferon-γ and lipopolysaccharide (LPS) then infected with amastigotes. The absence of ATP6V0d2 in the presence of NO increased amastigotes multiplication and induced the formation of individualized PVs, in contrast to communal PVs developed in non-silenced macrophages. The results demonstrate that V0d2 subunit knock down does not interfere with vacuolar acidification although this subunit appears to have an essential role in the biogenesis of spacious L. amazonensis PVs. **Supported by:**FAPESP

HP7 - CHARACTERIZATION OF FREE CERAMIDE IN *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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Leishmania (Viannia) braziliensis is the etiologic agent of cutaneous, mucosal and mucocutaneous leishmaniasis. Recent studies have demonstrated the importance of sphingolipid metabolism in *L. braziliensis* biology (Castro et al., 2013). The proposal of this work was to characterize in *L. braziliensis* the presence of free ceramide, that contains long-chain base and a fatty acid linked by an amide linkage. Lipids were extracted from promastigotes using chloroform:methanol (2:1, v/v) and isopropanol:hexane:water (55:20:25, v/v/v), the extracts were submitted to alkaline hydrolysis to remove glycerophospholipids. The intact ceramides and other sphingolipids were applied to a DEAE A-25 Sephadex column. Neutral and positive charged lipids, which passed through the column, were analyzed by electrospray ionization mass spectrometry. The following possible ceramides were detected in positive ion mode: 590, 592, 588, 466, 616, 594, 638, 614, 612, 596, 610, 622, 624 *m/z*. Presence of the sphingoid base was characterized in the three major ions described: 590, 592 and 588 m/z. The peak at 590 m/z corresponds to d39:1 or t38:2 ceramide, containing d20:0 or t19:1 sphingoid base; the peak at 592 *m/z* corresponds to d39:0 or t38:1 ceramide, presenting d20:0 or t19:0 sphingoid base; and the peak at 588 *m/z* corresponds to d39:2 or t38:3 ceramide, presenting d20:0 or t19:1 sphingoid base. Previous studies of our group demonstrated that major inositolphosphorylceramide species from *L. braziliensis* present d34:0 and d34:1 ceramide, containing mainly d20:0 sphingoid base. These results suggest that *L. braziliensis* exhibits two different ceramide pools. Ceramide fatty acid composition will be confirmed by gas chromatography mass spectrometry. It is the first time that free ceramide is described in *L. braziliensis* promastigotes. Expression of free ceramide in amastigotes and parasites under stress conditions are under investigation. **Supported by:**FAPESP, CAPES, CNPq

HP8 - INTERFERON GAMMA AND INTERLEUKIN 10 ARE CRITICAL FOR HOST HOMEOSTASIS DURING ORAL INFECTION BY *NEOSPORA CANINUM*

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Neospora caninum induces neuromuscular disorders in dogs and abortions in cattle, representing significant economic losses worldwide. Our aim in this study was to evaluate the systemic and organ-specific immune responses in mice, in a model that mimics natural exposure to the parasite. Mice infected with a parasite high dose by the oral route were resistant to the infection, while the same dose was lethal to all mice submitted to intraperitoneal inoculation. Analyzing the systemic immune responses induced by oral infection, we observed high concentrations of IFN-γ and TNF-α in serum samples after 7 days post-infection (dpi), which were still present at significant levels after 14 dpi, although accompanied by a sharp increase in IL-2, IL-4, IL-6, IL-10 and IL-17A. Surprisingly, none of the assessed cytokines were detected at 21 dpi. In situ analysis showed no histological changes in the small intestine during the experimental period, however parasite DNA was detected in different segments during 7 dpi, while changes in IFN-γ and IL-10 levels were observed only after 14-21 dpi. On the other hand, while peak detection of parasite DNA was also observed at 7 dpi in the liver, lungs and central nervous system, severe inflammatory lesions were observed at 7, 14 and 21 dpi, respectively, followed by in situ changes of the IFN-γ/IL-10 balance. In order to investigate the role of IFN-γ and IL-10 in the resistance to oral infection against this parasite, genetically deficient mice (IFN-$\gamma^{-/-}$ and IL-$10^{-/-}$) were submitted to the same inoculation protocol, which was lethal to both strains of mice. While IFN-$\gamma^{-/-}$ mice succumbed during the acute phase (<10 dpi), the infection protocol was fatal to IL-$10^{-/-}$ mice during the establishment of the chronic phase (15-30 dpi). In conclusion, IFN-γ and IL-10 are expressed in different tissues in response to the presence of *N. caninum*, and are critical for maintaining host homeostasis during oral infection by this parasite. **Supported by:**CAPES, Fapemig, CNPq

HP9 - MHCII KNOCKOUT IMPAIRS LEISHMANIA AMAZONENSIS EXPERIMENTAL PATENT INFECTION

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L. amazonensis, a species that causes cutaneous leishmaniasis in the new world, replicates within very large parasitophorous vacuole that continuously undergo fusion with lysosomes and phagolysosomes. Also, this specie is able to degrade major histocompatibility complex (MHC) class II within megasomes by cysteine proteases. Thus, suggesting means of circumventing the host's immune system. In this context, we aimed to verify the outcome of L. amazonensis infection in mhc ii -/- mice. Our results showed that MHCII deficiency led to a controlled infection. Thus, indicating that compensatory mechanisms may be triggered upon MHCII knockout leading to a non-patent Leishmania infection. **Supported by:**FAPEMIG; CAPES; CNPq.

HP10 - IL-27 SUPRESSES PARASITE CONTROL DURING VISCERAL LEISHMANIASIS THROUGH REGULATION OF IL-17 PRODUCTION

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Introduction: Visceral Leishmaniasis (VL) is a life-threatening disease that affects the liver and spleen of infected hosts. The disease is caused by *Leishmania infantum,* which is endemic in Brasil. Cytokines such as IFNγ and IL-17 play an important role in the induction of parasite control by infected macrophages, whereas IL-10 and IL-27 can facilitate parasite growth. Our group previously observed an important role of IL-17 and neutrophils in the parasite control. Thus, we aimed to understand the role of IL-27 in the regulation of the immune response during VL.
Methods and Results: We first observed high levels of IL-27 in the sera of VL pacients. In addition, we observed that *L. infantum* infection induces IL-27 production in the spleen and liver of C57BL/6 (WT) mice, 4 and 6 weeks post infection. This cytokine is also produced in vitro, and Toll-like receptors 3, 4 and 9, as well as TRIF adaptor are crucial for IL-27 production by bone-marrow dendritic cells. To address the role of IL-27 during infection, we infected WT and IL-27 knockout mice (IL-27-/-) with *L.infantum* and observed that IL-27-/- mice presented reduced levels of the parasite in target organs when compared to WT mice. In the next step, we evaluated the levels of CD4+ IFNγ+ T cells by flow cytometry in the spleen after infection, and observed that this cell population is reduced in IL-27-/- mice, compared to WT. The reduction of IFNγ production in IL-27-/- mice was confirmed by ELISA in tissue homogenate. However, IL-27-deficient mice presented elevated levels of IL-17 in the spleen and liver, 4 and 6 weeks post infection. Moreover, in the absence of IL-27, a higher influx of neutrophils could be observed in the spleen, which could be responsible for the increased parasite control observed in these animals.
Conclusion: Our work shows the regulation exerted by IL-27 on the IL-17-neutrophil axis, which could be exploited as a therapeutic approach. **Supported by:**FAPESP, CNPq, CAPES.

HP11 - *PLASMODIUM VIVAX*: PROFILE OF MEMORY AND NAïVE CD8+ T CELL DURING INFECTION

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CD8+ T cells are an important population of cytotoxic/suppressor T lymphocytes. During pre-erythrocytic phase of malaria, these cells act eliminating infected hepatocytes and producing cytokines. In humans, naïve T cells ($CD45RA^+$) can differentiate into memory T cells, which begin to express CD45RO molecule. These memory cells are important during a reinfection and respond promptly to the parasite products. Our hypothesis is that *P. vivax* infection modulates the immune response by reducing the differentiation of $CD8^+$ memory T. Thus, the aim of this study was to investigate $CD8^+$ naïve and memory T cells subsets and their cytokine production in subjects naturally infected with *P. vivax* from Porto Velho–RO. The immunophenotyping was performed using blood samples from 20 infected subjects and 12 healthy donors. Data acquisition was performed by flow cytometry. *P. vivax* infected subjects showed lower percentage of $CD8^+CD45RA^+$ and $CD8^+CD45RO^+$ T cells when compared to non-infected individuals ($p<0.01$). Interestingly, the cytokine production was significantly reduced in naïve T cells from infected individuals, observed by the lower percentage of TNF-α^+ ($p=0.01$) and IL-10^+ ($p=0.013$) T cells when compared to negative controls. In contrast, despite the reduction of percentage of $CD8^+$ memory T cells producing IFN-γ ($p<0.001$), P. vivax-infected individuals presented a significant increase of memory $CD8^+$ cells producing TNF-α^+ ($p=0.007$) and IL-10^+ ($p=0.004$). Finally, our data have shown that *P. vivax* infection results in an effector rather than central memory T cell phenotype as demonstrated by the reduction of $CD8^+CD45^+CD62L^+$ circulating cells ($p<0.001$) and augmentation of $CD8^+CD45^+CD62L^-$ peripheral blood cells ($p=0.004$). Taken together, our results suggest that *P. vivax* infection might impairs the differentiation of CD8 memory T cells and promotes a differential production of cytokines in naïve and in the remaining memory cells, which might contribute to the parasite survival.**Supported by:**CAPES; CNPq; FAPEMIG

HP12 - DRUG DELIVERY SYSTEMS POTENTIATES 17-(ALLYLAMINO)-17-DEMETHOXYGELDANAMYCIN (17-AAG) LEISHMANICIDAL EFFECT IN VITRO

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Leishmaniasis remains a major health problem world wiled. Current long-term treatments cause severe side effects and an increasing number of resistance cases. The 17-AAG is a less toxic analog of geldanamycin that has been demonstrated to have a strong leishmanicidal activity. In an attempt to increase the potency of 17-AAG and reduce its side effects, novel formulations were prepared: liposomes (LP)-containing 17-AAG (LP-17AAG) and bacterial cellulose membrane (CM)-containing 17-AAG (CM-17-AAG). LP-17-AAG activity against *Leishmania amazonensis* promastigotes was evaluated and showed that it was able to kill 76% of the parasites at a concentration as low as 1 pM, while empty LP showed no effect against promastigotes as evaluated by AlamarBlue assay. We also evaluated the efficiency of LP-17-AAG against intracellular *Leishmania*. Infected-macrophages were treated with different concentrations of LP-17-AAG for 24, 48 and 72h. Our results showed that doses, as small as 40 pM were able to promote complete clearance of parasite after 48h with no toxicity against macrophage cells. In a similar way CM-17-AAG had increased potency compared to soluble 17-AAG. CM-17-AAG containing as few as 5 nM of 17-AAG was able to kill 70% of *Leishmania*, while 5 nM of soluble drug wasn't able to kill parasites. CM containing 1.25 nM of 17-AAG was able to reduce in 83% the intracellular parasite viability. This indicates that efficiency of 17-AAG when impregnated in CM was remarkably increased. Our results show that new technologies and delivery systems can be used to increase drug efficiency and potency, as well as to directly deliver 17-AAG to infected cells, indicating those systems may become important therapeutic strategies against leishmaniasis, especially in cases of parasite resistance. **Supported by:**CNPq - 306672/2008-1, INCT-DT

HP13 - DUAL EFFECT OF IFN-γ DURING *LEISHMANIA AMAZONENSIS* INFECTION

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IFN-γ is a key factor in the control of intracellular parasites. Surprisingly, during L. amazonensis infection the role of IFN-γ is controversial. During in vitro infections IFN-γ can induce the proliferation of the intracellular amastigote form of the parasite, and during early in vivo infection it is not essential for parasite control. The reason why IFN-γ is dispensable during the initial weeks of infection is not clear yet. Thus, the aim of our work was to investigate the role of IFN-γ during early L. amazonensis infection. We infected C57BL/6 Wt and IFN-γ-/- mice with 104 metacyclics forms of the RFP+ parasite in the ear and followed the disease for six weeks. IFN-γ -/- mice showed equivalent lesion sizes and parasite numbers at the dermal site of infection for the first 4 weeks of infection, although they presented a higher expression of IL-4, IL-10 and arginase I whereas Wt mice had higher expression of iNOS and TNF-α starting at 2 wks p.i. Interestingly, we found a higher numbers of dermal CD4+T cells and monocytes in Wt at the beginning of the infection, which corresponded with pathology and parasite growth. Also, tracking the RFP+ signal we found neutrophils are the first infected cells, following by inflammatory cells, CD11b+Ly6G-Ly6C+, and finally by non-inflammatory cells, CD11b+Ly6G-Ly6C- during this infection. IFN-γ did not play a role in the transition of parasites through the different host cells. The enhanced host cell infiltrate observed in IFN-γ sufficient mice may counteract the protective role of IFN-γ. This could explain, in part, why IFN-γ cytokine seems not to be important in the beginning of the infection. We propose IFN-γ is playing a dual role at early time points in this model of infection, inducing both the expression of iNOS and parasite killing but also the recruitment of inflammatory cells which can support the parasite replication. **Supported by:**CNPq, CAPES, FAPEMIG, REDOXOMA, NIH

HP14 - DIFFERENTIAL DNA DAMAGE RESPONSE THROUGHOUT AFRICAN TRYPANOSOME LIFE CYCLE

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Trypanosoma brucei causes sleeping sickness in humans affecting seventy thousand people each year. The parasite has a complex life cycle infecting mammalians and tsetse flies. It displays different strategies to deal with each respective host resulting in important implications in how T. brucei controls several cellular process such as DNA replication and repair. Here we compare the DNA damage response (DDR) between T. brucei bloodstream and procyclic forms by assessing the DNA repair, through quantitative PCR, and cell growth after the treatment with genotoxic agents. Treating both forms with cisplatin and UV light, agents that causes DNA lesions mainly targeted by Nucleotide Excision Repair (NER), we observe that cisplatin adducts are more efficiently repaired in the bloodstream form either in mitochondrial (kDNA) and nuclear DNA. Both forms are incapable to tackle UV-induced DNA lesions. Conversely after treating parasites with agents that causes lesions targeted by Base Excision Repair (BER), we show that the repair of H2O2-induced lesions in kDNA is faster in bloodstream than in procyclic cells. After the treatment with MMS DNA lesions were only detected in the bloodstream form. When we looked at parasite growth after DNA damage with both cisplatin and MMS treatments, the bloodstream cells displayed a higher sensitivity and cell death rates, while procyclic parasites only stop growth without cell death. However both parasite forms showed similar cell death rates after hydrogen peroxide and UV treatments. Taken together, these results reveal a differentially regulated DDR throughout T. brucei life cycle. We suggest that the regulation of checkpoint and cell fate after DNA damage work favoring cell cycle blockage and down regulating DNA repair in procyclic form. However unrepaired lesions in bloodstream form do not block cell cycle progression, leading to cell death, despite the faster DNA repair rate presented by this form of the parasite. **Supported by:**CNPq, FAPEMIG, CAPES

HP15 - THE ROLE OF CLASS III PHOSPHATIDYLINOSITOL 3-KINASE (PIK) TCVPS34 IN *TRYPANOSOMA CRUZI* INVASION

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Many different proteins, including protein kinases, are involved in the regulation of response of *Trypanosoma cruzi* to environmental changes Among the protein kinases, phosphatidylinositol kinases (PIK) phosphorylate phospholipids regulating important cellular functions such as metabolism, cell cycle and survival. Vps34, a class III PIK, has been shown to regulate autophagy, trimeric G-protein signaling, and the mTOR (mammalian Target of Rapamycin) nutrient-sensing pathway. In T. cruzi, TcVps34 has been related to morphological and functional changes associated to vesicular trafficking. TcVps34 overexpression affects enzymatic activities related to acidification of intracellular compartments whereas its inhibition interferes with the parasite recovery after severe hypo-osmotic stress. The aim of this study is to characterize the TcVps34 role in the invasion of HeLa cells by metacyclic trypomastigotes (MTs) and extracellular amastigotes (EAs) overexpressing TcVps34. Invasion rates of parasites pretreated or not with 3-methyladenine, a Vps34 inhibitor, were compared to non treated parasites. EAs overexpressing TcVps34 showed significant increase in the invasion rate while parasites treated with TcVps34 inhibitor exhibited a lower invasion rate. By contrast, MTs overexpressing TcVps34 showed a lower invasion rate compared to control, suggesting a down regulation by TcVps34 in this developmental form. Taken together, these results suggest that TcVps34 plays key role in *T. cruzi* invasion. Further studies are needed to determine the mechanisms by which TcVps34 acts in the invasion of these different developmental forms. **Supported by:**FAPESP, CNPQ, FAPEMIG

HP16 - ENCAPSULATION OF CHALCONE CH8 IN LIPID CORE NANOCAPSULES (LNC) POSITIVELY MODULATE MICROBICIDAL MECHANISMS IN MURINE CUTANEOUS LEISHMANIASIS.

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The activity of the chalcone CH8 has been previously shown by our group against murine cutaneous leishmaniasis. Aiming at reducing the toxicity of CH8 and increasing its in vivo activity, the drug was encapsulated in lipid core nanocapsules of poly-□-caprolactone (LNC-CH8) and assessed for in vitro activity against L. amazonensis-GFP, toxicity to macrophages, induction of microbicidal mechanisms and evaluation of in vivo activity by intralesional route. To investigate the direct effect on the parasite, L. amazonensis-GFP promastigotes were incubated with different concentrations of free CH8, LNC-CH8 or empty LNC, for 72h at 26°C and cell viability was measured by fluorimetry. Infected and non-infected macrophages were incubated with varying drug concentrations for 48h at 37°C. Amastigote growth was quantified using optical microscopy and macrophage toxicity was assessed by released LDH. Nitric oxide (NO) production was assessed by the Griess method and reactive oxygen species (ROS) by plate fluorimetry and H2DCFDA dye. Intramacrophagic acidification was measured using Lysotracker red; and proteolytic activity by fluorimetry using funcionalizated beads with DQ-Green BSA. In vivo, BALB/ mice were infected in the ear and intralesionally treated with CH8 and LNC-CH8 twice a week, for 3 weeks. The results showed that the nanoencapsulation of CH8 did not change its antileishmanial activity, but reduced the cytotoxicity to uninfected macrophages. LNC-CH8 increased ROS production, cell acidification and proteolytic activity by infected macrophages, but did not alter the NO production. Nanoencapsulaton also led to increased CH8 efficacy in vivo, significantly reducing lesion sizes and parasite burdens. Our results show despite not directly affecting intracellular parasite growth, encapsulation of CH8 in LNC positively modulates important microbicidal mechanisms leading to improved infection control in vivo. **Supported by:**CAPES

HP17 - ARYL HYDROCARBON RECEPTOR (AHR) IN THE COURSE OF EXPERIMENTAL SEVERE MALARIA

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Introduction: Malaria is an important cause of death and illness and severe malaria(SM) is the most important complication of Plasmodium falciparum infection. The events that result in the development of SM are multi-factorial and could not be explained by one mechanism. Mice infected with P.berghei ANKA (PbA) faithfully recapitulate many of the characteristics of human SM. The Aryl Hydrocarbon receptor (AhR) is an intracellular receptor activated by ligands important in the modulation of the inflammatory response. The role of AhR in SM has not been explored. Objective: Investigate the role of AhR in the malaria pathogenesis. Methods: C57Bl/6(WT) and AhR-/- mice were infected with PbA, the parasitemia, survival were monitored daily. The production of several cytokines in the serum, brain and spleen was assessed by ELISA and flow cytometry. The expression of SOCS1,2,3 were evaluated by qPCR. Brain leukocyte recruitment and nitric oxide (NO) were evaluated by intravital microscopy and Griess method. Histopathology analysis was also performed in brain and liver. Results: PbA-infected AhR-/- mice showed increased parasitemia, lower hematocrit and earlier mortality when compared with WT. In the brain and spleen of PbA-infected AhR-/- mice there were a significant decreased expression of TNFα, IL1β, IFNγ, IL12 and IL10 when compared with WT counterparts. Also, there were increased levels of TGFβ, IL6, IL17 in the brain of infected AhR-/- mice when compared with WT mice. In PbA-AhR-/- mice, there was decrease expression of SOCS3 in the brain and increased level of alanine aminotransferase in the liver indicates a greater degeneration. The proportion of Th1, Th17 and Treg cells in the liver and he brain were deregulated in PbA-infected AhR-/- mice. Moreover, PbA-infected AhR-/- mice had increased brain blood barrier permeability. Conclusions: These findings indicate, the role for AhR in the immunopathogenesis of SM. **Supported by:**CAPES, CNPq, FAPEMIG

HP18 - DRUG RESISTANCE IS ASSOCIATED WITH DOWN MODULATION THE MACROPHAGE KILLING MECHANISMS IN ANTIMONY RESISTANT CLINICAL ISOLATES OF LEISHMANIA INFANTUM CHAGASI

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Introduction: Visceral leishmaniaisis is a life-threatning disease characterized by intense parasitism of the spleen, liver and bone marrow. Antimonials (Sb) have been the main antileishmanial therapy for decades, but increasing failure rates under antimonial treatment have challenged further use of these drugs. SbV is known to reinforce the killing mechanisms of macrophages, and is still unclear how it happens. We evaluated if Leishmania infantum chagasi (Lc) isolated from patients refractory to antimony treatment, relapse cases, show cross-resistance to SbIII, liposomal amphotericin b (L-Amb), Nitric oxide (NO), and also modulate macrophage infection. Methods and Results: Four Lc isolates from relapse cases and two from antimony treatment responsive patients, control group, were used in this study. The Lc promastigotes from relapse cases were SbIII and NO resistant, and, only, one isolate was L-Amb resistant. Amastigote forms from relapse cases showed a 10 to 25-fold increase in the macrophage infection as compared to control in the presence of Sbv. We evaluate whether the resistance phenotype of the isolates from relapse cases would enhance the infectivity and survival of amastigote in macrophages and macrophages killing mechanism. Macrophages were infected with isolates from relapse cases or control group in the presence of LPS/IFN-γ or without stimulus. The macrophage infection is higher with relapse cases Lc than the controls Lc and correlates with increment in Nitrite , IL1-β and IL-6 production. Conclusion: Taken together, the results suggest that isolates from refractory patients show cross-resistance to Sb, NO and, also, these parasites were able to stimulate inflammatory cytokines and down modulate the macrophage killing mechanisms. **Supported by:**FAPITEC/CNPQ , CAPES

HP19 - NOD/RIP2 SIGNALING CONTRIBUTE TO DEVELOPMENT OF A TH1 RESPONSE AND RESISTANCE AGAINST *L. MAJOR* INFECTION

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The receptors Nod1 and Nod2 have key role in the host responses. During activation, these proteins signals via the adapter molecule Rip2, favoring the production of cytokines and chemokines. Also, Nod1 and Nod2 participate in the detection/control of several pathogens, but its role during Leishmania infection is unknown. Here, we investigated the participation of Nod/Rip2 pathway in host response during L. major infection. BMDMs or BMDCs derived from C57BL/6, Nod1–/–, Nod2–/– and Rip2–/– mice were infected with L. major and cytokines (ELISA/flow cytometry) production and surface molecules expression (flow cytometry) were analyzed. The lesion development and parasite burden were measured in WT-, Nod1–/–-, Nod2–/–- and Rip2–/–-infected mice. Dendritic cells activation and cytokines production in vivo were evaluated at 1st, 2nd and 8th week p.i. by flow cytometry. Finally, we analyzed the susceptibility and cytokines production in chimeras generated by irradiating recipient mice. Nod1–/–, Nod2–/– and Rip2–/– BMDMs had an impaired induction of NF-κB–dependent products in response to infection. Moreover, IL-12p40 production and surface molecules expression were decreased in parasite infected-Rip2–/– BMDCs. Nod1 and Nod2 activation was crucial for in vivo parasite replication control and resolved cutaneous lesions. Rip2-dependent response was required for dendritic cells activation and induction of effective Th1 response in vivo. Additionally, Rip2-dependent signaling in radio-sensitive compartments was required for the control of the infection and induction of Th1 response. Our results indicate that Nod1/Rip2-dependent responses account for host resistance against L. major infection by mechanisms dependent of Th1 cytokine. Importantly, this study shows that the Nod-Rip2 axis effectively participate of the induction of adaptive immune responses against a Leishmania parasite, thus providing a novel function for Nod-like receptors family in parasite-host interactions. **Supported by:**FAPESP, INCTV/CNPq

HP20 - REVEALING A POTENTIAL P21-MEDIATED *TRYPANOSOMA CRUZI* EVASION MECHANISM

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a tropical neglected disease considered an important public health problem. Our group spends our efforts in trying to understand the dynamics of *T. cruzi* infection *in vitro* and *in vivo* in order to find potential targets for therapeutics. In this sense, we investigated the mechanisms underlying *T. cruzi* P21 activities. We have shown that rP21 induce host cell actin polymerization by binding to CXCR4 on host cell surface also suggests the possibility that P21 secreted in the cytosol of infected cells may likewise bind to regulatory proteins of the actin cytoskeleton polymerization machinery in the cell cytoplasm. If so, we can envisage a broad scenario involving P21 during disease progression. The chronic phase of Chagas disease is marked by not detectable parasitemia and parasite persistence. In this sense, which would be the cellular events beneath parasitemia control by host immune system? Would the parasite respond to host immune components in order to avoid its clearance? Our first attempts to test this hypothesis showed us that rP21 treatment of infected macrophages and myoblasts showed higher actin polymerization around intracellular parasites followed by a reduced parasite multiplication. In this context, we observed that treatment of infected myoblasts and macrophages with the cytokine IFN-γ similarly reduced parasite multiplication increased actin polymerization, as well as increased the transcription of the P21 gene by intracellular parasites. Corroborating these data, we observed that amastigotes of the low virulent G strain naturally express high levels of P21. Thus, we assume a possible direct relationship among host immune response, synthesis of P21 by the parasite, host cell actin cytoskeleton polymerization and the course of infection. Therefore, we believe that P21 may be an important component of the parasite to establish the chronic phase of Chagas disease. **Supported by:**FAPEMIG, CAPES, CNPq

HP21 - HIGH AVIDITY IGG ANTIBODIES ANTI-LEISHMANIA AS DETECTED BY ACID ELISA IN EXPERIMENTAL LEISHMANIASIS

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Caused by protozoan Leishmania (L.) infantum chagasi, visceral leishmaniasis is reemerging in some tropical countries such as Brazil. The main reservoir of disease is domestic dog and transmission occurs through phlebotomine sand flies bites between hosts. Diagnosis is usually parasitological, but serological diagnosis can be a helpful support. Humoral response is ineffective, and despite huge IgG amounts, circulating immune complexes are formed by excess leishmania antigens. Findings of specific antibodies by ELISA may be cautiously interpreted, due to frequent false positives from asymptomatic infections and false negatives due to immune complexes. Presence of circulating immune complexes directly affects the detection of antigens and antibodies, which may mask positive serology. An alternative would be to detect antibodies capable of blocking the formation of immune complexes, such as acid pH, which destabilizes immune complexes, freeing antibodies for ELISA. We evaluated the detection of IgG antibodies to pH 5 by ELISA in hamsters sera at 15, 30, 45, 60 and 90 days of infection. Those results fail to demonstrate time-response avidity antibody maturation. Results indicated that the detection of IgG anti-Leishmania antibodies in acid dilution selects a part of the total antibody population that is capable of reacting under conditions of stress, such as differentiating the highest avidity antibodies. Proportion of high avidity antibodies are usually proportional to whole amount of antibodies, but significant higher at 45 days of infection ($P < 0.001$ and $R2$ 0.98). Detection of high avidity antibodies in ELISA by acid dilution is revealed as an important tool to be exploited in the serological diagnosis of visceral leishmaniasis and could assist in specific situations where conventional serological techniques have indeterminate results. Aside to this, the avidity maturation of specific antibodies in leishmaniasis could be related to clinical staging of the disease. **Supported by:**CNPQ

HP22 - EXPRESSION OF 50-70KDA PROTEIN BY LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI AXENIC AMASTIGOTES UNRECOGNIZED BY IGG FROM INFECTED HOSTS.

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Visceral leishmaniasis due to Leishmania (Leishmania) infantum chagasi is widely distributed in Brazil. Transmission occurs by phlebotomine sand flies bites with domestic dog as reservoir, with potential for urban spreading. Serological tests are used for the detection of specific antibodies helpful in the diagnosis of visceral leishmaniasis. Sensitivity and specificity may vary in ELISA according to type of antigen used. Immunogenicity of an antigen is related to protein content and dimorphic L. (L.) infantum chagasi shows specific proteins or alteration of antigen expression levels according to life form or culture conditions. Promastigotes are widely used for the production of antigens due to ease cultivation while intracellular amastigotes are rarely used. Specific temperature and pH allows the transformation of promastigotes in axenic amastigotes in extracellular medium. We conducted the cultivation of axenic amastigotes for obtaining antigen and characterization of the proteins of different forms by electrophoresis profile. Axenic amastigotes presented a 50 and 70 kDa protein absent in similar promastigote antigenic extract. We devised to evaluate the efficiency of those antigens in Western blot with positive and negative serum for hamster, rabbits, dogs and Humans with visceral leishmaniasis. In the detection of IgG anti-Leishmania, axenic amastigotes extracts presented low immunogenicity, with absence of staining of the 50-70kDa protein, suggesting that axenic amastigotes could express antigens that are not expressed by intracellular amastigotes and therefore are unrecognized by infected host immune system. Our data suggest that caution may be used when mimicking intracellular conditions in Leishmania axenic cultivation, before ascribing a potential substitution of intracellular amastigotes as solid support in ELISA. The use of specific antigen preparations and their alternatives must be carefully explored before extrapolation for generic assays.

HP23 - EFFICIENT DUPLEX SOLID-PHASE FLUORESCENT IMMUNOSORBENT ASSAY (DFISA) FOR SIMULTANEOUS DETECTION OF SPECIFIC ANTI-*T. GONDII* IGG AND IGM BASED ON REFINED CONJUGATES

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Toxoplasma gondii infections are very common, causing occasional central nervous system and eye disease, and must be screened in prenatal care, for disease therapy. Serology is the main diagnosis with commercial antibody assays, but individual variation or low thresholds cause many inconsistencies. New solid-phase immunofluorescent assays (FISA) allows direct antibody quantification in microplates. Here, we developed a duplex solid-phase fluorescent immunosorbent assay (dFISA) for simultaneous detection of anti-*Toxoplasma gondii* IgG and IgM antibodies in prenatal care screening for toxoplasmosis. The efficiency of this method was initially analyzed in separate FISA for each immunoglobulin with commercial conjugates in 120 serum samples from pregnant women previously screened by IgG/IgM ELISA. We also validate the dFISA in 24 serum samples of adults at a large public hospital. IgG FISA showed substantial concordance (Kappa=0.786), 83.3% sensitivity and 96.3% specificity, while IgM FISA showed moderate concordance (Kappa=0.501), lower 66.6% sensitivity and similar 93.6% specificity. Assays using commercially available conjugates yielded poor results and high background fluorescence. New higher efficiency conjugated were prepared and tested in a same-well IgG/IgM dFISA. Compared to ELISA IgG/IgM, dFISA demonstrated concordance for IgG (Kappa=0.883; sensitivity=89.2%; specificity=100%,) and IgM (Kappa=0.918; sensitivity=85.7%; specificity=100%), with excellent reproducibility for anti-*T. gondii* IgG (84.8%) and IgM (80.5%) detection. dFISA is quick, inexpensive, easily performed and high throughput and the assay can be used for screening serum conversion in pregnant women, useful in large numbers applications as antenatal of public health care. **Supported by:**CAPES

HP24 - SUSCEPTIBILITY OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* ISOLATES TO MILTEFOSINE

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Leishmania (Viannia) braziliensis is the main causative species of leishmaniasis in Brazil with about 22,000 new cases per year. First line drug used for leishmaniasis chemotherapy is meglumine antimoniate, which is costly and requires parenteral administration. Miltefosine is an alkylphosphocholine already in use for the treatment of visceral leishmaniasis in Asia and Europe, but still not approved for use in Brazil. Recent clinical trials in Brazil indicate a higher efficacy for miltefosine as compared with antimony in the treatment of cutaneous leishmaniasis[1]. In this study, we evaluated the susceptibility of 21 clinical isolates of *L. (V.) braziliensis* from different regions of Brazil to miltefosine in vitro. We determined the inhibitory concentrations of miltefosine for promastigotes and amastigotes and identified significantly decreased susceptibility in 2 out of 21 clinical isolates. The molecular basis of these differences among the clinical isolates is under investigation. Phosphocholine uptake studies have shown a direct correlation with miltefosine susceptibility, suggesting that polymorphisms in the miltefosine transporter gene may be involved. Currently, we are sequencing the miltefosine transporter gene of these isolates in order to determine whether differences in susceptibility are due to polymorphisms in this gene. These findings will contribute to evaluate miltefosine's potential for leishmaniasis treatment in Brazil and also to elucidate the molecular basis involved in miltefosine susceptibility variations in *L. (V.) braziliensis*. [1] Machado PR et al. PLoS Negl Trop Dis. 2010;4:e912; Chrusciak-Talhari et al. Am J Trop Med Hyg. 2011;84:255-60. **Supported by:**FAPESP, CAPES and CNPq

HP25 - ECTONUCLEOTIDASE ACTIVITY OF E-NTPDASE-2 OF *LEISHMANIA AMAZONENSIS* IS ESSENTIAL TO REDUCE TNF-α, IL-12 AND NO PRODUCTION BY STIMULATED MACROPHAGES

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Given the relationship between *Leishmania amazonensis* ectonucleotidase activity and the ability of this parasite to cause injury in C57BL/6 mice, we decided to investigate the mechanism by which E-NTPDase-2 relates to parasite infection in macrophages. To this end, metacyclic promastigotes of *L. amazonensis* were incubated with ATP, ADP or AMP for 1 hour and the amount of inorganic phosphate was evaluated. The high ectonucleotidase activity correlates with the high expression of the E-NTPDase-2 in the membrane of these parasites. To verify if the activity of E-NTPDase-2 would interfere with the ability of the parasite to survive within activated macrophages, metacyclic promastigotes were pre-incubated with DIDS, an ectonucleotidase activity inhibitor and were used to infect J774 cells for 3 hours. The cells were washed to remove non-internalized parasites and cells were incubated for 48 hr in the presence of IFN-γ and LPS. Parasites pre-incubated with DIDS lose the ability to reduce NO production by stimulated cells and to survive within these cells. Interestingly, infection reduces IL-12 and TNF-α production by stimulated cells, but DIDS pretreatment prevents the parasite to modulate inflammatory cytokine production. To investigate whether E-NTPDase-2 of *L. amazonensis* acts providing substrate to adenosine production, infection was carried out in the presence of specific antagonists of adenosine receptors A2A or A2B. Indeed, blocking A2B receptors reduced parasite survival within stimulated cells. This was associated with an increase in NO, IL-12 and TNF-α production. Taken together, these results show that E-NTPDase-2 activity in *L. amazonensis* is important for generating substrate for adenosine production which, by acting on A2B receptors, reduces IL-12 and TNF-α production, cytokines that are essential for the release of NO by stimulated cells, thus favoring *L. amazonensis* infection. **Supported by:**CAPES; CNPq; FAPEMIG

HP26 - PERIPHERAL BLOOD MONOCYTES ACTIVATION MARKERS ON CANINE VISCERAL LEISHMANIASIS

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Peripheral blood monocytes are the largest group of circulating progenitor cells. Its functions have been revealed through the study of their subpopulations, especially in humans and mice in pathological processes. In Brazil, the parasite *L. infantum* is the cause of the canine visceral leishmaniasis. *Leishmania* spp. is an obligate intracellular parasite infecting mainly the mononuclear phagocyte system leukocytes. This study aimed to compare the expression of main monocytes activation markers from uninfected or *L. infantum* naturally infected dogs. Blood samples were collected from uninfected and infected (symptomatic and asymptomatic) dogs, labeled by antibodies against CCR2, CD11c, CD14, CD62L, MHC-II molecules and analyzed by flow cytometry. The $CD14^{+}$ cells were gated and the others surface markers expressions were compared among the groups. We noticed a marked reduction of the expression of molecules related to the monocytes activation (CCR2, CD11c, CD62L and MHC-II), especially in the group of symptomatic dogs, indicating a possible inhibition on the capacity of these cells to control the disease, evidenced by MHC II and CD11c reduced expression as well as their infection site migratory ability, stated by CCR2 and CD62L decrease. Our results suggest an important role of monocytes activation on the dog clinical state, which may also indicate the evolutionary state of the disease. **Supported by:**CNPq, UFMG

HP27 - CHARACTERIZATION OF THE INFECTION IN CANINE MACROPHAGES LINEAGE (DH82) BY *LEISHMANIA AMAZONENSIS* AND *LEISHMANIA CHAGASI.*

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Canine leishmaniasis occurs in many countries from both Old and New World. Latin America presents an estimated incidence of millions case of infected dogs, reaching up to 75% of the population in endemic areas. Canine infection by *Leishmania chagasi* is the most common, even though the reports of dogs infected by other *Leishmania* species are ever more often. In this study, we characterize the infection of canine DH82 macrophages cell line by *L. amazonensis* and *L. chagasi*. Our results demonstrated that DH82 interacts with promastigotes of both *Leishmania* species, however *L. amazonensis* interact 2,5 times more than *L. chagasi*. Furthermore, we observed that *L. amazonensis* promastigotes is more infective 4,48 or 6,6 times to DH82 macrophages than *L. chagasi* ones in 24h or 48h post infection, respectively. Specific antibody opsonization increases interaction in 12 times and, also, increases *L. chagasi* survival in 4,5 times when compared to non-opsonized *L. chagasi* in 24h post infection or 2,4 times in 48h post infection. The interaction and infection profile among DH82 and RAW 264.7 seems to be similar, although RAW 264.7 cells have been more permissive to both *Leishmania* species than DH82 cells. Interestingly, in 48 hour of infection DH82 cells were 2,6 times more infected by *L. amazonensis* than RAW 264.7 cells. MHC II and CD80 expression analysis on DH82 surface showed that these cells have a basal expression (33% and 28%, respectively) and that none of the *Leishmania* species modulate the expression of MHCII, but basal expression of CD80 seems to be increased in the presence of *L. amazonensis* (41% CD80+). In LPS-stimulated cells was observed 2 times more CD80+ cells and 1,8 times more MHCII + cells, however, the parasites do not appear to modulate the expression of both molecules in LPS-stimulated cells. Therefore, we propose that canine DH82 macrophage cell line may be a possible canine experimental model of infection *by Leishmania*. **Supported by:**CNPq

HP28 - IDENTIFICATION OF *RHODNIUS PROLIXUS* RELISH (RPRELISH) AND CHARACTERIZATION OF ITS ROLE AND OF THE ROLE OF RPDORSAL IN THIS INSECT IMMUNE RESPONSE

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In insects innate immunity is the first defense line against several microorganisms. Insect innate immune response comprises two levels: cellular and humoral. Humoral response is regulated mainly by two pathways, Toll and IMD. Both of these pathways have proteins from NF-kB family as key transcriptional factors. In Rhodnius prolixus, the Toll pathway has rpDorsal as its transcription factor (Ursic-Bedoya et al., 2009). In this work we report the identification of Rhodnius Relish homologue and characterize the role of both NF-kB factors (Dorsal and Relish) in this insect immune response. Aiming to understand the role of rpRelish in R. prolixus immune response, its expression was analyzed after challenging the insect with different stimuli: injection with gram-positive or negative bacteria, zymosan or blood feeding. We also knocked down, using RNAi, Relish, Dorsal or both genes. In knocked down insects, we analyzed several parameters: microbiota levels, egg posture and hatching and susceptibility to Trypanosoma cruzi infection. Our results showed that relish transcription is increased after a blood meal in midgut and fat body. Relish transcription is also activated in response to gram-positive bacteria in midgut and by all challenges in the fat body. Relish knockdown led to a decrease of defensin expression and an increase in bacterial levels in anterior and posterior midgut, suggesting that IMD pathway is responsible for control of the population of the symbiont Rhodococcus rhodnii. Egg posture was also reduced, but not effect on viability was noticed. Surprisingly, rpDorsal knockdown decreased bacterial levels in anterior and posterior midgut. In insects silenced for both transcription factors, although lysozyme-A, lysozyme-B and defensin were down-regulated bacterial levels also decreased. Knockdown of rpDorsal or rpRelish did not affect T. cruzi development, suggesting that Toll and IMD pathways are not involved in the control of this parasite by the insect. **Supported by:**CNPq

HP29 - ROLE OF E-NTPDASE OF *LEISHMANIA AMAZONENSIS* AND *LEISHMANIA BRAZILIENSIS* IN THE ADHESION AND MODULATION OF MACROPHAGES

