

Imunologia - Immunology

IM01 - DYNAMICS OF IMMUNE GRANULOMA FORMATION IN A *Leishmania braziliensis*-INDUCED SELF-LIMITING CUTANEOUS INFECTION IN THE PRIMATE *Macaca mulatta*

C Souza-Lemos¹, SN de-Campos¹, A Teva¹, S Côte-Real², EC Fonseca³, R Porrozi¹, and G Grimaldi Jr¹ (E-mail address: grimaldi@ioc.fiocruz.br)

¹Laboratório de Pesquisa em Leishmaniose,

²Laboratório de Biologia Ultraestrutural, Instituto Oswaldo Cruz/Fiocruz, Rio de Janeiro and

³Laboratório de Imunohistoquímica (SAP/HUAP), Universidade Federal Fluminense, Niterói, Brazil.

Leishmania braziliensis-induced granuloma formation at infection sites is a major histopathological feature of the human disease. The immunologically active granulomas are thought to restrain the infection, kill and remove the microbial target, and repair any accompanying tissue injuries. However, the overall antimicrobial efficacy of the granulomatous response appears to be variable, and it depends on host determinants and pathogen virulence. Even with an intense granulomatous response, leishmanial parasites are able to survive at low levels throughout the host's lifetime. In order to unravel the physiopathology of leishmaniasis in humans, it is necessary to better understand how *Leishmania* are able to survive for years within immunologically-active granulomas. In the present study, we used a macaque (*Macaca mulatta*) model of infection with *Leishmania braziliensis* as a means of assessing the usefulness of this primate system. This model more closely mirrors human protective immunity to *Leishmania* than the murine model; therefore, we used it to study the host inflammatory granulomatous response involved in the control of cutaneous leishmaniasis. Infected primates developed localized long-term skin ulcerations, but complete spontaneous clinical healing occurred in all infected animals. The infection induced the recruitment and activation of inflammatory mast cells, granulocytes, mononuclear phagocytes, and lymphocytes at the site of infection. During the acute reaction, polymorphonuclear leukocytes were more prominent than other cell types and apparently destroyed many parasites; macrophages then rapidly engulfed dying neutrophils together with their parasitic cargo. In the chronic phase, persisting parasites induced a typical T helper (Th)

cytokine, type 1-mediated, immunity-induced granulomatous reaction. By this time, more or less differentiated macrophage accumulations were found, and these evolved to become mature tissue granulomas consisting of all the specific cell types found within human granulomas. In the healing stage, fibroblasts proliferated at the periphery, and finally invaded the granulomas with fibrotic substitution. These findings point to the feasibility of using this model to elucidate the potentially disabling Th1-cell mechanisms that may eventually render the host granulomatous response inadequate for fighting *L. braziliensis* infections.

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IM02 - ORAL IMMUNIZATION WITH LOW DOSES OF PROTEINS OF *LEISHMANIA* INDUCES PROTECTION AGAINST *LEISHMANIA AMAZONENSIS*

ALVES, D.C.R.¹, MOREIRA, R.S.M.¹, OLIVEIRA, D.M.¹, RIBEIRO, C.C.¹, LIMA, M.P.¹, FERREIRA, A.A.C.¹, COSTA, L.E.¹, PFEIFFER, V.N.¹, COELHO, V.T.S.¹, FUMAGALLI, M.A.², MACHADO, C.M.², TAVARES, C.A.P.¹, CHÁVEZ-OLORTEGI, C.¹, MOTA-SANTOS, T.A.¹, COELHO, E.A.F.^{1,3}

1. Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.
2. Departamento de Farmacologia Bioquímica e Molecular, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.
3. Setor de Patologia Clínica, Coltec, UFMG, Minas Gerais, Brazil.