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Several studies have shown the importance of the E-NTPDase in the cell adhesion process of parasites to macrophages. Thus, we decided to evaluate the role of E-NTPDase-2 of *L. amazonensis* and *L. braziliensis* in adhesion and internalization of parasites in J774 macrophages. The ectonucleotidase activity was assessed by the hydrolysis of inorganic phosphate by incubating promastigotes of *L. amazonensis* or *L. braziliensis* with ATP, ADP or AMP. J774 macrophages were infected with *L. amazonensis* or *L. braziliensis* for 30 minutes or 3 hours and rates of adhesion and internalization were assessed by optical microscop y. After 3 hours, the cells were washed with PBS and stimulated for 48 hours with IFN-γ and LPS. NO production was determined by the Greiss method. Our results show that promastigotes of *L. braziliensis* have lower activity against all the nucleotides relative to promastigotes of *L. amazonensis*. Interestingly, promastigotes of L. braziliensis have lower adherence to J774 cells than *L. amazonensis* promastigotes. In *L. amazonensis*, pre-incubation of parasites with anti-NTPDase reduces rates of a dherence and internalization, while no effect was observed in *L. braziliensis*. Furthermore, *L. amazonensis* is able to resist to the inflammatory stimulus of LPS and IFN-γ, keeping the rates of infection in J774 cells. On the other hand, *L. braziliensis* is not resistant against inflammatory stimulus, which reduces its rate of infection. In part, this is explained by the ability of *L. amazonensis* reduce NO production by infected cells, unlike *L. braziliensis*. Our data demonstrate that E-NTPDase is important in adhesion, internalization and modulating of the immune response by infection initiated by *L. amazonensis*, but does not seem to be important in infectious initiated by *L. braziliensis.*
Supported by:FAPEMIG; CNPq

HP30 - EFFECT OF IRON CHELATOR ON THE ULTRASTRUCTURE, VIABILITY AND CYTOTOXICITY OF *TRICHOMONAS VAGINALIS*

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Trichomonas vaginalis is a flagellated parasite that causes Human Trichomoniasis, one of the most widespread STD in the world. The mechanisms by which parasites induce pathological alterations in their host are not completely understood. The aim of our study was to investigate the involvement of iron in the process of cytotoxicity, viability and adherence of the *T. vaginalis* to HeLa cells. The results demonstrate that withdrawal of iron from the culture medium affects the proliferation and ultrastructure of *T. vaginalis* and modulates the cytotoxicity. Using Scanning Electron Microscopy (SEM) and optic microscopy (OM) it was observed that T. vaginalis is highly plastic, acquiring amoeboid shape in contact with the host cell. However, the parasite losses such ability when treated with the iron chelator 2,2-dipyridyl. Analyses of the interaction of HeLa cells with parasites treated with 2,2-dipyridyl show decreased cytotoxicity and absence of apoptotic host cell phenotypes when compared with control cells. It was also observed that the iron chelator inhibits the intraspecific interaction of *T. vaginalis*, *i.e.* cell-cell adhesion, as well as inhibits cannibalism events detected in the control population cultured in iron-rich medium. Using specific markers for apoptosis, Annexin-V and TUNEL, parasites treated with 2,2-dipyridyl showed that chelator induces a cell death process different than apoptosis in part of the population of *T. vaginalis*. Together, these results suggest that iron is essential for the parasite survival and for the interaction with the host cell. **Supported by:**CAPES

HP31 - *TRYPANOSOMA CRUZI* INTRACELLULAR TRAFFIC: INFLUENCE ON HOST CELL RESPONSE AND PARASITE INTRACELLULAR DEVELOPMENT

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Previous work from our group has shown differential tissue tropism of two clonal populations of Trypanosoma cruzi, Col1.7G2 and JG (T. cruzi I and II, respectively), during mixed infections in BALB/c mice, with predominance of JG in the heart. Also, hearts of JG infected mice presented much more intense inflammatory process compared to hearts of Col1.7G2 infected mice. In vitro studies using embryonic cardiomyocyte cultures, showed that selection occurred due to higher intracellular multiplication rate of JG compared to Col1.7G2 in cardiomyocytes. The opposite was observed for infection in Vero cells. In an attempt to elucidate the mechanisms involved in this differential selection, we investigated distinct aspects of cellular response upon infection with these two T. cruzi populations along a 72 hour infection period in cardiomyocytes and fibroblasts isolated from neonate BALB/c mice. Our results corroborated previous data showing that, in cardiomyocytes, JG strain presented a higher rate of intracellular multiplication, while in fibroblasts this was observed for Col1.7G2. Cell supernatants of cardiomyocyte infected cultures were collected and we have found that infection induces the production of IL-6 in these cells with a substantial higher rate in cultures infected with JG. IL-6 production promotes inflammation in the heart by recruiting leukocytes, which could justify the more intense inflammatory process observed in hearts of JG infected mice. Production of other cytokines, such as TGF-β, which might explain the differences in parasite intracellular development, are under investigation. Additionally, we have evaluated the oxidative stress response generated in cardiomyocytes upon infection with these two parasite populations, but in the conditions tested we have found no difference between them. The same assays using shorter infection periods are being performed to evaluate whether parasite intracellular trafficking may also influence host cell response. **Supported by:**CAPES and CNPq

HP32 - EXPRESSION AND PROCESSING OF THE METACYCLIC STAGE-SPECIFIC SURFACE GLYCOPROTEIN GP82 OF *TRYPANOSOMA CRUZI*

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Infective forms of *Trypanosoma cruzi* are coated by a dense layer of GPI-anchored glycoproteins. Among those, the metacyclic stage-specific surface glycoprotein GP82 has been actively involved in the host invasion. A probe-derived screening on a metacyclic cDNA library allows us the isolation of a full-length cDNA (clone 5.4G6, GenBank EF154827) encoding a 726 aa protein with three in-frame methionines. The third methionine is surrounded by a Kozak consensus, followed by a canonical signal peptide, suggesting that the initiation of the translation begins at this point. The predicted mass of the polypeptide core after cleavage by the signal peptidase is 72 kDa which is in agreement with the experimental data obtained in metacyclic forms treated with tunicamycin. *T. cruzi* epimastigotes transfected with constructs encoding c-myc tagged GP82 proteins starting at the first or third methionines showed the same relative molecular size suggesting that those proteins were cleaved at the same position, although an alternative initiation on the third methionine cannot be ruled out. No differences were observed on western blots incubated with anti-c-myc or 3F6 monoclonal antibodies. Incubation of live transfected parasites with MAb3F6 showed that both constructs were expressed on the surface of epimastigotes through the entire cell. No signal was detected on live or fixed parasites incubated with anti-c-myc antibody, probably due to steric epitope hindrance. Triton X-114 extracts of transfected epimastigotes showed an enrichment of both tagged proteins in the detergent phase, similar to that obtained with native GP82 extracted from metacyclic trypomastigotes. Additional analyses are underway (e.g. PI-PLC digestion) to determine the nature of the attachment to the cell surface on both constructs. Mass spectrometry analyses are due to determine the signal peptide cleavage position and the GPI-anchor acceptor amino acid (ω). **Supported by:**FAPESP

HP33 - INTERACTION OF A NEWLY ATYPICAL BRAZILIAN STRAIN OF *TOXOPLASMA GONDII* WITH NEURONS AND GLIAL CELLS

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Background: Worldwide approximately two billion people are infected chronically with Toxoplasma gondii with unknown consequences. The infection is extremely successful, being able to cross the biological brain barrier and resulting in a chronical stage after the establishment of immune responses. The reactivation of the disease in immunocompromised patients causes toxoplasmic encephalitis with potential death. The toxoplasmosis treatment is limited varying with different strains. In Brazil T. gondii shows an uncommon genetic population which correlates to more frequently serious manifestations of the disease. We used a newly isolated and genotiped virulent strain obtained from the peripheral blood of infected human newborns (congenital toxoplasmosis) from 12 regions of Minas Gerais/ Brazil.
Objectives: To analyze in vitro infected neurons and glial cells by the virulent TgCTBr9 strain and stablish kinetics of the infected cells and neurites aspects
Methodology: The primary neuron and glial cells cultures obtained by the removal of the superior cervical ganglia of C57BL/6 mice were infected 1:10, host cell/parasite, for 24, 96 and 192 hours post-infection and then formalin-fixed for analysis of neuronal damage, infection rate and immunofluorescence.
Results: All the cultures showed neurons and glial cells containing parasitophorous vacuole with tachyzoites. At 192hs cultures we also found bradyzoites. At 96hs we observed decreased neurite density compared to the 24hs culture. As the time of cultures increased so did the infection rate. The survival rate of neurons did not change along the time.
Conclusion: Our data show that the virulent TgCTBr9 strain is able to infect neurons and glial cells in vitro. After 192hs of infection we see spontaneous encystment. This is an unprecedented result since there are no studies correlating this unusual strain and the pathogenesis of Toxoplasmosis in Brazil. **Supported by:**CNPq, FAPEMIG e CAPES

HP34 - HOST AUTOPHAGY REGULATES *TRYPANOSOMA CRUZI* INFECTION IN PRIMARY MACROPHAGES AND HEART MUSCLE CELLS

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The hemoflagellate protozoan *Trypanosoma cruzi* is the causative agent of Chagas disease. The outcome of the infection depends on both parasite internalization in mammalian cells and following fusion of the parasitophorous vacuole to the lysosome, key organelle related to endocytosis and autophagy. This process occurs in all eukaryotic cells to degrade macromolecules and cellular structures, but is still poorly understood during the *T. cruzi* infection. In the present work, we evaluated the participation of autophagy in infected macrophages and heart muscle cells (HMC). Starvation medium was used to exacerbate autophagy in host cells, before or after infection with bloodstream trypomastigotes (Y strain). Autophagy led to a decrease of the infection in macrophages (6 and 24h) and HMC (48h). Infection was also reduced when autophagy was induced after establishment of infection (up to 72h). Differences were also observed in parasite/ infected cell, suggesting different pathways in these cells, considering their phagocytic phenotype. By fluorescence microscopy, an increase of LC3 puncta was observed in all infected cells, corroborating that infection induces autophagy, much more exacerbated in starved cells. Ultrastructural analysis of starved cells showed autophagosome formation with typical concentric structures, surrounded by endoplasmic reticulum and close to lipid droplets (LDs) and intact parasites. Considering the role of LD in infection and autophagy, we evaluated the number of LDs, which decreased after starvation, and macrophages exhibited 5-fold more LDs than HMC, suggesting a role linked to inflammatory response during infection. Our data indicate that autophagy reduces infection and impairs *T. cruzi* proliferation with distinct profiles depending on the cell type. This may be an important mechanism to block parasite proliferation. **Supported by:**FAPERJ, CNPq and FIOCRUZ

HP35 - EFFECTS OF IRON DEPLETION BY 2,2-DIPYRIDYL IN *TRICHOMONAS VAGINALIS* PROTOZOAN DURING INTERACTION WITH EPITHELIAL CELLS

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The protozoan *Trichomonas vaginalis* is a pathogen commonly found inhabiting the human urogenital tract which causes trichomoniasis, a sexually transmitted disease (STD) that annually affects near 180 million people throughout the world. Although the cellular mechanisms of patogenicity not to be fully understood, it is known that the iron is an essential element, responsible for survival, growth, proteases expression, and adhesion in the host cells. The purpose of this work was to investigate the effect of iron depletion in the morphology and cytotoxicity of *T. vaginalis* isolates during interaction with epithelial cells. The parasites cultivated in complete iron-sufficient and iron-depleted medium were analysed by light and scanning electron microscopy. The interaction was analysed using HeLa cells monolayer and human vaginal epithelial cells. Trophozoites maintained in complete iron-sufficient medium were highly plastic, being able to interact with host cells, and assuming highly adherent amoeboid forms. The absence of iron resulted in dramatic changes in the morphology of *T. vaginalis*. Trophozoites underwent transformation from ellipsoid or amoeboid forms to rounded cells, whose flagella were internalized. Furthermore, the depletion iron strongly decreased the cytotoxicity of parasite in epithelial cells. Fresh isolates were far more cytotoxic than the well-established strain. Our data not only demonstrate that iron has a pivotal role in the biology of this microorganism, but also may indicate the direction to elucidate the mechanisms by which this metal regulates the expression of proteases and how it interferes in the virulence of this and other protozoa in the host-pathogen interaction. **Supported by:**UFSJ

HP36 - *TRYPANOSOMA CRUZI* BENZNIDAZOLE RESISTANCE: INVESTIGATION OF THE CONTRIBUTION OF ONE ABCG TRANSPORTER AND A MITOCHONDRIAL TYPE I NITROREDUCTASE

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Benznidazole (BZ) is usually adopted for first-line treatment of Chagas disease. However the drug has low efficacy in the chronic phase of the disease and regional differences in the treatment outcome have been reported. The reasons for treatment failures are unknown, but may be due to diverse BZ susceptibility among T. cruzi strains and/or characteristics of the immune system of the host. Previous evidence from our group indicated that a T. cruzi ABCG transporter single-copy gene (TcABCG1) is over-expressed in parasite strains with natural resistance to BZ. TcABCG1 amino acid variations between BZ-resistant TcI strains and BZ-susceptible CL Brener were detected (Araujo et al. 2011). A mitochondrial type I nitroreductase (TcNTR) has been involved in BZ-resistance by drug selection (Wilkinson et al. 2008). The goal of this study was to investigate the structure of TcABCG1 (1,995 bp) and TcNTR (939 bp) genes in parasite isolates obtained from four patients before and after BZ-treatment. All the isolates belonged to DTU TcII. The sensitivity to BZ was assessed in epimastigote forms. The IC50 values, corresponding to the drug concentration that caused 50% growth inhibition, varied from 10 to 55 µM BZ among the isolates. Comparison of BZ sensitivity between pre- and post-treatment isolates of each patient showed 2-fold increase of the post-treatment IC50 value in two patients. SNPs were observed in TcABCG1 gene of the isolates. However no amino acid changes were detected in the TcABCG1 proteins of pre- and post-treatment isolates of three patients. Three amino acid variations between the transporter proteins of the isolates of one patient were verified. The nucleotide sequence of TcNTR gene of the isolates of two patients was obtained. The nitroreductase protein sequence was conserved between pre- and post-treatment isolates of the two patients. Presently we are assessing the abundance of the TcABCG1 transporter protein in the isolates. **Supported by:**FAPESP and CNPq

HP37 - 5-NITRO-2-FURFURILIDEN DERIVATIVES ARE ACTIVE AGAINST *TRYPANOSOMA CRUZI* STRAINS BELONGING TO DISCRETE TYPING UNITS PREVALENT IN HUMAN PATIENTS.

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The search for new compounds for Chagas disease treatment is of high priority, because the only available drugs Benznidazole (BZ) and Nifurtimox (NFX) have low efficacy in the chronic phase of the disease. Nifuroxazide (NF) was used as lead compound to design and synthesize a set of fifteen compounds. The 5-nitro-2-furan group present in NF and NFX and considered as the pharmacophore was maintained in the skeleton of the NF analogues. The Lipinski's rule of 5' was taken into account, mainly regarding the hydrophobic property (ClogP), which was previously highly correlated with desirable IC50 values against Trypanosoma cruzi (Palace-Berl et al., 2013). The activity of the 5-Nitro-2-furfuriliden derivatives was assayed against epimastigote forms of three T. cruzi strains: Silvio X10 cl1 (TcI), Y (TcII) and Bug 2149 cl10 (TcV), that represent the discrete typing units more prevalent in human patients. TcI is a major agent of human infection in Amazonia, the Andean region, Central America, and Mexico; TcII in Brazil and TcV in other countries of the Southern Cone (Zingales et al., 2012). Very similar potency (log1/IC50) values for the three strains were verified for most of the NF analogues, which showed better anti-T. cruzi activity than BZ. In the cytotoxicity assay to LLC-MK2 cells only two compounds had IC50 values lower than 160 µM, which was the maximum drug concentration used. A time-kill assay (Moraes et al., 2014) was conducted to estimate how long intracellular amastigotes of the Y strain should be exposed to compound concentrations to reduce cellular infection to undetectable levels. NF analogues abolished intracellular infection at 3 – 10 µM with at least 96 h of compound exposure, while displaying minimal host cell cytotoxicity. These results suggest that the novel nitroderivatives have trypanocidal activity in vitro against both insect and mammalian replicative stages of divergent T. cruzi strains. **Supported by:**Fapesp e CNPq

HP38 - THERAPY WITH INFLIXIMAB DURING CHRONIC *TRYPANOSOMA CRUZI* INFECTION AMELIORATES ELECTRICAL ABNORMALITIES AND CARDIAC TISSUE DAMAGE IN ASSOCIATION WITH REDUCED TNF AND INCREASED IL-10 EXPRESSION

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Chagas disease (CD) is characterized by parasite persistence and immunological unbalance favoring inflammation. Chronic chagasic cardiomyopathy (CCC), the main clinical manifestation of CD, occurs in a TNF-enriched inflammatory milieu and frequently progresses to heart failure. To challenge the hypothesis that TNF plays a key role in *Trypanosoma cruzi*-induced immune deregulation and CCC pathogenesis, we tested the effect of anti-TNF antibody (Infliximab) on immune response, electrical abnormalities and heart injury in chronic experimental Chagas' heart disease. The results showed that C57BL/6 mice chronically infected with the Colombian *T. cruzi* strain presented immunological, electrical and histopathological abnormalities resembling patients with Chagas' heart disease. Treatment with Infliximab during chronic infection did not alter parasite load but repositioned the immune response. Anti-TNF therapy reduced chronic splenomegaly, decreased TNF mRNA expression in the cardiac tissue and plasma TNF and IFNγ levels. Further, anti-TNF therapy diminished TNF but increased IL-10 production by Ly6C+ and F4/80+ cells. Importantly, Infliximab therapy restored normal average heart rate and decreased *T. cruzi*-induced prolonged PR and QRS intervals and the frequencies of mice afflicted by arrhythmias and second degree atrio-ventricular blocks. Moreover, anti-TNF treatment decreased the deposition of fibronectin in the cardiac tissue of chronically infected mice. Anti-TNF therapy has beneficial effects in the immune response, which was positioned towards a more balanced profile, and in CCC improving heart electrical abnormalities and tissue damage. Therefore, these data support the hypothesis that in chronic *T. cruzi* infection TNF is a crucial player in the pathogenesis of Chagas' heart disease (i) fueling an inflammatory milieu and the immunological unbalance and (ii) contributing to cardiac tissue damage. **Supported by:**FAPERJ/CNPq

HP39 - TEMPERATURE AND PH ARE IMPORTANT SIGNALING IN REGULATING *LEISHMANIA (L.) AMAZONENSIS* AMINO ACID TRANSPORTER AAP3 EXPRESSION.

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The L-arginine plays an important role in the Leishmania mammal infection since it is a common substrate for both parasite arginase and host inducible nitric oxide synthase, directing the success or the control of infection. There are two copies of the amino acid transporter AAP3 coding gene arranged in tandem in the parasite genome. Promastigotes starved from arginine regulate the expression of the 5.1 AAP3 copy. While promastigotes live in the midgut of the sandfly vector (25°C – pH7.0), Leishmania amastigotes are found in the mammal macrophage phagolysosome (pH5.0 - 34°C). Here we evaluated if those signals also trigger the AAP3 regulation. Then, we treated *L. (L.) amazonensis* log-phase promastigotes or amastigotes-like ($5x10^7$) at 25°C or 34°C in pH5.0 or pH7.0 for 4 hours in medium EBSS without or with 400 µM of arginine to analyze both AAP3 mRNA expression by Real Time PCR. We confirmed that the AAP3 5.1 transcript is more abundant than the AAP3 4.7 in starvation condition and besides, the amount of the AAP3 5.1 transcript increased during arginine starvation at 34°C in both pHs compared to 25°C. The increase of the temperature also led to a raise of AAP3 4.7 expression. We also carried out arginine uptake assays in promastigotes or amastigotes, submitted to the same treatments. In promastigotes, the L-arginine uptake correlates with the increase of AAP3 5.1 transcript. Amastigotes submitted to starvation of arginine at 25°C, pH7.0 reverted the AAP3 5.1 expression to the same profile observed for promastigotes but there was not an increase in arginine uptake. The AAP3 4.7 transcript did not change significantly in any amastigote treatment. Our results indicated that the change of temperature and pH, besides starvation of arginine, might be important signals to regulate the differential expression of AAP3 5.1 transcript in promastigotes in stationary phase to assure the parasite success during the early stages of infection. **Supported by:**FAPESP and CNPq

HP40 - MOLECULAR KARYOTYPE OF TRYPANOSOMA RANGELI: INTEGRATION OF IN SILICO SEQUENCE ASSEMBLIES WITH THE CHROMOSOMAL BANDS SEPARATED BY PULSED FIELD GEL ELECTROPHORESIS

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Trypanosoma rangeli and Trypanosoma cruzi share vectors and mammalian hosts over a wide geographical area in Latin America. T. rangeli is considered non-pathogenic to mammalian hosts but harmful to triatomine vectors. The sequencing of T. cruzi and T. rangeli genomes has opened the possibility to link information from mapping studies to the underlying sequences of these trypanosomes. The karyotypes of Choachí and SC-58 isolates of T. rangeli are composed of 16 chromosomal bands varying from 0.40 Mb to 3.44 Mb: 2 to 3 bands with a large size ranging from 2.19 to 3.5 Mb, and 13 to 14 smaller bands, between 0.40 and 1.48 Mb (Stoco et al., 2014). Most of chromosomal bands hybridized with probes from protein encoding genes. To examine the level of synteny among T. rangeli and T. cruzi, markers from two large T. cruzi syntenic groups previously mapped on the chromosomal bands XX (3.27 Mb) and XVI (2.09 Mb) of clone CL Brener were used. Our preliminary results confirm the linkage of most of T. cruzi markers to T. rangeli chromosomes, indicating that the syntenic blocks are well conserved in both trypanosomes. However, we found a break of synteny at the chromosomal ends of T. rangeli. Despite these differences, T. rangeli and T. cruzi seem to exhibit a conserved chromosome structure. We are currently mapping other T. cruzi syntenic groups on T. rangeli karyotype. **Supported by:**Capes

HP41 - CURE OF RESISTANT *TRYPANOSOMA CRUZI* STRAIN WITH A TERPENE IN NANOSTRUCTURED FORMULATION IN THE CHRONIC PHASE OF MICE INFECTION

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Chagas disease (CD), originally present in Latin America, nowadays is neglected and autochthone in other Continents. The only drugs available to treat CD are benznidazole (BZ) and nifurtimox (NF). Terpene (TP) is a lipophilic substance active in vitro against T.cruzi isolated from Lychnophoras (Arnica), a Brazilian plant, that when used in nanostructured (NS) formulations as a carrier improves its therapeutic efficacy and reduces toxicity. TP was isolated and characterized previously, as well as its NS polymeric formulation (TPNS) with stealth polymer. This work evaluates the activity of TP compared to BZ in the treatment of the chronic phase of the infection in murine model. Young female Swiss mice, 20g of weigh were i.p inoculated with 500 blood trypomastigotes of the VL-10 strain (resistant to BZ and NF). The oral treatment started in the 90th day of infection for 20 consecutive days. The mice were divided in groups: TPNS1(8mg/kg/day), TPNS2(12mg/kg/day), BZ(100mg/kg/day) and negative controls (Infected untreated, NS unloaded). The treatment efficacy was assessed by parasitological (hemoculture, PCR) and serological (ELISA) tests. Animals infected and treated in with NSTP2 showed 88% of parasitological cure whereas the treatment with TPNS1 cured 43% of the animals and mice treated with BZ were not cured. Free TP reduced the parasitemia when compared to controls but no cure was observed. Therefore NS formulations were able to slowly release the new substance TP, strongly active against T.cruzi in the chronic phase of infection. These nanoparticles were capable of to change the pharmacokinetics of the active substance leading to an increased therapeutic efficacy at low doses, achieving cure rates of 43-88% of experimental animals infected with strain totally resistant to BZ in function of the doses used. These findings represent a great perspective for treatment of CD which constitutes one of the most serious neglected parasitic diseases in the word. **Supported by:**FAPEMIG/Rede TOXIFAR, CAPES, CNPq, UFOP

HP42 - DIFFERENTIAL ANALYSIS OF L-ARGININE METABOLISM BETWEEN *LEISHMANIA INFANTUM* STRAINS AND THEIR RELATION WITH INFECTIVITY *IN VITRO*

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Leishmaniasis is a group of diseases with a variety of clinical manifestations, which are highly dependent on *Leishmania* species, the immunological status of the host and the infectivity of the parasite strain. In this sense, the L-arginine metabolism is related with this infectivity, since this amino acid is shared by two enzymes nitric oxide synthase (NOS) and arginase that may act differently in the death or survival and multiplication of the parasite in macrophages. The nitric oxide ($^{\cdot}$NO) production by NOS and urea by arginase are present in the parasite and host cell causing a mix of enzymatic activities. Based in previous observations from our group, that two *Leishmania infantum* strains, one strain from Old World (*Li*OW) and another from New World (*Li*NW) demonstrated different profiles in the *in vivo* infection, the aim of this work was to study the L-arginine metabolism on these parasites. It was studied the $^{\cdot}$NO and urea production during the parasite growth, the cell cycle and the infectivity on BALB/c mice peritoneal macrophages. The evaluation of the parasitic growth curves showed a significant difference between the two strains. The Green method was used to analyse the $^{\cdot}$NO production but any significant value was not found in the parasite culture. However, the major arginase activity was showed on the third day for both *L. infantum* strains, those values were found at the same time the parasitic growth curves begin to enter in the stationary growth phase. The cell cycle and the *in vitro* infectivity of *L. infantum* strains are being investigated, however, the results up to now indicate that *Li*OW showed higher infection index *in vitro* compared to *Li*NW in 24 and 72 hours post-infection. The metabolism of L-arginine in the parasite can be considered a good therapeutic target, considering that the enzymes may modulate the host response to infection. **Supported by:**CNPq, FAPERJ, Fiocruz-RJ

HP43 - EFFICACY OF LACTONE IN NANOSTRUCTURED FOR TREATMENT OF THE EXPERIMENTAL CHRONIC PHASE OF CHAGAS DISEASE

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Chagas disease (CD) is a neglected zoonosis endemic in Latin America. The only drug currently available in Brazil is benznidazole (BZ) which causes severe side effects and presents low therapeutic efficacy, especially in the chronic phase of the infection. TP is a lactone isolated from Lychnophoras (Arnica) which presents in vitro and in vivo tripanocidal effect (acute phase). This substance is cytotoxic and highly lipophilic demanding a pharmaceutical formulation to carry it. Nanostructured (NS) allows controlled release of substances, selectivity for the target tissue and reduction of toxicity. This work evaluated the therapeutic efficacy of TP in NS formulations for treatment of the chronic experimental CD in murine model. The NS formulations containing TP (TP-NS) were produced and physic-chemically characterized. Swiss female mice were i.p. infected with 500 blood trypomastigotes of the Y strain of T.cruzi. Mice were treated by oral and iv routes for 20 consecutive days with Conventional TP-NS(oral dose 5.0mg/kg/day, iv dose 2.0mg/kg/day), Stealthy TP-NS(oral 5.0mg/kg/day; iv 2.0mg/kg/day) and BZ(oral 100mg/kg/day; iv 50mg/kg/day) and controls. Treatment was started 90 days post-infection. Both, NS formulations showed physic-chemical characteristics suitable for iv and oral administrations. After treatment mice were evaluated to assess parasitological cure. Treatment efficacy was verified by hemoculture, PCR and ELISA. Infected mice treated with conventional-NS showed 33% of parasitological cure (iv) and 30% (oral).Infected mice treated with stealthy TP-NS showed 50% of parasitological cure (oral) and 55% (iv). Infected mice treated with BZ were not cured. Data demonstrated the effect of TP-NS in vivo on the chronic CD and the high efficiency of NS formulations. This work demonstrates that TP is a potent substance for treatment of CD and improved therapeutic regimes must be explored in oral route, the most applicable. **Supported by:**FAPEMIG/Rede TOXIFAR, CAPES, CNPq, UFOP

HP44 - EFFECTS OF ORAL ADMINISTRATION OF PROBIOTICS IN EXPERIMENTAL MURINE TOXOPLASMOSIS

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Introduction: Probiotics are live micro-organisms capable of improving the intestinal microbial balance and when administered in sufficient quantities, can produce beneficial effects on the health of the host, resulting in an increased resistance against pathogens. Recently, probiotics have been used successfully to treat diseases caused by various protozoa. However, there have been no studies on the effects of probiotics in T. gondii infection. Objective: This study aims to investigate the effects of microbiota in T. gondii infection and the effects of probiotic treatment with regard to the survival and development of murine toxoplasmosis. Methods and Materials: Conventional, germ free Swiss mice will be utilized and infected orally with T. gondii cysts for analysis of intestinal permeability. For both the survival curve and the analysis of the effect of treatment on murine toxoplasmosis, the animals were inoculated via gavage with 10 cysts of strain TgCTBr07. After development of the survival curve, we selected probiotics (S. boulardii and E. coli Nissle 1917) for the analysis of weight gain and macrophage migration. The protocol was approved by CETEA/UFMG (394/2013). Data were analyzed adopting a significance level of $p < 0.05$. Results: Increased intestinal permeability in conventional animals indicated increased susceptibility to infection compared to Germ-free mice. Analysis of the survival curve, the weight development and recruitment of macrophage demonstrated that S. boulardii treatment was able to reduce the pathological effects of the infection in conventional mice but not it GF. Conclusion: The intestinal microbiota influences in the development of toxoplasmosis. The probiotic plays an important role in modulating the microbiota immune systems, helping limit the initial spread of the parasite through the recruitment of immune cells and preventing the loss of animal weight. Histological studies of the organs are in progress.

HP45 - MICRORNA REGULATES NO PRODUCTION IN *LEISHMANIA (LEISHMANIA) AMAZONENSIS* INFECTED MACROPHAGES

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The microRNAs (miRNAs) are non-coding RNAs (21-24 nt) that can modulate gene expression by the complementary binding of the initial 6 to 9 nucleotides of 5' region to the 3'UTR of target mRNA. The miRNAs can modulate inflammatory mechanisms during immune response by post-transcriptional regulation of genes involved in these pathways. Recent studies have described the role of miRNAs in modulation of Nitric Oxide (NO) production during inflammatory responses. Here, we determine if L. (L.) amazonensis infection can subvert the miRNAs profile of mouse macrophages as well as the production of NO. We analyzed the miRNA profile of total RNA from BALB/c mice Bone Marrow-Derived macrophage (BMDM) infected by L. (L.) amazonensis WT and L. (L.) amazonensis knockout for arginase (Arg-/-). We verified a modulation on 32% of the 84 miRNAs analyzed in BMDM infected with L. amazonensis WT compared to non-infected BMDM, whereas 60% were up-regulated. The infection with the Arg-/- parasite increased the percentage of total miRNAs modulated (45%) compared to WT, but only 35% of the miRNAs were up-regulated. Moreover, we showed the up-regulation of miR-721 and miR-294-3p in L. (L.) amazonensis WT infected macrophages, but not in Arg-/- infection, fact that corroborate with the increase in NO production and reduction on parasite infectivity. In silico analysis indicated nitric oxide sintase-2 (nos2) mRNA as a possible target for those miRNAs. Indeed, the NO production is higher in macrophages infected by Arg-/- when compared to WT. We showed that blocking the binding of these miRNAs to nos2 mRNA, the NOS2 expression and NO production is increased in macrophages during L. (L.) amazonensis infection. As expected, infection with L. amazonensis Arg-/- did not induce these miRNAs from BMDM, We concluded that L. amazonensis infection alters the miRNA profile of macrophages to subvert the host immune responses. **Supported by:**FAPESP and CNPq

HP46 - MOLECULAR CHARACTERIZATION AND GENE EXPRESSION PROFILE OF NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE-1 (LANTPDASE-1) IN LEISHMANIA AMAZONENSIS

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Leishmaniasis is a widespread disease, with approximately 12 million cases in the world (WHO 2011). The current forms in humans are tegumentar and visceral leishmaniasis. Leishmania amazonensis is the etiological agent of mucocutaneous leishmaniasis. Ecto-NTPDases are enzymes located at the external surface of the cells. They hydrolyze tri- and/or di-phosphate nucleotides and have a key role during the purines acquisition by trypanosomatids. Several studies have shown a correlation between ecto-NTPDase activity and infectivity of parasites. Therefore, this work aims to perform molecular characterization and analysis of NTPDase1 gene expression. To develop it, the central portion, comprising conserved regions of apyrases 1-5, besides the initial and terminal portions of the CDS were amplified by PCR, from DNA and cDNA of L. amazonensis. The PCR products were cloned and sequencing, in order to obtain the complete sequence from NTPDase1 gene. The CDS presented 1278 nucleotides, which encodes a protein with 425 amino acids. The sequence was deposited in GenBank under number of access JQ697233.2. The alignment performed with the predicted amino acid sequence showed a high identity between NTPDase1 sequences from other species of Leishmania genus, showing 98% to L. mexicana, 88% to L. infantum, 88% to L. donovani, 87% to L. major and 83% to L. braziliensis. From the gene sequence, we built a molecular model of the predicted protein and designed primers for the NTPDase1 gene expression analysis by RT-qPCR, during the axenic cultivation and in distinct evolutive parasite forms. There was a significant increase in NTPDase1 gene and protein expression from the 4th day of cultivation. Furthermore, expression of NTPDase1 between different evolutionary forms indicated a significant increase of 5.2±0.8 and 7.3±1.1 times to axenic amastigotes and amastigotes regarding the promastigotes. So far, that results suggest the role of this enzyme on infectivity. **Supported by:**CNPq PAPES VI

HP47 - PARASITE DENSITY AND HAEMATOLOGICAL CHANGES IN PATIENTS DURING AND AFTER VIVAX MALARIA

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Haematological changes are among the most common complications during episodes of malaria. Some blood parameters have been suggested as good markers of the severity of the disease, including suggestive for diagnosis of infection even before visualization of parasites on microscopy. In this study, 18 individuals from the Brazilian Amazon were sampled, 66.6% (12/18) were male, with mean age 33.5 years old. The diagnosis was first performed by conventional thick smear microscopy and subsequently confirmed by RT-PCR. The molecular diagnosis detected only episodes of malaria caused by *P. vivax*, confirming the results previously obtained by microscopy. The average parasitaemia of patients was 3266 (90 - 28500) parasites/mm^3 in the blood. To evaluate the possible hematologic changes during and after vivax malaria, blood samples were obtained during acute infection and on days 30 and 60 after infection. Regarding the parameters investigated, 44% (8/18) of patients proved to be anemic during malaria, with mean hemoglobin levels 12.9 (10.7 - 15.0) g/dL, increasing to 13.6 (11.4 - 15.3) g/dL during the monitoring period. Lymphopenia was observed in 38% (7/18) of individuals during the episode of illness, with a mean value of 1.31 (0.4 - 3.0) x 10^3/mm^3 and 2.0 (0.3 - 3.7) x 10^3/mm^3 during follow-up. Most significant changes were observed in relation to the platelets. During the acute cases, 77% of participants were thrombocytopenic, showing an average level of 98.2 (12 - 191) x 10^3/mm^3. During convalescence this index was normalized, with 207.3 (146 - 297) and 217.7 (148 - 292) x 10^3/mm^3 on days 30 and 60, respectively. No individual showed up thrombocytopenic during the follow-up period. Thus, become evident haematologic changes during vivax malaria and these changes were reversed after appropriate chemotherapy of malaria cases. **Supported by:**FAPEMIG, CNPq and UFJF

HP48 - PROFILE OF INTRACYTOPLASMIC CYTOKINE SYNTHESIS IFN-γ AND IL-4 BY NEUTROPHILS AND EOSINOPHILS IN PERIPHERAL BLOOD OF DOGS NATURALLY INFECTED BY *LEISHMANIA INFANTUM*

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Visceral leishmaniasis (VL) has great impact on public health and dogs are considered the main domestic reservoir of the parasite. In this context the search for immunological biomarkers is essential for understanding the mechanisms of resistance and susceptibility to human and canine disease. In this study, 124 dogs naturally infected by *L. infantum* from an endemic area were divided into four groups according to their clinical forms and the results of serological and molecular diagnosis: (i) asymptomatic dogs seronegative/PCR+ for *L. infantum* (AD-I), (ii) asymptomatic dogs seropositive (AD-II), (iii) symptomatic dogs seropositive (SD) and (iv) non infected animals as control group (CD). In addition, the *in vitro* studies were conducted employing biomarkers of resistance and susceptibility to canine visceral leishmaniasis (CVL), analyzing the intracytoplasmatic production of IFN-γ and IL-4 by neutrophils and eosinophils after stimulation with soluble *L. infantum* antigen (SLA). Our results confirm that after stimulation, the neutrophils increased the synthesis of IFN-γ in AD-II and SD groups, and presented a decreased production of IL-4 in the AD-I group. In relation to eosinophils in the control culture, a significant increase in the synthesis of IFN-γ and IL-4 was observed in AD-II and SD groups. These results suggest that the animals of AD-II and SD groups exhibit a mixed immune profile (Type 1 and 2). In contrast, animals of AD-I group do not differ from the CD dogs. The animals of AD-II and SD groups were unable to control parasite replication. **Supported by:**UFOP, FAPEMIG, CNPq, PPSUS/MS and DECIT/MS

HP49 - THE ACTIVITY OF CROTALIC VENOM AND ITS PURIFIED COMPOUNDS ON MURINE *LEISHMANIA (LEISHMANIA) AMAZONENSIS* - INFECTED MACROPHAGES

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Leishmaniasis threaten more than 350 million people worldwide specially in tropical and subtropical regions. Due to their relevance and morbidity leishmaniasis are considered an important public health problem by World Health Organization. The treatment of leishmaniasis comprises compounds which exhibit high toxicity, high cost, and in some cases parasite resistance and this scenario has motivated the search for new therapeutic approaches. Some studies have successfully reported the leishmanicidal properties of snake venoms as well as their purified compounds. The South American rattlesnake *Crotalus durissus* spp. contains several biologically active proteins such as gyroxin, crotamine, and convulxin. In the present study the activity of these three fractions purified from *Crotalus durissus terrificus* snake venom was tested on BALB/c bone marrow macrophages previously infected with *Leishmania (Leishmania) amazonensis*. Preliminary results showed that in the presence of 20 µg/ml of either gyroxin, convulsin or crotamine for 48 hours there was a reduction of 48, 42, and 30%, respectively, of intracellular amastigote growth. The cytotoxicity of the three venom fractions was tested by MTT method and data showed that in the presence of 50 µg/ml for 48 hours there was 100% of macrophage viability, opening perspectives to test the leishmanicidal effect of the three fractions at higher concentrations. The possible macrophage activation induced by the crotalic venom fractions through the production of nitric oxide, reactive oxygen species and cytokines in the supernatants of treated macrophage cultures is also under investigation. **Supported by:**FAPESP

HP50 - REPEATED AMINO ACID SEQUENCES IN TRYPANOSOMA CRUZI ANTIGENS AS VIRULENCE FACTORS DURING PARASITE INFECTION

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Using an immunoscreening approach, several antigens derived from a Trypanosoma cruzi amastigote cDNA library that react with sera from chagasic patients were isolated. Among them, 70% were found to contain repeated amino acid sequences. The antigen presenting sequence homology to the eukaryotic L7a ribosomal protein and containing an AKP repetitive domain at N-terminus was characterized. We investigated the role of amino acid repeats present in the T. cruzi L7a antigen by generating recombinant versions of the complete antigen (TcL7a) as well as truncated versions containing repetitive (TcL7aRep) or non-repetitive domain (TcL7a∆Rep) and immunizing mice with these antigens. Whereas mice immunized with TcL7a produced IgG antibodies against the complete protein as well as against the repetitive domain, they produced low levels of antibodies against the non-repetitive domain. On the other hand, immunization with TcL7aRep did not generate antibodies against any of the antigens. Whereas mice immunized with TcL7a produced high levels of IFN-γ, low levels of IFN-γ were detected in mice immunized with repeats. After challenging with trypomatigotes, we observed a partial protection in mice immunized with the TcL7a whereas immunization with TcL7a∆R did not alter parasitemia levels compared to controls. In contrast, same assay with TcL7aRep resulted in exacerbation of parasitemia compared to other groups and in 100% mortality rates. Our results suggest repetitive domains present in T. cruzi antigens may be used by parasite to modulate the host immune response, most likely by inducing B cell tolerance. To verify whether immunization with other T. cruzi antigens containing amino acid repeats also result in exacerbated parasitemia after challenging with trypomastigotes, we produced recombinant versions of complete trans-sialidase (TS) antigen and without the sequences encoding the C-terminal Shed Acute Parasite Antigen (SAPA) repeats, as well as TS antigen made of SAPA repeats. **Supported by:**CAPES, CNPq and INCTV

HP51 - HISTIDINE DEGRADATION PATHWAY IS DEPENDENT ON THE FORMATION OF A BI-ENZYMATIC COMPLEX IN *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is able to catabolize carbohydrates and amino acids as carbon and energy sources. Our group has been investigating the metabolism and biological roles of several amino acids including histidine (His). We showed that this amino acid is able to maintain the parasite viability under metabolic stress, and it can be oxidized producing CO_2 when used as an unique carbon source. We showed also that His can be incorporated in epimastigotes and metacyclic trypomastigotes forms in an ATP-dependent transport. Once in the cytoplasm, His is converted to urocanate and then to 4-imidazolone-5-propionate (IPA) by two sequential enzymatic steps catalyzed by His ammonia lyase and urocanase (TcUH), respectively. IPA can be converted into α-ketoglutarate (α-KG) in a fast non-enzymatic decomposition reaction, or act as the substrate of imidazolonepropionase (TcIP, EC: 3.5.2.7), which could lead to the production of glutamate (Glu). As IPA is extremely unstable in physiological conditions, its metabolic fate (a-KG or Glu) depend on its intra-molecular channeling from TcUH to TcIP. In this work we used the recombinant IP to demonstrate that TcIP is able to associate with TcUH forming the macromolecular complex that allow the production of Glu from His. We also obtained transfected lineages of *T. cruzi* expressing both TAP-*Tagged* TcIP and TcUH (TAP-*Tagging* methodology). The transfection process was confirmed by *western-blotting* and immunofluorescence revealed with anti-calmodulin, anti-UH and anti-IP antibodies. The transfected parasites showed no differences in growth rates when compared with wild type strain. We showed that the UH-IP complex occurs in vitro and we are confirming that it also occurs in vivo. From our results, we propose that the dynamic of such complex is able to regulate His metabolic fate: the Krebs cycle or to the biosynthesis of other amino acids. **Supported by:**CAPES

HP52 - THE ROLE OF IL-18 IN *LEISHMANIA AMAZONENSIS* INFECTION

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IL-18 is an important pro-inflammatory cytokine during Th1 immune responses due to its ability to induce IFN-γ production by NK and T cells. In addition, depending on the immunological environment, it can be associated with Th2 immune responses. Since the role of IL-18 during L.amazonensis infection is not clear yet, the aim of our work was to evaluate the importance of this cytokine during infection in C57BL/6 mice. For that, we infected Wt and IL-18KO mice in the ears with 103 metacyclic forms of the parasite and followed the development of the disease until 12 weeks post-infection. The infection with low doses of L. amazonensis induced much smaller lesions in IL-18KO mice from the third week comparing with Wt mice. The development of smaller lesions was associated with reduced parasite loads in the ears and in the draining lymph nodes at 8 and 12 weeks post-infection. Because macrophages are the mainly host cells for this parasite, we investigated if macrophages were involved in IL-18-mediated susceptibility to L. amazonensis infection. We showed that macrophages obtained from Wt or IL-18KO responded similarly to L. amazonensis infection in vitro. Moreover, we showed that C57BL6 Wt macrophages do not respond to IL-18, since they do not express IL-18R. In addition, we evaluated the kinetic of expression of IL-18 and IL-18R in lesions of Wt mice at 2, 4, 6, 8, 10 and 12 weeks after infection. We detected the expression of that cytokine and its receptor, but we did not find differences between the different time-points. In conclusion, our data points to a role played by IL-18 in partially promoting susceptibility in L. amazonensis infection. The way this cytokine is involved in this phenotype is not fully understand, but it is not through macrophage activation and it seems to be dependent on the interaction of IL-18 and other cytokines in activation of T and NK cells. Now we want to find what cytokines are involved in this infection in early and late time-points. **Supported by:**CAPES

HP53 - EVALUATION OF PROSTAGLANDIN F2-ALPHA SYNTHASE EXPRESSION IN *LEISHMANIA BRAZILIENSIS*

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Prostaglandin (PG) production is not restricted to mammals; recent studies have shown parasites able to synthesize PG from metabolites of arachidonic acid (AA). Prostaglandin F2a synthase (PGF2S) of *Leishmania* shares 34% identity and 51.4% similarity with the mammalian homolog (AKR1C3) and has been detected in the secretome of *L. braziliensis* and in the exosome of *L. donovani*. According to the TDR Targets Database the *L. major* PGF2S homolog has 13 putative antigenic epitopes, with 77.8% antigenicity and druggability index of 0.8 (range:0-1), making it one of the most antigenic proteins of L. major and a candidate drug target. We have shown that *Leishmania braziliensis* promastigotes are able to express Prostaglandin F2a Synthase (*Lbr*PGF2S) during all phases of axenic growth and that arachidonic acid, added to the culture, is a substrate for the synthesis of Prostaglandin F2a. Also, we have generated transfectants of *L. braziliensis* overexpressing *Lbr*PGF2S, which was confirmed by Southern and Western blotting, and this mutant displayed a higher survival rate within the macrophage when compared to the control transfectant. We observed the localization of *Lbr*PGF2S dispersed in the cytoplasm but with a high concentration near the flagellar pocket in promastigotes, by immunofluorescence. The analyses of *Lbr*PGF2S secretion and production of PGF2a and TH1/TH2 cytokines in infected host cell culture are underway. Our preliminary results show that *Lbr*PGF2S is constitutively expressed in promastigotes and demonstrates its catalytic activity to produce PGF2a. In addition we observed a correlation between the levels of the enzyme and survival rate within macrophages *in vitro*. Overall, our results indicate that *Lbr*PGF2S may be relevant for host-parasite interaction and a better understanding of its role must be pursued. **Supported by:**FAPESP - 2011/02040-4

HP54 - THE MAJOR ROLE OF LYSOSOMAL ASSOCITED MEMBRANE PROTEIN-2 (LAMP-2) IN TRYPANOSOMA CRUZI INFECTION OF HOST CELL .

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Trypanosoma cruzi enters host cells by inducing lysosomal exocytosis, which in turn leads to compensatory endocytosis events that drive parasites into host cells. Parasite initially resides in a vacuole containing lysosomal membrane, but later escapes and gets free in the cytosol, where it will start replicating. Sialic acid from host cells and parasite trans-sialidases have been implicated in both parasite invasion and vacuole escape by inducing the formation of a tight vacuole. We have previously shown that absence of both LAMP-1 and 2, major components of lysosomal membranes and also highly sialilated, decreases invasion and increases parasite intracellular multiplication. To test whether sialic acid present in both LAMP isoforms or intrinsic characteristics of each protein was important for the observed phenotype, we performed cell infection assays using LAMP-2 and LAMP-1/2 knock out (KO) cells, comparing to wild type cells. Absence of LAMP-2 alone was enough to reproduce both infection and intracellular multiplication phenotypes of LAMP-1/2 KO cells, indicating that LAMP-2 plays a major role in this process. Decreased invasion was due to two events. First, parasite adhesion on host cell surface was compromised in cells lacking LAMP-2. Additionally, these cells showed decreased efficiency in compensatory endocytosis after lysosomal secretion. On the other hand, no change in vacuole morphology was detected, indicating that protein itself rather than its sialic acid modifications was determinant for decreased invasion. We have also observed that in LAMP-2 KO cells parasites showed a tendency for a faster rate of escape from its vacuole. Since escape from parasitophorous vacuole precedes and is important for parasite multiplication, the higher rate of parasite intracellular development observed in LAMP-2 KO cells might be the result of a faster parasite escape from its vacuole. Our data strongly support a major role for LAMP-2 in T. cruzi infection of host cells. **Supported by:**CNPq e CAPES

HP55 - LINEAR B-CELL EPITOPE MAPPING OF MAPK3 AND MAPK4 FROM LEISHMANIA BRAZILIENSIS: IMPLICATIONS FOR SERODIAGNOSIS OF HUMAN AND CANINE LEISHMANIASIS

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The correct and early identification of humans and dogs infected with Leishmania are key steps in the Leishmaniasis control. Also, a method with high sensibility and specificity and low cost that allows screening of large number of samples is extremely valuable. In this study, we analyzed the potential of MAPK3 and MAPK4 proteins from L. braziliensis as antigen candidates for the serodiagnosis of human visceral and tegumentary leishmaniasis, as well as canine visceral disease. Additionally, we mapped linear B-cell epitopes in these proteins and select those epitopes which sequences are divergent in their orthologs in Homo sapiens, Canis familiaris and in Trypanosoma cruzi and compared the performance of these peptides with the recombinant protein using ELISA. Both MAPK3 and MAPK4 recombinant proteins showed better specificity in the immunodiagnosis of human and canine leishmaniasis than soluble parasite antigens and EIE-LVC kit. Furthermore, the performance of the serodiagnosis assay was improved using synthetic peptides corresponding to B-cell epitopes derived from both proteins. The methodology used to in this study can be applied to other pathogens as an attempt to identify novel antigens. **Supported by:**CAPES, CNPQ, FAPEMIG

HP56 - *LEISHMANIA (V.) BRAZILIENSIS* OBTAINED FROM MUCOSAL LESIONS ARE MORE RESISTANT TO HYDROGEN PEROXIDE AND NITRIC OXIDE

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Leishmania (Viannia) braziliensis is the major parasite present in cutaneous (CL) or mucosal (ML) leishmaniasis in Brazil. It is not clear how mucosal lesions appear after *L. (V.) braziliensis* infection, however, parasite factors that increase *Leishmania* survival and/or dissemination may be involved. To evaluate the resistance of parasites isolated from CL and ML to nitric oxide (NO) and reactive oxygen intermediates (ROIs), three CL (JCJ8c, RPL5c e CSA7c) and three ML (ASL9m, PPS6m e JBC8m) isolates were maintained in IFNgama Knockout (IFNgKO) mice and cultured in Grace´s Insect medium for three or eight days to obtain promastigotes in logarithmic phase or stationary phase, respectively. The ability of parasites to produce NO was evaluated by the Griess assay and the viability of parasites cultured in presence of different amount of NO donor (sodium nitroprussiate) or hydrogen peroxide (H_2O_2) was evaluated by the MTT assay. Additionally, it was investigated the ability of parasites to convert arginine to urea. Parasites from ML harvested in logarithmic phase of growing are more resistant to NO donor 1 mM SNP (viability: ML = 90.2 ± 4.5 % x CL = 75.8 ± 3.6 %; $p< 0.01$) and 5mM SNP (viability: ML= 81.5 ± 5.2 x CL = 58.7 ± 6.1%; $p< 0.05$) and 1mM H_2O_2 (viability: ML= 80.4 ± 22.2 % x CL = 49.7 ±4.6 %CL; $p<0.05$). Parasites from ML in stationary phase of growing were more resistant to 5mM H2O2 (viability: ML = 51.04±10.2 x CL = 28.4 ± 2.95 %; $p<0.05$). All parasites showed similar arginase activity and ability to produce NO. These results suggest that resistance to ROI or NO can be important for parasite maintenance in the host and dissemination to mucosal areas. **Supported by:**CAPES, FAPEG e CNPq

HP57 - EFFECT OF THE TREATMENT WITH THE PALADACYCLE COMPLEX DPPE 1.2 ASSOCIATED TO THE RECOMBINANT CYSTEINE PROTEINASE RLDCCYS1 ON *LEISHMANIA (LEISHMANIA) AMAZONENSIS* INFECTION IN A MURINE MODEL.

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Leishmaniasis affect 12 million people worldwide and 350 million are at risk of infection. Treatment of leishmaniasis is limited by toxicity and parasite resistance to the drugs currently in use, validating the need to develop new leishmanicidal compounds. Previous data from our laboratory showed the in vitro and in vivo activity of the palladacycle complex DPPE 1.2 against Leishmania (Leishmania) amazonensis. The leishmanicidal effect of DPPE 1.2 was followed by immunomodulation characterized by a significant increase of CD4+ and CD8+ lymphocyte expression in treated L. (L.) amazonensis-infected BALB/c mice. In the present study the leishmanicidal effect of DPPE 1.2 associated to a recombinant Leishmania cysteine proteinase, rLdccys1, was tested in BALB/c mice infected with L. (L.) amazonensis. Preliminary results showed a small but not significant reduction of lesion size in animals treated with either DPPE 1.2 alone or associated to rLdccys1. However, the evaluation of parasite load by limiting dilution showed a reduction of 96% in both treated groups and of 42% in mice treated with rLdccys1 alone compared to mice which received PBS. Analysis of CD4+ and CD8+ lymphocytes in the draining lymph nodes by FACS showed 26-35% of CD4+ and 11-13% of CD8+ in all tested groups. The evaluation of parasite load 20 days after the end of treatment showed a reduction of 82% either in animals treated with DPPE 1.2 alone or associated to rLdccys1. It was also demonstrated a small but significant increase of CD4+ expression in mice treated with DPPE1.2 plus rLdccys1 compared to the other groups, whereas there was no difference of CD8+ expression among all groups tested. The small but significant recovery of parasite load 20 days after the end of treatment led us to increase the DPPE 1.2 concentration used. These experiments as well as the cytokine dosages in foot lesions of treated mice are currently in progress. **Supported by:**FAPESP

HP58 - *TRYPANOSOMA CRUZI*: INCREASED PROLINE UPTAKE AND OXIDATIVE STRESS

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Trypanosoma cruzi, the etiological agent of Chagas' disease, has a metabolism largely based on the consumption of glucose and proline. Additionally this amino acid is involved in differentiation processes and resistance to several stresses, oxidative, metabolic and osmotic among them. In this work, we studied the importance of proline uptake in oxidative stress and against trypanocidal drugs. We generated a transgenic model of *T. cruzi* that overexpresses the proline transporter Tc069. These parasites showed a significant increase on the proline transport rate and also had a higher intracellular proline concentration when compared to controls. The uptake was inhibited by all the proline analogues tested, but no by other natural amino acids except D-proline, that competitively inhibited the transport. This lack of stereospecificity results interesting since *T. cruzi* has two functional proline racemases. We also tested the effect of an increased proline transport in oxidative stress responses (hydrogen peroxide and nitric oxide) and also in trypanocidal drugs resistance (nifurtimox and benznidazol). We found that Tc069 parasites were more resistant than controls in all the cases. Taken together, our results show that decreasing intracellular proline levels, e.g. through blockage of proline transporter Tc069, could affect the survival ability against host natural defenses or pharmacological treatments. **Supported by:**Fapesp

HP59 - THE TRANSCRIPTION MODE OF *P. FALCIPARUM SURF* GENES AND FUNCTIONAL ANALYSIS OF A CONSTITUTIVELY EXPRESSED SURFIN

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Blood stage forms of the protozoan parasite *Plasmodium* cause malaria and express virulence factors at the surface of the infected red blood cell (IRBC) and many of them are encoded by multigene families. In *Plasmodium falciparum*, the most important disease-related IRBC exposed antigens are the PfEMP1 antigens encoded by the *var* gene family. *Var* gene expression is controlled in tight manner termed allelic exclusion. Another, smaller gene family which appears to be not as variant as the *var* genes comprise the 10 *surf* (*surface-associated interspersed genes*) genes with yet unknown function. Herein we monitored the transcription mode of *surf* genes using continuous blood culture of tightly synchronized *P. falciparum* parasites, followed by RT-qPCR analysis of *surf* transcripts after 20 and 40 reinvasion cycles. While four out of ten genes showed differing transcript quantities, transcription of PF3D7_07_0402200 (*surf*4) was reproducibly stable throughout the experiment. The remaining *surf* genes were transcriptionally silent during all time points. In order to elucidate the biological role of the corresponding protein we genetically tagged the protein with GFP and a destabilizing domain (DD24). First experiments using a cloned parasite line expressing GFP and DD24 showed a differential growth rate when destabilizing the *surf*4-GFP-DD24 protein and detailed phenotype analyses are currently under way. **Supported by:**FAPESP

HP60 - EFFECT OF *TRYPANOSOMA CRUZI* INFECTION ON CARDIAC FIBROBLASTS *IN VITRO* AND *IN VIVO*

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Cardiac fibrosis is an important manifestation of Chagas cardiomyopathy. The involvement of myofibroblast in this process is unknown. Here we investigated the role of cardiac fibroblast (CF) on generation of cardiac fibrosis induced by *Trypanosoma cruzi* Y strain using models of infection *in vitro* and *in vivo* (Swiss Webster mice). We studied the effect of *T. cruzi* infection (Y strain) on *in vitro* activation of primary CF cultures, in a kinetic study from 6 to 72 hours of infection (hpi) analyzing proliferation, myofibroblast differentiation and extracellular matrix (ECM) expression. In our culture system, 30% of CF were infected by *T. cruzi* using a multiplicity of infection (MOI) of 10:1 parasite:cell. Our preliminary data suggest that *T. cruzi* is not able to induce CF proliferation as evaluated by ki-67 expression. However, Western blot (WB) analysis of alpha smooth muscle actin (α-SMA) expression revealed cell differentiation. At 6hpi, increased expression of α-SMA was detected in *T. cruzi*-infected CF, when compared with non-infected (NI) controls. Further, at 48 hpi increased expression of the ECM components fibronectin (FN) and laminin (LN) was detected in *T. cruzi*-infected compared with NI primary CF cultures. Moreover, in an *in vivo* model we verified that acute infection (15 days-post infection) induced increased expression of FN and α-SMA in the cardiac tissue, paralleling the peak of heart parasitism and inflammation. Therefore, our *in vitro* results suggest an effect of *T. cruzi* on activation of CF. Also, the parallelism between CF activation and heart parasitism/inflammation raises the idea that inflammatory cells may also contribute to cardiac fibroblast activation and genesis of fibrosis in Chagas' heart disease. These cues are presently under investigation. **Supported by:**CNPq

HP61 - MURINE SEVERE MALARIA: EVALUATION OF PROTECTIVE IMMUNITY AFTER IMMUNIZATION WITH *PLASMODIUM BERGHEI* STRAIN OF LOW VIRULENCE

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Given the large number of clinical cases and deaths attributed to malaria, efforts have been focused on trying to develop an effective malaria vaccine. Historically, the best results in the induction of immunity were achieved using living sporozoites, attenuated or not. However, combined with other factors, difficulties in obtaining these forms have hampered the use of this methodology. Currently, vaccine studies using mature blood stage parasites have shown good results. However, considering that some strains of Plasmodium are more virulent than others, inducing a more severe form of clinical presentation, this study aims to assess whether malaria induced by *Plasmodium berghei* NK-65 (strain of low virulence in mice C57/BL6) is capable of inducing protection against severe malaria induced by murine *P. berghei* ANKA (causative of cerebral malaria in C57/BL6 mice). For this purpose, animals were immunized once or twice with 10^3 *P. berghei* NK-65 infected erythrocytes and subsequently challenged with 10^5 *P. berghei* ANKA infected erythrocytes. As shown by ELISA, the immunized animals showed high levels of IgG antibodies (reactivity index / RI = 1.05 to 24.22) in the first cycle of immunization. Furthermore, immunizations reduced parasite burden in up to 85% (6.39% to 0.76%) when the challenge was done with homologous strain and 49.5% (1,98% to 0,98%) when the challenge was done with the heterologous strain. Interestingly, 25% when challenged with heterologous strain that used in the challenge (*P. berghei* ANKA) remained alive at day 7 after challenge. Our results also showed that levels of anti-inflammatory (IL-10) and pro-inflammatory cytokines (TNF-α and IFN-ɣ) were concentrated in higher levels in the lung than in the brain. However, the correlation between cytokine levels and development of lesions in lung and brain tissues are still under investigation. **Supported by:**CNPq FAPEMIG UFJF

HP62 - CYTOKINES INVOLVED IN CARDIAC LESIONS ASSOCIATED WITH ORAL INFECTION BY TRYPANOSOMA CRUZI IN MICE

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It is suggested that oral infection by T. cruzi could lead to a distinct parasite and/or immune response profile compared with other routes of infection. Thus, the objective of the project was to characterize the cardiac lesions and the immune response against oral infection in experimental Chagas' disease in mice, quantifying the pro-and anti-inflammatory cytokines in the heart as well as analyzing the cardiac tissue parasitism. There were used 40 Swiss mice infected with Berenice-78 (Be-78) strain, equally divided into 2 groups, one infected intraperitoneally (IR) and other by oral route (OR) and made an assessment of the relative mRNA expression of pro -inflammatory (IFN-γ and TNF-α) and anti-inflammatory cytokines (IL-10 and TGF-β) by Real Time - PCR and the quantification of cardiac parasitism by immunohistochemistry. The Intraperitoneal infection had higher levels of IFN-γ compared to the OR and control (C) groups on days 14 and 28 after inoculation of the parasite, which was held on the 35th day, where there was a significant increase in cytokine on RO animals. For TNF-α and IL-10 cytokines there was a significant increase expression in the animals orally infected on the 28th day of infection when compared to IR and C groups, this increase was maintained at day 35 but compared only to Control group. The analysis of cardiac parasitism demonstrated a low tissue parasitism in animals infected with Be-78 strain by both intraperitoneal and oral routes. Thus, it can be seen that there was an increase in the relative expression of mRNA for the cytokines studied first in the intraperitoneal route and later in the oral route. In this sense, it can be inferred that this delay in cardiac cytokine expression occurs due to the gastric barrier that has several mechanisms that holdup the penetration and multiplication of T. cruzi and retards activation of the immune response in the heart. **Supported by:**CNPq, Fapemig e UFOP

HP63 - SEROPREVALENCE OF TOXOPLASMOSIS IN CAMELS IN LIBYA

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Toxoplasmosis is a worldwide contagious disease of humans and other warm-blooded animals including birds caused by coccidian parasite *Toxoplasma gondii*. The aim of this study was to determine the prevalence of antibodies to *T*. *gondii* among camels by latex agglutination test (LAT) in the central area in Libya.
A total of 1344 camel blood samples were collected, 809 blood samples were obtained from the jugular vein of camel, and 535 blood samples were collected from abattoirs from June 2008 to April 2009. Those samples were collected randomly from the central area of Libya.
Out of 1344 serum samples which were screened for the seropositivity to *T*. *gondii* antibodies, a total of 269 (20.0%) were found to be seropositive for *T*. *gondii* infection. The prevalence of *T*. *gondii* antibodies among males and females was (19.8%), and (20.3%), respectively. The prevalence rate was higher in the age of 1-3 years (63.5%). The present serological results indicated a widespread exposure to *T*. *gondii* among camels in the central area of Libya.
Supported by: Libyan National Center for Diseases Control in Tripoli-Libya

HP64 - CHARACTERIZATION OF THE ANTIOXIDANT SYSTEM ENZYMES IN *TRYPANOSOMA RANGELI*

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It is well known that the antioxidant system of trypanosomatids plays a major role on the establishment of the infection. However, there is no description of the antioxidant defenses of *Trypanosoma rangeli,* a hemoflagellate protozoan parasite considered as non-pathogenic to mammals and whose course of infection in these hosts is still controversial. In this study we report the initial characterization of *T. rangeli* genes encoding for trypanothione reductase (TrTRed) and for a cytosolic (TrTRPxcit) and a mitochondrial (TrTRPxmit) tryparedoxin peroxidase. *In silico* analysis of sequences retrieved from the *T. rangeli* genome pointed out a 77% identity between TrTRPxcit and TrTRPxmit and a single complete copy of each gene was found on the haploid genome. PCR amplification from gDNA using primers designed based on the *T. rangeli* genome sequencing resulted in amplicons of ~0.6, 0.7 and 1.2Kb, for TrTRPxcit, TrTRPxmit and TrTRed, respectively. Identity with the *T. cruzi* orthologous genes was of 89% for TrTRPxcit, 84% for TrTRPxmit and 82% to TrTRed. The cysteine-rich domains (FTFVCPTE) involved in mediating the peroxidase activity are present in both tryparedoxin peroxidase sequences, being divergent in the region II. While TrTRPcit is identical to that found in most other peroxiredoxins 2-cys (HGEVCPA), TrTRPxmit reveals to be identical to *T. cruzi* (NGDVIPC). Polypeptides binding sites that are involved on the formation of dimers and decamers are also observed. Also, a dimerization domain and the pyridine nucleotide-disulphide oxidoreductase domain, a small NADH binding domain within the larger FAD binding domain, were observed in TrTRed. This study confirms the presence of these genes in *T. rangeli*, having identified the conservation of the active sites as observed in *T. cruzi*. Heterologous expression and functional studies are in progress in order to extend the characterization of the antioxidant system in *T. rangeli.* **Supported by:**CAPES, CNPq, FINEP and UFSC

HP65 - COMPARISON OF DIFFERENT EXERCISE PROTOCOLS ON THE MICROBICIDAL ACTIVITY OF MACROPHAGES: LEISHMANIA MAJOR AS AN EXPERIMENTAL MODEL

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Physical exercise could be beneficial for health through responses and adaptations promoted in diverse biological systems, including the immune system. Moderate-intensity exercise can reduce the risk of infection by intracellular microorganisms, by directing the immune response towards a Th1 pattern. Present study investigates the effect of 12 weeks of swimming (chronic exercise) and a single exercise session (acute exercise) on nitric oxide production by Balb/C peritoneal macrophages. Balb/C mice were divided into 2 groups to chronic exercise (sedentary control and moderate exercise) and 3 to acute exercise (sedentary control, moderate exercise and vigorous exercise). Chronic moderate-intensity exercise consisted of 3 sessions per week of moderate-intensity swimming. Acute exercise consisted of a single session of moderate-intensity exercise and a single intense session. Macrophages isolated from animals submitted to swimming activity for 12 weeks (chronic exercise), when stimulated by LPS, showed a significant increase in NO production compared to macrophages isolated from the sedentary group under the same conditions. A single session of moderate exercise (acute) promoted a significant increase in NO production by macrophages stimulated with LPS and infected either with Leishmania major or Leishmania braziliensis. A single session of moderate and intense exercise (acute exercise) resulted in a decrease of the infection index Leishmania/macrophage infection in vitro by Leishmania major and Leishmania braziliensis. These data suggest that chronic and acute physical exercise promotes an improvement in macrophages microbicidal capacity, which may facilitates the resolution of intracellular infections. **Supported by:**FAPERJ

HP66 - AMITRIPTYLINE AND CYCLOBENZAPRINE ARE EFFECTIVE IN VITRO ANTILESHMANIALS AND SUPPRESS PRO-INFLAMMATORY CYTOKINES

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Based in the drug repurposing approach, two chemically related drugs, amitriptyline (AMT) and cyclobenzaprine (CBP) have been clinically used in the treatment of depression and as a skeletal muscle relaxant, respectively. With the aim to identify new therapeutic alternatives for Visceral Leishmaniasis (VL), this study was carried out to investigate the anti-*Leishmania infantum* activity and their possible activation of the cellular immune response. Both drugs exerted a leishmanicidal effect, with IC50 values of 21 µM (CBP) and 8 µM (AMT) against promastigotes and 112 µM (CBP) and 38 µM (AMT) against intracellular amastigotes. No toxicity was observed for NCTC cells. By using flow cytometry analysis, AMT incubated with infected macrophages (peritoneal) from BALB/c mice induced suppression of TNF, IFN-γ, MCP-1, IL-10, IL-6, but when co-cultivated with lymphocytes, a high production of IFN-γ was observed, demonstrating a cooperation between these cells. CPB demonstrated a similar effect in infected macrophages, but when co-cultivated with lymphocytes did not elicit production of IFN-γ. In addition, AMP and CBP induced no NO production. Considering the structural differences between AMT and CBP, it is clear that the presence of a double bound in the aromatic ring of CBP conferred a reduce effectiveness. The leishmanicidal effect promoted by both drugs was independent of NO production. Considering that the high levels of IL-6 and IL-10 are correlated with an elevated parasite burden, the AMT-suppression of these cytokines, with the increased IFN-γ levels in the presence of lymphocytes, suggest AMT as a promising drug candidate for future pre-clinical studies. **Supported by:**FAPESP 2013/07275-5.

HP67 - DIFFERENTIAL ACTIVATION OF PROTEIN TYROSINE KINASES IN HUMAN EPITHELIAL CELLS DURING INVASION BY DIFFERENT *TRYPANOSOMA CRUZI* STRAINS IN NUTRIENT-DEPLETED MEDIUM

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Metacyclic trypomastigotes (MT) of *T. cruzi* G and CL strains differ markedly in their ability to enter cultured mammalian cells. In serum-containing DMEM, the number of CL strain MT that invade human epithelial HeLa cells is about 3-fold higher than G strain MT. Such a difference is presumably related to the fact that CL strain invasion of HeLa cells in serum-containing DMEM is facilitated by actin cytoskeleton disruption whereas G strain invasion is apparently associated with actin recruitment. The difference in infectivity between CL and G strains observed in full nutrient condition decreases when the parasites interact with HeLa cells in PBS^{++}, a solution devoid of macromolecules or aminoacids, which induces lysosome exocytosis required for parasite internalization. Under this condition, the evidences are that both strains invade cells in a manner mediated by metacyclic-stage-specific surface molecule gp82, which triggers actin cytoskeleton disorganization and lysosome mobilization to the cell periphery. However, MT of CL and G strains may be inducing distinct signaling pathways in host cells, one dependent and the other independent of protein tyrosine kinase (PTK). Pretreatment of HeLa cells with genistein, a PTK inhibitor, markedly reduced the susceptibility to G strain MT, but not to CL strain MT. By confocal microscopy, we examined whether CL and G strain MT were internalized in a parasitophorous vacuole containing lysosome markers. Most internalized CL strain MT were positive for lysosome markers (LAMP-1, LAMP-2) whereas many G strain MT lacked the referred markers, suggesting that these parasites may be entering through plasma membrane invagination. Experiments are under way to clarify whether G strain MT-containing membranes are positive or not for Rab5, which defines early endosomes, or for Rab7, which is associated with late endosome and is a main factor in endosomal membrane trafficking. **Supported by:**CNPq e FAPESP

HP68 - CHARACTERIZATION OF NEUTROPHILS EXTRACELLULAR TRAPS INDUCED BY *LEISHMANIA (VIANNIA) BRAZILIENSIS*.

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Neutrophils release extracelullar traps (NET), a DNA structure decorated with granular and cytoplasmic proteins which ensnare and kill many *Leishmania* species. *Leishmania* susceptibility to NET killing can be subverted by the expression of 3'-nucleotidase by the parasites. Because NET induction by *L. (V.) braziliensis* (Lb) has not yet been demonstrated, we characterized NET induction by 3 different Lb strains. Neutrophils from healthy blood donors were isolated and incubated with promastigotes of Lb isolated from cutaneous (CL), mucocutaneous (ML) or disseminated (DL) leishmaniasis patients. NET was measured as free DNA in the supernatants of promastigotes-stimulated neutrophils. Data is expressed as mean ± SEM of the fold-change related to control values. We observed that the 3 strains differently stimulate NET release: CL induced significantly more NET (4.13 ± 0.81 N=12) than ML (2.55 ± 0.25 N=11) and DL (1.87 ± 0.19 N=12) Lb. These differences were not associated with the neutrophil ROS production induced by each strain or ratio of neutrophil-parasite interaction. Next, we determined the percentage of NET-killed parasite by XTT assay. We observed a higher susceptibility of the CL (24.02 ± 3.56 N=3) compared to ML (8.12 ± 5.45 N=3) and DL (12.98 ± 0.12 N=3) promastigotes. Then, we evaluated the 3'-nucleotidase activity of the Lb strains by measuring the Pi released from 3'-AMP substrate. DL and ML parasites displayed the highest 3'-nucleotidase activity. Our results show that Lb induces and is killed by NETs, although it may vary between strains. These differences might be explained by the levels of 3'-nucleotidase activity from each strain. Taken together, our data suggests that differences in NET induction and parasite escape through 3'nucleotidase might be determinant in the development of leishmaniasis clinical forms. **Supported by:**NIH AI 30639, FAPERJ, CAPEs and CNPq

HP69 - LAMININ INDUCES NEUTROPHIL EXTRACELLULAR TRAPS (NETS) RELEASE BY HUMAN NEUTROPHILS AND CO-LOCALIZES WITH NETS INDUCED BY *LEISHMANIA AMAZONENSIS.*

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Neutrophils upon various stimuli such as *Leishmania amazonensis* (La), release extracellular traps (NETs), composed of decondensed chromatin associated with granular and cytosolic proteins. La promastigotes are inoculated by its insect vector into a blood pool where it enters in contact with neutrophils and the extracellular matrix proteins (ECM). Here, we study the interaction neutrophils with laminins (a major ECM glycoprotein) and La, either isolated or in association. Initially, neutrophils from healthy blood donors were stimulated with La and NETs extruded were assessed for co-localization with different laminins isoforms by fluorescence microscopy. Neutrophils express α1 and α5 and have a mild expression of α4 laminin chain. These cells are negative for α2. Interestingly, α1 and α5 co-localize with NET-DNA. Next, the expression of the laminin receptor integrin α6 (CD49f) was evaluated by flow cytometry, and our results showed that around 70% of the neutrophils expressed this receptor. Following we verified if different laminin isoforms (111, 211, 411, 421 and 511) stimulate NET release by neutrophils. Our results showed that neutrophils stimulated with all different laminin isoforms tested were able to release NETs in a dose dependent manner. However, any of the tested laminin isoforms modulate the production of NETs by neutrophils stimulated with La promastigotes. Together, these results show that neutrophils express different laminin isoforms and its receptor and laminin induces NET extrusion. Moreover, endogenous laminin is released in association with NETs when neutrophils were stimulated by La.

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HP70 - TRANSCRIPTOME ANALYSIS OF TRYPANOSOMA CRUZI DURING INFECTION OF CULTURED CELLS

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Chagas' disease is a debilitating disease that affects millions of people in several countries. Trypanosoma cruzi, its causative agent, is a digenetic protozoan parasite that undergoes several differentiation steps during its life cycle, and the success and virulence of infection is dependent on host cell invasion. Several key steps are necessary for a successful invasion by the parasite, from binding to cell surface receptors to escape from the phagolysosome to differentiation and cell division. Our aim is to identify parasite genes relevant to host cell invasion and differentiation by large-scale transcriptome analysis and thus increase our understanding of T. cruzi's biology. Next-generation sequencing of RNA transcripts (RNA-seq) has been described as the preferred approach to quantify transcript levels. The transcriptome of trypomastigotes before and after host cell invasion was obtained by deep sequencing. Total RNA was extracted, depleted of rRNA, fragmented, and used for whole transcriptome sequencing with the Ion Total RNA-seq kit (Life Technologies). The RNA fragments were converted to cDNA and amplified by emulsion PCR in an Ion OneTouch System (Life Technologies), and then subjected to DNA sequencing in an Ion Torrent Personal Genome Machine (Life Technologies). Between 2-3 x 106 reads per sample were generated. In a reference-based strategy, reads were mapped to the publicly available genomic sequence of T. cruzi using the CLC Genomics Workbench software package. Differential gene expression analysis of parasite cells at different time points during invasion is currently being performed.

Supported by:CNPq, FAPERJ and CAPES

HP71 - QUALITY OF TYPE 1 IMMUNE RESPONSE INDUCED BY LEISHMANIA (L.) DONOVANI NUCLEOSIDE HYDROLASE (NH36) PEPTIDES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OBTAINED FROM CUTANEOUS LEISHMANIASIS PATIENTS.