Leishmaniasis is a disease caused by protozoan parasites of the genus *Leishmania*. The pathology of the infection is determined not only by the parasite species, but also by genetics background and immune factors of the host. In this work, we investigated the effect of immunization with low doses of soluble *Leishmania* antigen (SLA) of *L. amazonensis* in the course of murine infection with this parasite. Groups of BALB/c mice (n=10, per group) received three oral doses of SLA (1µg per mouse), SLA plus alum (1µg of SLA plus 1µg of alum, per mouse) or PBS, as control. Thirty days after the last dose, mice were challenged subcutaneously with 1x10⁵ promastigotes of *L. amazonensis*. Measures of the footpad swelling,

quantification of the parasite load, cytokine and serum antibodies levels were performed. Significant reductions in the footpad swelling and parasite load of the BALB/c mice immunized with SLA or SLA plus alum were observed as compared to control group. This immunization schedule also induced a high level of IFN- γ and low levels of IL-4 and IL-10 by spleen cells in response to soluble *L. amazonensis* antigen. Low levels of total IgG and IgG1 against SLA were observed in the sera of immunized groups. In conclusion, oral immunization of BALB/c mice with low doses of SLA was able to induce protective immunity against *L. amazonensis* infection.

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IM03 - CYTOKINE PRODUCTION IN SPLEEN AND GUT AFTER ORAL IMMUNIZED WITH IRRADIATED TACHYZOITES OF *Toxoplasma gondii* RH STRAIN IN C57BL/6J AND BALB/c MICE

Galisteo Jr., A.J.^{1,2}, Vieira, D.P.¹ & de Andrade Jr., H.F.¹

¹Lab. de Protozoologia (IMTSP – Inst. de Medicina Tropical de São Paulo/USP); ²Centro de Biologia Molecular (IPEN- Inst. de Pesq. Energéticas e Nucleares/CNEN SP)

Toxoplasma gondii can infect most mammals and birds, sometimes causing severe pathology. Infection with *T. gondii* naturally occurs through ingestion of raw or undercooked meat containing cysts or oocysts from cat feces. Vaccines must induce a good mucosal immunity that could result in local control of infection, preventing systemic disease. Oral vaccines must induce intestinal immunity to prevent infection and irradiated *T. gondii* tachyzoites induce significant protection to mice, similar to chronically infected animal. We studied mucosal cytokine response of C57Bl/6j and BALB/c mice, immunized with 10⁷ tachyzoites radiation-sterilized (255Gy/⁶⁰Co) *T. gondii* RH strain (oral or parenteral route), with 3-biweekly doses. Real-Time PCR reactions were performed to detect and quantify expression of cytokine mRNA (IFN- γ , IL-2, IL-4 and IL-10) in splenocyte and Peyer's patches cultures. After 2 weeks, immunized and control animals were challenged with 10 cysts of ME49 strain p.o. Protection was determined at the 30th day by brain cyst counting. It was possible to observe in our models a remarkable increase of the IFN- γ mediated

response in splenocytes obtained from all groups, despite lower in oral-immunized C57Bl/6j mice. Parenteral immunized mice presented high levels of IL-4 and IL-10 expression, not observed in ME49 infected group. In Peyer's patches cells, samples obtained from oral-immunized groups showed higher expression of IFN- γ and IL-2, despite all cytokine levels were superior than observed in ME-49 infected control group. All immunized groups presented significant protection when challenged with ME-49 viable agents; with high protection in BALB/c mice both oral or parenterally immunized. Our data also suggests that increased mucosal expression of IFN- γ and IL-2 could be related to effective immune response against parasite infection, as expressed in low numbers of cerebral cysts. This pattern of gut immune response could be a desirable effect for a good vaccine in toxoplasmosis.

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IM04 - THE ROLE OF CYTOKINES AND TRANSCRIPTION FACTORS IN DERMIS OF DOGS NATURALLY INFECTED WITH *L. CHAGASI* WITH DIFFERENT PARASITE DENSITY

Menezes-Souza, D.^{1,2,3}, Guerra-Sá³, R.; Giunchetti, R.C.^{1,2}; Teixeira-Carvalho, A.⁴; Martins-Filho, O.A.⁴; Corrêa-Oliveira, R.¹ and Reis, A.B.^{1,2}

¹Laboratório de Imunologia Celular e Molecular, IRR-FIOCRUZ-Belo Horizonte/MG

²Laboratório de Imunopatologia, ICEBII-NUPEB/UFOP-Ouro Preto/MG

³Laboratório de Bioquímica e Biologia Molecular, ICEBII-NUPEB/UFOP-Ouro Preto/MG

⁴Laboratório de Biomarcadores de Diagnóstico e Monitoramento, IRR-FIOCRUZ-Belo Horizonte/MG