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Leishmaniasis is a group of diseases caused by different species of parasites from the genus Leishmania. The diverse clinical presentations of the disease depend upon which Leishmania species is involved and also upon host-related factors. A bivalent vaccine that could generate protective immunity to the agents of visceral and cutaneous forms would be useful for control of leishmaniasis in countries where both diseases are endemic. The nucleoside hydrolase of Leishmania donovani (NH36) is the main component of the licensed vaccine against canine visceral leishmaniasis, Leishmune®, with high homology among Leishmania parasites, being thus a good candidate for a bivalent Leishmania vaccine. Recombinant peptides of NH36 were generated (F1, F2 and F3). Previously studies showed that mice immunized with F3 were protected against L. chagasi infection. Protection was mainly mediated by CD4T. The F1 and F3 vaccines also decreased the footpad lesion caused by L. amazonensis. We are characterizing the quality of type 1 immune response induced by L. donovani NH36 peptides, in peripheral blood mononuclear cells (PBMC) obtained from localized cutaneous leishmaniasis patients. After treatment, there is an increase in TNFα single positive cells, followed by IL2 and IFNγ single producers. An increase in multifunctional CD4T cells (IFNγ TNFα IL-2 triple producers) was observed for NH36, F1 and F2. These results suggest that peptide domains F1 and F2 are good inducers of CD4 T cells, corroborating the epitope prediction data. Stimulation with NH36, F1, F2 and F3 peptide domains induced mainly CD8T that were single producers of IFNγ, followed by TNFα and IL2 single producers. Interestingly, IFNγ+TNFα+IL2+ CD8T cells were observed in healed patients only in response to F3 peptide. These preliminary results show that the whole NH36 molecule and all 3 peptide domains are recognized by PBMCs from patients with localized cutaneous leishmaniasis, caused by L. braziliensis. **Supported by:**CNPq, FAPERJ, CAPES

HP72 - INVASION OF TRYPANOSOMA CRUZI METACYCLIC TRYPOMASTIGOTES IS INFLUENCED BY INCREASED HOST CELL LYSOSOME BIOGENESIS

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BACKGROUND: Mobilization of host cell lysosomes from the perinuclear region to the cell periphery and subsequent exocytosis are key events for T. cruzi metacyclic trypomastigotes (MT) invasion. Lysosome exocytosis contributes to the parasitophorous vacuole biogenesis. Short time incubation of human epithelial HeLa cells in PBS++, a solution without nutrients, increases lysosome exocytosis and susceptibility to infection by MT. Whether this increase reflects an increased lyososome numbers is unclear. Here we addressed that question using HeLa cells induced to effectively generate lysosomes.
METHODS AND FINDINGS: MT were incubated for 1 h in Hela cells in full-nutrient medium or in PBS++, and the number of internalized parasites was counted. HeLa cells maintained for 1 h in PBS++ were two to three-fold more susceptible to MT invasion than cells in full-nutrient medium. Analysis of HeLa cells after incubation in PBS++, by confocal immunofluorescence microscopy using anti-LAMP2 antibodies, showed a large number of lysosomes scattered near the cell periphery. To increase the number of lysosomes, HeLa cells were grown for 36 hours in complete medium with 100 mM sucrose. This resulted in increased lysosome numbers. Cells were subsequently plated on coverslips and maintained overnight in sucrose-free medium for use in MT invasion assays. MT invasion was significantly higher in cells pretreated with sucrose than in untreated controls. Fluorescence confocal images using anti-LAMP2 showed swollen lysosomes (sucrosome), in greater numbers than in untreated cells. Consistent with this observation, we detected increased levels of membrane and luminal proteins of lysosomes, such as LAMP1, LAMP2 and cathepsin D. As compared to untreated controls, fewer parasites were LAMP-2 positive at 30 min post-infection, acquisition of LAMP2 increasing at a later time (1 hour). CONCLUSIONS: Our results demonstrated a close association between the quantity of host-lysosomes and MT invasion. **Supported by:**FAPESP, CNPQ

HP73 - *TITYUS SERRULATUS*' VENOM POTENCIAL EFFECTS AGAINST *TRYPANOSOMA CRUZI* INFECTION

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Introduction: *Trypanosoma cruzi* (Tc) is the etiologic agent of Chagas' disease. Chemotherapy for this neglected tropical disease is available, but it's limited and often presents side effects and high toxicity. Nitric oxide (NO) is one of the most important molecules, produced by several cells, including macrophages (MO), in response to cytokines' (CK) signaling that impair the parasites' growth and dissemination. *Tityus serrulatus*' venom (TsV) or its fractions (Ts) modulate inflammatory mediators produced by MO, although the pathway by which TsV acts is so far unknown. Mitogen-activated protein kinases (MAPK) are involved in many cellular processes regulating several signaling transduction pathways. Our aim here was to unravel the mechanism by which TsV acts as an immunomodulator during Tc infection and determine its potential beneficial effects in Chagas' treatment. Methods and Results: Peritoneal MO from C57Bl/6 mice were stimulated with Tc antigen (AgTc - 10µg) and/or TsV (400µg/ml). Cells lysates were analyzed by Western blot with equal protein amounts. MO were also stimulated with Ts (100|50|25|12,5|6,25µg/ml) and supernatant was collected after 24 and 48 hours for NO (GRIESS). TsV didn't interfere in Tc's uptake, but increased MO microbicidal potential through a higher NO production, which resulted in decreased trypomastigotes' release. TsV also induced proinflammatory (IL-6, TNFα) and decrease regulatory CK levels (IL-10) produced by infected MO. We found that TsV could acts synergistically with AgTc regulating some MAPK, increasing its phosphorylation (as seen to p38) or reducing it (for JNK and ERK1/2) in a time-dependent manner. TsV fractions, Ts6 and Ts7, were able to induce great amounts of NO, suggesting two groups of potential key molecules present in TsV able to modulate the immune response to Tc infection. Conclusion: Together, these results suggest that TsV is a candidate to act as a potential immunoregulator during experimental Chagas' disease. **Supported by:**CNPq, CAPES, FAPEMIG

HP74 - SOCS2 MODULATES NEUTROPHIL APOPTOSIS AND B CELLS FUNCTION DURING TRYPANOSOMA CRUZI INFECTION

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Background. Trypanosoma cruzi causes Chagas Disease and, during this infection, the innate immune response is important to control the pathogen growth and progress of pathology. Supressor of cytokine signaling (SOCS)2 is induced by Lipoxin (LXA) production and is crucial for the regulation of immune response. Here we investigated the role of SOCS2 in the modulation of neutrophil apoptosis and B lymphocytes function during T. cruzi infection. Methods and results. WT and SOCS2 KO mice were infected with T. cruzi (Y strain) and in different days post infection, serum samples were obtained and immunoglobulin production was analysed. Serum from WT and SOCS2 KO mice was incubated with T. cruzi antigen (1h) and than with anti IgG. T. cruzi infection induced an increased IgG production by SOCS2 KO when compared with WT mice. Also, we observed a reduced parasitemia and high mortality in uMT KO mice (mice deficient in B cells) when compared with WT counterparts. In addition, bone marrow neutrophils from WT and SOCS2 KO mice were purified and incubated with LXA (1ug/ml) (8hs) and/or with TNF (100 ng/ml) (20hs) and the levels of Caspase-3and Bax was analysed by Western Blot. SOCS2 deficient neutrophils results in elevated levels of Caspase 3 Total, caspase 3 cleaved and reduced Bax levels when compared with WT cells. Conclusions. Taken together, the results indicated that SOCS2 is important in the regulation of immunoglobulin production and signaling pathway associated with neutrophil apoptosis during T. cruzi infection. Keywords: SOCS2, innate immunity, B cells, Trypanosoma cruzi. **Supported by:**CNPq and FAPEMIG

HP75 - DEFICIENCY OF ARYL HYDROCARBON RECEPTOR RESULTS IN CLEARANCE OF *TRYPANOSOMA CRUZI* ASSOCIATED WITH REDUCTION OF REACTIVE OXYGEN SPECIES

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Introduction: *Trypanosoma cruzi* is etiological agent of Chagas disease, and the balance of immune response (IR) is essential to control parasite growth. Reactive oxygen species (ROS) has a dual role during trypanosomatids biology and the aryl hydrocarbon receptor (AhR), a transcription factor, generates metabolites that lead to ROS release. However, is still unknown the role of AhR and the consequences of its absence in the control of parasite replication. Methods and Results: To know the relation among AhR, ROS and *T. cruzi*, we infected WT and AhR knockout (KO) peritoneal macrophages (MΦ). The amastigote rate was evaluated at 4h and 48h post infection. Parasite growth was also evaluated by daily counting of released trypomastigotes in the supernatant. Our results demonstrated no difference in the uptake of parasite by AhR KO- and WT cells. However, we found that the amount of amastigotes 48h post infection and realize of trypomastigotes were lower in AhR KO MΦ. To investigated which was the mainly factor responsible for the radically increased of parasite clearance in the AhR KO MΦ we performed a real time chemiluminescence assay for ROS detection. For this WT and AhR deficient MΦ were isolated and cultured as described above. After overnight culture, luminol, zymosan A, and parasites were added to culture. Light emission was quantified every two minutes during three hours. We observed that unstimulated AhR-deficient MΦ produced similar levels of ROS than WT cells. However, the deficiency of AhR resulted in dramatically decreased in the production of ROS by MΦ stimulated with Zymosan and infected with *T. cruzi.* Conclusion: These results suggest that AhR controls the levels of ROS production by *T. cruzi*-infected MΦ, which are probably necessary for the parasite replication. This is the first report about the role of AhR in the modulation of IR and ROS production during *T. cruzi* infection. **Supported by:**FAPEMIG and CNPq

HP76 - TRYPANOSOMA CRUZI CYSTEINE PROTEINASE DEGRADES FIBRONECTIN THAT FUNCTION AS BARRIER FOR METACYCLIC TRYPOMASTIGOTE INVASION OF HOST CELLS

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Extracellular matrix (ECM) proteins, which serve as substrates for diverse adhesion molecules, may mediate cell attachment and/or invasion of pathogenic microorganisms. Invasion of tissue culture trypomastigotes of T. cruzi is modulated by diverse ECM components, such as fibronectin (FN), collagen and laminin. The role of these proteins in metacyclic trypomastigote (MT) internalization is unclear. Here we examined the involvement of FN in MT invasion. Cell invasion assays, performed using HeLa cells and CL strain MT, showed that FN present in DMEM containing 10% FBS did not impair parasite internalization, but higher FN concentrations had an inhibitory effect. Unlike human alveolar epithelial A549 cells, HeLa cells do not express FN on the surface. In serum-free medium, CL strain MT invaded A549 cells and HeLa cells pretreated with TGF-β to induce FN expression, at significantly lower numbers than untreated HeLa cells. Fibronectin present in cell culture medium bound to CL strain MT and was digested by cruzipain, the major T. cruzi cysteine proteinase, as indicated by the observation that treatment of parasites with E-64, a cysteine proteinase inhibitor, increased the association with FN. Unlike CL strain, G strain MT, with negligible cruzipain activity, displayed FN on the surface upon incubation in cell culture medium. Gp82, the metacyclic stage-specific surface molecule implicated in cell invasion and expressed in both CL and G strains, bound to FN. More direct evidence of FN-digesting activity of CL MT cruzipain was provided by and assay in which the parasites, pretreated or not with E-64, were incubated with FN, which was analyzed by SDS-PAGE and silver staining of the gel. FN was extensively degraded in a manner inhibitable by E64. By contrast, G strain MT exhibited negligible activity toward FN. Taken together, our results indicate that cruzipain plays a role in gp82-mediated internalization of T. cruzi MT, through its digesting effect on FN that functions as a barrier rather than as bridge for the parasite interaction with the host cell receptor. **Supported by:**CNPq e FAPESP

HP77 - MODULATION OF INNATE IMMUNE RESPONSE FUNCTION BY 5-LIPOXYGENASE INHIBITOR (ZILEUTON) DURING EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION

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Introduction. Chagas' disease is an illness, highly neglected, caused by the protozoan Trypanosoma cruzi, and is the major cause of heart disease in the endemic areas. The search for new treatment is important for find ways to control T. cruzi infection. The innate immunology is very important for infection control through cellular and humoral immunities. Cells like dendritic cells and macrophages act secreting cellular mediators and molecules that contribute with cellular activation and/or producing molecules against T. cruzi. Use of drugs that affect this cells can leads to modulation of immune system and consequent change in infection and immune profile. Thus we used a marketed 5-lipoxygenase inhibitor, enzyme responsible for lipid mediators production like leukotrienes (LT) and lipoxins (LXA), to evaluate its effects in T. cruzi infection. LT e LXA acts in innate immune cells upregulating or downregulating them activities. Methods and results. WT mice were infected and treated with Zileuton (30mg/kg) and the parasitemia, levels of cytokines in serum and the expression levels of mRNA for cytokines were verified in the heart, spleen and liver. Splenic cells were analyzed by cytometry flow. Our results showed that treatment with Zileuton interfered in immunity profile and resulted in decrease of parasitaemia in treated mice. The serum levels of IL-12 decreased 9 and 15 days after infection in Zileuton treated mice. Cells CD11c+CD8+IL-12p70+ and F4/80+IL-12p70+ increase in spleen 15 days after infection in treated mice compared with non treated mice. mRNA expression of IFN-γ and IL-6 decreased in splenic tissue 9 days after infection. mRNA expression of IFN- γ decrease and IL-6 increase in heart 9 and 15 days after infection in treated mice. Conclusion. In summary, our results suggest that the treatment with Zileuton could be a tool in the therapeutic pathway to modulate immune response during experimental Chagas' disease. **Supported by:**CNPq, FUNDEP/Santander and FAPEMIG.

HP78 - ANTIGENICITY EVALUATION OF MASP MULTIGENE FAMILY OF *TRYPANOSOMA CRUZI*

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The *Trypanosoma cruzi* genome contains a large number of multigene families that encode surface proteins, among them the Mucin-Associated Surface Protein (MASP) family. MASP genes are clustered internally in the chromosomes, in regions that lack of synteny with *Trypanosoma brucei* and *Leishmania major* genomes. MASP proteins are highly polymorphic and are mainly expressed at the surface of trypomastigote stage, suggesting that this family may participate in host-parasite interactions. We speculate that MASP may be involved in host cell invasion and/or may participate in immune evasion mechanisms. To better characterize the immune response to this polymorphic family, it is necessary to select a set of members that may represent the MASP overall sequence variability. To this end, we have clustered its protein sequences within seven subgroups based on their sequence similarity and selected members representative of each subgroup to obtain the corresponding recombinant proteins. The selected genes were amplified from CL Brener genome, cloned into the pET-28a-TEV vector and expressed in *Escherichia coli* BL-21Star. The antigenic profile of MASP subgroups is currently been evaluated by ELISA using three panels of sera. First, sera from mice infected with *T. cruzi* CL Brener strain from the 2, 6, 10, 19 and 22 passages were used to investigate whether MASP antigenic profile changes during successive passages in mice. Second, sera from C57BL/6 mice at 5, 10, 15, 20, 25 and 30 days post-infection were tested to analyze MASP recognition profile during the acute phase of experimental infection. Third, sera from C57BL/6 mice collected after three months of experimental infection were used to determinate MASP antigenic properties during the chronic phase of *T. cruzi* infection. Preliminary results suggest that MASP members derived from distinct subgroups are differently recognized during the acute *T. cruzi* infection. **Supported by:**CNPq, FAPEMIG, CAPES, INCTV

HP79 - INTERLEUKIN-17 DOES NOT INTERFERE WITH *LEISHMANIA AMAZONENSIS* INFECTION OF THYOGLYCOLATE-ELICITED PERITONEAL MACROPHAGES FROM C57BL/6 MICE.

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IL-17 is a proinflammatory cytokine secreted mainly by activated T cells that has been implicated in several inflammatory diseases. This cytokine stimulates a variety of cells, including macrophages, to produce inflammatory mediators; nevertheless, the role of IL-17 in the microbicidal function of macrophages is unclear. We investigated whether IL-17 is able to interfere with the ability of murine macrophages to produce nitric oxide (NO), IL-12p40, IL-10 and to kill Leishmania amazonensis. Thyoglycolate-elicited peritoneal macrophages from male C57BL/6 mice were primed with IL-17 and/or IFNγ before the stimulus with lipopolysaccharide (LPS) or infection with *L. amazonensis* promastigotes. Culture supernatants were harvested 48 h after stimulus to assess NO and cytokine production. The cells were analyzed 24 and 72 h after *Leishmania* infection to analyze leishmanicidal activity. IL-17 alone did not induce NO or IL-12p40 production, but increased IL-12p40 and IL-10 production in LPS stimulated macrophage. Additionally, IL-17 was able to synergize with IFNγ in macrophages to increase NO production. The IL-17 does not interfere with the parasite burden in cells infected with *L. amazonensis* 72 hours after infection. These results show that IL-17 alone is unable to interfere with activation of C57BL/6 macrophages, but IL-17 can interact with other products present in the site of infection to interfere with microbicidal capacity of macrophages. **Supported by:**FAPEG, CNPQ, CAPES

HP80 - ASSESSMENT OF SUBCELLULAR LOCALIZATION AND GENE KNOCKOUT OF TWO PROMISING *LEISHMANIA INFANTUM* ANTIGENS

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Repetitive proteins are known to be highly immunogenic, due to their capacity to stimulate the B lymphocytes, independent of T cells presentation. Our group has previously identified a group of 13 new antigenic proteins from Leishmania infantum, that are rich in repetitive motifs and classified as hypothetical in gene databases. Two of these antigens (Lci10 and Lci12) displayed high sensitivities when tested with dog and human sera. This work aimed to better characterize these antigens, investigating aspects of their subcellular localization and phenotype after single and double knockout. The selected proteins were analyzed through immunofluorescence and by cell fractionation using gradients of digitonine and freeze/thawing, to investigate their localization in promastigote and amastigote forms. The knockouts were performed through the homologous recombination method, with silencing cassettes generated by phusion PCR. The resulting strains were tested to confirm the deletion of the endogenous genes and to evaluate their growth in vitro. The results from the immunofluorescence and the cell fractionation showed that the Lci10 was present in the cytoplasm, associated to proteins that interact with cell membranes or nucleus. The Lci12 was found in membrane/organelar fractions and in the cytoplasm. After single knockout, all strains (Lci10 and Lci12) grew less than the wild-type controls. Cultures were recovered from the double knockout, and the growth curves displayed the same results as the single knockout. Confirmation PCR performed on the double knockout strains, showed the presence of the target gene, as well as, the silencing cassetes. These results indicate that the assessed proteins are essential to the survival of L. infantum, due to the presence of the target gene after the double knockout. Due to their immunogenicity and the essentiality to the cell, these proteins are strong candidates to the development of new serological tests and vaccine targets. **Supported by:**CNPq/CAPES

HP81 - USE OF PCR IN MONITORING THE CHAGASIC CARDIAC TRANSPLANTED PATIENTS: A TOOL FOR EARLY IDENTIFICATION OF INFECTION REACTIVATION.

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The Chagas disease (CD) is considered the third most common parasitic infection in the world. It is estimated that 6 to 8 million people are infected causing 12 thousand deaths per year. About a third of those infected develop cardiac or digestive complications and 20-30% of the cardiac forms can lead to heart failure or sudden death. Heart transplantation is a valid therapeutic option for patients with chronic CD. However, after transplantation patients are submitted to periodic endomyocardial biopsies (EB) to monitor transplant but the differential diagnosis between cellular rejection and CD reactivation has been considered difficult delaying the correct treatment. In this study, we evaluated the possibility of PCR use in the follow up EB, focused on nuclear and mitochondrial markers for early diagnosis of the presence of *T. cruzi* (Tc) reactivation. From 2009 to 2014, we analyzed 412 EB from 57 chagasic cardiac patients who underwent heart transplantation at the Hospital das Clínicas da UFMG. The *T. cruzi* DNA was found in 93 samples and approximately 60% of patients have shown at least one positive result for the molecular diagnosis tests performed. Positive results were detected in the firsts held EB, 1–18 months before the clinical reactivation, which indicates that PCR contribute to the early diagnosis of CD reactivation with potential to assist in treatment decisions. Although the factors that determine disease reactivation in some patients, but not in others, are not yet completely understood, certainly a relevant contribution is attributed to the genetic aspects of the parasite. Therefore, we characterize the populations of Tc into I-VI in positive samples using sequential PCR tests based on 3 markers: the COII gene, the intergenic spacer of miniexon gene and rDNA 24Sα gene. Most of our patients had reactivation by TcII supporting the idea that this is the main strain associated with the cardiac form of CD reactivation, at least in this geographical region. **Supported by:**FAPEMIG, CNPq, CAPES

HP82 - TYPE I STRAINS OF *TOXOPLAMA GONDII*, BUT NOT TYPE II, INDUCES INOS DEGRADATION, INCREASES ARG1 EXPRESSION AND PERSISTS IN ACTIVATED MACROPHAGES

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Macrophages activated with interferon-gamma (IFN-γ) and lipopolysaccharide (LPS) express inducible nitric oxide (NO) synthase (iNOS) and produces NO. NO control *Toxoplasma gondii* growth by inhibiting some biochemical pathways. However, *T. gondii* has evolved evasion mechanisms such as the degradation of iNOS and consequently the inhibition of NO production. Another evasion mechanism of *T. gondii* is the induction of arginase 1 (ARG1) expression in macrophages after infection. ARG1 down modulates NO production by competing with iNOS for the substrate, arginine. However, there are few studies on the induction of ARG1 in macrophages infected with different strains of T. gondii. Thus, the investigation of the kinetics of iNOS and ARG1 expression in macrophages infected with different strains of *T. gondii* is important to possibly develop new treatments for toxoplasmosis. For this, peritoneal macrophage was cultured with DMEM supplemented with 10% FBS. RH strain of *T. gondii* was maintained in the peritoneal cavity of Swiss mice and the ME-49 strain in infected Vero cells. Macrophages was activated for 24h with LPS and IFN-γ, and infected with *T. gondii*. Cells attached to coverslips and supernatant were collected after the infection and iNOS and ARG1 expression was assayed by immunofluorescence and NO production determined by the Griess reagent. *T. gondii* of the RH strain degraded iNOS and inhibited NO production right after 2h of infection not allowing its return after 6, 12, and 24 h of infection, and an increase in ARG1 expression was observed. However, *T. gondii* of the ME-49 strain degraded iNOS and inhibited NO production only in the first hours of infection, and was not capable of maintain its degradation; an increase in ARG1 expression was also seen. These results may help to explain how the virulent strains of *T. gondii*, as RH strain, can persist in activated macrophages. **Supported by:**UENF, CAPES, CNPq, FAPERJ

HP83 - *TRYPANOSOMA CRUZI* INDUCES TROPHOBLAST DIFFERENTIATION: A POTENTIAL LOCAL ANTIPARASITIC MECHANISM OF THE HUMAN PLACENTA?

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During congenital transmission of Chagas disease, *Trypanosoma cruzi (T. cruzi)* breaks down the placental barrier formed by the trophoblast, basal laminae and villous stroma. The trophoblast, first placental tissue in contact with maternal blood, undergoes continuous epithelial turnover (meaning cellular proliferation, differentiation and cell death), which is considered part of innate immunity. Considering the low congenital transmission rate, we propose that *T. cruzi* induces differentiation in the trophoblast as part of a possible local antiparasitic mechanism of the placenta.
We analyzed β-human chorionic gonadotropin (β-hCG) and syncytin expression in human placental chorionic villi explants (HPCVE) and BeWo cells (trophoblast cell line) using immunofluorescence and western blotting. Additionally, β-hCG secretion into the culture medium was measured by ELISA. We assessed the differentiation of trophoblastic cells in BeWo cells using the two-color fusion assay and by determining desmoplakin re-distribution.
T. cruzi trypomastigotes induce β-hCG secretion and expression as well as syncyntin expression in HPCVE and BeWo cells. Additionally, the parasite induces the trophoblast fusion of BeWo cells.
We conclude that *T. cruzi* induces differentiation of the trophoblast. Our results suggest, that the turnover of the trophoblast may be a component of local antiparasitic mechanisms in the human placenta. **Supported by:**FONDECYT 1120230, CONICYT-PBCT Anillo ACT 112 CONICYT / MINCYT 2011-595-CH/11/08

HP84 - *TRYPANOSOMA CRUZI* INDUCES TROPHOBLAST PROLIFERATION: THE TROPHOBLAST TURNOVER IS PART A LOCAL ANTIPARASITIC MECHANISM OF HUMAN PLACENTA?

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Congenital Chagas disease is caused by the protozoan *Trypanosoma cruzi (T. cruzi)*. The parasite reaches the fetus through the placental barrier formed by trophoblast, fetal connective tissue and basal laminae. However, congenital transmission rate is relatively low, so that there may be local antiparasitic factors. The trophoblast, first placental tissue in contact with maternal blood, undergoes continuous epithelial turnover (meaning cellular proliferation, differentiation and cell death). The epithelial replacement is considered part of innate immunity. We propose that *T. cruzi* induces cell proliferation in the trophoblast. BrdU incorporation was analyzed in human placental chorionic villi explants (HPCVE) and BeWo cells (trophoblast cell line), using immunohistochemistry and spectrophotometry. Additionally the cell cycle phases was analyzed in BeWo cells by flow cytometry and the mitotic index was determined. In HPCVE immunohistochemically quantified as Ki67 and PCNA nuclear proliferation markers. Low concentrations of the parasite induces increased proliferation of the trophoblast. We conclude that the induction of trophoblast proliferation as part of mechanism trophoblast turnover may constitute part of the innate immune mechanisms against parasitic aggression. **Supported by:**FONDECYT 1120230, CONICYT-PBCT Anillo ACT 112 CONICYT / MINCYT 2011-595-CH/11/08

HP85 - ANALYSIS OF "EXPRESSION CURVE" OF PTERIDINE PATHWAY AND ANTIOXIDANT ENZYMES OF *LEISHMANIA (V.) BRAZILIENSIS* STRAINS NATURALLY RESISTANT/SUSCEPTIBLE TO NITRIC OXIDE

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Leishmania (V.) braziliensis is associated with various clinical outcomes of American Tegumentary Leishmaniasis (ATL) including self-healing localized cutaneous lesions, multiple disseminated lesions and metastasis to oropharyngeal mucosa. The production of cytokines, reactive oxygen species (ROS) and nitric oxide (NO) by host macrophages normally leads to the destruction of phagocytosed parasites. The inducible nitric oxide synthase (iNOS) catalyzes the synthesis of NO from arginine, but the enzyme arginase competes with iNOS for the substrate, resulting in the production of polyamines and failing to control the intracellular replication of Leishmania. The *L. (V.) braziliensis* strains used in this study were previously characterized as being naturally resistant or susceptible to NO. The resistant strain was also related to higher number of lesions than the susceptible strain, as well as to resistance to pentavalent antimony, the first-line drug for ATL. However, the molecules and/or metabolic pathways of the parasite that contribute to such resistance are unknown. The aim of this work is to identify molecules associated with NO resistance that ultimately may be related to the different clinical manifestations caused by these polar strains. Evaluation of parasite response to ROS showed similar susceptibility of both strains to exogenous H_2O_2 and Menadiona, indicating that ROS isn't a mandatory part of the molecular mechanism involved in the observed phenotype. Time course analysis of mRNA expression of arginase 1, pteridine pathway enzymes and antioxidant enzymes showed an increase in the levels of such enzymes at the end points of the growth curve, when the cultures are rich on metacyclic parasites. Fold change ratio of the gene expression between resistant and susceptible strains showed that the resistant one presented 2 fold expression for tryparedoxin peroxidase. Our results suggest that higher levels of antioxidant enzymes could be related to higher ROS and RNS natural resistance in this species. **Supported by:**CAPES, FIOCRUZ

HP86 - STUDY OF GENETIC POLYMORPHISMS IN THE TGF-BETA 1 GENE IN PATIENTS WITH CHRONIC CHAGAS DISEASE

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Studies developed by our group demonstrated the involvement of TGF-b in the development of Chagas heart disease. It was already observed that TGF-b circulating levels are increased in patients with cardiac damage. After ten years follow-up, patients with higher levels of circulating TGF-b1, progressed Chagas disease with worse prognosis. Recently, it was reported that the polymorphism at codon10 of the gene encoding TGF-b1 might influence the production of this cytokine. In populations of Colombia and Peru, the same polymorphism is also involved in susceptibility to T. cruzi infection. Thus, the aim of this study is to evaluate the polymorphism of the alleles of the TGF-b1 gene in patients in the chronic phase of Chagas disease correlating the expression of different alleles of the TGF-b1 gene, serum levels of this cytokines and the clinical outcome of Chagas disease. For this, 180 individuals were invited and consented to participate in the study. Analysis of five single nucleotide polymorphisms were performed by PCR methods and sequencing the fragments of TGF-b1 gene. Furthermore, serum levels of this molecule were measured by ELISA. In the promoter region, a discrepancy was observed between the analyzed polymorphisms: the polymorphism in the-800G>A region was present in 10% of patients while the polymorphism in the-509T>C region was found over 90% of patients. In exon1, two polymorphisms were also evaluated and only one (+10C>T) was present at about 60% of patients; and in exon5, the polymorphism was not observed in the analyzed samples. Measurement of circulating TGF-b1 levels showed that approximately 70%of patients analyzed to date have higher levels of this cytokine. Because this study is conducted in blind, correlations could not be realized by the moment. Therefore, we intend to identify if there are frequent genotypic patterns in patients with more advanced stages of cardiac involvement to propose TGF-b1 as a biomarker of the development of Chagas disease. **Supported by:**CNPq, DECIT, FAPERJ, IOC/ FIOCRUZ

HP87 - CLINICAL, PARASITOLOGICAL AND HISTOPATHOLOGICAL EVALUATION OF DOGS NATURALLY INFECTED WITH *LEISHMANIA INFANTUM* FROM GOVERNADOR VALADARES - MG.

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The canine visceral leishmaniasis (CVL) is a zoonosis of major impact on public health worldwide. In order to evaluate the role of dogs in the infection cycle of the visceral leishmaniasis (VL), the manifestations of the disease should be known as well as the extent and progression of lesions in several compromised organs. The aim of this study was to make a clinical, parasitological and histopathological evaluation of dogs naturally infected with *Leishmania infantum* from Governador Valadares - MG. Samples from 60 dogs with positive serology were collected. The animals were anesthetized with thiopental sodium and after, euthanized with potassium chloride. The animals were examined for the most prominent clinical signs of CVL. Thus, they were separated into two groups: asymptomatic dogs, and symptomatic dogs. Aspiration of bone marrow in the sternum was performed and prepared slides (smears) that were stained by the use of type Panoptic ® stain for analysis of parasitism. Liver fragments were collected, fixed in formol solution of 10% buffered and processed by routine histopathology techniques. Paraffin sections were mounted on slides and stained with hematoxylin and eosin (H & E). The technique of immunohistochemist for parasitological analysis was used. The results showed dogs 70% symptomatic and 30% asymptomatic. Symptomatic animals showed that the most frequent changes were onychogryphosis 61%, cachexia 43% and ulcers 37%. Animals with parasital spinal cord 72% were symptomatic and 28% asymptomatic. Statistically significant difference was found in symptomatic dogs that presented a higher number of *leishmania* compared to asymptomatic ones ($p < 0.0001$). The average size of granulomas in the livers of naturally infected animals showed to be higher in asymptomatic animals than in symptomatic where ($p = 0.0003$). As conclusion has been asymptomatic animals had lower parasitism associated with the largest area of granulomas, suggesting a greater response to infection. **Supported by:**FAPEMIG

HP88 - ACTIVITY AND IMMUNOLOCALIZATION OF NITRIC OXIDE SYNTHASE CONSTITUTIVE IN *LEISHMANIA (LEISHMANIA) AMAZONENSIS* AND *LEISHMANIA (VIANNIA) BRAZILIENSIS* PROMASTIGOTES

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The American tegumentary leishmaniasis is an infectious parasite, endemic in several countries in Latin America and, caused by various species of *Leishmania*. This parasite is an obligate intracellular parasite, uses mechanisms subversion of microbial activity of macrophages, for example, regulating the production of superoxide radicals and nitric oxide (NO). During Leishmania infection, nitric oxide plays a crucial role in the death of the parasites in vitro and in vivo. In this study, we investigated the activity of constitutive nitric oxide synthase (cNOS) and NO production by the species *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) amazonensis*. The cNOS enzyme was identified in promastigotes using indirect immunofluorescence reaction (RIF) by confocal microscope analysis and immunolocalization of the enzyme cNOS by transmission electron microscopy (TEM) using antibody anti-cNOS (SIGMA). The production of NO was measured of promastigotes cultures by Flow Cytometry using the fluorescent indicator DAF-FM (4-Amino-5-methylamino-2′,7′-difluorofluorescein). These results showed that promastigotes of *L. braziliensis* and *L. amazonensis* are able to express cNOS. Analysis by Flow Cytometry showed that *L. amazonensis* produce more NO than L. braziliensis. Immunofluorescence assay showed that the cNOS enzyme is co-located in glycossomes compartments. Immunolocalization by TEM of cNOS was predominantly observed inside organelles glycossomes-like. In conclusion, correlation between the expression of cNOS and NO production by *L. amazonensis* and *L. braziliensis* suggest a possible virulence factor, which is able to regulate the mechanism of the NO production by the host cell, that confers resistance to the parasites against NO damages.

HP89 - EFFECT OF PUTRESCINE TREATMENT IN THE ENTRANCE AND DEVELOPMENT OF PHOSPHATIDYLSERINE POSITIVE SUBPOPULATION OF *TOXOPLASMA GONDII* IN ACTIVATED MURINE MACROPHAGES

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Toxoplasmosis is a disease caused by *Toxoplasma gondii* with high relevance to veterinary and human health. A subpopulation of tachyzoites exposes phosphatidylserine (PS) on the outer membrane mimicking apoptotic cells. PS is a phospholipid with negative charge usually localized in the inner leaflet of the plasma membrane of non-apoptotic cells. The exposure of PS by apoptotic cell induces the secretion of transforming growth factor beta1, inducing the phosphorylation and nuclear translocation of the transcription factor SMAD. This inhibits nitric oxide (NO) production that is an important microbicidal agent of the immune response. The polyamine putrescine is a molecule related mainly to cellular growth. However, it is also describe by its cationic charge capable of establish strong ligation with membrane phospholipid. The aims of this work were to verify the effect of putrescine treatment in the entrance of *T. gondii* in murine macrophages and possible blockage of PS. The subpopulations of *T. gondii* that exposes PS (PS+) or does not (PS-) were isolated. Putrescine treatment was performed with part of the PS+ subpopulation (PUT). The viability of parasite was not affect by putrescine treatment. After 1 h, the PS+ subpopulation showed higher interiorized parasites rate than the PUT subpopulation. It was observed that PS+ tachyzoites were located in tight-fitting vacuole, indicating active cell invasion. The PS- subpopulation and PUT showed high rate of phagocytic loose-fitting vacuole. The PS- subpopulation infection failed to inhibit NO production in activated murine macrophage. In contrast, the PS+ subpopulation inhibited NO production with high rate of infected macrophage and induction of SMAD 2 phosphorylation and nuclear translocation, after 1 and 24 h of infection. PUT was not effective in inhibiting NO production by macrophages. The similar behavior of the subpopulation PS- and PUT suggests that putrescine may interfere in the recognition of PS by macrophage. **Supported by:**CAPES, UENF, FAPERJ, CNPq

HP90 - *LEISHMANIA*-INFECTED ADIPOCYTES: A POSSIBLE MODEL FOR PARASITE PERSISTENCE?

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Leishmania amastigotes reside inside phagolysosomal compartments of phagocytic mononuclear cells. However, there are studies reporting that *Leishmania* is also capable of infecting different cell types including fibroblasts, melanocytes, neuronal and muscle cells. It has been recently proposed that adipose tissue-derived mesenchymal stem cells are permissive to old World *Leishmania* species infection, although the cellular aspects that orchestrate this process have not yet been elucidated. The aim of this work is to evaluate the susceptibility of adipocyte-derived 3T3.L1 fibroblasts to *L. (L.) amazonensis* and correlate infection rates and parasite maintenance throughout the infective process. Briefly, 3T3.L1 fibroblasts were differentiated into adipocytes after incubation in DMEM medium with appropriate concentrations of dexamethasone, isobutyl-1-methylzanthine, and insulin for 4 days. Later, cells were cultured in insulin-containing DMEM for 3 days. After 12 days, fully transformed adipocytes presenting lipid droplets were infected with GFP-expressing *L. (L.) amazonensis* amastigotes. Adipocytes were infected for 1, 24, 48, 72, and 144h (MOI=5) and parasite number was determined by fluorescence microscopy. Our results show that adipocytes are infected by amastigotes that are able to replicate in a time-dependent manner up to 48h. However, growth is not sustained after later time points, even though GFP-parasites can be found in adipocytes 144h post-infection. Electron microscopy studies are being carried out to analyze the ultrastructure of the infected adipocyte and will provide evidence of *Leishmania* persistence in non-phagocytic cells. Our data suggest that the adipose tissue is a potential site for maintenance of *Leishmania*. This hypothesis will be further investigated in an attempt to explore the role of adipocytes as host cells in latent leishmaniasis. **Supported by:**FAEPEX-UNICAMP

HP91 - CORRELATION BETWEEN CHAGAS DISEASE CLINICAL MANIFESTATIONS AND THE BIOENERGETIC METABOLISM OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES

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Chagas disease clinical manifestations vary between individuals, and the geographic region. The acute phase of the disease when left untreated progresses to the chronic indeterminate form or with cardiac, digestive and cardiodigestive symptoms. The purpose of this work is to establish a possible correlation between the clinical form of the disease and parasite mitochondrial bioenergetic metabolism. In this sense, epimastigotes derived from trypomastigotes isolated from the blood of four patients with Chagas disease: cardiac (MAMA), digestive (AP), indeterminate (MJFL) and cardiodigestive (SAO) were obtained. The growth curve was performed in LIT medium and the number of cells determined in a Neubauer chamber. Oxygen consumption rates were determined in a Hansantech Oxygraph. In relation to the profile of the growth curve, significant differences were observed among isolates. The stationary phase was reached on the 6th day for AP; on the 4th day for MAMA and MJFL and on the 3rd day for SAO. Only SAO had a long stationary phase, while the others declined rapidly after reaching this phase. The MAMA, SAO and MJFL isolates had similar growth rates (GR) and doubling times (DT) (GR = 1.02, 1.13, 0.92 and DT = 28.44h, 26.97 h, 28.31 h, respectively), while for for AP the GR and DT were: 5.79 and 41.8 h, respectively. Oxygen consumption rates were: 10.5 ± 0.6; 9.4 ± 0.2; 7.0 ± 0.8; 8.7 ± 0.5 nmoles O2 consumed/min.108 cells for AP, SAO, MAMA and MJFL respectively. Up to now, the results suggest differences in the bioenergetic metabolism of the isolates from patients with different clinical forms of Chagas disease. **Supported by:**FAPESP, CNPq

HP92 - CHARACTERIZATION OF A SURFACE SRS PROTEIN OF *NEOSPORA CANINUM* (SAG-RELATED-NCLIV_060700)

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Neospora caninum is a parasite of the phylum Apicomplexa related to abortion and losses of fertility in cattle. As part of its intracellular cycle, the first interaction of the parasite with the target cell is performed with the surface proteins such as the SRS superfamily (Surface Antigen Glycoprotein – Related Sequences). SAG related or SRS proteins have been target of intense research due to its immunodominant pattern, exhibiting potential as diagnostic and/or as vaccine candidates. Thus, this study initiated the molecular characterization of the surface protein NcLIV_060700 of *Neospora caninum*. The coding sequence of NcLIV_060700 (without the signal peptide and the GPI anchor) was cloned, expressed in TOPO/NcLIV_060700 plasmid (developed and patented by our research group) containing a histidine tail and purified in a nickel column. BALB/c mice were immunized with subcutaneous injection of 50µg of the recombinant protein or 50µg of protein extract *N. caninum* tachyzoites (positive control) or urea (negative control), in complete (week 0) and incomplete Freund's adjuvant (weeks 3, 6 and 9) and at week 10 the blood was harvested. In an enzyme-linked immune sorbent assay the serum anti-NcLIV_060700 recognized the recombinant protein but did not react with the native form present in the protein extract of *N. caninum*. In contrast to the ELISA, by western blot the native NcLIV_060700 protein was detected as a single band of 35 kDa, in agreement with the predicted molecular weight. More experiments are being performed to further characterize this new SRS protein of *N. caninum*, including confocal localization and mass spectrometry identification. **Supported by:**Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES

HP93 - HOW DOES TRYPANOSOMA RANGELI CIRCULATES AMONG TRIATOMINE BUGS AND ITS VERTEBRATE HOSTS?

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Trypanosoma rangeli is a protozoan parasite broadly found in South America infecting several species of triatomines and an extensive variety of mammals. The classic mechanism of parasite acquisition by the insect occurs during the feeding on an infected mammal. Nevertheless, the lack of reporting of multiplication of the parasite in the vertebrate host suggests the existence of alternative forms of transmission. In this study we evaluated different ways by which T. rangeli could be acquired by Rhodnius prolixus. Initially we evaluated the transmission through hemolymphagy behavior. In this assay 2nd instar uninfected starved nymphs were maintained together with one 5th instar infected fed nymph for 15 days. After this period, nymphs were fed and after moulting to 3th instar examined for parasite search. In these conditions no infection was observed in the exposed nymphs. In another experiment, one donor infected was placed into a container with one or five uninfected recipients. One anesthetized mouse or pigeon (just one recipient was tested with this host) were placed ventral side down on the container as food source. The recipient nymphs were examined for parasite presence 21 days after feeding. After taking a blood meal on mice together with infected co-specifics 76.9% of the 5th instar nymphs became infected by T. rangeli. Even in those assays where one donor nymph shared the mouse host with five recipient ones a similar percentage of infection (63.1%) was observed. Surprisingly, 20% of the recipient nymphs became infected when pigeons were used as hosts. In the assays where mice were used as hosts, the vertebrates were able to transmit the parasite to new nymphs up to four months after being infected. The number of parasites found in the intestinal tract of insects was variable being lower in insects that fed on mice with 30 days post infection. This is the first time an avian has been implicated in Trypanosoma transmission.**Supported by:**FAPEMIG, CPQRR, INCT

HP94 - CLONING AND EXPRESSION OF A SURFACE SRS PROTEIN OF *NEOSPORA CANINUM* (SAG-RELATED NCSRS57)

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Neospora caninum is an apicomplexan parasite that nowadays continues to be a major abortifacient in cattle production worldwide. As any apicomplexan, the adhesion is mediated by surface proteins such as the superfamily SRS (Surface Antigen Glycoprotein – Related Sequences). This work was developed with the initial aim to clone and express the surface protein NcSRS57 (SAG3/NcLIV_060660) of *Neospora caninum*, not yet described in the literature. The sequence lacking the signal peptide and the GPI anchor site (1074 bp) was amplified using PCR and ligated to the pGEM-T-easy vector. The plasmid was sequenced and confirmed the identity of NcLIV_060660 (NcSRS57). The alignment with homologues resulted in an identity/similarity to *Toxoplasma gondii* TgSAG3 (45%/61%) (GenBank ADK27784.1); NcP36/SAG1 (*N. caninum*) (22%/38%) (GenBank AAF32519.1); NcLIV_060700 (21%/38%) (GenBank AAO85715.1) and NcP29/SRS2 (*N. caninum*) (20%/34%) (GenBank AAD39486.1). The NcSRS57 fragment was subsequently ligated in pET-32 expression plasmid, transformed in E. coli BL21 (DE3) and induced with 1mM IPTG at 37°C for 3 hours. The expression of the NcSRS57 recombinant protein was visually confirmed as a 20kDa band (in contrast to the 28 kDa predicted without the signal peptide) in a SDS-PAGE. The fragment in pET32 was also sequenced to check for the identity of the NcSRS57coding sequence. After confirmation, the NcSRS57 was purified in a nickel column and production of polyclonal antibodies is being performed for future assays. **Supported by:**Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES

HP95 - L-PROLINE IS ESSENTIAL FOR TRYPANOSOME COLONIZATION IN THE TSETSE FLY.

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The tsetse vector (*Glossina morsitans*) transmits African trypanosomes. L-proline is abundantly present in distinct fly tissues and in vitro studies suggest that it is the major carbon source of the procyclic stage of *Trypanosoma brucei*. However, its importance for parasite colonization of the tsetse has not been proven. Here, we report the expression and essentiality of delta-1-pyrroline-5-carboxylate dehydrogenase from *T. brucei* (TbP5CDH), the second enzyme in the proline catabolism pathway. TbP5CDH is highly expressed in the midgut (procyclic) and proventricular stages, but not in bloodstream forms, as seen by in vitro tests. Knocked-down of TbP5CDH by induction of double-stranded RNA resulted in: i) growth deffects in defined media with low-glucose concentration (SDM80), ii) high susceptibility to P5C when used as the only carbon source, iii) alterations in EP-procyclin expression, iv) differences in ATP levels and v) inability to colonize the tsetse midgut. Furthermore, during a trypanosome infection, the content of L-proline changed in the tsetse haemolymph, and the expression of enzymes involved in the proline biosynthetic pathway in this insect was down regulated. The ability to enzymatically produce proline by *T. brucei* was also studied. Taken together, our data show evidence that the free proline in the fly (either released by the fat body or supplied by the bloodmeal) is essential for energy production and tsetse colonization by *T. brucei* procyclics. Moreover, we suggest that expression of EP-procyclins may be linked to proline metabolism. **Supported by:**FAPESP

HP96 - TC95, A POTENTIAL DRUG CANDIDATE AGAINST LEISHMANIA SP.: ANTIPROLIFERATIVE EFFECTS AND MECHANISMS OF ACTION.

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The protozoan parasites of the Leishmania genus cause leishmaniasis, a disease with a large spectrum of clinical manifestations. .The current chemotherapy is based on antimonials, amphotericin B and pentamidine. Miltefosine was recently introduced as first line treatment for visceral leishmaniasis in India. However, there is an urgent need for new therapeutic regimens that are safer, accessible and more efficacious. An interesting approach in drug development is the combination of different inhibitors with known activity against the parasites. Thus, the aim of this work was to study the effects of TC95, a hybrid molecule between trifluralin and miltefosine, in different species of Leishamania that are relevant for the epidemiology of the leishmaniasis in Brazil. We also studied the mechanisms of death induced by the treatment. The antiproliferative effects showed that TC95 has a high leishmanicidal activity against L. amazonensis, L. mexicana, L. guyanenesis, L. brasiliensis, L. donovani and L. infantum, with just 24h of treatment. Studies of mechanisms of action in L. amazonensis and L. donovani demonstrated that TC95 induced an increase in ROS production, loss of mitochondrial membrane potential, and reduction in ATP production, indicating that mitochondrion is one of the first targets of the treatment. Transmission electron microscopy confirmed these effects showing dramatic lesions in the mitochondrial ultrastructure. In L donovani, after treatments for just 2 and 4h, different cell death phenotypes were observed: 1) increase in early necrotic cells stained with Anexin V/PI; 2) DNA fragmentation; 3) release of Cytochrome C. In axenic amastigotes isolated from murine lesions, TC95 also presented a potent effect with IC50 value of 200nM. Taken together, these results indicate that TC95 affect important cell targets during the treatment and is a potential compound against Leishmania sp. **Supported by:**CNPq, FAPERJ, CAPES

HP97 - IRF1 PARTICIPATION DURING INFLAMMATION INDUCED BY L. INFANTUM EXPERIMENTAL INFECTION

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Visceral leishmaniasis (VL) is a chronic and potentially fatal disease caused by protozoan Leishmania infantum in Brazil. During infection, the dendritic cells (DCs) recognize antigenic products through pattern recognition receptors (PPRs) and then orchestrate the development of a Th1-dominated granulomatous response, characterized by high IFN-ɣ production and appropriate neutrophils recruitment to inflammatory foci, which is related to resistance against VL. It has been shown that MyD88 signaling interacts with interferon regulator factor 1 (IRF1) inducing regulation both type I and II IFN and proinflammatory cytokines. In addition, we demonstrated that TLR9/MYD88 signaling in dendritic cells participates in the restriction of Leishmania infantum by regulating neutrophils recruitment to infection foci. However, the role of IRF-1 in the inflammation developed during VL is unknown. Herein, we aimed to evaluate the participation of IRF1 during experimental VL. Our results demonstrated that IRF1-/- infected mice were more resistant to infection, displaying lower parasites numbers in spleen and liver, at 4th and 6th weeks post-infection (wpi). Evaluating the pattern of immune response, IRF1-/- infected mice presented higher number of CD4+T lymphocytes producing IFN-ɣ at 6th weeks post-infection. In addition, an intense neutrophilic infiltration was observed into target organs from IRF-1-/-. Interestingly, spleeny DCs from IRF1-/- infected mice presented a raised-mature stage, expressing higher MHCII and CD86 molecules. Taken together, the results suggested that IRF1 participates in the regulation of inflammatory response during L. infantum infection, however its immunomodulatory mechanisms remain to be elucidated. **Supported by:**CAPES FAPESP CNPq

HP98 - AN OPTIMIZED NANOPARTICLE DELIVERY SYSTEM BASED ON CHITOSAN AND CHONDROITIN SULPHATE MOLECULES REDUCES THE TOXICITY OF AMPHOTERICIN B AND IS EFFECTIVE IN TREATING TEGUMENTARY LEISHMANIASIS.

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Amphotericin B (AmpB) is active against leishmaniasis, but its use is hampered due to its high toxicity observed in the patients. In this study, a nanoparticle delivery system for AmpB (NQC-AmpB) containing chitosan (Cs) and chondroitin sulphate (ChS) was evaluated in BALB/c mice against Leishmania amazonensis. An in vivo biodistribution study, including biochemical and toxicological evaluations, was performed to evaluate the toxicity of AmpB. Nanoparticles were radiolabeled with technetium-99m and injected in mice. The products presented a similar biodistribution in the liver, spleen, and kidneys of the animals. Free AmpB induced alterations in the body weight of the mice, which, in the biochemical analysis, indicated hepatic and renal injury, as well as morphological damage to the kidneys of the animals. In general, no significant organic alteration was observed in the animals treated with NQC-AmpB. Mice were infected with L. amazonensis and treated with the nanoparticles or free AmpB; when parasitological and immunological analyses were performed. The NQC-AmpB group, as compared to the control groups, presented significant reductions in the lesion size and in the parasite burden in all evaluated organs. These animals presented significantly higher levels of IFN-□ and IL-12, and low levels of IL-4 and IL-10, when compared to the control groups. The NQC-AmpB system was effective in reducing the infection in the animals, and proved to be effective in reducing in significant levels the toxicity evoked by AmpB, which was observed when it was administered alone. In conclusion, the NQC-AmpB could be considered a viable possibility for future studies in the treatment of leishmaniasis. **Supported by:**INCT NANOBIOFAR

HP99 - TESTICULAR IMMUNOLOGICAL PROFILE DURING EXPERIMENTAL CHRONIC INFECTION OF LEISHMANIA CHAGASI.

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Visceral leishmaniasis (VL) is an anthropozoonosis caused by a protozoan of Leishmania donovani complex transmitted mainly by hematophagous phlebotomine vectors; however, vertical transmissions also occur. It has been reported the presence of Leishmania in semen of infected animals, causing tissue damage leading to azoospermia and testicular atrophy in chronic stage. The aim of this study was to characterize lesions in the male genital system of golden hamisters and elucidate the testicular immune profile generated by this animal during experimental infection. 30 males hamisters (Mesocricettus auratus) were infected with amastigotes of L. chagasi intraperitoneally with parasite load of 1 x 10^6 parasites/animal. They were euthanized at 07, 10, 13, 16, 19 weeks after infection. Control groups were not infected animals. At each time points, spleen, testis and epididymis were removed. Macroscopic analysis of spleen and PCR for Leishmania kDNA was performed to confirm the experimental infection. Tissues were placed in protease inhibitor, macerated and cytokines IL1-β, IL4, IL10, TNF and IFN-g were detected by ELISA. All spleens were positive for the presence of Leishmania kDNA, except in the control group. In early stages of infection, no increase in proinflammatory cytokines IL1-β, TNF, INF-g was observed in testis until the 10th week of infection. With low parasite load, the testis maintains high levels of IL4 and IL10, leading to a Th2 type response with immunosuppression, characterizing a suitable place for the parasite. On the other hand, when high parasite load was observed, immune profile changed to Th1 leading to testicular atrophy. As the testis is an immunological privileged organ, we believe that the parasite lodges in it remaining protected from the host immune system, favoring the survival of the parasite and facilitating the spread of venereal LV. **Supported by:**FAPEMIG, CAPES e CNPq.

HP100 - MONITORING HUMORAL RESPONSE DURING EXPERIMENTAL INFECTION IN MICE WITH TRYPANOSOMA CRUZI AND TRYPANOSOMA RANGELI BY IMMUNOBLOTTING TESTS

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Trypanosoma rangeli is a non-pathogenic parasite that infects many mammals in the Americas, including humans. Due to its morphological and antigenic similarities with *Trypanosoma cruzi*, etiologic agent of Chagas disease, and the co-existence of these parasites in the same natural habits, mixed infections may occur causing misdiagnosis of the disease. In Chagas disease formation of IgM antibodies occurs during the acute phase, but with the course of infection the level of parasitemia is mainly modulated by IgG antibodies. For infections caused by *T. rangeli* the IgG isotype is present in lower titers than in *T. cruzi* infection. Therefore, this study aims to evaluate the humoral immune response based on the serologic reactivity of IgG obtained by experimental infection in mice with T. cruzi and T. rangeli. Parasites were cultured in vitro using LIT medium and trypomastigotes of *T. rangeli* Choachí and *T. cruzi* Y were obtained in DMEM culture and in aging cultures, respectively. Approximately 1,000 trypomastigotes were inoculated intraperitoneally and antisera obtained through sequential sampling for 64 days, with accompanying parasitemia. The serological reactivity was measured by immunoblotting assays in which we observed an increase in serum levels of IgG antibody around the 14th day remaining high until about the 64th day. Whereas the total proteins of epimastigote and trypomastigote forms of both parasites were recognized more strongly by homologous than heterologous antisera. Beside the recognition of *T. cruzi* proteins by heterologous antisera indicates the serological cross-reactivity for the diagnosis of Chagas disease this recognition decrease during infection indicating that the risk of cross-reactivity decreased when antisera from animals with chronic *T. rangeli* infections are tested. Thus, other tests are being conducted to identify the antibody class involved in serological cross-reactivity response and if it remains in prolonged chronic infections. **Supported by:**FAPESC; Unoesc

HP101 - A COMPREHENSIVE PROTEOMIC ANALYSIS OF G STRAIN EPIMASTIGOTES OF *TRYPANOSOMA CRUZI* ISOLATED FROM AN OPOSSUM OF AMAZONIC REGION

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Trypanosoma cruzi G strain (DTU TcI) was isolated from an opossum of Brazilian Amazon and is characterized by low infectivity and non-hybrid genome. Within our broad project to the characterization of G strain, LC-MS/MS analysis of peptides generated by in-gel digestion of epimastigote protein lysate and after a database automated search were performed. The search using CL Brener and Sylvio X10/1 strains database and PEAKS6 software (FDR ≤1%) allowed the identification of 2,947 proteins, of which 1,091 protein[illegible] uncharacterized in the database. Thus, this is the most abran[illegible] been revealed so far regardless the life stage of the [illegible] largest amount of proteins was amino acid[illegible] and energy metabolism cate[illegible] metabolism, which is [illegible] followed by [illegible] ways. Nine of the ten [illegible] database search resulted in the p[illegible] by non-classical pathway and in many p[illegible] Finally, several drug target candidates studied by our gr[illegible] aminopeptidase, oligopeptidase B, prolyl oligopeptidase, me[illegible] phosphorylase and cathepsin B. Moreover, a large amount of members of trar[illegible], mucin-associated surface protein, retrotransposon hot spot protein and dispersed gene family protein 1 multigenic families were also identified. This exhaustive proteome analysis represents a significant advance in understanding the set of proteins expressed in *T. cruzi* and also an important step towards elucidation of the genome sequence of G strain. **Supported by:**CNPq, CAPES, FAPDF, PRONEX, Finep

HP102 - DIFFERENTIAL EXPRESSION OF HEPARIN-BINDING PROTEINS IN DISTINCT GENOTYPES OF *TRYPANOSOMA CRUZI* AND THEIR INVOLVEMENT IN ESTABLISHING EFFICIENT INFECTION

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Heparin-binding proteins (HBPs) play an important role in the recognition and invasion of intracellular pathogens. The recognition between HBPs and heparan sulfate proteoglycans (HSPG) on the surface of mammalian cells mediates invasion of amastigote and trypomastigote forms of *Trypanosoma cruzi* in cardiomyocytes. The mechanism of invasion triggered by HBP-HSPG interaction, as well as the presence of HBPs in different genotypes of *T. cruzi* has not been demonstrated. In this study, we evaluated the expression of HBPs in metacyclic (MT) and culture-derived trypomastigote (TCT) forms of *T. cruzi* of Y strain and SMM36, a sylvatic isolate from Santa Maria Madalena, RJ, Brazil, compared with the gp82, gp90 and gp35/50. To evaluate the expression and subcellular localization of HBPs, trypomastigotes were incubated for 1 h on ice with 20µg/ml of biotin-conjugated glycosaminoglycans (heparin or heparan sulfate) and processed for flow cytometry and fluorescence microscopy. Flow cytometry analysis showed that trypomastigotes (TCT and MT) of different DTUs have distinct expression profiles of these proteins. TCTs have higher expression of gp35/50 and HBPs while MTs have higher levels gp82, gp35/50 and gp90. In TCTs, HBPs are located in the flagellar membrane, as shown in clone Dm28c, while GPs are distributed along the body of the parasites (TCT and MT), except that gp90 is negative in the TCTs. Additionally, our data showed a 30% reduction of infection for TCTs in glycosaminoglycan-deficient cells (CHO-745) when compared to wild type cells (CHO-K1), demonstrating that the HBPs-HSPG recognition is important for the invasion of different *T. cruzi* strains in target cells. **Supported by:**Fiocruz, PAPES, CNPq and FAPERJ

HP103 - EXPRESSION PROFILE OF SPECIFIC MASP MEMBERS OF *TRYPANOSOMA CRUZI*

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The annotation of the *Trypanosoma cruzi* genome revealed a new multigene family composed of about 1,400 genes, which became known as mucin-associated surface protein (MASP). The high level of polymorphism of the MASP family associated with its localization at the surface of the parasite infective forms suggests that MASP participates in host-parasite interactions. We speculate that the large repertoire of MASP sequences may contribute to the ability of *T. cruzi* to infect several host cell types and/or participate in host immune evasion mechanisms. In a previous study, we demonstrated that MASP peptides are recognized by sera from acutely infected mice. We also observed a variable level of reactivity against MASP peptides in sequential passages in mice and significant variation in expression levels of MASP transcripts in the trypomastigote population. In the present study, we investigated the expression profile of specific MASP members during *in vivo* and *in vitro T. cruzi* infections. Monoclonal antibodies raised against B5 and H5 peptides, each one present in a single MASP member and known to be antigenic, were generated and used in immunofluorescence assays. Approximately 30% of the parasites were labeled, indicating that the expression of a MASP member containing one of these peptides is limited to a subset of the population. These antibodies were also used to carry out the enrichment of the parasite population expressing the B5 or H5 peptide. We obtained an enrichment of approximately 3-fold relative to wild type (WT) population. The enrichment effect on the infectivity profile *in vivo* was evaluated by infection of C57BL/6 mice with WT trypomastigotes and the population enriched for each one of the MASP variants. Preliminary results suggest alterations in the parasitemia in mice infected with H5-enriched trypomastigote population compared with the WT group. Cell invasion assay are underway to evaluate the enrichment effect on the parasite infectivity *in vitro*. **Supported by:**CNPq, FAPEMIG, CAPES, INCTV

HP104 - INTRASPECIFIC VARIABILITY AND HETEROLOGOUS EXPRESSION OF TRYPANOSOMA RANGELI FLAGELLAR CALCIUM BINDING PROTEIN (FCABP)

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Chagas disease is still a major public health problem in Central and South Americas. The etiological agent, *Trypanosoma cruzi*, shares geographic regions, hosts and antigens with *Trypanosoma rangeli* leading to mixed and/or misdiagnosed infections. Due the serological cross-reactivity between these two parasites, false-positive diagnosis of Chagas disease should be underreported. Thus, the uses of molecular biomarkers that differentiate these parasites are highly recommended. Based on a comparative proteomic assessment, in this study we address the *T. rangeli* Flagellar Calcium Binding Protein (TrFCaBP) as a specific biomarker for differential diagnosis from *T. cruzi*. Initially, the FCaBP gene from four *T. rangeli* strains was sequenced and compare to the homologous *T. cruzi* protein sequence. After cloning and heterologous expression, TrFCaBP expression was assessed by qPCR and western blotting using an antisera generated in mice. In silico analysis of the TrFCaBP gene from *T. rangeli* Choachí, SC58, Macias and LDG strains showed high conservancy (>99% similarity). Regardless the similarity (88%) with TcFCaBP, there was a lack of six aminoacid residues (TSDKGL) at the N-terminal region in all *T. rangeli* strains when compared to the *T. cruzi* homologous protein, consisting in a unique *T. rangeli* epitope. Analysis by qPCR and western blotting showed no difference in the mRNA levels and protein expression between *T. rangeli* epimastigotes and trypomastigotes. In conclusion, TrFCaBP is a potential marker for specific *T. rangeli* detection in serological diagnosis. ELISA and immunoblotting assays are being conducted using both the recombinant TrFCaBP and synthetic peptides. **Supported by:**FAPESC; CNPq; CAPES; PIBIC/Unoesc/Art. 170

HP105 - POSSIBLE ROLE OF AUTOPHAGY IN THE PROLIFERATION INHIBITION OF TOXOPLASMA GONDII IN INTESTINAL EPITHELIAL CELLS, IEC-6, ACTIVATED WITH INTERFERON-GAMMA

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Autophagy is a self-regulated degradation process of cellular components in response to external or intracellular stresses or infections caused by pathogens. Autophagy also acts as a microbicidal mechanism against microorganisms including Toxoplasma gondii. T. gondii is the etiologic agent of toxoplasmosis, a zoonosis of veterinary and medical importance. This parasite actively penetrates host cells, residing in a parasitophorous vacuole (PV). The association of autophagic vesicles with the PV and subsequent fusion with lysosomes causes degradation of T. gondii. Association of autophagic vesicles with the PV is seen when cells are activated with IFN-γ. This causes death of T. gondii by inducing rupture of the PV. Enterocytes are the first line of defense in T. gondii oral infection. After ingestion, the parasite passes through the gastrointestinal tract and infects enterocytes. The present study aimed to verify whether the activation of IEC with IFN-γ may inhibit the replication of T. gondii by the association of autophagic vesicles around the PV. IEC-6 were activated with IFN-γ for 24h and infected with T. gondii for 2h. The cells were washed, collected and further cultured for 24 and 48h. The development of T. gondii, after Giemsa staining, and association of autophagic vesicles, reveled with antibody against LC3A, with the PV were evaluated. Autophagic vesicles were seen associated to the PV in great quantities in activated IEC-6. Furthermore, it was possible to observe higher amounts of parasites on non-activated cells. The increase of autophagic vesicles in activated cells and its association with the PV suggests that autophagy may be involved in the inhibition of T. gondii replication in IEC-6, similar to the events reported in macrophages. Autophagy in IFN-γ activated IEC-6 was pharmacologically inhibited with 3 methyladenine and T. gondii growth will be assayed. Preliminary results showed that the autophagy inhibitor causes no damage to enterocytes. **Supported by:**CAPES, UENF, FAPERJ, CNPq

HP106 - ANTI-LEISHMANIA BRAZILIENSIS ACTIVITY OF PHENANTHROLINE AND ITS METAL-BASED COMPLEXES

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Peptidase inhibitors/chelating agents such as 1,10-phenanthroline and its substituted derivatives, either the metal-free state or as ligands coordinated to transition metals, interfere with crucial functions of several biological systems. In previous works, our group described that L. braziliensis produced gp63 molecules sensible to 1,10-phenanthroline. Herein, we initially studied the cellular distribution of gp63 in a virulent strain of L. braziliensis by biochemical and immunocytochemical analyses. After that, we reported the inhibitory effects of three 1,10-phenanthroline derivative compounds, 1,10-phenanthroline-5,6-dione (phendio), [Cu(phendio)2]and [Ag(phendio)2], on both cellular and extracellular metallopeptidase activities produced by L. braziliensis promastigotes as well as their actions on the parasite viability and on the interaction with murine macrophage cells. The gp63 molecules were detected in several parasite compartments, including cytoplasm, membrane lining the cell body and flagellum, and flagellar pocket. The treatment of L. braziliensis promastigotes for 1 hour with 1,10-phenanthroline and its derivatives resulted in a significant inhibition of cell viability. The pre-treatment of promastigotes with metallopeptidase inhibitors induced a reduction on the expression of surface gp63 as well as a significant reduction on the association index with macrophages. In parallel, the treatment of L. braziliensis-infected macrophages with the 1,10-phenanthroline and its derivatives promoted a powerful reduction on the number of intracellular amastigotes. Collectively, the 1,10-phenanthroline and its metal-based drugs present a good perspective for prospective studies to the development of new anti-L. braziliensis drugs. **Supported by:**CNPq

HP107 - ANALYSIS OF THE SYSTEMIC IMMUNE RESPONSE AND CARDIAC INFLAMMATION ASSOCIATED WITH ORAL INFECTION WITH TRYPANOSOMA CRUZI IN MICE

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After controlling the vectorial and transfusional infection of Chagas disease in Brazil, the oral contamination became the main mechanism of transmission indifferent regions. Based on this, the aim of this study was to evaluate the systemic immune response and cardiac inflammatory process in Swiss mice orally (VO) or intraperitoneally (VI) infected with Berenice-78 *Trypanosoma cruzi* strain. Thus, five animals from each group were euthanized at 7, 14, 28 and 35 days after infection and blood and heart were collected for further analysis. Infection by VO showed lower parasitemics levels, being the highest peak of parasitemia on day 22 after infection. Conversely, infection with VI showed higher parasitemics levels, being the highest peak of parasitemia on day 20 after infection. Evaluation of WBC showed an increase in the number of total leukocytes and lymphocytes on days 14, 28 and 35 after infection by the VO via. The animals infected by VI showed this increase only at day 35 after infection. In the analysis of immunophenotyping of peripheral blood mononuclear cells a reduction in the percentage of CD4+ T lymphocytes was observed on days 28 and 35 after infection on animals of VI group. On the other hand, it was observed a decrease in CD8+ T lymphocytes in the VO group on days 7, 14 and 28, restoring baseline levels at day 35 after infection. In VI group this reduction occurred in 7 and 14 days, returning to basal levels at day 28.There was no inflammation observed in the hearts of VO group animals, however the VI group showed inflammation on the 35th day. Accordingly, these data suggest that oral infection has a distinct parasitological/immune response profile compared to the intraperitoneal route, however these systemic changes seem not to influence in a greater cardiac injury in the acute phase. **Supported by:**CNPQ

HP108 - THE NATURAL CYSTEINE PEPTIDASE INHIBITOR (ICP) OF *TRYPANOSOMA BRUCEI RHODESIENSE*: REQUIRED FOR VIRULENCE AND THE CONTROL OF INNATE RESPONSES?

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Inhibitors of cysteine peptidases (ICP) are protein inhibitors belonging to the chagasin-family, which inactivate papain-like cysteine peptidases (CP). In Sleeping sickness, T. brucei rhodesiense penetrates the central nervous system by unknown mechanisms, leading to meningoencephalitis. The parasite's cathepsin L-like peptidases have been implicated in the penetration of the blood brain barrier. We generated ICP null mutant lines in T. rhodesiense (Δicp), which have higher CP activity and induce adhesion molecules in endothelial cells. In mice infected with WT parasites, the first wave showed high parasitemia, while those infected with Δicp or the ICP re-expressor line displayed 8-fold lower parasitemia. At day 3, we found 60% higher numbers of CD11b+ cells in the spleen of mice infected with Δicp, suggesting a more prominent innate response. After the remission of the first wave, mice infected with WT or the ICP re-expressor line displayed a second wave of high parasitemia, while infection with Δicp was controlled and almost no parasites were detected in the blood. At day 13, mice infected with WT or with the ICP reexpressor line exhibited splenomegaly, higher cellularity but reduced numbers of $CD4^+$, $CD8^+$ and $CD19^+$ cells, as compared to uninfected control mice. In contrast, mice infected with Δicp had no increase in the number of splenocytes and unaltered numbers of CD4+, CD8+ and $CD19^+$ cells. We found significantly higher levels of TNFα, IL6 and IL10 in the spleen of mice infected with WT or the ICP re-expressor line, as compared to those infected with Δicp or control mice, while elevated IFNγ was similar in all infected mice. In contrast, the serum of mice infected with Δicp had 10-fold higher levels of TNFα, and 4-fold lower IL10, suggesting that sustained increased inflammatory response unrelated to the spleen is associated with reduced parasite burden. We propose that ICP helps to downregulate innate responses that contribute to the control of infection. **Supported by:**FAPERJ, CAPES e CNPq

HP109 - MITOCHONDRIAL PEROXIDOXIN IS AN IMPORTANT VIRULENCE FACTOR FOR *LEISHMANIA BRAZILIENSIS* PARASITE

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Peroxidoxin belongs to a family of proteins called peroxiredoxins (PRXs), which play a vital role in detoxifying reactive oxygen species in the parasites – an activity that is particularly relevant for Leishmania. These factors classify PRXs of these organisms as important virulence factors. In the present work, the role of mitochondrial Peroxidoxin was investigated along the Leishmania braziliensis life cycle. After the expression analyses in the distinct developmental stages of the parasite we observed an increased expression of Peroxidoxin in the metacyclic forms and especially in intracellular and axenic amastigotes, when compared to less not infective stage promastigote (log phase). Western blot analyses revealed higher expression of the protein after 24 hours of the axenic culture, which was further confirmed by flow cytometric when percentage of positive cells and medium fluorescence intensity was assessed. In vitro infection assays used specific inhibitors for functional domain of the Peroxidoxin reduced intracellular parasite burden compared to control groups. Mutant parasite overexpressing Peroxidoxin gene showed an increase of parasite load in in vitro assay using peritoneal macrophages and significant lesion development in mice when compared to infection with wild-type parasites. From these data, we conclude that expression of Peroxidoxin is an important virulence factor for L. braziliensis. **Supported by:**CAPES/REUNI, CNPq e FAPEMIG

HP110 - PRODUCTION OF IL-12P40, IL-23 AND NO BY A MURINE CELL LINE CHARACTERIZED AS DENDRITIC CELL AFTER STIMULUS BY TOXOPLASMA GONDII

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Introduction: Dendritic cell (DC) is the major antigen presenting cells that activate naïve T lymphocytes. Recently, we characterized the murine cell line AP284 as DC subtype. Here we investigate the ability of Toxoplasma gondii tachyzoite stimulate production of IL-12p40, IL-23 and NO by AP284. AP284 cell line were cultured for 24 h in 1 mL RPMI medium and stimulated with Lipopolysaccharide (LPS); heated killed Escherichia coli (HK E.coli) or live T. gondii tachyzoite (RH strain). Tachyzoite parasites were obtained in coculture with L929 cells. Cytokine and NO production were assayed by ELISA or Griess reaction respectively. After stimulation of AP284 with 0,2 µg/ LPS/ mL; 1x 10^7/ HK E. coli/ mL and 1 x 10^4 tachyzoite/mL it was observed high production of IL-12p40 (40±7.81; 60±1.13 and 23±8,69 ng/mL respectively), IL-23 (16±6.33; 24±4.77 and 1.3±0.52 ng/ml respectively) and NO (13±1.32; 12±3.38 and 1.3±1,12 µM. It was not observed production of IL-12p70 by AP284 after any stimulus and addition of IFNg inhibited the IL-12p40 and IL-23 and increases NO production. Our results suggest that AP284 cell line is useful cell to analyze the ability of different antigens of pathogens to stimulate IL-23 production and to search for IL-23 antigen inducers. **Supported by:**Fapeg

HP111 - MAPPING B-CELL EPITOPES FOR THE PEROXIDOXIN OF LEISHMANIA (VIANNIA) BRAZILIENSIS AND ITS POTENTIAL FOR THE CLINICAL DIAGNOSIS OF TEGUMENTARY AND VISCERAL LEISHMANIASIS

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The search toward the establishment of novel serological tests for the diagnosis of leishmaniasis and proper differential diagnosis may represent one alternative to the invasive parasitological methods currently used to identify infected individuals. In the present work, we investigated the use of recombinant Peroxidoxin of Leishmania braziliensis as a potential antigen for the immunodiagnosis of human tegumentary (TL) and visceral leishmaniasis (VL) and canine visceral leishmaniasis (CVL). Linear B-cell epitope mapping was performed to identify polymorphic epitopes when comparing orthologous sequences present in Trypanosoma cruzi, the agent for Chagas disease (CD), and the Homo sapiens and Canis familiaris hosts. ELISA demonstrated that TL, VL and CVL individuals showed high levels of antibodies against rPeroxidoxin, allowing identification of infected ones with considerable sensitivity and great ability to discriminate (specificity) between non-infected and CD individuals (98.46% and 100%; 98.18% and 95.71%; 95.79% and 100%, respectively). ELISA also showed a greater ability to discriminate between vaccinated and infected animals. A depletion ELISA assay using soluble peptides of this B-cell epitope confirmed the recognition of these sites only by infected individuals. Moreover, this work identifies two antigenic polymorphic linear B-cell epitopes of L. braziliensis. Specific recognition of TL and VL patients was confirmed by significantly decreased IgG reactivity against rPeroxidoxin after depletion of peptide-1- and peptide-2-specific antibodies (peptide 1: reduced by 32%, 42% and 5% for CL, ML and VL, respectively; peptide-2: reduced by 24%, 22% and 13% for CL, ML and VL, respectively) and only peptide-2 for CVL (reduced 9%). Overall, rPeroxidoxin may be a potential antigen for the diagnosis of TL, VL or CVL, as it has a higher agreement with parasitological assays and is better than other reference tests for diagnosing CVL in Brazil. **Supported by:**CAPES/REUNI, CNPq e FAPEMIG

HP112 - OVEREXPRESSION OF *LEISHMANIA BRAZILIENSIS* HSP83.1: EFFECT ON PARASITE VIRULENCE

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Leishmania (V.) braziliensis is a parasite responsible, in Brazil and other countries of the Americas, for most cases of human cutaneous leishmaniasis, a serious public health problem. Using data mining methods, it is possible to identify parasite proteins or peptides that exhibit similar biochemical and functional roles than host molecules associated to defense mechanisms. Among them, it has been identified parasite virulence factors that lead to the suppression of host inflammatory response. In the present work, we have investigated the role of *Leishmania* Heat Shock Protein 83.1 protein in *in vitro* and *in vivo* infectivity. HSP83.1 is a highly conserved molecule in prokaryotes and eukaryotes that plays important roles in protein folding, assembly of protein complexes, translocation of proteins across cellular compartments and modulation of immune response. Sequence analysis revealed high similarity among orthologs and conservation of functional domains. Expression analyses in the distinct developmental stages of the parasite revealed increased expression in the metacyclic forms and especially in intracellular and axenic amastigotes. Infection assays using specific inhibitors for a functional domain of HSP83.1 reduced intracellular parasite burden compared to control groups. Mutant parasite overexpressing HSP83.1 was obtained. We observed an increasing in the parasite load in macrophages infected with mutant compared to wild-type parasites and similar results were observed by monitoring lesion development in mice. **Supported by:**CAPES/REUNI, CNPq, FAPEMIG

HP113 - *LEISHMANIA* (L.) *AMAZONENSIS* PROMASTIGOTE ACTIVE CELL INVASION VERSUS PHAGOCYTOSIS: WHICH WAY SUIT IT MOST?

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Leishmaniasis is a disease caused by protozoa from the genus *Leishmania*. The parasite has two developmental stages: promastigotes, which are the extracellular flagellated forms, and amastigotes, which are intracellular multiplicative forms found into the mammalian hosts. Although dendritic cells and macrophages are considered the main host cell for *Leishmania* invasion, the infection of non-phagocytic cells has been reported. Thus, the aim of this study was investigate the potential of active invasion of *Leishmania amazonensis* in non-phagocytic cells. Then, 105 immortalized macrophages from C57BL/6 were plated in 24 well plates, and fixed for 40 minutes in formaldehyde 0,01%. Also, non-fixed and non-phagocytic cell lines such as, VERO, HeLa and C2C12 were plated. After, cells were washed with PBS and metacyclic promastigotes of *L. amazonensis* were incubated in the amount of 10 parasites/cell during 4 hours. Next, cells were fixed in Bouin solution for 15 minutes and stained with Giemsa for analysis by light microscopy. One hundred infected cells were counted and the number of parasites internalized. We observed the presence of L. amazonensis into prefixed macrophages from C57BL/6 and into the non-phagocytic cell lines. Thus, we showed here additional evidences sustaining the idea that *Leishmania amazonensis* promastigote forms are able to actively invade host mammalian cells. **Supported by:**CAPES/CNPq/ FAPEMIG/ UFU

HP114 - *LEISHMANIA AMAZONENSIS* PHAGOLYSOSOME FUSION: A MAP-KINASE SIGNALING-DEPENDENT EVENT.

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Introduction: *Leishmania* spp are causative protozoa of leishmaniasis, which affects millions of people worldwide. The host-pathogen interaction involves the parasite surface molecules and cellular receptors that culminate in phagocytosis. One mechanism that promastigotes use to evade the microbicidal effect of phagocytosis is to inhibit phagolysosome biogenesis by delaying lysosome fusion using LPG. However, the molecular mechanisms underlying the *Leishmania*-mediated inhibition of phagosome-lysosome fusion are still poorly understood. This study aimed to understand which signaling pathways are involved in the formation of the phagolysosome process, the invasion and multiplication of the parasite in the host cell. Methods: Kinetics of recruitment to the phagosome vesicles, invasion and multiplication assays were performed using specific inhibitors of cell signaling PI3K, Akt, MEK1/2, ERK2, mTOR and NRAS. In addition, western blot of invasion kinetics at different times were performed to understand what pathways are activated in each step of the invasion. Results: In the invasion assay there was a reduction in the rate of invasion and parasite load in murine peritoneal macrophages when were treated with inhibitors of PI3K, MEK1/2, ERK2 and AKT signaling pathways. In addition, inhibition of signaling involving AKT, ERK, MEK1/2, mTOR and RAS delayed phagolysosome maturation. Furthermore, western blot showed that the AKT pathway was activated by *L. amazonensis* entry into the host cell 15 and 30 hours post *L. amazonensis* invasion. The ERK pathway was activated at 15 minutes, 30 minutes and 1 hour after invasion. However, the intracellular multiplication was not affected by inhibition of the pathways. Conclusion: *L. amazonensis* cell invasion and phagolysosome fusion relies on different host cell signaling pathways. It also appears likely that different pathways temporally regulate fusion of lysosomes, and that ERK-2 signaling is a major contributor during both processes.
Supported by:CAPES/CNPq/FAPEMIG/UFU

HP115 - IDENTIFICATION OF THE LEISHMANIA PHOSPHOLIPID INVOLVED IN MACROPHAGE PHAGOCYTOSIS WITHOUT TRIGGERING ITS INFLAMMATORY RESPONSE

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Amongst the mechanisms presented by the protozoa of the genus *Leishmania*, to escape from the immune response of the vertebrate host, it was proposed that *Leishmania* uses apoptotic mimicry to promote a silent entry into the vertebrate macrophage. Data in the literature suggest that one of the signals recognized by the macrophage is the exposure of the phospholipid phosphatidylserine (PS). However, a recent publication reported the absence of PS in *Leishmania* species. The aim of this communication is to identify the phospholipid presented in the external plasma membrane layer that lead the macrophage to perform the parasite phagocytosis without triggering an inflammatory response.

As a strategy, we performed a competition between liposomes constructed with a single phospholipid class, at concentrations of 0.2 µM, 20 µM or 2 mM, and promastigotes of *L. (L.) amazonensis* in a macrophage (MOI 5:1) infection assay. After 4 and 24 hours of infection, we determined the amount of amastigotes/macrophage and the rate of infected macrophage by counting 100 macrophages, in each of four random fields of each well of the chamber slide. The infectivity rate was then calculated by the multiplying both parameters.

In the presence of phosphatidylcholine (PC) liposomes, we observed a dose dependent decrease in the rate of infected macrophage that reflected in the infectivity rate, in both 4h and 24h of infection. This profile was not observed in the presence of liposomes of PS. In the assays, the number of amastigotes/macrophage was not affected. The results suggest that PC liposomes are blocking the receptor in the macrophages and thus inhibiting the entry of some parasites. Another explanation could reside in a literature data indicating that the PC can reverts the signaling cascade that results in apoptosis. As the number of amastigotes/macrophage was not altered, we can say that the *Leishmania* that is phagocytosed by macrophages replicates normally. **Supported by:**FAPESP and CNPq

HP116 - POLYAMINES BIOSYNTHETIC PATHWAY IN DIFFUSE CUTANEOUS LEISHMANIASIS

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Diffuse Cutaneous Leishmaniasis (DCL) is a rare clinical manifestation of tegumentary leishmaniasis caused by Leishmania amazonensis. It is characterized by an inefficient parasite-specific cellular response and heavily parasitized macrophages. It has been demonstrated “in vitro” that polyamines and their derivatives are essential to Leishmania proliferation. Interference with polyamine biosynthesis has been successfully applied to the clinical treatment of African sleeping sickness. However, the relevance of these mediators for DCL pathogenesis remains unknown. Here, we evaluate systemic release and in situ expression of enzymes linked with polyamines synthesis and the role of arginase inhibition in vitro. Plasma from 12 DCL and 29 Localized Cutaneous Leishmaniasis (LCL) patients were evaluated by ELISA. The plasma levels of arginase and ODC were higher in DCL than those from LCL patients. Immunohistochemistry confirmed that arginase and ODC expression were higher in DCL than in LCL lesions. Additionally, in situ transcriptomic analyses revealed that expression of spermine synthase, S-adenosylmethionine Transport and CAT2A (transporters from S-adenosylmethionine and arginine, respectively) were elevated in DCL lesions compared with LCL. Interestingly, enzymes from parasites present in the lesion, arginase and glutathionylspermidine synthase were also increased in DCL when compared with LCL. In order to know if arginase inhibition alters Leishmania infection, human macrophages were infected with L. amazonensis and treated with NOHA (arginase inhibitor). The infectivity index 72h post-infection in the group treated with NOHA was lower than the unstimulated infected group. Our data suggest that L. amazonensis subvert polyamine biosynthetic enzymes as an important strategy for parasite proliferation in DCL. Investigating the involvement of polyamine pathway in DCL pathogenesis can contribute to the development of new therapeutic strategies for human Leishmaniasis. **Supported by:**FAPESB, CNpQ, FIOCRUZ

HP117 - THE ROLE OF UBIQUITIN FOR TARGETING AMASTIGOTES FORMS OF TRYPANOSOMA CRUZI FOR DEGRADATION IN LAMP1+ VACUOLES IN MURINE MACROPHAGES

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Eukaryotic cells target intracellular pathogens to acidified phagosomes that trigger pathogen degradation. Phagosomes of intracellular bacteria such as Salmonella spp. and Mycobacterium turberculosis are enveloped by conjugated ubiquitin chains and targeted to acidified phagosomes for degradation and infection control. However, the participation of ubiquitin signaling in intracellular T. cruzi targeting to acidified phagosomes has never been reported. In this context, we evaluated whether degradation of the intracellular amastigotes of T. cruzi in macrophages is dependent on ubiquitin signaling. To assess this question, bone marrow macrophages from C57BL/6 (WT) mice were infected for 1h, 12h, 24h and 48h and submitted to immunofluorescence for detection of ubiquitin chains. We observed that infected macrophages show ubiquitin associated with T. cruzi amastigotes in all time analyzed. About 5% of parasites associated with ubiquitin after 1h of infection. This percentage increased to 25% and 20% after 12h and 24h, respectively. After 48h of infection we observed 11% of parasites associated with ubiquitin. We also noted that the association of ubiquitin with parasites occurred as vesicles around the whole parasite and as agglomerate side of the parasite. We observed higher number of ubiquitin as agglomerated side of the parasite in compared to ubiquitin as vesicles around the whole parasite. In addition, we analyzed whether T. cruzi amastigotes associated with ubiquitin could be target to lysosomes for degradation. We detected that parasites with ubiquitin colocalizes with LAMP1 positive vesicles. All together, our data suggest that in macrophages, T. cruzi amastigotes associates with ubiquitin chains to target the parasites to LAMP1+-acidified vacuoles for degradation. This process may be critical for infection control. **Supported by:**CNPq

HP118 - SNARES OF THE ENDOCYTIC PATHWAY ARE INVOLVED IN THE BIOGENESIS OF THE *TRYPANOSOMA CRUZI* PARASITOPHOROUS VACUOLE.

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Trypanosoma cruzi is the protozoan parasite that causes human Chagas disease. As an obligate intracellular parasite, *T. cruzi* resides transiently in a parasitophorous vacuole (TcPV). It is well established that TcPV must fuse with lysosomes of the host cell to establish a productive intracellular infection. SNAREs proteins are key molecules of the vesicle fusion machinery. The goal of this study is to identify SNAREs proteins involved in the parasite infection of non-professional phagocytic cells. Our results indicated that, after 3 hours of infection, large amount of the TcPV population were decorated with EGFP-Vamp7 in a patchy pattern (68.7% ± 12.2%). It is worthy to note that, Vamp7 is localized on lysosomes and is involved in the fusion of these organelles with different target membranes. However, EGFP-Vamp3, which regulates fusion of recycling/early endosomes with plasma membrane, was only detected in a minor fraction of the vacuoles (30.2% ± 15.0%). EGFP-Vamp8, involved in the homotypic fusion between late endosomes, was not detected. The kinetics of Vamp3 and Vamp7 association with TcPV showed different pattern throughout the course of the infection. Although both SNAREs were highly recruited at early infection times (15 min), Vamp7 increased during the first 6 hours whereas Vamp3 declined. Interestingly, Vamp7 overexpression increased twofold the parasite infection rate while siRNA silencing of Vamp7 caused a marked decrease in the infection rate. In addition, we have detected some Vamp7 cognate partners (Vti1b, Snap23 and Stx3) in the vacuole membrane. Taken together, these results indicate that Vamp7 plays a major role in TcPV biogenesis, probably by enabling the interaction with the endolysosomal compartment.