Dogs naturally infected by *Leishmania chagasi* represent the main domestic reservoir of the visceral leishmaniasis considering intense skin parasitism in different clinical forms. The skin is the first point of contact with organisms of the genus *Leishmania* from sand fly vectors, and apparently normal skin of sick dogs harbours amastigote forms of *L. chagasi*. The present study was therefore designed to investigate the relationship between cytokines (IFN- γ , IL-4, IL-5, IL-10, IL-12, IL-13, TGF- β and TNF- α), transcription factors (T-

bet, GATA-3 and FOXP3) and parasite density as seen in the ear skin of dogs naturally infected by *L. chagasi*. Thirty-five infected dogs were subdivided according with the tissue parasite density using Leishman Donovan Units (LDU), as follows: Low Parasitism (LP; n=12), Medium Parasitism (MP; n=11) and High Parasitism (HP; n=12). Sixteen non-infected dogs (CD), used as control group, were serologically and parasitologically negative for *L. chagasi*. Our results showed an increase of pro-inflammatory cytokines IFN- γ , TNF- α and transcription factor T-bet in all infected groups (LP, MP and HP) in comparison with the CD. The HP group presented a low expression of IL-12 in comparison with LP and a negative correlation ($r=-0.5928/p=0.0002$) between IL-12 and high parasite density. In HP, was found a high expression of immunoregulatory cytokines IL-10 and TGF- β in relation to CD, LP and MP groups. Furthermore, a positive correlation ($r=0.4240/p=0.0245$) between skin parasite density and IL-10 expression was observed. The increase of IL-12/IL-10 ratio in dogs with lower parasite density was observed. These findings reinforced the important of pro-inflammatory cytokines mainly IL-12 in the control of parasitism. Herein, we confirmed the involvement of IL-10 and TGF- β in the suppression of appropriate immune response in the control of parasite replication in the skin.

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IM05 - ASYMPTOMATIC DOGS NATURALLY INFECTED WITH *L. CHAGASI* PRESENT HIGH EXPRESSION OF IFN- γ , TNF- α , IL-13 AND GATA-3 IN DERMAL COMPARTMENT

Menezes-Souza, D.^{1,2,3}; *Guerra-Sá*³, *R.*; *Giunchetti, R.C.*^{1,2}; *Teixeira-Carvalho, A.*⁴; *Martins-Filho, O.A.*⁴; *Corrêa-Oliveira, R.*¹ and *Reis, A.B.*^{1,2}.

¹Laboratório de Imunologia Celular e Molecular, IRR-FIOCRUZ-Belo Horizonte/MG

²Laboratório de Imunopatologia, ICEBII-NUPEB/UFOP-Ouro Preto/MG

³Laboratório de Bioquímica e Biologia Molecular, ICEBII-NUPEB/UFOP-Ouro Preto/MG

⁴Laboratório de Biomarcadores de Diagnóstico e Monitoramento, IRR-FIOCRUZ-Belo Horizonte/MG

Canine visceral leishmaniasis (CVL) manifests itself as a broad clinical spectrum ranging from asymptomatic infection to patent severe disease.

In the last decade, the search for new immunobiomarkers in CVL has been intensified. However, it still remains to be elucidated that mechanisms of the acquired immune response in dermal compartment. The aim of this study was to investigate the detail cytokines (IFN- γ , IL-4, IL-5, IL-10, IL-12, IL-13, TGF- β and TNF- α) and transcription factors (T-bet, GATA-3 and FOXP3) profiles by real time PCR in dermis biopsies of dogs naturally infected by *L. chagasi* with distinct clinical forms. Thirty-five infected dogs were subdivided according with clinical form, as follows: Asymptomatic (AD; n=10), Oligosymptomatic (OD; n=10) and Symptomatic (SD; n=15) and sixteen non-infected dogs (CD) were used as control group. Our major findings indicated that asymptomatic animals showed high expression of pro-inflammatory cytokines IFN- γ and TNF- α in relation to SD and CD groups. Also a negative correlation between high levels of IFN- γ and TNF- α ($r=-0.3988/p=0.0263$; $r=-0.5496/p=0.002$) with clinical evolution. Interestingly, IL-13 showed a similar profile with the IFN- γ and TNF- α , associated with a negative correlation ($r=-0.6879/p<0.0001$) with the clinical manifestations intensity. The IFN- γ /IL-4 ratio values were higher in the AD and OD groups in relation to SD group. Furthermore, GATA-3 presented a negative correlation ($r=-0.6654/p<0.0001$) with clinical progression. Together, our results suggest that IL-13 can promote an increase of IFN- γ production during asymptomatic disease as describe by other authors. Further, the high expression of pro-inflammatory cytokines in asymptomatic animals contributes to control the evolutions of the clinical signs.