HP119 - *TRICHOMONAS VAGINALIS* INHIBITS NITRIC OXIDE PRODUCTION BY PHOSPHATIDYLSERINE EXPOSURE IN IEC-6 ACTIVATED WITH INTERFERON - γ

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Trichomonas vaginalis, an obligatory extracellular parasite of human urogenital tract, is the etiologic agent of Trichomoniasis, considered nowadays the sexually transmitted non-viral disease most common worldwide. More serious cases of this disease can lead to infertility or abortion. Thus, studies about the evasion mechanism of this parasite are in need. Our group showed that subpopulation of the parasite Toxoplasma gondii exposes phosphatidylserine (PS), inducing TGF-β1 secretion by infected macrophages activated with interferon γ. This PS exposure by T. gondii, leads to degradation of iNOS and therefore the inhibition of nitric oxide (NO) production and consequently persistence of infection on these cells. This escape mechanism through the PS exposure, known as apoptotic mimicry, has also been described on others protozoa. On this study, it was observed by flow cytometry that 37% of T. vaginalis population exposes PS on its surface. Interactions of T. vaginalis with IEC-6 activated by interferon γ were performed. NO production was assayed in the supernatant of IEC-6 activated after T. vaginalis infection, with T. vaginalis incubated with Annexin-V or only IEC-6 (control). Using confocal microscopy, the presence of iNOS was observed after 24 and 48h of infection and compared to non-infected cells and the ultrastructure of the interaction between both cells was also analyzed by Scanning Electron Microscopy (SEM). T. vaginalis was capable of degrading iNOS, and inhibiting NO production of activated IEC-6. SEM images showed, after 24h of infection, attachment of parasite on host cell and after 48h the spread of T. vaginalis, also noticed changes on parasite structure. These data suggest that T. vaginalis was able to infect activated IEC-6 causing iNOS degradation and inhibition of the NO microbicidal system, via apoptotic mimicry, therefore, allowing infection persistence, indicating that T. vaginalis has the same escape mechanism presented in others protozoa. **Supported by:**CAPES, UEZO, UENF, CNPq, FAPERJ

HP120 - PHENYLPRONANOID DERIVATIVES ISOLATED FROM *NECTANDRA LEUCANTHA* (LAURACEAE) AS NOVEL COMPOUNDS AGAINST *LEISHMANIA DONOVANI*

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Natural secondary metabolites are potential sources for the treatment of visceral leishmaniasis (VL). Phenylpropanoids are low molecular weight metabolites produced by plants for protection against infections, ultraviolet irradiation, wounding and herbivores. Here we reported the antileishmanial activity and the possible immunomodulation of three phenylpropanoid derivatives isolated from leaves of *N.leucantha* (Lauraceae):1,2-dimethoxy-6-[2'-methoxy-4'-(8'-propenyl)phenoxy]-4-(8-propenyl)benzene (1), dehydrodieugenol B (2) and 1,2-dimethoxy-6-[2'-methoxy-4'-(7'-hydroxy-8'-propenyl)phenoxy]-4-(8-propenyl)benzene (3). Despite no effectiveness against extracellular promastigotes, compounds 1 – 3 eliminated intracellular amastigotes, with IC50 values of 9 µg/mL, 5 µg/mL and 34 µg/mL, respectively.These compounds also showed toxicity against peritoneal macrophages, with IC50 values of 109 µg/mL, 36 µg/mL and 100 µg/mL respectively. When incubated with infected bone marrow-derivated macrophages (BMDM), the phenylpropanoid derivatives1 – 3 suppressed the secretion of IL-6 and IL-10, with an increased but non-significant NO production. Considering that elevated levels of IL-6 and IL-10 are correlated with a worse prognostic and a higher parasite burden in LV infection, the immunoregulatory action of the studied phenylpropanoid derivatives could be an important pathway for parasite clearance. Furthermore, these unsophisticated chemical structures could represent promising scaffolds for drug design studies for leishmaniasis. **Supported by:**FAPESP 2013/07275-5 and 2013/50318-7

HP121 - HOST-PARASITE INTERACTION IN CHAGAS DISEASE: GENETICALLY UNIDENTICAL ISOLATES OF A SINGLE *TRYPANOSOMA CRUZI* STRAIN IDENTIFIED *IN VITRO* VIA LSSP-PCR

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Factors intrinsic to *Trypanosoma cruz*i and/or other host-related act in Chagas disease pathogenesis. In this context, the parasite genetic polymorphism seems to have a critical role in disease prognosis, and have been demonstrated an association between the distincts clinical forms development and geographic distribution of *T. cruzi* subpopulations. Polyclonal strains are common in the natural infections, and may involve specific interactions parasite-organ. Therefore, this project consisted in biological (cellular and acellular growth media culture) and molecular (Low-Stringency Single-Specific Primer Polymerase Chain Reaction/LSSP-PCR) profile characterization of parasite subpopulations obtained from blood cultures (3, 6, 12 months after the infection) in 24 mice experimentally infected with 5000 blood trypomastigotes forms of Berenice-78 (Be-78 parental strain) *T. cruzi* strain. It was possible to identify isolates with *in vitro* behavior, as well as gene signature profile, distincts from that observed for the Be- 78 parental strain. In some cases, the isolates showed some degree of resemblance with the Berenice-62 (Be- 62) strain, isolated from patient Berenice in Chagas disease chronic phase, 16 years before the xenodiagnostic that originated the Be- 78 strain. These data suggest the presence of distinct subpopulations in Be-78 strain and underline the plasticity of the parasite against the selective pressure exerted by the parasite-host interaction. In the next stage, infection with the isolates previously selected will be compared to infection with Be-78 parental and Be-62 strains in isogenic murine model for further lesions characterization in an attempt to clarify aspects proposed by clonal-histotropic model, comparing possible behavioral, related to the specific histotropism and pathogenicity. Financial Support: CAPES, CNPq, FAPEMIG and UFOP. **Supported by:**CAPES, CNPq, FAPEMIG and UFOP

HP122 - INFECTION OF CHICKENS AND CANARIES WITH DIFFERENT BRAZILIAN LINEAGES OF AVIAN PLASMODIUM

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Protozoans of the genera Plasmodium are etiologic agents that cause avian malaria. Few information about the potential risk and consequences of such infections to domestic avian hosts in Brazil, including the impact on animal production. This work aimed to study avian malaria in captive wildbirds in the state of Minas Gerais, Brasil. This research was approved by Ethical Committee of Universidade Federal de Minas Gerais, UFMG, and Instituto Estadual de Florestas de Minas Gerais, IEF. Blood samples of 83 passerine birds were collected. Stained blood smears were performed to confirm infection. Moreover, 20 microliters of blood were used to DNA extraction and sequencing of the cytochrome b gene (cytb). The diagnostic screening was performed by Nested PCR and associated to microscopy. PCR positive samples were used to obtain sequences which were aligned using CLUSTALX. The phylogenetic relationships were inferred using known lineages sequences available in Genbank and MalAvi databases. Parasites were detected by microscopy in ten donor animals while PCR amplified 12 samples. After sequencing, five lineage sequences were obtained, CAOBS01, TURUF01, PADOM09, MOBON01 and PHPAT01. In a first step, we selected the CAOBS01 lineage to infect three chickens. Rare small trophozoites were observed on 10 and 12 days post infection (dpi) in two of the three infected chickens, but chickens controlled the infection and no parasites were observed in a period of 60 days. Indeed, all chickens had no clinical signs of avian malaria at that time. Three canaries were infected with the same lineage, CAOBS01 and other three with PHPAT01. One canary from each group became infected on 3 dpi and maintained detectable parasitemia until 9 dpi. These preliminary data indicate that is possible to standardize a susceptible animal model to study infection of new Brazilian avian malarial parasites. This approach could improve the knowledge about parasite biology. **Supported by:**FAPEMIG

HP123 - DEPRESSION IN CHRONIC EXPERIMENTAL CHAGAS DISEASE IS REVERSED BY FLUOXETINE AND BENZNIDAZOLE

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The existence of the nervous form of Chagas disease (CD) is a matter of discussion since the description of neurological, behavioral and learning by Carlos Chagas. In most patients, the clinical manifestations of acute phase, including neurological disorders disappear without apparent sequels in the chronic phase of infection. Patients chronically infected exhibit behavioral changes such as memory disorders and depression. Little is known about the pathophysiology of behavioral changes in CD. These changes may result of (i) the presence of the parasite in the central nervous system, (ii) sequels of cardiac abnormalities, (iii) inflammatory tissue injuries, and/or (iv) systemic inflammatory profiles. Previously, we showed that C57BL/6 mice are resistant to acute meningoencephalitis, exhibit depressive-like behavior in the chronic phase of infection, indicating that this behavioral change is independent of the prior existence of acute meningoencephalitis, thus not being a sequel to this. In the present study, we showed that mice infected with the Colombian strain of T. cruzi exhibit characteristic behavioral changes in depression in late chronic phase (120 and 150 days post infection, dpi), such as increased immobility in the tail suspension test (TST), in absence of temperature changes and weight loss. Aiming to understand the pathophysiology of chronic behavioral changes in CD, we initially approached the process of reuptake of serotonin, using fluoxetine (Fx) and the participation of the parasite, using the trypanocidal drug benznidazole (Bz), treating chronically infected mice when behavioral changes are already installed (120 dpi) for 30 consecutive days. Fx therapy improved depressive profile, suggesting the contribution of a neurological component. Treatment with Bz in the acute phase of infection prevented the depression in chronic phase therapy in the chronic phase was beneficial in reducing the immobility time of animals in TST. **Supported by:**CNPq/ FAPERJ/ CAPES

HP124 - GENE EXPRESSION PROFILE OF CYTOKINES IN GOLDEN HAMSTERS (MESOCRICETUS AURATUS) INFECTED WITH LEISHMANIA (VIANNIA) BRAZILIENSIS

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American tegumentary leishmaniasis is widely spread in Brazil and represents a public health issue, in which Leishmania (Viannia) braziliensis stands out as the most prevalent specie. Studies using experimental models are of great importance to evaluate immunopathogenesis and efficacy of vaccine candidates and new drugs. However, most strain of mice are resistant to L. braziliensis infection, while golden hamster is highly susceptible, becoming one of the most suitable models. Despite this, the broad use of golden hamsters in studies is impaired by the lack of available immunological and molecular supplies, Accordingly, new tools that address the immune response in hamsters are required The aim of this study was to develop and evaluate a SYBR Green RT-qPCR assay to estimate the cytokine gene expression profile in distinct tissues of hamsters infected with L. braziliensis. We first optimized RNA extraction from skin and lymph nodes, by using a protocol based on Trizol and RNeasy Mini Kit (Qiagen). cDNA was synthesized from 2.0 µg RNA, previously treated with DNAse I. cDNA concentration was estimated by fluorescence, using the Qubitt ssDNA assay (Life Technologies). RT-qPCR assays were standardized using primers designed to cytokines (IFN-γ, IL-10, TNF-α, TGF-β) and enzymes (arginase and iNOS) involved in pathogenesis of leishmaniasis. Therefore, gene expression assays by the $\Delta\Delta$CT method were validated using skin and lymph node samples from uninfected and infected hamsters. This study established the best PCR cycling conditions, primers concentration (100nMX100nM Fw/Rv) and cDNA sample concentration (20ng/well) for all assays. Observed PCR efficiency varied from 93-105%, and specificity was assured by the observation of a single peak at melting curves. These results indicate the applicability and first description of a broad panel of cytokine gene expression assays in hamster model of L. braziliensis infection, using a SYBR Green RT-qPCR system. **Supported by:**CAPES, CNPq, FIOCRUZ/IOC,FAPERJ

HP125 - ENZYMATIC CARACTERIZATION AND IMMUNOCYTOLOCALIZATION OF PROLYL OLIGOPEPTIDASE FROM *LEISHMANIA CHAGASI*

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Leishmaniasis represents a group of diseases caused by protozoan of the genus *Leishmania* and is endemic in 98 countries and the available treatment for this disease has its side effects. This project focused on the initial studies of the [illegible] *Leishmania chagasi*, a serine protease, through its [illegible] gene was cloned and purified by nickel affinity [illegible] the best activity of rPOPLc was found [illegible] pH 7.5. The N-Suc-Gly-Pro [illegible] among all tested in this study [illegible] pM and 4.651,16 mEU/[illegible] and TLCK, although it was inhibited by Z-Pro-prolinal, a specific inhibitor [illegible] to immunocitolocalization, it was observed that rPOPLc [illegible] allowed an initial characterization of POPLc and the understanding [illegible] in the biology of the parasite may contribute to the search of new alternatives [illegible] for leishmaniasis.

HP126 - ROLE OF B-1 CELLS IN THE INFECTION BY *LEISHMANIA (L.) AMAZONENSIS*

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Protozoa of the genus *Leishmania* are responsible for causing leishmaniasis, a disease transmitted by the bite of an infected sandfly. These protozoans have developed different strategies to survive within important cells of the host immune system. Despite advances in the study of *Leishmania* immunobiology, there are still many gaps about the role that different cell types play during the infection. In the immune system, B-1 cells are primarily found in the peritoneal and pleural cavities. They are part of a subset of B cells responsible of producing large quantities of interleukin-10 (used as self-renewal factor) and circulating immunoglobulin M. Some studies showed that B-1 cells have the ability to migrate to inflammatory sites and differentiate into mononuclear phagocytes, while its absence results in improvement of the infection by various pathogens. Therefore, the aim of this study is to characterize the involvement of B-1 cells in the immune response to *Leishmania (Leishmania) amazonensis* infection using a murine model. In *in vitro* studies, B-1 cells, peritoneal macrophages (PM) and bone marrow-derived macrophages (BMM) from C57BL/6 mice were infected with *L. (L.) amazonensis* promastigotes for 2 to 48 hours, showing that the B-1 cells have phagocytic capacity and allow the growth of intracellular parasites, comparable to infection in PM and BMM. Thus, our results suggest that B-1 cells may contribute to disease progression infections by *L. (L.) amazonensis.* **Supported by:**CNPq

HP127 - EUPATHDB: AN ONLINE GENOMICS RESOURCE FOR EUKARYOTIC PATHOGENS.

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The Eukaryotic Pathogen Database Resource (EuPathDB) is a family of free online databases that integrate genomic data with functional genomics and clinical/field isolate data for numerous eukaryotic pathogens within Amoebazoa, Apicomplexa, Diplomonadida, Microsporidia, Trichomonadida and Kinetoplastida. EuPathDB also integrates data informing upon host-parasite interactions from datasets that analyze mixed parasite-host samples such as human cells infected with parasites. An interactive data exploration platform, EuPathDB provides data mining and visualization tools for discovering meaningful relationships between genomic features to support hypothesis-driven research. The databases are updated and expanded bimonthly with data ranging from genome sequence and annotation to expression data, to parasite field isolates, to host data in response to infection. Despite the breadth of data (140 genomes, 150 functional datasets), it is easy to mine, visualize, download and browse different data types. Data is mined using the Strategy System to search within and between datasets, developing in silico experiments that identify features with similar biological characteristics. Search strategies and results can be downloaded, saved and shared with colleagues. Data may be visualized in the context of the genome sequence and annotation using an interactive and configurable browser. Individual record pages that compile all available data for a feature (e.g. gene, isolate, genomic sequence) provide a comprehensive view of the feature. Our extensive user-support system includes video tutorials, a rapid-reply email question hotline, and hands-on workshops at locations worldwide. Attend our poster and exhibit table for an overview of this NIH/NIAID-funded resource. Or visit one of our sites: AmoebaDB.org, CryptoDB.org, EuPathDB.org, GiardiaDB.org, MicrosporidiaDB.org, PiroplasmaDB.org, PlasmoDB.org, ToxoDB.org, TriTrypDB.org, TrichDB.org, OrthoMCL.org, and HostDB.org.

HP128 - *LEISHMANIA INFANTUM* ECTO-NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE-2 IS AN APYRASE INVOLVED IN MACROPHAGE INFECTION AND EXPRESSED IN INFECTED DOGS

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Background: Visceral leishmaniasis is an important tropical disease, and *Leishmania infantum chagasi* is the main pathogenic agent of visceral leishmaniasis in the New World. Recently, ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases) were identified as enablers of infection and virulence factors in many pathogens. Two putative E-NTPDases have been found in the *L. infantum* genome. Here, we studied the ~45 kDa E-NTPDase from *L. infantum chagasi* to describe its natural occurrence, biochemical characteristics and influence on macrophage infection. Methodology/Findings: We used live *L. infantum chagasi* to demonstrate its natural ecto-nucleotidase activity. We then isolated, cloned and expressed recombinant rLicNTPDase-2 in bacterial system. The recombinant rLicNTPDase-2 hydrolyzed a wide variety of triphosphate and diphosphate nucleotides (GTP>GDP=UDP>ADP>UTP=ATP) in the presence of calcium or magnesium. In addition, rLicNTPDase-2 showed stable activity over a pH range and was partially inhibited by ARL67156 and suramin. Microscopic analyses revealed the presence of this protein on cell surfaces, vesicles, flagellae, flagellar pockets, kinetoplasts, mitochondria and nuclei. The blockade of E-NTPDases using antibodies and competition led to lower levels of parasite adhesion and infection of macrophages. Furthermore, immunohistochemistry showed the expression of E-NTPDases in amastigotes in the lymph nodes of naturally infected dogs from an area of endemic visceral leishmaniasis.Conclusions: In this work, we demonstrated that LicNTPDase-2 it functions as a genuine enzyme from the CD39 family. We showed that E-NTPDases are present on the surface of promastigotes and in other intracellular locations. We showed, for the first time, the broad expression of LicNTPDases in naturally infected dogs. Additionally, the blockade of NTPDases led to lower levels of in vitro adhesion and infection, suggesting that these proteins are possible targets for rational drug design. **Supported by:**CAPES, CNPq, FAPESP, UFV, FAPEMIG

HP129 - *LEISHMANIA* (*LEISHMANIA*) *AMAZONENSIS* AP-ENDONUCLEASE (LAMAP): INVOLVEMENT IN PARASITE RESISTANCE TO OXIDATIVE AGENTS

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During the intracellular stage, parasites from *Leishmania* genus are constantly challenged with free radicals produced by phagocytic cells damaging the DNA. These damages can generate abasic sites through the parasite genome and must be repaired to assure its survival. AP-endonuclease family enzymes are actively involved in the repairing of these lesions. Our group previously identified and molecularly characterized the Ap-endonuclease gene from *L*. (*L*.) *amazonensis* (*lamap*). In this current work, we overexpressed lamap and evaluated the possible modifications in parasite biology. L. amazonensis strains were challenged with different concentrations of menadione (MD), an oxidative inductor agent. After one hour of treatment the parasites were harvested in PBS and incubated in Schneider's medium before the counting of survived cells. In another assay, the parasites were pre-treated with a chemical inhibitor of Ap-endonuclease known as methoxyamine (MXE) for 4 hours before proceeding with the stages described above. To evaluate possible changes in host-parasite interaction macrophage from Balb/C mice were incubated with *L*. (*L*.) *amazonensis* stationary phasis promastigotes with or without previous treatment of the parasites in 20 mM of MXE. Macrophages were grown in RPMI medium for three days and in each day the corresponding glass slices were stained and analyzed in microscopy. After 48 hours parasites overexpressing the lamap gene were able to survive in MD concentrations varying from 62,5µM to 1mM while the control group survived from 62,5µM to 500 µM. Pretreatment with MXE did not change replication levels in all the groups, while in non-treated ones overexpressed amastigotes replicated approximately ten times more than the control group. Overexpression of lamap seems to confer resistance to MD in transfected *L*. (*L*.) *amazonensis* when compared to wild type showing the importance of its enzyme in DNA repair and its use as a target in vaccines. **Supported by:**CNPq, CAPES, FAPERJ, IOC/FIOCRUZ

HP130 - MELITTIN PEPTIDE KILLS AND INDUCES MORPHOLOGICAL CHANGES IN *LEISHMANIA AMAZONENSIS* METACYCLIC FORMS

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Leishmania amazonensis is the causative agent of American cutaneous leishmaniasis, an important neglected tropical disease. Leishmaniasis chemotherapy is based on drugs that exhibit toxic effects, high cost, difficult administration and is far from satisfactory due to increases in drug resistance. Thus, new chemotherapeutic agents from natural sources, such as animal's venoms, are a line of research to be exploited. Melittin is a naturally occurring antimicrobial peptide, which comprises to 40-50% of the dry weight of *Apis melifera* venom and is lethal to all developmental forms of *T. cruzi*. In this study we investigated the mechanism of death pathway induced by mellitin on *Leishmania amazonensis* promastigotes. Mellitin treatment with a LC50 (2.3 µg/ml for 24 h) induced loss of parasite viability as verify by propidium iodide as well as depolarization of the mitochondrial membrane, which was quantified by Rh 123 staining. Scanning and transmission electron microscopy images showed profound alterations on the parasite morphology including cell swollen and surface membrane blebs, mitochondrial and nucleus alterations and an increase in the number and size of lipid droplets compared to control cells. The data suggested that mellitin induces apoptosis in promastigotes of *L. amazonensis*. The analysis of the effect of mellitin on amastigote forms is in progress. The results obtained in the present study provide a rationale for further exploration of the mechanism of action of mellitin on *L. amazonenis* promastigote and amastigote forms. **Supported by:**CNPq, CAPES, FAPERJ and Pronex

HP131 - COMPARATIVE EVALUATION OF LESION DEVELOPMENT, TISSUE DAMAGE AND CYTOKINE EXPRESSION IN GOLDEN HAMSTERS (MESOCRICETUS AURATUS) INFECTED BY INOCULA WITH DIFFERENT CONCENTRATIONS OF LEISHMANIA (VIANNIA) BRAZILIENSIS

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The golden hamster (Mesocricetus auratus) is a susceptible model to Leishmania (Viannia) spp. but available studies employ different infection protocols accounting for differences in clinical and pathological presentations. Herein, preparations of L. (V.) braziliensis were standardized to contain 104, 105 or 106 parasites to determine an optimal inoculum that ensures cutaneous lesions without causing a disseminated infection in hamsters. Lesion development were followed for 105 days by size measurement and then skin, draining lymph node, spleen and sera were investigated to parasite load, spleen visceralization, cytokine expression, histopathological alterations and anti-Leishmania IgG levels. The time for lesion emergence was inversely proportional to the number of parasites in the inocula. Animals infected by 104 inoculum presented mostly with nodular lesions while 106 often exhibited ulcerated lesions. Differences in the final lesion size were observed between 104 and 105 inocula or 104 and 106 but not between 105 and 106 inocula. High levels of IFNG expression, anti-Leishmania IgG and parasite load occurred independently of the inocula used. A mild inflammatory skin involvement was seen in animals infected with 104 parasites while extensive tissue damage and parasites spleen visceralization occurred with 105 and 106. These results indicate that inoculum with 104 parasites generated skin lesions with less systemic commitment, despite high IFNG expression and parasite load. This suggests that a modulation in the immune response to different numbers of parasites occurs in an early phase of the infection that could dictate the establishment and magnitude of the chronic phase of the disease. **Supported by:**CAPES, CNPq, FIOCRUZ/IOC,FAPERJ

HP132 - STUDY OF MURINE MONOCYTE SUBPOPULATIONS AS POSSIBLE BIOMARKERS OF RESISTANCE AND SUSCEPTIBILITY DURING EXPERIMENTAL INFECTION WITH *LEISHMANIA (LEISHMANIA) MAJOR*

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Monocytes are important circulating leukocytes that migrate to tissues during the inflammatory response. Recently, two major subpopulations of monocytes have been described based on the expression of surface marker GR1(Ly6C). Previous results of our group showed that the population of GR1+ monocytes plays an important role in the early stages of the disease, being rapidly recruited to the lesions, efficiently eliminating *L. major* parasites. The objective of this study is to evaluate the changes of GR1+ subpopulation of monocytes in peripheral blood, during experimental infection of Balb/c mice treated with Amphotericin B and the association between the frequency of this subpopulation with the development of the lesion. BALB/c and C57BL/6 mice were, infected in the left footpad with $2x10^6$ promastigotes of L. major. After 2 weeks of infection, were applied daily injections of 5mg/kg of Amphotericin B via intraperitoneal for 2 more weeks. Blood samples were collected and stained for F4/80, CD11b and GR1 and acquired on a BD FACSCanto. The results were analyzed by FlowJo software. The development of the paw was measured with a caliper every week. Our results show that the frequency of GR1+ monocytes in the blood increases during infection by *L. major* and that this increase is directly related to the size of the lesion. C57BL/6 mice showed a decrease in the frequency of GR1+ monocytes after 8 weeks of infection being related to a decrease of lesion size. In addition, we observed that BALB/c treated for 4 weeks with amphotericin B showed less damage and therefore decrease in frequency of monocytes in the blood. These results indicate that the frequency analysis of monocytes in the blood can be used as a prognostic indicator in treated animals, since decreasing the frequency of these cells in the blood during treatment is directly related to the improvement in the clinical condition of the animal. **Supported by:**CNPq FAPEMIG, CAPES e NIH GRANT

HP133 - CELLULAR MAP KINASE PATHWAY IS DIFFERENTLY INDUCED BY TRYPANOSOMA CRUZI EXTRACELLULAR AMASTIGOTES (EAS) OF G AND CL STRAINS

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EAs are alternative infective forms of *Trypanosoma cruzi* and together with bloodstream trypomastigotes, sustain the parasite cycle in mammalian hosts. Differences in the infectivity of EAs from two strains, G (derived from sylvatic type I) and CL (derived from the vector Triatoma infestans, type VI) have been widely acknowledged: G strain EAs are much more infective *in vitro* than CL parasites. Mitogen-Activated Protein kinase (MAPK) pathways are activated in mammalian cells in response to a variety of extracellular stimuli and mediate signal transduction from the cell surface to the nucleus. There are four MAPKs cascades: the extracellular signal-regulated kinases (ERK1/2), c-Jun NH2-terminal kinases (JNK-1/2/3), p38 (p38α/β/γ/δ) and ERK5. We have analyzed host proteins phosphorylation in extracts of HeLa cells previously incubated with EAs from both strains, using a phospho-kinase array kit®, and Western blots assays. G strain EAs induced a bimodal phosphorylation of MAPKs (p38, ERK) (increased activation followed by rapid decline and increased again, ending in a further decline of activation). The phosphorylation of protein phosphatase MKP1 was also observed, at the opposite points of greater ERK activation. CL parasites, however, did not induce MAPK bimodal activation. G strain also activates JNK1, MKK3 and MKK6 (p38 activators) and MSK2 (activated by p38 and ERK1/2) whereas CL not. Taken together these results could raise two hypotheses: first, G and CL strains induce different MAPK signaling responses in Hela cells; second, G strain EAs -but not CL- seem to induce the MAPK-based danger response pathway which ultimately might lead to immune activation and secretion of proinflammatory cytokines. Further studies are needed to verify these hypotheses. **Supported by:**FAPESP, CNPQ, PRPQ

HP134 - CALCIUM INDEPENDENTE PHOSPHOLIPASE A2 (IPLA2) OF *LEISHMANIA AMAZONENSIS* REGULATED INFECTION AND INTRACELULAR BEHAVIOR OF AMASTIGOTE FORMS

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Infection of host cells by intracellular parasites involves an initial contact followed by the recognition of specific ligands located on the surface of both cells. The number and distribution of such molecules depend on exocytosis processes that are mostly dependent on the fusion of compartments of the endo/exocytotic pathways with the plasma membrane. Previous work of our group we observed that the inhibition of calcium-independent phospholipase (iPLA2) by bromoenolactone (BEL) in promastigotes of *L. amazonensis* caused a reduction in the rate of endocytosis of transferrin, a classical marker of endocytosis, and interfered in the exocytosis of gp63, molecule engaged in Leishmania-macrophage interaction. In this study we verify whether the inhibition of iPLA2 from *L. amazonensis* forms could modify the rate of macrophage infection, interfere with the intracellular differentiation of the promastigotes and the proliferation of intracellular amastigotes. Metacyclic forms were incubated for 1h with 2.5 µM of BEL, an irreversible inhibitor of iPLA2. After treatment parasites were incubated with macrophage for analysis of the adhesion, internalization and survival of the parasite within the macrophage for 96 hours, at each time analyzed, the slides were stained with gienmsa and quantified by counting total of 200 cells in three independent experiments. The interaction were analyzed by scanning electron microscopy to analysis the parasitophorous vacuole. We observed that BEL significantly reduced the adhesion (70%) and infection (80%) at 96 hours post infection (hpi). We also observed a reduction in the rate of proliferation of amastigote forms. Microscopic analysis of infected macrophages at 24 and 48 hpi showed a significant change in the morphology of the vacuole containing treated parasites. We believe that treatment with BEL modifies important cellular signaling pathways that control the infection and the formation of the parasitophorous vacuole. **Supported by:**CNPq, CAPES, FAPERJ, Pronex

HP135 - THE TNFRP55 MODULATE THE INFLAMMATORY RESPONSE IN *LEISHMANIA AMAZONENSIS* INFECTION

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Introduction: The cytokine tumor necrosis factor (TNF) is required for resistance to several pathogens, such as *Listeria monocytogenes, Candida albicans, Trypanosoma cruzi* and Leishmania major. One protective function of this cytokine is the ability to synergize with IFN-y to induce the expression of iNOS by macrophages, leading to NO production and the killing of parasites. Two cognate receptors for TNF have been described: the TNFR1 (TNFRp55) and the TNFR2 (TNFRp75). TNFR1 promotes cell survival and inflammation or, alternatively, can induce apoptosis. Although many studies had demonstrated that TNF plays a central role in the outcome of many infection models, the role of this cytokine in L. amazonensis infection remains to be completely understood. The objective of this study was to evaluate the role of TNFRp55 in infection by L. amazonensis.
Methods and Results: Our data did not show differences in parasite load, lesion size and production of TNF-α, IFN-γ and IL-10 by lymph node cells stimulated in vitro with the parasite antigen, between C57BL/6 wild-type and TNFRp55-/- mice, 8 weeks post infection. After 16 weeks, an increase in lesion size was seen in the TNFRp55-/- mice, but the parasite load was not different between the groups. At this time of infection, the production of TNF-α, IFN-γ and IL-10 were the same for both groups, but knockout mice showed a higher arginase activity in the footpad, which can reflect a higher inflammatory infiltration. Interestingly, at the beginnig of infection, larger lesions were seen in wild type mice.
Conclusion: These data suggest that TNFRp55 plays an immunomodulatory effect in this infection model that may be is important for the resolution of the inflammatory process, be by mediating may apoptosis, but TNFRp55 was not essential for the control of the parasite replication. **Supported by:**Capes, CNPq e FAPEMIG

HP136 - PARASITOPHOROUS VACUOLES INDUCED BY *LEISHMANIA AMAZONENSIS* AND *LEISHMANIA MAJOR* INTERACT DIFFERENTLY WITH THE AUTOPHAGIC PATHWAY.

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Compartments induced by intracellular microorganisms can interact with vesicles of the autophagic pathway. Few studies have evaluated the participation of autophagic compartment membranes in the formation of *Leishmania*-induced parasitophorous vacuoles (PVs). Previously, it was demonstrated that *Leishmania mexicana*-induced PVs acquire macromolecules from host cell cytoplasm by microautophagy. PVs induced by L. mexicana and L. amazonensis are large and share similar features. Herein, we hypothesize that the large PVs induced by *L. amazonensis* fully interact with autophagic compartments, while the tight PVs induced by *L. major* interact to these compartments in a limited extent. Thus, the present study aimed to determine the role autophagic compartments play in the biogenesis of *Leishmania*-induced PVs. Initially, mouse macrophages (MΦ) were infected with *L. amazonensis* or *L. major* and incubated with the markers of acidic degradative compartments, LysoTracker® or DQ™-BSA. Then, we examined the expression in PVs membranes of the LC3 marker of autophagic compartments. We found that *L. major* in comparison with *L. amazonensis* colocalizes in a higher extent with either LysoTracker or DQ-BSA. On the other hand, LC3 colocalizes more frequently with *L. amazonensis* inside PVs than with L. major. These findings demonstrate that PVs induced by *L. amazonensis* or *L. major* interacts differently with compartments of the autophagic pathway. The role autophagy plays in parasite infection was investigated in RAW MΦ cell-line overexpressing LC3 (RAW-LC3) submitted to infection with *L. amazonensis* or *L. major*. We found a significant reduction in the percentage of *Leishmania*-infected RAW-LC3. Interestingly, RAW-LC3 didn't loose the ability to bind parasites, indicating overexpression of LC3 reduced parasite entry by decreasing its internalization. Further studies are under way to clarify the role autophagic process plays in PVs biogenesis, as well as in parasite infection. **Supported by:**CNPq; FAPESB; CAPES; INCT-DT

HP137 - RNAI KNOCK DOWN OF A *LEISHMANIA BRAZILIENSIS* AMASTIN GENE REVEALS ITS ROLE AS A MAJOR PARASITE VIRULENCE FACTOR

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Leishmaniasis, a human parasitic disease with manifestations ranging from cutaneous ulcerations to fatal visceral infection, is caused by more than 20 Leishmania species. The study of parasite proteins involved with promastigotes/amastigote differentiation and intracellular survival is critical for our understanding of this complex disease and the interactions b[illegible]en this parasite and its mammalian host. Amastins are surface glycoproteins [illegible]e gene families present in the genome of *T. cruzi* and several *Leish*[illegible]n other trypanosomatids and are among the most imm[illegible] antigens. Here we showed that the genome of [illegible] to all four previously described ama[illegible] up-regulated in *L. brazilie*[illegible]ause, in contrast to *T*[illegible]way has been identif[illegible]ction by knocking-down its e[illegible] of siRNA derived from one specific d[illegible]% of δ-amastins whereas transcript levels of α, β[illegible] *L. braziliensis* such as gapdh were not affected by the RN[illegible]*sis* cells lines in which δ-amastins gene expression was knocked-dow[illegible]mpaired growth as intracellular amastigotes in mouse macrophages and com[illegible]ely fail to produce infection when inoculated in BALB/c mice footpads. In both infection assays, this attenuated phenotype was reverted by the re-expression of an RNAi-resistant amastin gene, thus highlighting the role of amastins as a virulence factor essential for Leishmania infection. **Supported by:**CAPES

HP138 - PUTATIVE *TRYPANOSOMA CRUZI* P21 ANTAGONIST PEPTIDES AND SCFV ANTIBODIES *BINDING* SITES: AN *IN SILICO* ANALYSIS

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Introduction: *Trypanosoma cruzi*, etiologic agent of Chagas disease, secrete a protein called P21. Previous studies using the recombinant form P21 of *T. cruzi* showed that this protein is involved in host cell invasion, enhanced macrophage phagocytosis and actin polymerization. The use of biotechnology tools as molecular *phage display* technique has shown promise and provides a selection of bioactive peptides and monoclonal antibodies (scFv) expressed on filamentous phage. In vitro assays using this genetic engineering allow the selection of binder clones in various types of antigens through the phage libraries. The aim of this study is to find peptides and antibodies *binding* rP21 protein and evaluate the effect of these antagonists on the biological activities of the protein. Methods: The *phage display* technique was applied for the selection of peptides and monoclonal antibodies (scFv fragments). Expression of clones to enzyme immunoassays, conventional DNA extraction kit and QIAprep Spin Miniprep, sequencing and bioinformatics analysis, large scale purification in HPLC and lyophilization using IGblast tool were used. Results: The selection proved effective at the end of each biopanning, immunoassays showed that the expressed clones were able to recognize the recombinant protein P21 by using antibody HA. With the sequencing of the heavy and light chains of the antibody and using the IG blast characterize the sequences of scFv selected and in vitro studies will be performed to further evaluate the proposed biological activities. In addition, two peptides also showed strong affinity for P21 protein. Invasion assays showed an inhibitory effect of the peptides on the pro-phagocytic activity of p21. Conclusion: The use of these molecules will be promising for further studies. **Supported by:**CAPES/CNPq/UFU

HP139 - CHARACTERIZATION OF THE CASEIN KINASE 2 (CK2) AND ITS INFLUENCE ON THE *LEISHMANIA BRAZILIENSIS* - MACROPHAGES INTERATION.

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The cells interaction, in leishmaniasis, activates signal transduction pathways inducing numerous biological activities, including protein kinase CK2. CK2 has been observed in all eukaryotic cells, presents essential cellular functions and recognizes serine/threonine or tyrosine residues in target proteins. In a L. braziliensis virulent strain, studies show CK2 activity 23-fold greater than in a non-virulent strain. Initially, the ck2 gene from L. braziliensis was cloned into a recombinant protein expression vector. This clone, confirmed by sequencing, was used to characterize the genomic organization and to produce recombinant CK2α used for antibody production. The anti-CK2α obtained was able to recognize recombinant and native parasite CK2, both secreted and cytoplasmatic. Antibodies anti-CK2α inhibited the association index between an infective Leishmania strain and macrophages by 61%. Spermine was able to stimulate the native secreted CK2 (133.15%) but was not able to stimulate the recombinant CK2α activity. The recombinant CK2α specific activity was inhibited by heparin (84.22%) and TBB (97.6%). Secreted CK2 was also inhibited by heparin (87.57%) and TBB (96.64%). Recombinant CK2 increased the association index (11%), where the process was reversed by heparin (43.45%) and TBB (53.55%). Recombinant CK2 increased the association index (96%), with reversal by heparin (59.1%) and TBB (66.4%). No differences were observed in quantifications of ck2α gene transcripts between virulent and avirulent strains. These findings demonstrate the CK2 enzyme importance in the process of host - parasite interactions. In this way, studies involving this enzyme can be interesting for development of new drugs or vaccines. **Supported by:**CNPq, FAPERJ and PIBIC-UERJ

HP140 - TRYPANOCIDAL AND LEISHMANICIDAL ACTIVITY OF ISOBENZOFURANONES

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The neglected tropical diseases (NTDs) represent some of the most common infections of the poorest people living in the Latin American. Chagas disease and leishmaniasis figure between the most important NTDs with great burden. There are few drugs available, with extremely limited efficacy and safety, leading to an urgent demand for new treatment. The search for new drugs becomes ever more necessary due the fact that the parasites have developed resistance to the available drugs. The isobenzofuranones are heterocycles with diverse spectrum of biological activity. It has been reported a wide activity of this family of compounds against microorganisms, especially fungi. In the present work, we investigated the effect of compounds of the class of isobenzofuranonas on *Trypanosoma cruzi*, *Leishmania* and macrophages infected and uninfected. Epimastigotes of *Trypanosoma cruzi* and *Leishmania infantum chagasi* promastigotes were grown in the presence of 10 compounds at different concentrations. The calculation of EC_{50} was based on the viability of the parasites after 24, 48 and 72 hours. For *T. cruzi* a visual assessment showed that compounds 3, 8 and 13 caused changes in morphology and death of the parasites at the end of 72h. Compounds 9 and 10 caused different morphological changes, but its effect on the viability has not been determined, and is apparently low. For *Leishmania*, compounds 8 and 9 were able to kill more than 50% of the parasites in the first 24h. At the end of 72h all compounds significantly reduced the number of parasites. Cytotoxicity assay on macrophages showed that, in the concentration tested, there was no loss in cell viability for both compounds. Macrophage infection by *Leishmania* showed a decreased number of infected cells for both compounds, with macrophages morphologically preserved for compound 9. These studies will open a new range of possibilities, leading us in the search for new drugs to be applied in Chagas disease and leishmaniasis chemotherapy. **Supported by:**FAPEMIG, Capes, FAPESP, UFV e CNPq

HP141 - REGULATION OF THE PROLINE DEPENDENT DIFFERENTIATION PROCESSES IN *TRYPANOSOMA CRUZI* BY BRANCHED CHAIN AMINO ACIDS

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T. cruzi has the ability to metabolize amino acids and use them as carbon and energy sources. It is know tha leucine as other amino acids like Asp, Glu, Asn, Gln, Pro and Ileu can be metabolized by epimastigotes of *T. cruzi* and it is well established that amino acids play several roles in the trypanosomatids biology, i.e., Pro, Glu and Asp are involved in the metacyclogenesis. Furthermore, Leu and Ile were reported as regulators of proline dependent metacyclogenesis process (Homsy 1989).
Due to the absence of putative genes for branched chain amino acids (BCAAs: Leu, Ile and Val) biosynthesis in *T. cruzi*, their availability should depend on both, their uptake from the extracellular medium, and protein degradation. In the present work, we studied the BCAAs uptake system in epimastigotes forms of *T. cruzi* (CL-14) and its importance as regulators of the proline-induced differentiation process. Our results showed that BCAAs maintain the parasites viability of epimastigotes after 24 hr and 48 hr of starvation in PBS. The presence of extracellular BCAAs in a proline based differentiation medium (TAU-Proline), diminished the amount of metacyclic tripomastigotes. The effect of BCAAs was also evaluated at the cellular level infection, in wich was observed that when infected CHO-K1 cells are cultured in RPMI medium supplemented with leucine (1mM and 5mM) the tripomastigotes burst is diminished by 60%, additionally it was observed that this supplementation with leucine (5mM) at different infection times (infection, amastigotes and intracellular-epimastigotes), affects the number of total tripomastigotes liberation. We propose that those amino acids contributes to the metabolic regulation of the proline-dependent differentiation processes in *T. cruzi.* **Supported by:**CNPq

HP142 - AROMATIC DIAMIDINES,A DNA/RNA BINDING MOLECULE, INHIBIT THE GROWTH OF TRYPANOSOMA CRUZI EPIMASTIGOTES

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The mitochondrial genome of trypanosomas, called kinetoplast DNA (kDNA) is a complex and unique structure. It has been shown that aromatic diamidines, which fit snugly into the minor groove of DNA, exhibited an antiparasitic activity (Stolic et al 2009). The mechanism of action of aromatic diamidines is not fully elucidated. However it has been proven that some of their activities are related to DNA binding and subsequent inhibition of DNA-dependent enzymes (topoisomerases, polymerases, nucleases) as consequence of the complex structure and regulation of kDNA (Wilson et al 2008). Thus, we hypothesized that it also affects the kinetoplast. In the present work we tested the effect three DNA binders (named here in as MB17, MB19 and MB38) on the epimastigotes growth. Epimastigote forms of Trypanosoma cruzi CL14 were cultured under normal conditions and exposed to a range of drug concentration. Briefly, the cell number was followed along nine days by absorbance reading at 620 nm, comparing to a calibration. The assays were carried out in four replicates and the inhibition percentage was calculated with respect to the middle exponential phase, which happens in the 5th day. We had observed that both the three DNA binders were able to inhibit the growth of T. cruzi epimastigotes with IC50 0.5 µM (MB17); 3.7 µM (MB19) and 8.3 µM (MB32). Further fluorescent microscopy assays show that MB compounds bind specifically kinetoplast and disturb kinetoplast replication and therefore the parasite division. Further studies on the intracellular stages are being conducted to validate these compounds as a potential antiparisitic drug. **Supported by:**Croatian Ministry of Science, Education and Sport ; FAPESP; CNPQ

HP143 - NEUTROPHIL ELASTASE ACTIVITY IS REQUIRED FOR SUCCESSFUL INFECTION OF MURINE MACROPHAGES BY LEISHMANIA DONOVANI

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The comparison of the genomes of different Leishmania species revealed that only a few genes are species-specific, suggesting that gene expression regulation and/or specific interactions can be determinant for pathogenesis. L. major has three genes sharing similarity to bacterial ecotin: ISP1, ISP2 and ISP3. Ecotin is a bacterial serine peptidase inhibitor that interacts with S1A family enzymes such as neutrophil elastase (NE). We have previously shown that deletion of the ISP2 gene in L. major leads to reduced parasite survival in macrophages, due to the triggering of a pathway associated with NE, TLR2 and TLR4. In L. donovani, we could not detect the expression of ISP2 in promastigotes. To investigate if the absence of ISP2 expression plays a role in the infection by L. donovani, we generated transgenic L. donovani lines in which the L. major ISP2 gene was introduced in the ribosomal locus. ISP2 expression in the mutant lines was confirmed by Western blot. Infection assays of mouse peritoneal macrophages in vitro show that the ISP2-expressing lines have largely reduced intracellular growth. In agreement with a role for NE activity, intracellular growth of WT L. donovani was reduced by the addition of a synthetic NE inhibitor, and the parasites survived poorly in macrophages from elastase knock-out mice. C57B/6 mice infected with L. donovani ISP2-expressing lines showed decreased parasite load in the spleen. Assays in macrophages from TLR2 or TLR4 knock-out mice, showed that the absence of TLR4 led to a reduced parasite uptake and prevented their intracellular growth, whereas the absence of TLR2 decreased only parasite growth. We suggested that the activation of the pathway involving NE-TLR4-TLR2 is beneficial in L. donovani infection **Supported by:**CNPq

HP144 - SERO-EPIDEMIOLOGICAL SURVEY AND MOLECULAR SURVEILLANCE OF CANINE VISCERAL LEISHMANIASIS IN MUNICIPALITY NOT ENDEMIC

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Canine visceral leishmaniasis (CVL) is a chronic, systemic disease, and the dog is considered the main domestic reservoir of the parasite. Control programs predict the realization serological surveys to assessing the prevalence of the disease and, at the same time, the identification of serologically positive dogs, since the canine infection precedes the human. This study is a survey for investigation of CVL in the municipality of Alfenas-MG, located in area considered non-endemic for the disease, but with large flow of health service demand and students from different regions of the country, including endemic for this disease. The detection of infection by *L. chagasi* in dogs was conducted through serological methods (Immunochromatographic rapid test DPP® Bio-Manguinhos and ELISA *in house* employing specific antigen), in serum samples, and by molecular methods conventional PCR (RV1/RV2) and Real-Time PCR (Linj31, Ldon and DNAPOL) in whole blood samples. The results show that of the 64 samples analysed, six (9%) reacted in the DPP® test, but only one (0.15%) was confirmed by Elisa test. The molecular tests indicate that all animals were negative. Because it is not endemic area, negative results found suggest that apparently, no transmission is occurring in animals resident in kennel. The positive results in serology, unconfirmed by PCR, may be due to cross-reactivity with other diseases (Chagas disease, blood parasites as *Babesia canis* and *Ehrlichia canis*), since in this study, all animals were asymptomatic. The Ministry of Health currently recommends the DPP as screening and confirmatory ELISA where canines, but other tests are required for conclusive diagnosis of CVL, whereas serologic tests may show cross-reactivity and can generate false positives. **Supported by:**FINEP CAPES, UNIFAL-MG, FMUSP

HP145 - *LEISHMANIA AMAZONENSIS*: STUDY OF THE C3H/HE RESISTANT AGAINST IN PERITONEAL MACROPHAGES

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Leishmaniasis is a spectral disease where several different clinical manifestations can occurs. Different species of parasite produce different clinical manifestations, but the genetic background as well as the immune status of the host can modulate the development of disease. The strain C3H/He is resistant to infection by *L. amazonensis*. When infected, these animals develop a small lesion which is self-resolutive. In infection with *Leishmania,* the main cell of the innate immunity to perform the initial containment and modulation of the adaptive immune response is the macrophage, which is also responsible for the development of infection. Thus studies to understand the mechanisms of action of this cell are important for a better comprehension of the disease. Therefore, this work aims to study the control of *L. amazonensis* comparing the response to infection of primary cells obtained from mouse strains with different degrees of sensitivity. Peritoneal macrophages from BALB/c, C57BL/10 and C3H/He mice, which have different degrees of susceptibility to *Leishmania*, were infected with promastigotes of *L. amazonensis* (2MOI). The peritoneal macrophages were observed at 24, 48, 72 and 96 hours after infection, to evaluate the multiplication of intracellular amastigotes, analysis the production of nitric oxide and the production of Th1/Th2 citokynes as well as the arginase activity. The results of multiplication of intracellular amastigotes showed no difference between strains in the early stage of infection. However, from 72 hours C3H/He cells presented lower number of intracellular amastigotes and less amount of infected when compared with BALB/c mice macrophages. Analysis of the production of nitric oxide, arginase activity and cytokine production in progress. In conclusion, our results suggest that amastigotes have more difficulty to multiply or are eliminated primarily by macrophages in C3H/He mice. **Supported by:**Capes and IOC

HP146 - TNF-α AND TGF-β CYTOKINES EXPRESSIONS IN AMERICAN CUTANEOUS LEISHMANIASIS ARE DIFFERENTLY MODULATED BY LEISHMANIA (V.) BRAZILIENSIS OR LEISHMANIA (L.) AMAZONENSIS

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Leishmania (V.) braziliensis and Leishmania (L.) amazonensis are the most pathogenic agents of American cutaneous leishmaniasis in Brazil. However, we have already demonstrated a dichotomy between these Leishmania sp. with the human T-cell immune response; L. (V.) braziliensis leads the infection from the localized cutaneous leishmaniasis (LCL), a moderate T-cell hypersensitivity form, toward to the mucocutaneous leishmaniasis (MCL) at the T-cell hypersensitivity pole, L. (L.) amazonensis drives the infection to the anergic diffuse cutaneous leishmaniasis (ADCL) at the T-cell hyposensitivity pole.This work examined the role of the TNF-α and TGF-β cytokines expressions in biopsies of skin and mucosal lesions from 13 ACL patients due to these parasites; a) 7 LCL cases, 4 by L. (V.) braziliensis (DTH+) and 3 by L. (L.) amazonensis (DTH-); b) 3 LCM cases by L. (V.) braziliensis (DTH+++); and c) 3 ADCL cases by L. (L.) amazonensis (DTH-). Biopsies of skin and mucosal lesions were carried out for immunohistochemical analysis of immunostained cells using TNF-α (52B83 ab1793) and TGF-β (SC-146) antibodies. A Zeiss image analysis system was used to quantify reactive macrophages in 5 to 8 fields/histological section (400x). Results showed an opposite progression between the TNF-α+ and TGF-β+ cell densities; while the TNF-α+ cell density showed an increased expression from the T-cell hyposensitivity pole (ADCL) to the T-cell hypersensitivityone(MCL):ADCL=254cells/mm2→LCL/L.a=257cels/mm2→LCL/L.b=999cels/mm2→MCL=1474cels/mm2 ($P<0,05$), the TGF-β+ cell density showed an increased expression from the T-cell hypersensitivity pole (MCL) to the T-cell hyposensitivity one (ADCL): MCL=890cells/mm2→LCL/L.b=1101cells/mm2→LCL/L.a=1405cells/mm2→ADCL=1633cells/mm2($P<0,05$). These findings evidence the role of L. (V.) braziliensis and L. (L.) amazonensis driving the modulator effect of the inflammatory (TNF-α) and anti-inflammatory (TGF-β) cytokines in the cellular immune response of ACL. **Supported by:**PARASITOLOGY DEPARTMENT, EVANDRO CHAGAS INSTITUTE, BELÉM-PARA STATE,BRAZIL.

HP147 - EFFICACY OF INTRANASAL IMMUNISATION WITH LACK DNA AGAINST *LEISHMANIA (VIANNIA) BRAZILIENSIS* INFECTION USING THE GOLDEN HAMSTER MODEL

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In the murine model, LACK DNA vaccine administrated by parenteral routes has shown protective against *Leishmania (Leishmania) major* infection, but not against *L. donovani* and *L. amazonensis* infections. On the other hand, intranasal (IN) immunisation with LACK DNA protects mice against *L. amazonensis* and *L. infantum* infections. However, vaccine studies against *L. (Viannia) braziliensis* have been largely neglected mainly due to high resistance of most mouse strains to infection. Recently, we demonstrated that the golden hamster (*Mesocricetus auratus*) is an appropriate model for immunopathogenesis studies of cutaneous leishmaniasis caused by *L. (V.) braziliensis* and that the IN immunisation with whole *L. amazonensis* antigens (LaAg) can protect golden hamsters against *L. braziliensis* infection. In this study, we investigated whether the protective effect of IN immunisation with LACK DNA can be extended to *L. braziliensis* infection using the golden hamster model. We also evaluated the heterologous prime-boost (HPB) vaccination strategy with LACK DNA and LaAg as vaccine. Golden hamsters received two intranasal doses of 50µg LACK DNA or 50µg LACK DNA (1st dose) + 10µg LaAg (2nd dose). Controls received PBS. Two weeks post-vaccination, the hamsters were challenged with $1x10^5$ promastigotes of *L. braziliensis* in the hindpaw. The results showed that IN immunisation with LACK DNA did not reduce lesion growth. A positive correlation was observed between lesion size and *IFNG* and *IL10* gene expression in skin lesion. On day 114 of infection, the non-protected animals presented cytokine gene expression and anti-Leishmania IgG and IgG2 levels that were comparable to the non-immunized control group. A tendency was observed for lesion size reduction in animals immunized by HPB strategy. These preliminary results obtained with HPB vaccination strategy could be promising in the search for an effective vaccine against *L. braziliensis* infection. **Supported by:**CNPq, FAPERJ, IOC-Fiocruz

HP148 - IMMUNOPATHOLOGICAL ASPECTS OF CHRONIC INFECTION BY BLOOD OR METACYCLIC TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI IN MICE.

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Even presenting a distinct repertory of surface molecules, infective forms of Trypanosoma cruzi, metacyclic trypomastigotes – MT and blood trypomastigotes – BT, are functional in relationship to host-parasite relations. Previous studies of our group showed that in acute phase the initial interaction between the BT forms and the vertebrate host leads to a different immunological response in comparison with that observed in infection by MT forms. In this sense the aim of this project was to evaluate changes related to immunological and pathological parameters on chronic phase of experimental infection in Swiss mice by MT or BT forms of Berenice-78 strain. The results showed a peripheral blood reduction of total leukocytes and lymphocytes in both infected groups and a decrease of monocytes on BT group. The TM-form infected animals presented an increased number of NK and $TCD4^+$ circular cells. The evaluation of production of intracytoplasmic cytokines in spleen demonstrated an augment of $TCD4^+$cells producing TNF on a BT group and IL-10^+on MT group. It was also verified a significant percentual increase of the TNF-α, IFN-γ and IL-10^+ cytokines produced by the lymphocytes B on the MT group. The evaluation of the cytokine expression in the heart showed reduction of mRNA expression of TNF-α on TM group. On the other hand it was observed an increase of IL-10^+ and TGF-β by the BT group. Moreover, it was observed a bigger fibrosis area on the MT animals when compared to BT group. In face of these results, it is suggested that the infection by MT forms has been resolved after 180 days, due to a profile of anti-inflammatory cytokines and an evolution to an expressive heart inflammatory process resolution chart by fibrosis been more expressive. On the BT group there is a profile of pro-inflammatory cytokines, suggesting that probably the infection by BT forms develops a more severe and late resolving infection. **Supported by:**Fapemig, CNPq, UFOP

HP149 - INTRANASAL VACCINATION WITH LEISHMANIAL ANTIGEN PROTECTS GOLDEN HAMSTER AGAINST *LEISHMANIA (VIANNIA) BRAZILIENSIS* INFECTION

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Previous results have shown that parenteral immunisation with whole *Leishmania (Leishmania) amazonensis* antigens (LaAg) promoted disease aggravation in mice challenged with *L. amazonensis.* On the other hand, oral and intranasal (IN) immunisation with LaAg protects mice against *L. amazonensis* infection. However, studies regarding vaccine against species of the subgenus *Viannia*, the main causative agent of cutaneous and mucosal leishmaniasis in America, are hampered by the lack of easily handle bio-models that accurately reproduce the human disease. Recently, we demonstrated that the golden hamster (Mesocricetus auratus) is an appropriate model for immunopathogenesis studies of cutaneous leishmaniasis due to *L. (Viannia) braziliensis.* In this study we investigated whether the protective effect of IN immunisation with LaAg can be extensive to *L. braziliensis* infection using the golden hamster model. Golden hamsters receiving two IN doses of 10 µg LaAg or intramuscular (IM) doses of 20 µg LaAg and were challenged two weeks post-vaccination with *L. braziliensis* in the hindpaw. The results showed that IN immunisation with LaAg significantly reduced the lesion growth which was accompanied by reduction in parasitic load at the site of the lesion and by reduction in IgG and IgG2 serum levels. On day 114 of infection, IN immunized hamsters that were considered protected presented mRNA gene expression for IFNG and IL10 similar to uninfected skin, [i.e., without correlation with protection], at least at the endpoint of the experiment. Unlike the nasal route, IM immunisation did not protect. These results demonstrate for the first time the feasibility of inducing cross protection against *L. braziliensis* infection using the nasal route of immunisation. **Supported by:**CNPq, FAPERJ, IOC-Fiocruz

HP150 - CONVERSION OF TOXOPLASMA GONDII BRADYZOITES INTO TACHYZOITES IN INTESTINAL CELLS

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Toxoplasmosis is a worldwide disease caused by Toxoplasma gondii. This protozoan parasite can infect any nucleated cells of warm-blooded animals. Two of the most common forms of transmission of T. gondii is by the ingestion of undercooked meat containing encysted bradyzoites or contaminated food and water with sporulated oocysts. Intestinal cells are thus the primary site for bradyzoite to invade and start infection in a new host. Since bradyzoites are slow replicating forms, the ability to convert to tachyzoites in the new host is a key event for estabishing the pathogenesis of toxoplasmosis. In order to understand how and when this conversion occurs and if it is different according to the host cell type, we used bradyzoites isolated from the brain of mice infected with cystogenic ME49 strain of T. gondii. Bradyzoites were allowed to interact with intestinal cells (IEC-6) previously plated on round coverslips in 24 well plates. The samples were fixed at progressive time intervals and labeled with anti-SAG1 and anti-BAG1 antibodies, which specifically recognize tachyzoite and bradyzoite proteins, respectively; and with anti-CST1, a cyst wall marker. Observations were carried out in a ZEISS Axioplan fluorescence microscope. Bradyzoites started to divide 15 hours post infection. Parasites positive for SAG-1 and BAG-1 were seen after 24 hpi and up to 120hpi, indicating that the conversion is a slow and asynchronous process. Besides that, at 72hpi parasitophorous vacuoles containing SAG-1 positive tachyzoites presented deposition of CST1 protein on the membrane. Analysis of the ultrastructural aspects of PV, formation of cyst wall, dividing parasites and the proximity of the host cell organelles with the PV membrane by SEM and TEM corroborated the fluorescence microscopy results. These initial data show that conversion of bradyzoites to tachyzoites in a new host is not complete in the early divisions of the parasite. **Supported by:**CNPq e FAPERJ

HP151 - INTERACTION BETWEEN HUMAN NEUTROPHILS AND DENDRITIC CELLS DURING L. BRAZILIENSIS INFECTION

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Neutrophils are able to modulate immune response against pathogens by cytokine and chemokine production, release of granules enzymes and interaction with other cells. Dendritic cells are the major cells types recruited in response to cytokine and chemokine produced by neutrophils. Objective:This study evaluated the expression of surface molecules in human neutrophils infected with Leishmania braziliensis, release of granules enzymes and production of reactive oxygen species. We also evaluated the interaction between L. braziliensis infected neutrophils and DCs, in order to observe the effect of this interaction concerning the maturation of DCs and changes in their cytokine profile. Methodology:Cells were purified from peripheral blood of healthy donors. Neutrophils were promptly used and DCs were generated in vitro. Neutrophils were infected or not with L. braziliensis and co-cultured with DCs. Neutrophils and supernatants were harvested 3 hours after the culture to evaluate the release of myeloperoxidase and metalloproteinase 9. Cell phenotype and function were analyzed by the expression of Mac-1 and ROS production. Infected neutrophils were added to DC culture to assess their rate of infection. Sorting of infected neutrophils (based on GFP expression by L. braziliensis) was performed and cultured with DCs to assess expression of surface molecules on DC. Dendritic cells were also treated with supernatant from neutrophils cultures and the expression of CD1a, DC-SIGN and HLA-DR was evaluated, as well as cytokine production. Results:Neutrophil infection with L. braziliensis induced the production of MPO, MMP-9, IL-8 and ROS. We also observed increase in Mac-1 and decrease in CD16 and CD62L expression indicating neutrophils´activation after L.b. infection. Considering the phenotype of DCs after co-culture, we observed increase in the expression of CD1a and HLA-DR, as well IL-8 production suggesting that the presence of neutrophils could mediate DCs activation. **Supported by:**CNPQ

HP152 - EXPERIMENTAL REINFECTION OF BALB/C MICE WITH GENETICALLY DISTINCT *TOXOPLASMA GONDII* STRAINS

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Toxoplasmosis is usually asymptomatic in immunocompetent individuals, but can cause severe symptoms in AIDS patients and congenitally infected fetuses. Several authors have reported that primary infection with *T. gondii* causes an effective immunity, preventing the host against reinfection. However, cases of congenital toxoplasmosis were reported in immunocompetent women chronically infected, indicating the possibility of reinfection in humans. Recent studies have demonstrated the occurrence of reinfection of BALB/c mice by recombinant Brazilian strains and experimental studies have shown that animals chronically infected with ME 49 (type II) strain are not reinfected when challenged with virulent RH strain (type I), but there is no information about induced protection by primary infection with avirulent or intermediate virulence strains. In this study, we evaluated the infection of Balb/c mice with genetically distinct *T.gondii* strains, using ELISA with strain -specific synthetic peptides and real time PCR. Our data showed that primary infection with ME49 strain (type II) protects the host against the colonization of CNS with cysts of VEG strain (type III) used in challenge, but the primary infection with VEG strain (type III) does not protect the host of challenge by ME 49 (type II) and RH (type I) strains. The quantification of brain cysts by real time PCR showed that animals infected with VEG strain have a higher number of tissue cysts in relation to mice infected with ME49 strain. In addition, the humoral immune response against synthetic peptides was more pronounced in animals infected with VEG strain (type III). These results show that serotyping with synthetic peptides is a promising tool for the study of the protective response induced by infection with different *T.gondii* genotypes, contributing to therapeutic or vaccine approaches for toxoplasmosis.

HP153 - EXPERIMENTAL INFECTION OF FIRST STAGE RHODNIUS PROLIXUS NYMPHS WITH BACTERIAL SYMBIONT AND TRYPANOSOMA CRUZI

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Chagas disease is transmitted by members of the triatomine subfamily. One of those inscets, Rhodnius prolixus, is also a classical model used to study insect physiology and host-parasite interactions. However, most of the studies were performed with adult insects or late nymphal stages due to their relative greater size and therefore, first stage nymphs are almost completely neglected. Here, two main reasons led us to characterize the time course of infection of first instar nymph with either *Rhodococcus rhodnii* or *T. cruzi*: (1) they hatch from eggs as germ-free insects, so they are excellent to introduce pathogens and symbiotic bacteria and study the response of a naïve insect. (2) Our lab is seeking to develop a highthroughput RNAi screening strategy for Rhodnius, using oral delivery of dsRNA. This approach needs the use of a large number of insects, which is only feasible with 1st stage nymphs. Infection of N1 insects with bacterial symbiont was made by exposition of insects to feces from adults or to an artificial mimetic of triatomine feces mixed with known amounts of *R. rhodnii* suspension. With *T. cruz*i, insects were infected by feeding with decomplemented blood in an artificial feeder with 10^7 epimastigote parasites/mL of blood. Distinct patterns for growth of the symbiont in the gut after a blood meal were observed for N1 and N2 insects. N1 insects were more permissive for *T. cruzi* than adult females. In addition, a preliminary screen for gene silencing with orally administered dsRNA was performed with N1 insects, demonstrating feasibility of a large scale screening. **Supported by:**CNPq, Faperj

HP154 - BEHAVIORAL ASSESSMENT OF BALB/C MICE INFECTED WITH GENETICALLY DISTINCT *TOXOPLASMA GONDII* STRAINS

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The behavioral manipulation is the main theoretical current presently used to explain the behavioral changes present in intermediate hosts, mainly rodents, infected by *Toxoplasma gondii*. Several studies suggest that parasitism by *T.gondii* causes metabolic, immunological and neuropathological changes that allow the development of changes in the behavior of infected individuals. However, there is no information about the effect of the *T.gondii* genotype in behavioral changes. We evaluated the effect of chronic infection with genetically distinct *Toxoplasma gondii* strains in the behavior of Balb/c mice. The infection models were evaluated by strain specific IgG humoral response, quantification of brain cysts by real time PCR and behavioral tests as Passive Avoidance, Barnes Maze, Elevated Plus Maze, Open Field aiming for evaluation of learning and memory, aversion to predator odor, anxiety and locomotor and exploratory activity respectively. Our data showed that infection with the VEG strain induced higher humoral immune response and number of brain cysts than the infection with ME49 strain. In addition, the infection by VEG strain showed higher reduction in the recoverability of memory than the ME49 strain. On the other hand, infection by ME49 strain caused a reduction in aversion to predator odor (cat). These data suggest that factors inherent in the pattern of immune response and number of brain cysts induced by the infecting strain are directly related to behavioral alterations. Our data show that the study of the effect of *T.gondii* genotype about animal behavior is a promising approach to understanding the mechanisms and factors involved in the complex relationship parasite-host.

HP155 - ANALYSIS OF SERCA AND PLN DISTRIBUTION DURING *T. CRUZI*-CARDIOMYOCYTE INTERACTION

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Calcium ions play a pivotal role in the host-pathogen interaction, including *Trypanosoma cruzi* invasion and intracellular development. Transient increase in intracellular Ca2+ levels was previously reported during *T. cruzi* invasion process (Garzoni et al., 2003). Also, depletion of Ca2+ causes reduction in parasite entry and alteration in its intracellular development in cardiomyocytes (Meirelles et al., 1999). The maintenance of intracellular Ca2+ homeostasis in cardiomyocytes occurs mainly by Ca2+ATPase of sarcoplasmic reticulum (SERCA), an enzyme that transport 70% of Ca2+ inside the sarcoplasmic reticulum (SR) lumen (Winther et al., 2013). This enzyme regulation occurs by phospholamban (PLN), a reversibly phosphorylated and transmembrane protein. The regulation of Ca2+ is also critical for maintaining the exciting-relaxation coupling of the cardiac cells. Thus, the model of primary culture of cardiomyocytes was used to evaluate the distribution and expression of SERCA and PLN during *T. cruzi* (Y strain) interaction (24-72 hours).
To analyze the distribution of SERCA and PLN by indirect immunofluorescence, the cells were fixed in 4% paraformaldehyde, permeabilized with Triton X-100 and incubated with anti-SERCA or anti-PLN antibody. After washing, cells were incubated with anti-mouse IgG conjugated to Alexa 488 or anti-rabbit IgG conjugated to Alexa 546. DNA was detected with DAPI dye. As negative control, the primary antibody was omitted and samples were analyzed using a Zeiss Axio Imager fluorescence microscope.
Immunofluorescence analysis revealed an intense positive staining for SERCA and PLN, visualized in the nuclear envelope and SR, after 24h of infection. Further observations at later time of infection (72h) showed absence of staining for SERCA 2a and PLN in regions where the intracellular parasites were located. These preliminary results suggest alteration in the SERCA 2a and PLN during *T. cruzi* intracellular development. **Supported by:**Fiocruz, PAPES, CNPq and FAPERJ

HP156 - ROS INVOLVEMENT IN MACROPHAGE INFECTION BY *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is an obligatory intracellular parasite that replicates into macrophages which produce reactive oxygen species. However, the parasite survival and development in this potentially harmful environment remains poorly understood. Thus, we investigated the contribution of the redox status in the *T. cruzi*-macrophage infection. For that, we used molecules with antagonic redox status, such as the antioxidant NAC and the NADPH oxidase inhibitors DPI and apocynin (APO), as well as, the prooxidants H_2O_2 and menadione. In the first approach, we concomitantly infected the cells with trypomastigotes and treated with the molecules for 72 h. We observed that the NADPH oxidase inhibitors and NAC greatly impaired the macrophage infection, decreasing the endocytic index. Moreover, the prooxidant molecules, menadione and H_2O_2 increased the endocytic index, although only H_2O_2 led to an increment of the number of parasites per infected macrophages. Using a different protocol, macrophages were previously incubated with *T. cruzi* and after 24 h, treated with DPI, APO, NAC, menadione or H_2O_2 for additional 48 h. DPI, APO and NAC decreased the macrophage infection. On the other hand, menadione and H_2O_2 enhanced parasite infection. We also evaluate the role of redox balance during amastigogenesis *in vitro* and observed that the parasites differentiated faster in the presence of H_2O_2. Despite of that, menadione greatly impaired amastigogenesis, showing the importance of H_2O_2 in the intracellular cycle. Surprisingly, although NAC diminished macrophage infection, it did not alter *T. cruzi* differentiation. In this scenario, the oxidant environment promoted by menadione and H_2O_2 improved macrophage infection, in contrast to DPI, APO and NAC that reduced the number of intracellular amastigotes. Taken together, our results strongly suggest that NADPH oxidase activation favors the parasite establishment in the intracellular milieu, modulating the success of the infection. **Supported by:**CNPq, UERJ, FAPERJ, INCT-EM

HP157 - DELTA-AMASTIN PROTEIN TARGETING AND PHENOTYPIC CHANGES ON *TRYPANOSOMA CRUZI* METACYCLOGENESIS

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Plasma membrane proteins (PMP) are associated with several functions such as morphology, host parasite interaction, or transport of molecules. Functional characterization of *T. cruzi* PMPs is appealing, since they are the first line of direct host parasite interaction, thus constituting potential drug targets and/or vaccine candidates. Despite of its importance, little is known about PMPs functions. Here, it is shown additional data towards the delta-amastin characterization. This amastin subfamily includes highly hydrophobic proteins containing 4 transmembrane domains, which are located at cell surface and are upregulated in amastigote stage. Since delta-amastins were described as surface proteins potentially O-glycosylated at threonines residues located at the first hydrophilic region, it was decided to overexpress and compare wild type delta-amastin (AF::GFP) and its mutated form containing 5 Thr codons replaced by Alanine (5TmAF::GFP) fused to GFP. These mutations partially impaired protein targeting to cell membrane compared to AF::GFP, and intriguingly AF::GFP and 5TmAF::GFP migrated as unmodified proteins. Additionally to this amastin mutant, we mutated the first cysteine codon (Cys to Ala) generating 1CmAF::GFP, which seems to not interfere with protein targeting. When metacyclogenesis was analyzed, 5TmAF::GFP expression reduced the epimastigote differentiation into metacyclics, compared to AF::GFP expression in G strain. Based on in silico analysis amastin contains a potential signal peptide, thus to corroborate its function, GFP was expressed fused at the c-terminus of the first 59 aa (A-SP::GFP). The amastin SP efficiently targeted the protein to perinuclear location, which is compatible with ER, however this SP do not seems to be cleaved since A-SP::GFP was not detected at supernatant but only at parasite insoluble fraction. To better characterize amastin function different PPI approaches are being tested to identify its partners. **Supported by:**CAPES, CNPq, Fundação Araucária

HP158 - ANALYSIS OF PHOSPHOLIPID DIFFERENTIAL EXPRESSION IN *LEISHMANIA (VIANNIA) BRAZILIENSIS* PROMASTIGOTES AND AMASTIGOTES BY TANDEM MASS SPECTROMETRY

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In the life-cycle of *Leishmania* the parasite changes its morphology and metabolism according to different host environments and thus, promastigotes are found in sandfly vector and amastigotes in mammalian hosts. Plasma membrane of *Leishmania* contains a complex array of lipids, and the metabolism of glycerophospholipids (PLs) and sphingolipids (SL) in *Leishmania* is the subject of a number of studies. In this study it was analyzed in *Leishmania (Viannia) braziliensis* promastigotes and axenic amastigotes, the major PL and SL species using the electrospray ionization mass spectrometry (ESI-MS). By ESI-MS, in both positive and negative ion modes, it was identified more than 200 molecules of PLs and SLs comprising phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and inositolphosphoryl ceramide (IPC) from total lipid extracts from cultured promastigotes and axenic amastigotes. The mass spectra exhibited substantial heterogeneity in the PL population, and showed significant differences between promastigote and axenic amastigote lipid profiles. Axenic amastigotes showed an increase in the intensity of peaks at *m/z* 537 (LysoPI 14:3), *m/z* 539 (LysoPI 14:2), *m/z* 567 (LysoPI 16:2); and a decrease in the intensity of peaks at *m/z* 778 (d34:1), *m/z* 780 (IPC d34:0 or t33:1), *m/z* 806 (IPC d36:1 or t35:2) and *m/z* 808 (IPC d36:0 or t35:1) in relation to promastigotes. It was also observed in axenic amastigotes a decrease in the intensity of ions at *m/z* 728 (PC 32:3), *m/z* 778 (PC 36:6), *m/z* 780 (PC 36:5), *m/z* 806 (PC 38:6), *m/z* 808 (PC 38:5), *m/z* 828 (PC alkenylacyl 40:1 or alkylacyl 40:2), *m/z* 830 (PC alkenylacyl 40:0 or alkylacyl 40:1) and *m/z* 832 (PC alkylacyl 40:0). These results regarding PL and IPC expression may contribute to better understand about changes in lipid pathways during differentiation of promastigote to amastigote, and identification of new targets for chemotherapies against leishmaniasis. **Supported by:**CAPES, CNPq and FAPESP

HP159 - ASSOCIATION OF ANTI - *LUTZOMYIA LONGIPALPIS* SALIVARY GLAND LYSATE IGG WITH ASYMPTOMATIC INFECTION BY *LEISHMANIA (L.) INFANTUM* IN DOGS

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Aim of this study was to evaluate the presence of IgG anti-salivary glands lysate of *Lutzomyia longipalpis* (SGL) in serum of dogs from endemic areas and its correlation with different clinical forms of canine visceral leishmaniasis (CVL). One hundred and ninety dogs from dogs with deltamethrin impregnated leash (DIL) and 95 dogs clinical examination, serological examination (direct parasitological examination (real-time PCR dogs were classified as uninfected, sick (n = 50), severe captured *Lu. longipalpis* in direct ELISA assay to the quantities of anti-LGS IgG be of anti-LGS IgG were higher in serum of exp asymptomatic infected dogs ($p \leq 0.01$) in relation to sick dogs. Fro suggest that humoral immunity against *Lu. longipalpis* saliva can cont protection from further infection by *Leishmania (L.) infantum* and preventing deve of CVL symptoms. **Supported by:**FAPESP 2012/05847-9 and 12/50285-9, LIM-FMUSP

HP160 - L1-POSITIVE CELLS (CALPROTECTIN) ARE RELATED TO INTENSE PARASITISM IN THE GRATROINTESTINAL TRACT (TGI) OF DOGS NATURALLY INFECTED BY LEISHMANIA INFANTUM

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The gastrointestinal tract (TGI) contains the largest number of immune cells in the body. Appropriate interactions between these different cell types are essential in generating immune responsiveness or tolerance to a large amount of antigens. The complex of S100A8/S100A9 (L1 antigen) is actively secreted during the stress response of phagocytes and it is as important endogenous DAMPs. Studies in Canine Visceral Leishmaniasis have described a chronic inflammatory exudate throughout, where parasites are most found in the large intestines, specially colon. Our study aims to evaluate the chronic cellular exsudate in lamina propria and the parasite load in the jejune and colon TGI segments of dogs with LVC with the expression and localization of L1. For this study, twelve symptomatic infected and six uninfected dogs were used. Dogs were euthanized with a lethal dose of thiopental (1 ml/kg) and after necropsy, fragments of jejune and colon were collected and fixed in 10% buffered formalin. Gross and microscopical exams showed no severe alterations of the mucosa. Amastigotes forms of Leishmania were distributed in the lamina propria (mucosa) in both small and large intestines, but, interestingly, the parasitism was diffuse throughout the lamina propria in small intestine and closely distributed to the muscularis mucosa in large intestine. Plasma cells, macrophages, and lymphotes are the predominant mononuclear cells. Polymorphonuclear neutrophils and/or eosinophils are rarely found. L1 calprotecin positive cells were easily found in the jejunum and colon of infected dogs when compared to controls. Otherwise, colon segments showed more positive L1 cells than jejune and it was directly correlated to the parasite load. However, we did not observe any correlation among inflammatory cells intensity and L1 positive cells in both segments. This data could indicate that the parasite can take advantage of innate immune response using these L1 positive immature cells. **Supported by:**CAPES, CNPQ, FAPEMIG

HP161 - A HIGH THROUGHPUT ANALYSES OF CYTOKINES DETECTION AND ACTIVITIES ALONG *TRYPANOSOMA CRUZI* EXPERIMENTAL INFECTION

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Host immune response against *Trypanosoma cruzi* is highly complex and involves many components, both regulators and effectors. This is the firs report of a high throughput kinetics analysis of a broad cytokines and chemokines expression in different organs from mice infected by the virulent *Trypanosoma cruzi* CL strain compared to the non virulent G strain. the results showed that pro-inflammatory cytokines IL-12, IFN-gamma and TNF-alpha were highly expressed in stomach and spleen during the acute and chronic infection with both strains. IL-2, IL-7 and M-CSF were specially expressed during chronic phase, unlikely from IL-3, that was expressed at acute infection. KC and MIP-2 was expressed at acute and chronic phase. MIP-1alpha and beta, IP-10, RANTES, MIG, MCP-1 were expressed at a chronic phase of both strains. IL-4 and IL-9 were less expressed, but where detected in the spleen, at the chronic phase of CL infection, and in the stomach, at the acute phase of infection. In cardiac tissue, the majority of cytokines and chemokines were downregulated; only IL-10 was detected during the acute infection by CL strain. Considering infection by G strain, this cytokine was only observed in samples from chronically infected animals. Furthermore, mice were infected with virulent *T.cruzi* CL or Y strains and submitted to treatments with IL-3, IL-7 or IL-9 cytokines to check there role in the immune response. Treatment with IL-3 in infected animals with either CL or Y strain appeared to have emproved mice clinical condition. Also treatments controlled parasitemia in mice inoculated with Y strain. Thus we have observed that the pattern of cytokines released along *T. cruzi* infection depends on *T. cruzi* strain and host organs. **Supported by:**FAPEMIG/CAPES/CNPq

HP162 - IMMUNOHISTOCHEMICAL ASPECTS OF HEPATIC INTRALOBULAR GRANULOMA IN DOGS NATURALLY INFECTED WITH *LEISHMANIA INFANTUM*

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Canine Visceral Leishmaniasis (CVL), in Brazil, is a zoonosis and a systematic chronic disease caused by protozoa of the genus Leishmania. In dogs, the chronic hepatitis is well characterized by a presence of intralobular granulomas constituted by epithelioid cells, macrophages, lymphocytes and plasma cells. Macrophages could appear parasitized or not with amastigotes forms of Leishmania. The aim of this study was to characterize by immunohistochemistry the hepatic granuloma cells of twenty-five dogs, both sexes, naturally infected with Leishmania infantum. Dogs were clinically defined in groups of 8 asymptomatic animals without any clinical manifestation and 17 symptomatic with clinical signs of the disease as lymphadenopathy, skin lesions and weight loss. Fourteen dogs with negative serological and parasitological exams to Leishmania were used as controls. Dogs were euthanized with a lethal dose of thiopental (1 ml / kg) and after necropsy, fragments of liver were collected and fixed in 10% buffered formalin. Microscopic and morphometric analyzes showed significantly higher granulomas in symptomatic compared to asymptomatic animals, but no differences between the groups regarding the number of granulomas and the presence of Leishmania amastigotes. We also observed a greater number of calprotectin positive cells (cytoplasmic antigen termed "L1" that indicates that immature macrophages) in the granulomas of symptomatic animals. On the other hand, there is a higher expression of iNOS in granulomas of asymptomatic dogs than symptomatic ones. These findings suggest that the granulomatous inflammatory response in livers of symptomatic dogs occurs extensively where they are constituted by immature macrophages. Further analysis should be performed to evaluate the phenotype and quantification of other cell types to better characterize the hepatic intralobular granulomas in CVL. **Supported by:**CNPQ, FAPEMIG, CAPES

HP163 - HOST CELL INVASION BY TRYPANOSOMA CRUZI DM28 DOES NOT REQUIRE LYSOSOME EXOCYTOSIS AND SPHINGOMYELINASE ACTIVITY, BUT IS CRUZIPAIN-MEDIATED AND INFLUENCED BY CHAGASIN LEVELS

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Penetration of nonphagocytic cells by T. cruzi is a complex event involving multiple pathways, which vary according to the host cell, parasite strain, developmental form and phylogenetic lineage. Molecular pathways engaged in the invasion of fibroblasts and cardiomyocytes by tissue culture trypomastigotes (TCT) of the Y strain are well established. Raises in the host cell intracellular Ca2+ resulting from of mechanical cell surface wounding by TCT lead to synaptotagmin VII-dependent lysosome migration and their fusion at the parasite synapse. Release of acid sphingomielinase (ASM) following lysosome exocytosis promotes rapid endocytosis and parasite internalization. Accordingly, invasion by Y-TCT is favoured by the disruption of host cell actin cytoskeleton and depends on ASM activity. The invasion of endothelial cells or cardiomyocytes by Dm28-TCT is also associated with Ca+2 transients and relies on cruzipain activity. Invasion was linked to the activation of G-protein-coupled kinin receptors and more recently, endothelin receptors were also described as part of such multi-signaling platform in smooth muscle. The availability of active cruzipain can be tuned by the endogenous inhibitor chagasin. We found that cruzipain-mediated invasion of smooth muscle cells is impaired in a Dm28 transgenic line with elevated chagasin expression (pCHAG). Lysates of pCHAG, but not WT, did not induce Ca2+ transients in the host cell. Invasion by Dm28-TCT was unaffected by ASM inhibitors, potentiated by BAPTA-AM and markedly reduced by cytochalasin D. Furthermore, Dm28-TCT were unable to induce lysosome exocytosis, as compared to Y-TCT, in assays of hexosaminidase release. Cruzipain irreversible inhibitors drastically reduced invasion by Dm28-TCT while had little effect in the uptake of Y-TCT. We propose that Dm28-TCT has adapted to engage alternative invasive routes and distinct host cell machineries that are unrelated to triggered lysosome exocytosis and mechanical wounding. **Supported by:**CNPq