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IM06 - HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF EARS SKIN BIOPSIES OF DOGS NATURALLY AND EXPERIMENTALLY INFECTED WITH LEISHMANIA (LEISHMANIA) CHAGASI

*FIGUEIREDO, M.M.*¹, *MOURA, E.P.*¹, *SOUZA, C.C.*¹, *MELO, M.N.*², *TAFURI, W.L.*¹, AND *TAFURI, W.L.*¹

¹ Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Canine visceral leishmaniasis is a severe systemic disease caused by *Leishmania chagasi*. The aim of

this study was to evaluate the intensity of inflammatory process and the parasite load of skin of naturally and experimentally infected dogs with *L. chagasi*. Dogs were divided in four groups: (1) 20 uninfected mongrel dogs; (2) 65 infected dogs obtained from Santa Luzia-MG; (3) 10 beagles, experimentally infected with *L. chagasi* (5×10^7 i.v. promastigotes) from HERTAPE-CALIER, Betim-MG; (4) 53 infected dogs obtained from Santo Agostinho Veterinary Clinic, Belo Horizonte-MG. All ears biopsies were fixed in formalin (10%) for histological and parasitological studies. Qualitative and quantitative studies of the histological alterations and the tissue parasite load were carried out under optical microscope and morphometrical analysis. In general, a chronic inflammatory reaction was observed in all infected animals. The cellular exudate was diffuse in the upper dermis and localized mainly in the deep dermis and predominately composed by plasma cells, macrophages and lymphocytes. Under these microscopically analysis, in experimental group the inflammatory response and the parasite load was less intense than the other infected animals groups. However in the quantitative analysis we did not find any statistical difference concern to the inflammatory response, but the parasite load was significantly lower than group 4. Moreover, dogs from group 4 showed higher numbers of parasites in skin tissues than group 2. Symptomatic dogs showed higher parasite load than asymptomatic only in the group 2. These results could be indicating that the experimental infection induce a distinct histopathological and parasitological scenario.

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IM07 - IMMUNOHISTOCHEMICAL ANALYSIS OF LAMININ, AN EXTRACELLULAR MATRIX COMPONENT, IN LIVERS OF DOGS NATURALLY INFECTED WITH *Leishmania (Leishmania) chagasi*

MELO, F. A.^{1,3}, MOURA, E. P.¹, RIBEIRO, R. R.¹, ALVES, C.F.¹, LIMA, W.G.², MICHALICK, M.S.M.¹, TAFURI, W. L.¹, ABREU-SILVA, A. L.³, CALABRESE, K. S.⁴, TAFURI, Wg. L.¹.

¹ Universidade Federal de Minas Gerais, Minas Gerais, Brazil; ² Universidade Federal de Ouro Preto, Minas Gerais, Brazil; ³ Universidade Estadual do Maranhão, Maranhão, Brazil; ⁴ FIOCRUZ-IOC, Rio de Janeiro, Brazil.

Leishmania species protozoa that alternately parasitize their sand fly vectors and mammalian macrophages. Parasites are deposited in the mammalian skin by infected sand flies and thereafter must interact with and overcome a variety of obstacles, including extracellular matrix and basement membrane proteins, to establish infection within macrophage. Structural and functional changes of the liver in canine disease have been studied by workers in rodent models of *Leishmania* infection, human visceral leishmaniasis, experimentally and naturally infected dogs. We have described laminin expression in livers of 30 mongrel dogs naturally infected with *Leishmania chagasi* from Belo Horizonte, Metropolitan area. Dogs were sacrificed with 2.5 percent intravenous Thionembutal™ and intravenous T61™ (INTERVET Laboratory). During necropsy, liver samples were obtained and fixed in 10% neutral-buffered formalin. All tissue samples were dehydrated, cleared, embedded in paraffin, cut into 4-5 micrometers thick sections, and stained by Hematoxylin and Eosin (HE). Also, these paraffined tissues were used for immunohistochemical detection of amastigotes forms of *Leishmania* in tissues. Other livers samples were embedded in tissue-freezing medium and cut in criostat for immunohistochemistry assays (laminin detection). The tissue images were transferred to a computer video screen by means of the software KS300 and relayed to a computer-assisted image analysis system (Kontron Elektronik/Carl Zeiss, Germany) for morphometrical analysis. Liver laminin were detected mainly in the walls of central veins and perisinusoidal space in discontinuous displacement. Infected dogs showed higher laminin deposition in livers than non-infected animals ($p < 0,001$). Spearman Rank Correlation between parasite load and laminin deposition showed positive correlation ($r = 0,4838$, $p = 0,068$). In the literature, a high expression of laminin could be related to an inflammatory and degenerative processes in tissues. Thus, our results could have been demonstrated a strict correlation between the parasite tissue load and extracellular matrix components alterations in canine visceral leishmaniasis.