HP164 - ANTIBODY RESPONSES OF MICE TO SALIVARY ANTIGENS OF *RHODIUS PROLIXUS* (HEMIPTERA, REDUVIIDAE)

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Rhodnius prolixus is a haematophagous insect with medical importance once it is a vector of *Trypanosoma cruzi*, the etiologic agent of Chagas disease in Americas. Due to the direct contact between the saliva and the host immune system during hematophagy, monitoring the levels of antibodies anti-saliva in the sera from persons from endemic areas could be a good alternative for entomological inquiries. Therefore, the present work aimed to evaluate the recognition of salivary antigens of *R. prolixus* by immunoglobulins from the serum of mice used as blood source from *R. prolixus*, other triatomine species and other haematophagous arthropods. Groups of mice were exposed to low (two females) or high (four females) infestations by different triatomine species or other haematophagous arthropods. ELISA assays were performed to evaluate the production of anti-saliva IgG and IgM, as well as for checking cross reactivity with saliva of other blood sucking arthropods. After a single challenge with *R. prolixus*, IgM and IgG anti-saliva were detectable in saliva after approximately 2 and 6 days, respectively. There was no difference in the level of immunoglobulins from animals exposed to high or low infestations. By immunizing mice with four infestations, the IgG levels gradually increased up to eight weeks after the first infestation and started to decrease 56 days (week 14) after the last infestation, again with no difference between groups immunized with high or low rates of infestation. IgG present in the serum of mice immunized with *R. neglectus* partially recognized salivary antigens from R. prolixus (~50% of the values of *R. prolixus*) but no cross-reaction was observed with sera from animals immunized with other blood sucking arthropods as *Pantrongylus megistus, Triatoma infstans, Triatoma brasiliensis, Aedes aegypty, Lutzomyia longipalpis, Stomoxy calcitrans, Ornithodoros rostratus* and *Amblyomma cajennense*. Our results indicate that saliva of *R. prolixus* is a promising contact marker for the triatomine infestations. **Supported by:**CAPES, FAPEMIG, INCT-EM

HP165 - EVALUATION OF THE ANTIGENICITY OF REPETITIVE DOMAINS OF TWO NOVEL *LEISHMANIA INFANTUM* PROTEINS

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An effective way to control visceral leishmaniasis is the improvement of diagnostic methods, vaccination or immunotherapy. For this reason, repetitive proteins have been studied, due to their high immunogenic capacity. Our group has identified some novel antigens containing repetitive motifs through serological screening. Two of them (Lci2 and Lci12) were further evaluated in this study, aiming to assess the repetitive motifs' ability to diagnose visceral leishmaniasis, in human and dog sera, and improve methods of serological diagnosis of the disease. To perform this evaluation, synthetic genes were designed with the repetitive and non-repetitive motifs of the proteins flanked by restriction enzymes sites. The different coding sequences were cloned into expression vectors and the corresponding his-tagged recombinant proteins, produced in *Escherichia coli* plysS, were purified and quantified. Two sets of protein were generated equivalent to the complete proteins or repetitive/non-repetitive regions. Immunoassays (ELISA) were then performed with infected sera from humans (50 positive and 50 negative) and dogs (46 positive and 15 negative), with the positive ones confirmed through prior parasitological examination. Both Lci2 and Lci12 displayed a better sensitivity for dog sera than for human sera. When the complete Lci2 was compared to its repetitive region it displayed sensitivities of 93% and 80% for the dog sera, respectively, compared to 73% and 67% for the human sera (p=0,0001). For Lci12, the complete protein was compared to its non-repetitive region, displaying sensitivities of 91% and 93% for the dog sera (p=0,001) compared to 46% and 17% for the human sera (p value = 0,06), respectively. These results show the importance of the evaluation of repetitive motifs in antigenic proteins, leading to the development of new diagnostic strategies, such as chimeric proteins, which could be designed in order to improve diagnostic methods. **Supported by:**CNPq

HP166 - EVALUATION OF THE ROLE OF TRIALYSIN IN THE VIABILITY OF MICE PERITONEAL MACROPHAGE AND IN THE FEEDING PROCESS OF TRIATOMA INFESTANS

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Triatoma infestans are hematophagous insects and vectors of Trypanosoma cruzi. They produce a number of molecules in their saliva that neutralize host responses and ensure success in feeding. Previous results showed that his saliva induces death in peritoneal macrophages of mice and trialysin, a protein that form pores in the membrane of T. cruzi, human erythrocytes and bacteria, is the main candidate of the activity. The objectives of this study were to evaluate the possible role of trialysin in the mortality induced in peritoneal macrophages and its importance for blood feeding. For such, second instar nymphs had the trialysin knocked down by RNA interference (injection of trialysin dsRNA) and insects or their saliva were tested in different assays in comparison to controls (insects injected with saline or injected with non-specific dsRNA). The levels of trialysin mRNA after RNAi were measured by qPCR. The action in peritoneal macrophages of C57BL/6 mice was evaluated by the MTT method. Knock down insects had their hematophagy evaluated by the electromyogram of the cibarial pump. Knock down insects had the trialysin levels decreased by approximately 99% and the mortality of macrophage induced by their saliva was 49.4 to 60% lower ($p<0.05$) in comparison to controls. Insects with reduced salivary trialysin ingested 40% less blood ($p<0.05$) that insects injected with saline, but all other feeding parameters evaluated (probing time, total contact time, ingestion rate, non-ingestive time, number of interruptions, frequency of the cibarial pump) were similar to controls. Results confirm that trialysin induces mortality in peritoneal macropages and its presence in the saliva is important to fully engorge during blood feeding by triatomine bugs. **Supported by:**CAPES, FAPEMIG, PRPq-UFMG e INCT-Entomologia molecular

HP167 - DIFFERENTIAL ROLE OF TGF-β IN EXTRACELLULAR MATRIX REGULATION DURING *TRYPANOSOMA CRUZI*-HOST CELL INTERACTION

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The most severe manifestations of Chagas disease are cardiomyopathy and cardiac fibrosis, but skeletal muscle is also affected by *T. cruzi* infection, resulting in necrosis and miositis. Accumulation of extracellular matrix (ECM) during fibrosis is involved in the pathogenesis of Chagas disease, but their regulatory mechanisms during infection have not been elucidated. Thus, we propose to compare the response of cardiomyocytes (CM), cardiac fibroblasts (CF) and skeletal myoblasts (L6E9) infected with *T. cruzi* after TGF-β stimulation, which is an important fibrogenic cytokine is involved in the development of chagasic cardiomyopathy, and also analyze the mechanisms of the signaling pathways involved in this process. To achieve these goals, normal and *T. cruzi* infected (Y strain) cultures of MC, CF and L6E9 were treated for 1h or 48h with TGF-β (1-10 ng / ml) and the expression of fibronectin (FN), phosphorylated SMAD 2 (PS2) and p38 was analyzed by indirect immunofluorescence and Western blot (WB) after 72h of infection. CF and L6E9 showed an increased expression of FN from 1 ng/ml TGF-β in contrast to CM, which was only observed with increased ECM with 15ng/ml, a high concentration of cytokine. After *T. cruzi* infection, FN fibril disorganization and reduction of expression was observed in CM, CF and L6E9 while adjacent uninfected cells showed similar FN profiles to the controls. WB analysis revealed that the differential response to TGF-β stimulus results from different intracellular signaling pathways, since L6E9 and CF have higher triggering of the SMAD pathway when compared with CM, while the p38 pathway (SMAD independent) is less stimulated in FC and L6E9 when compared to CM. Thus, our data suggest new perspectives on the mechanisms of regulation of matrix during *T. cruzi* infection, paving the way for identification of novel therapeutic targets against chagasic fibrosis. **Supported by:**CNPq, FAPERJ, CAPES, FIOCRUZ

HP168 - PARTICIPATION OF TLR-2 AND TLR-6 IN THE SKIN FIBROBLASTS IMMUNE RESPONSE DURING EXPERIMENTAL INFECTION BY *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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Initial moments of infection by *Leishmania* are crucial to the disease evolution and involve the host and parasite factors, such surface molecules, parasite species and genetic background. Several studies have shown the TLRs involvement in response to Leishmania infection. We demonstrated that the absence of TLR-2 induce a lower susceptibility to *L. (L.) amazonensis* (L.a.) infection, which controlling the parasite load and cellular profile alterations of the inflammatory infiltrate at the site, however, the immune response developed during infection is not known. To evaluate the participation of the TLR-2 and the skin fibroblasts (SF) in modulation and recruitment of inflammatory cells, we evaluated the production of inflammatory mediators by SF in the early stages of infection. TLR-$2^{-/-}$and TLR-$6^{-/-}$ mice were inoculated in the ear with 10^5 *L. amazonensis* promastigotes. After 1, 7, 15 and 30 days of infection, the cellular profile analysis was performed by light and electron microscopy and the production of inflammatory mediators evaluated by flow cytometry. Our results showed that the absence of TLR-2 induced a distinct cellular response, effective in reducing the parasite load and infection control. Furthermore, it was observed that the SF on site of inoculation of producing cytokines, contributing to the initial response to infection. In TLR-$2^{-/-}$ were found significant increase in SF producers of IL-4 and IFN-γ on the first day of infection when compared with WT mice, where high production of IL-4 was observed after 30 days of infection. These results suggested the likely SF participation in the intense recruitment of eosinophils to the site of infection in the absence of TLR-2. In conclusion, the TLR-2 modulation may be a crucial factor for the development of a more efficient immune response in controlling infection, and a pathway in the search for alternatives in the development of new therapies for the treatment. **Supported by:**CNPq, FAPERJ, IOC/FIOCRUZ

HP169 - THE RATES OF PREDATION ENDURED BY *RHODNIUS PROLIXUS* ARE AFECTED BY INFECTION WITH *TRYPANOSOMA CRUZI* OR *TRYPANOSOMA RANGELI*

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Triatomines live inside shelters aggregate with co-specifics. At dusk, they eventually leave the refuges searching for hosts and return to them mostly before dawn. As insect predators are commonly their hosts, triatomines can be killed during host search and the subsequent feeding process. In this study, we evaluated predation rates in *Rhodnius prolixus* infected with either *Trypanosoma cruzi* or *Trypanosoma rangeli* and compared them with those recorded with healthy insects. Assays were performed in square glass arenas presenting one central refuge. A mouse kept in a cage that allowed insect contact, was placed inside each arena at the start of assays. Insects of this species avoid staying outside refuges during daylight hours. Therefore, the percentage of insects that remained exposed outside shelters at the end of a 96h acclimatization period was recorded and shown to be 8 and 6% for *T. cruzi* insects and those in control groups, respectively. In the presence of a host, insects of both groups showed an intense shelter-related activity, i.e., leaving and entering shelters. However, a higher proportion ($p<0.05$) of insects from the infected group were predated when compared with non infected ones (35 and 20%, respectively). In assays with *T. rangeli*, insects infected showed a significant increase in exposure levels and rates of predation, when compared with those of the control group. After the acclimation period, 26% of *T. rangeli* infected insects were found outside the arena against 14% of control bugs ($p<0.05$). In the presence of a host, predation rates were 49 and 27% for the infected and control groups, respectively ($p<0.05$).These results showing that the infection by *T. cruzi* or *T. rangeli* alters shelter use by *R. prolixus*, inducing a higher exposure risk and consequently higher predation rates. In a natural context, the increase observed in the predation rates of infected triatomines may have implications on the transmission rates of both trypanosomes. **Supported by:**FAPEMIG

HP170 - *PLASMODIUM CHABAUDI* ALTERS LIPID METABOLISM OF THE HOST: WHAT ARE THE SIGNALLING PATHWAYS INVOLVED?

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Malaria is the most important neglected disease for public health in tropical and subtropical countries, with 300-500 million of cases and 600 000 deaths/year. The causative agent, *Plasmodium spp*., is transmitted by the bite of *Anopheles spp.* mosquitoes, and the disease occurs by the invasion and destruction of erythrocytes of the vertebrate host. We aimed to clarify possible adaptive mechanisms of host-parasite interaction regarding lipid metabolism. For this, male mice (n=12; ~40g) were infected with *P. chabaudi* (105; via IP) and euthanized on day 5, with collection of spleen, liver, adipose tissue and plasma. The Thin Layer Chromatography technique was used to verify the lipid profile. There was a significant increase of esterified cholesterol, fatty acid and monoacylglycerol in the spleen ($p=0.007$, $p=0.01$ and $p=0.01$), liver ($p=0.02$, $p=0.01$ and $p=0.009$) and adipose tissue ($p=0.01$, $p=0.02$ and $p=0.0003$), respectively. In addition, there was a decrease of triacylglycerol in the spleen ($p=0.0006$) in opposition to the increase observed in liver ($p=0.02$) and adipose tissue ($p=0.04$), after infection. Furthermore, there was a decrease of plasmatic lysophosphatidylcholine ($p=0.02$) and significant reduction in hepatic mRNA expression of PPARα ($p=0.0002$) and PGC1α ($p=0.01$). We demonstrate, therefore, that the *Plasmodium* infection can severely influence on the host lipid metabolism in an organ-dependent manner, possibly by manipulating key enzymes in the synthesis and degradation of these macromolecules. **Supported by:**Capes; Faperj, CNPq

HP171 - FIBROCYTES AND THE INVOLVEMENT OF TLR-2 IN *LEISHMANIA AMAZONENSIS* INFECTION

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Fibrocytes as important part to understand the progression of many diseases because they have identified in areas related lesions generated in these processes. The morphology of fibrocytes and their behavior when interacting with parasites is poorly understood. Fibrocytes may have an important role in the innate immune response and development of acquired response, since they have Toll receptors "like" (TLRs), stimulate T limphocytes and produce cytokines. In this study, we detected the presence of TLR–2 and examined the morphology of fibrocytes and their interaction with *Leishmania (L.) amazonensis*. For this, we evaluate the infection of primary cultures of fibrocytes by *L. amazonensis* and the involvement of TLR-2 in the internalization through the optical microscopy and scanning and transmission electron microscopy, using ultrastructural immunocytochemistry assays. Through ultrastructural analysis, we describe the presence of TLR-2 in the plasmatic membrane and in parasitophorous vacuole containing promastigote in initial moments of infection. We verified details of the fibrocytes morphology and ability to rapidly internalize *Leishmania* promastigotes. Parasites were differentiated in amastigotes after 2h of interaction in phagolysosomes. Since TLRs signaling after the invasion of microorganisms confers specificity to the immune cells in response to different pathogens and are present in the phagosome of *Leishmania* in fibrocytes, perhaps these may be involved in the development of leishmaniasis, and are target cell studies for the production of therapies most effective and least toxic to the control and treatment of leishmaniasis. **Supported by:**CNPq, FAPERJ, IOC/FIOCRUZ

HP172 - IDENTIFICATION OF PROTEINS ON THE SURFACE OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES

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Worldwide the entire human population is at risk of infectious diseases of which a high degree is caused by pathogenic protozoans, worms, bacteria and virus infections. One of the most devastating pathogens is the human malaria parasite *Plasmodium falciparum* which invades red blood cells and during proliferation modifies the constitution of the erythrocytic membrane. In this sense, a variety of proteins are exported by the parasite to the surface of the erythrocyte guaranteeing survival in the human host. Among methods for identification of surface proteins the CELL-SELEX technology (Systematic Evolution of Ligands by Exponential enrichment) is a novel tool. CELL-SELEX DNA uses ligands with high-affinity and specificity, denominate as aptamers which are processed by high-content iterative cycles against *P. falciparum*-infected erythrocytes. Nine cycles were already performed against infected or non-infected red blood cells and aptamer binding has been visualized by live cell fluorescence microscopy. Further deriving aptamers were cloned and the consensus sequence was determined. In the following selected aptamers will be applied to real-time- and cytometric assays in order to monitor the progress of aptamer in vitro-selection and subsequently proliferation of this deadly pathogen. **Supported by:**FAPESP

HP173 - ROLE OF CD200 IN THE INFECTIVITY OF LEISHMANIA (LEISHMANIA) AMAZONENSIS ISOLATES ASSOCIATED TO LOCALIZED CUTANEOUS LEISHMANIASIS (LCL) AND DIFFUSE CUTANEOUS LEISHMANIASIS (DCL)

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Leishmaniasis is a tropical disease caused by the intracellular protozoan of the genus Leishmania. A spectrum of clinical manifestations of the disease is caused by heterogeneous group of parasites and its correlated with the specie of Leishmania and the host immune response. Recently, it was described the role of the immunomodulatory molecule called CD200 in bone marrow macrophages (BMM) from C57BL/6 mice infected with L. (L.) amazonensis IFLA/BR/67/PH8. In this study, we examined the difference in infection rate and expression of CD200 of two isolates of L. (L.) amazonensis associated to Localized Cutaneous Leishmaniasis (LCL) and Diffused Cutaneous Leishmaniasis (DCL). BALB/c and C57BL/6 mice were used as animal models of infection. BMM were infected with axenic amastigotes and different time points were analyzed. Samples were processed for parasite quantification and analysis of CD200 expression by quantitative PCR. Animals were infected with 106 stationary phase promastigotes from each isolate in the footpad of C57BL/6 and BALB/c mice; parasite burden and lesion size were measured. Each experiment was compared to our reference isolate (PH8). In vitro, a marked difference was observed: while PH8 and LCL infections were able to grow in BMM from C57BL/6 and BALB/c mice, DCL only grew in BMM from Balb/c mice. In vivo, PH8 and LCL infections produced large lesions with high amount of parasites and DCL showed comparatively smaller lesions with significantly less parasites. Future experiments will validate whether the expression of CD200 in infected BMM is associated with the virulence of different isolates of Leishmania in vivo. **Supported by:**FAPESP

HP174 - LEISHMANIA (L.) AMAZONENSIS INDUCE TOLL-LIKE RECEPTOR EXPRESSION IN MACROPHAGES

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Parasites of the Leishmania genus are the causative agents of leishmaniasis. Its transmission occurs through the bite of the female sandfly and once inside the host, the parasite is phagocytosed by immune cells, such as macrophages. Leishmania can survive inside parasitophorous vacuole of these cells, subverting the immune response. Macrophages have on their surfaces toll-like receptors (TLR), the first defensive systems against invasive microorganisms. TLRs recognize pathogen-associated molecular patterns (PAMPs) and initiates signal transduction through the adaptor proteins MyD88 and/or TRIF, resulting in immune response production. The knowledge of how these receptors are activated by Leishmania is still poorly understood. Studies have shown that Leishmania induces TLRs expression in macrophages. However, it is unclear whether TLR pathway is required to induce the expression of its own receptor during the Leishmania infection. Thus, the aim of our study was to evaluate if the TLR adaptor protein MyD88 is required to TLR2 and TLR4 expression in macrophages infected with L. (L.) amazonensis. For this, bone marrow macrophages (BMMs) from wild-type (WT) or MyD88-/- mice were infected with axenic amastigotes of L. (L.) amazonensis. Western blot analysis from infection assay showed that TLR2 is expressed just when BMMs (WT or MyD88-/-) are infected by L. (L.) amazonensis for 1 and 96 hr. Infection of WT macrophages by amastigotes of L. (L.) amazonensis did not alter the expression of TLR4. However, the absence of MyD88 adapter protein decreased the expression of TLR4. These results suggest that L. (L.) amazonensis induces TLR4 expression in macrophage for a mechanism MyD88-dependent. Further experiments will be performed to address in more detail the importance of MyD88 and TRIF adaptor on the modulation or TLRs after Leishmania infection. **Supported by:**FAPESP

HP175 - PATHOGENESIS OF CHAGAS DISEASE: DIFFERENCES BETWEEN INTRAGASTRIC AND INTRAPERITONEAL INFECTION.

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Chagas disease is a worldwide public health problem and affects approximately 10 million people. Although the vectorial transmission of Chagas has been controlled in Brazil there are other ways of transmission, such as the ingestion of food contaminated with *Trypanosoma cruzi*, which ensures the continuation of this zoonosis. Recent outbreaks of the disease from the consumption of foods and beverages contaminated with *T. cruzi* have emphasized the importance of this transmission route in humans. Therefore due to the need to elucidate the mechanisms involved in oral infection by *T. cruzi*, this study aims to provide knowledge concerning the pathology of oral Chagas disease. Groups of Swiss mice were infected intragastrically (IG) or intraperitoneally (IP) with *T. cruzi* trypomastigotes forms of strain SC2005, derived from an outbreak of oral Chagas disease in Santa Catarina. To evaluate the mouse infection parasitemia levels, histopathology, PCR and mortality were analyzed. The parasitemia levels showed differences between the two groups. Just 36% of animals infected by intragastric route showed parasiteamia. The parasitemia peaks were later and less intense in mice infected by IG. Mortality of infected animals by the IP route was more intense and earlier when compared to the IG route. Histopathological analysis revealed a myotropic pattern of strain SC2005, with the presence of inflammatory infiltrates and parasites in different organs of the animals infected by the two routes. There was an intense activation of the immune system organs, except the thymus, which showed no change. *T. cruzi* DNA associated with the presence of inflammatory infiltrates were detected by PCR in the esophagus, stomach and intestine of all infected mice. The results of this study show the influence of the inoculation route on the establishment and development of the *T. cruzi* SC2005 strain infection in mice. **Supported by:**capes

HP176 - COMPARATIVE PROTEOMIC ANALYSIS OF AN ENVIRONMENTAL ACANTHAMOEBA POLYPHAGA ISOLATE BEFORE AND AFTER EXPERIMENTAL INFECTION OF RAT

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Acanthamoeba spp. are free-living protist pathogen, capable of causing a blinding keratitis and fatal granulomatous encephalitis. The damage caused by trophozoites in human corneal or brain infections is the result of several different pathogenic mechanisms not elucidated at the molecular level so far. To identify possible virulence factors of A. polyphaga, we started investigating changes in the proteome of a long-term in vitro cultivated low[illegible]nt environmental strain before and after experimental infection. The A. poly[illegible] 30872 proteome was assessed by two-dimensional gel electr[illegible] identification of protein spots by in tandem mass spect[illegible] (ApPI) and after (ApAI) a passage in rat exp[illegible] investigation of the repertoires of [illegible] ApAI samples, the res[illegible] the pH range of 3-10[illegible] spots for the ApPI s[illegible] and for the ApAI sample, a to[illegible] expressed proteins upregulated in ApAI, iso[illegible]ase, enolase, peroxiredoxin, CBS, LIM, sinapsin, tra[illegible]u), Heat shock protein 90 alpha, hypothetical proteins and oth[illegible]dentified. Our preliminary results indicated that the analyzed envi[illegible] and low virulent A. polyphaga ATCC 30872 strain undergoes changes in its expressed protein repertoire upon a single passage in rat hosts, during which it acquires competence to cause disease. These alterations involve several biological processes, since upregulated proteins include some involved in stress response, proteolysis, energetic metabolism, phosphorylation, cell cycle control and proliferation. **Supported by:**CAPES E CNPQ

HP177 - INNATE IMMUNE RESPONSE EXPRESSION IN THE CLINICAL-IMMUNOLOGICAL SPECTRUM OF HUMAN *LEISHMANIA (L.) INFANTUM CHAGASI*-INFECTION IN AMAZONIAN BRAZIL

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The clinical-immunological spectrum of human L. chagasi-infection in Amazonian Brazil has been defined in five profiles with basis on the IFAT-IgG and DTH reactions: Asymptomatic infection (AI: DTH+/++++/IFAT-), Subclinical resistant infection (SRI:DTH+/++++/IFAT+/++), Indeterminate initial infection (III: DTH-/IFAT+/++), Symptomatic infection (SI=AVL) and Subclinical oligosymptomatic infection (SOI), both with the same (DTH-/IFAT+++/++++) reaction profile. The innate immune response of these profiles was investigating by the IL-17A, IL-4, IL-1β, IL-6, IL-8, TNF and IL-10 cytokine expression. There were examined by flow cytometry 101 serum samples of individuals (both genders and with ≥1 year old) from an AVL endemic area in Barcarena, Pará state, Brazil, belonging to the clinical-immunological profiles: SI=AVL (10), III (28), SRI (13), SOI (12), AI (28) and, 10 non-infected (DTH-/IFAT-) individuals (control group). The flow cytometry cytokine analysis was realized by a BDTM system Cytometric Bead Array in a FACS Canto II. There were no differences concerning the IL-1β and IL-8 expressions among the III, SRI, AI and SI profiles; but IL-1β expression of SI profile was higher than that of the control group. There were higher IL-6 and IL-10 expressions in SI profile compared to those in the other profiles. There were also higher expression of IL-17A in III profile compared to those in ISO and IA profiles, as well as that in ISO compared to those in SRI and AI profiles. However, it should also be highlighted the higher TNF expression in SI profile than those in the other profiles, as well as that in III profile than those in the other profiles. These results represent strong evidence on the role of IL-6 and IL-10 cytokines on the susceptibility of human *L. chagasi*-infection, as well as indicate the ambiguous role of TNF, being moderately expressed in III profile, and decreasing its expression towards the AI profile or increasing towards the susceptible SI profile.

HP178 - BIOLOGICAL CHARACTERIZATION OF *LEISHMANIA (VIANNIA) LINDENBERGI* IN BALB/C MICE MACROPHAGE INTERACTION

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L. (V.) lindenbergi was originally described with isolates from human cases of cutaneous leishmaniasis occurring in the forest peri-urban area of Belém, Pará state, north Brazil. The parasite was distinguished from the other *L. (V.) spp*. based in their morphologic, biologic behavior in experimental infection of hamsters and enzymatic characteristics, but till now there is no, however, any observation on its interaction with the BALB/c mice macrophage. The present study aims to investigate the *L. (V.) lindenbergi* macrophage-interaction looking for the resistance against the serum complement (complement meditate-lysis assay), the composition analysis of carbohydrates (agglutination test), the infection index and the production of nitric oxide by the macrophage-infection of BALB/c mice. The results of the lysis assay, there was no difference between the human serum and guinea pig serum in the lysis of promastigotes of *L. (V.) lindenbergi*. Promastigotes incubated in the presence of the lectins (Con A and RCA), revealed the presence of the manose and galactose residues on its surface. In the interaction of stationary phase promastigotes of *L. (V.) lindenbergi* with peritoneal macrophages, approximately 77% of macrophages were infected with amastigotes, after 24 h. With 48 h, the proportion fell to 68%, and 72 h, reached 67% of the total. Regarding the internalization, the average was three parasites/cell 24 h after and of a parasite/cell in periods of 48 and 72 h. The production of NO was observed in the controls (peritoneiais only macrophages) and inhibited in the presence of the parasite. *L. (V.) lindenbergi* stationary-phase promastigote forms showed to be resistant against to the complement lysis and presented high levels of manose and galactose in its surface. There was no difference on the macrophage-infection indexes among the time-points studied and the nitric oxide expression was inhibited during the host cell-infection.

HP179 - CLINICAL, PARASITOLOGICAL AND HISTOPATOLOGICAL PROFILE OF DOGS NATURALLY INFECTED WITH *LEISHMANIA INFANTUM* IN GOVERNADOR VALADARES - MG

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This work aim to evaluate the clinical, parasitological and histological profile of naturally infected dogs with *Leishmania infantum* of Governador Valadares City Hall (MG - Brazil), an endemic area to visceral leishmaniasis.60 RIF positive mongrel dogs were submitted to euthanasia in accord of Decree Law 51.838 of Ministryof Health of Brazil. In necropsy, the animals were clinically evaluated being watch evident signals of CVL. Thus, the animals were clinically classified in asymptomatic (no signals of disease) and symptomatic (presenting any clinical signal of disease). Bone medullar aspiration was prepared to confirmation of parasite infection. Spleen fragments were collected to histological and parasitological analyses. 70% of total of serological positive were symptomatic and 30% were asymptomatic dogs. Symptomatic dogs showed onychogryphosis (61%), cachexia (43%), skin ulceration (37%), alopecia (28%), seborrhea (26%) and lymphpathy (24%) as mainly clinical signals. 77% of animals were positive in parasitological test and 23% were negatives. 46 animals were parasite positive in bone medullar evaluation. 72% of bone medullar positive dogs were symptomatic and 28% asymptomatic. Spleen parasitism tissue was higher in symptomatic than asymptomatic dogs. Histological analyses showed increase of capsule thickness of symptomatic dogs compared to control ones but no difference between infected animals. Naturally infected dogs increased the splenic changes compared to control animals. The hyperplasia and hypertrophy of the white pulp, congestion, hemossiderosis and inflammation of capsule and the capsule and trabecular thickening were the most common histological findings in naturally infected animals. Furthermore, differences were not found in histological damage between symptomatic and asymptomatic dogs. **Supported by:**FAPEMIG

HP180 - IL-6, IL-27 AND SCD14 ARE ASSOCIETED WITH SEVERITY IN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL), also known as kala-azar, is a disease caused by an intracellular protozoa of the genus Leishmania. VL is ranked as second in mortality and fourth in morbidity among tropical diseases, with 20,000 deaths per year. Some subjects that are infected with Leishmania do not develop the classic form of the disease, considered asymptomatic, others have classical VL e and few of these can die despite treatment, considered severe VL. To understand the mechanisms involved in VL and identification of the biomarkers for assessment of disease severity, we evaluated cytokines levels in the DTH+ and VL patients before and after treatment. As expected, our results confirm presence of serum cytokine storm in active VL patients, when compared with healthy and asymptomatic individuals. These levels of cytokine decrease significantly after of the treatment, especially IFNα (1053 to 24), IL-10 (46 to 5), IL-6 (32 to 3), IL-27 (2675 to 895) and TNFα (94 to 20). We observed correlation (more than 50%) between IL-10, IL-6, IL-27, IL-23 and TNFα with spleen and liver sizes ($p \leq 0.0005$). Hematological disorders was negatively correlational with cytokines levels. There is a strong association with high levels of IL-6 and VL severity, the probability of the patients that show IL-6 levels <200 pg/mL and die is 48x more that patients with less level (p=0,01). We also suggest that IL-27 may be orchestrating this response since it is associated with IL-6, IL-10 and TNF-a. We also evaluated if the severity of VL is associated with other pathogen recognition molecules, such as sCD14. The elevated sCD14 serum levels (2882 ± 784,8) did not associate with LPS concentration and bacterial infections, but it is associated with clinical and laboratorial manifestation of VL and IL-27, IL10 and IL-6. Together, our data suggest that Leishmania may triger sCD14 and start an inflammatory response, stimulating IL-27, IL-10 and IL-6 production and disease severity. **Supported by:**iii, CNPq, CAPES, FAPITEC

HP181 - ROLE OF MACROPHAGE CYTOSKELETON IN LEISHMANIA (L.) AMAZONENSIS INFECTION

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Leishmaniasis comprises a group of diseases caused by protozoa of the genus Leishmania, which are transmitted to mammalian hosts by phlebotomine insects. During infection the parasites are phagocytosed mainly by macrophages and come to reside within parasitophorous vacuoles. Parasites of complex Leishmania mexicana leads to formation of giant vacuoles which can fuse with other vacuoles, vesicles and organelles of the host cell. Traffic and fusion of vesicles depend directly on the cytoskeleton of the cell. Microtubules are essential in the intracellular organization, communication between organelles and transport of vesicles. The actin filaments act in cell motility, migration and phagocytosis. Thus, the objective of this proposal is to analyze molecules of the cytoskeleton of the host cell, such as tubulin and actin and study their association with the formation of the parasitophorous vacuole. **Supported by:**FAPESP

HP182 - ROLE OF PKC, MAPK AND PROTEIN TYROSINE PHOSPHATASE DURING INVASION OF MAMMALIAN CELLS BY METACYCLIC TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI*.

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Mammalian cell invasion by metacyclic trypomastigotes (MT) of *T. cruzi* involves complex molecular interactions that trigger different signaling pathways in host cells and parasites. Previous studies implicated the participation of target cell phosphoinositide-3 kinase, the mammalian target of rapamicin (mTOR) and protein kinase C (PKC), as well as of parasite protein tyrosine kinase (PTK) and phospholipase C. The role of protein tyrosine kinases (PTPs) during cell invasion by MT remains to be investigated. In addition to addressing that question, we analyzed the phosphorylation profiles associated with PTK and PKC activation during MT invasion of genetically divergent *T. cruzi* strains CL and G, which differ markedly in their infective properties. Upon 1-15 min interaction, MT of both strains induced in HeLa cells the activation of PTK that resulted in increased phosphorylation levels of its substrates. Within the same time frame, CL strain MT, but not G strain MT, triggered an increase in HeLa PKC subtrate phosphorylation levels that by 60 min interaction diminished to intensities comparable to controls. As PKCs are upstream to MAP kinases that are related with mTOR activation, ERK and p38 MAPKs were analyzed and found to be phosphorylated in HeLa cells interacting with CL strain, but not with G strain. As regards the response in parasites, the phosphorylation levels of PKC and PTK substrates were increased In G stain MT, but not in CL strain, upon 1-60 min contact with HeLa cells. The role of PTPs was determined using PTP inhibitor BPV(phen). Treatment of cells with BPV increased invasion of G strain MT, but not of CL strain, whereas the treatment of parasites inhibited the infectivity of CL strain that presented more ecto-phosphatase activity than G strain. Taken together, these data reinforce the notion that distinct signaling pathways are triggered during internalization of *T. cruzi* strains with different invasive capacities. **Supported by:**CNPQ e CAPES

HP183 - DEVELOPMENT AND STANDARDIZATION OF METHODS FOR DETECTION OF TRYPANOSOMA CRUZI CONTAMINATION IN AçAí (EUTERPE OLERACEA)

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INTRODUCTION/BACKGROUND: Despite of the achievement of the control of vectorial transmission for Chagas Disease (CD), in endemic regions, alternative forms of transmission have emerged such as oral transmission. The oral transmission occurs by ingestion of food contaminated with triatomines. Suspicion has fallen on Açaí (Euterpe oleracea) as the main vehicle of foodborne CD transmission in some areas of Brazil and other South American countries. OBJECTIVES: To develop and evaluate molecular techniques and cultivation methods to detect T. cruzi contamination in paste and beverage of açaí (Euterpe oleracea). METHODS: For the detection of T. cruzi parasites in açaí by PCR assays, we used the primers for TCZ1 and 28Sα rRNA genes. Ten mL of açai were inoculated with 107 cells of T. cruzi epimastigotes, which were further isolated by flotation in zinc sulfate followed by the DNA extraction and PCR. The minimum amounts of cells in ten mL of açaí were also evaluated. Alternative protocols of DNA extraction were also tested. RESULTS: The minimum limit of detection using the 28Sα rRNA primers was achieved in the order of 12 pg of T. cruzi DNA, while TCZ1 primer was 20 fg. The minimum numbers of cells in ten mL of açaí detected were: 17 epimastigotes cells for 28Sα rRNA primers and 1.7 epimastigote cells for TCZ1 primers. DISCUSSION: The methodology of T.cruzi isolation based on zinc sulfate flotation, together with the high sensitivity of the 28Sα rRNA and TCZ1 primers showed promising results. Now we are evaluating the viability of trypomastigotes in açaí, as well as, determining the minimum limit of T. cruzi detection by real-time PCR. **Supported by:**CNPq/MCTI/ANVISA

HP184 - GENERATION OF RECOMBINANT ANTIBODIES (SCFV) AGAINST *TRYPANOSOMA CRUZI* SURFACE PROTEIN: A POTENTIAL IMPROVEMENT TO FUNCTIONAL GENOMICS AND/OR DRUG DELIVERY.

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Most of molecular tools to do functional genomics by ablating gene expression is poorly effective in *T.cruzi,* because many genes are encoded by multi gene families, which compromise the conventional gene knock outs. To circumvent this limitation, we decided to test the expression of scFv to specifically target protein expression (intrabodies). Additionally to this application, the immortalization monoclonal antibodies (mAb) through scFv engineering are important by itself, and the scFvs can also be used for better drug delivery design. Chagas disease treatment has questionable effectiveness and serious side effects is worthy to highlight. Thus, the mAbs-10D8, mAbs-2B10, mAbs-3F6 and mAbs-2C2 hybridomas, that express anti-GP35/50, anti-GP35/50, anti-GP82, and anti-2C2 respectively, were selected to create scFvs anti-T.cruzi. Here, the technology of recombinant antibodies was used to obtain the regions encoding the variable light and heavy chains of mAb-10D8 by RT-PCR using specific primers and total RNA from hybridoma cells. The fragments were sequenced and used for the synthesis of a synthetic gene of scFv (scFv- 10D8) optimized for E. coli expression. The scFv-10D8 gene was subcloned fused to a histidine tag into a prokaryotic expression system (pET22b). After testing some induction conditions and enriching a fraction containing scFv-10D8, it was demonstrated that the recombinant antibody is able to recognize in the same fashion as mAb-10D8 the parasite protein. These results encourage us to express this scFv anti-T.cruzi gp35/50 and check if it can interfere with protein function on host parasite interaction, this procedure is underway. Based on these experiments the other hybridomas will be carried out. **Supported by:**CAPES, CNPq e Fundação Araucária

HP185 - EXPRESSION OF ALFA SMA, VIMENTIN, CK AND TGF-β IN LIVERS OF DOGS NATURALLY INFECTED WITH LEISHMANIA INFANTUM

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Visceral leishmaniasis (VL) is an endemic disease in Brazil. Changes in hepatic extracellular matrix, mainly collagen deposition are described in both human and dog. , However the mechanisms have not been elucidated, mainly in canine visceral leishmaniasis (CVL). The aims of this work was to investigate the differentiation of hepatic progenitor cells and hepatic stellate cells into myofibroblasts and their role in hepatic fibrosis pathogenesis . LFragmentsof livers s were obtained from 20 naturally infected dogs with Leishmania infantum and 8 non-infected dogs. For histopathological evaluation,was performed HE and Gomori Ammoniacal Silver for labeling eticular fibers. To evaluate markers of fibrogenesis, we performed α-SMA, vimentin, and TGF-β CK immunostaining. Histopathological changes were:observed as , portal and intralobular capsule inflammation with, intralobular granulomas; sinusoidal congestion; Kupffer cells hypertrophy and hyperplasia; and steatosis. We observed a higher deposition of reticular fibers in infected animals than uninfected dogs. In parallel, α-SMA, TGF-β and CK increased expressionwere also observed. . The marking of vimentin did not differ among the groups. Positive correlation among parasitie load and collagen deposition was observed in infected animals. Our results suggest fibrosis and parasite load correlation in t CVL. We could infer that myofibroblasts-derived hepatic progenitor cells mainly demonstrated by α-SMA and CK labeling might play an important role in fibrosis supported by the TGF-B data.. However other mechanisms is necessary to check as the monocytes-macrophages transition wicth may also be involved in tissue fibrosis. **Supported by:**CNPQ

HP186 - REVERSE GENETIC APPROACHES TO IDENTIFICATION OF ESSENTIAL GENES INVOLVED WITH THE EXPORT OF MRNA IN *TOXOPLASMA GONDII*

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Toxoplasma gondii is an important pathogen because the symptoms of the disease are more aggressive for Brazilian patients in comparison to patients from the EUA and Europe. The nucleo-cytoplasmic RNA export is an essential post-transcriptional pathway for gene expression control in eukaryotic cells, but it is still poorly understood in this parasite. Recently, our group have demonstrated that TgUAP56, a high conserved protein and specific component of mRNA export in metazoans, is an essential component of mRNA export pathway in *T. gondii*. However, other proteins involved in mRNA export are less conserved or even absent in species of Apicimplexa group. The question arose if less conserved proteins would plays a similar role in mRNA export in those species in comparison to the components described for metazoans. In the present work, the main goal is to investigate the function of less conserved proteins that might be components of mRNA export in *T.gondii*. Searching for orthologous *T. gondii* were performed using as seed the *S. cerevisiae* proteins known to be related to the export of mRNAs. Comparative analysis by alignments led to the choice of three target proteins (RanBP, Gle2 and PAB1) for further functional analysis by localization, proteomic and genetic reverse approaches. We have obtained a *T.gondii* strain expressing the HA-Tagged RanBP, showing it is a major cytoplasmic protein. Furthermore, we intend to identify the RanBP protein complex by immunoprecipitation assays followed by mass spectrometry. We are currently working on obtaining *T. gondii* strains for expression of HA-tagged Gle2 and PAB1 proteins in addition to construction of plasmids in order to perform knockout and overexpression analysis. We believe that this work will allow the identification of specific components in mRNA export pathway of *T.gondii* and the results will be useful to search for similar proteins in other parasites. **Supported by:**CAPES, CNPq, FIOCRUZ