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IM08 - Clinical importance of mielogram and hemogram in dogs naturally infected by *Leishmania (Leishmania) chagasi*

Raquel Trópia de Abreu¹; Samuel Leôncio Braga¹; Maria das Graças Carvalho², Rodolfo Cordeiro Giunchetti^{1,3}; Cláudia Martins Carneiro¹; Rodrigo Dian de Oliveira Aguiar¹; Rodrigo Corrêa Oliveira³; Olindo Assis Martins-Filho⁴; Alexandre Barbosa Reis^{1,3}.

1-Laboratório de Imunopatologia, NUPEB/UFOP

2-Laboratório de Hematologia Clínica, Faculdade de Farmácia/UFMG

3-Laboratório de Imunologia Celular e Molecular, IRR/FIOCRUZ

4-Laboratório de Biomarcadores de Diagnóstico e Monitoração, IRR/FIOCRUZ

Hematological measurements in *L. chagasi*-infected dogs have limited applications for disease diagnosis but can be very important in evaluating the clinical status of the infected-animals, as well as in the understanding of VL pathogenesis. Herein, a group of 48 dogs: asymptomatic (AD, $n=10$), oligosymptomatic (OD, $n=9$) and symptomatic (SD, $n=12$) compared with non-infected dogs (NID, $n=17$) was evaluated by bone marrow smears analysis and hemogram to investigate the association between bone marrow alteration and parameters of the peripheral blood (haematological status). The results showed that peripheral blood alterations were related to those shown by bone marrow, mainly in SD group. Indeed, SD group dogs demonstrated a significant decrease in erythropoietic lineage cells number and erythrocytes, hemoglobin and hematocrit resulting in a nonregenerative anemia. Moreover, there were observed increase in neutrophils and their precursors, decrease in band eosinophils and eosinophils and significant increase in lymphocytes number in bone marrow associated to leukopenia with left shift, decrease in eosinophils, lymphocytes and monocytes number in peripheral blood. The present study showed that the clinical evolution of CVL in naturally infected dogs promotes clear alterations in the bone marrow and peripheral blood. So, the data analysed together allow to conclude that the assessment of these parameters constitute an useful and additional tool for CVL prognosis, as well as for parasitological diagnosis in two specific situations, namely: a) to clarify cases with a strong suspicion of CVL, however not confirmed by sorologic tests, by using bone marrow analysis; and b) to orientate

complementary laboratory tests to investigate CVL suspicion, based on clinical and hemogram data.

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IM09 - IMMUNOHISTOCHEMISTRY STUDY OF CALPROTECTIN (L1 PROTEIN) IN LIVERS AND SPLEENS OF DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) CHAGASI*

MOURA, E.P.¹; FIGUEIREDO, M.M.¹; MELO, F.A.¹; AMORIM, I.F.G.¹; MUDADO, A.M.¹; BIASIZZO, R.L.¹; SILVA, L.C.¹; SOUZA, C.C.¹; MICHALICK, M.S.M.¹; TAFURI, W.L.¹

¹ Universidade Federal de Minas Gerais, Belo Horizonte-MG, BRAZIL.

Calprotectin (L1 protein) represents a cytosolic antibacterial protein present on membrane of monocytes and is involved in their recruitment to inflammation site by adhesive interactions with the endothelium. In a murine leishmaniasis model, that accumulation of L1-expressing cells in an early course of infection is related to susceptibility to the disease. Those L1+ cells are less capable of phagocytosing and killing parasites, suggesting that possible association with disease exacerbation. In this study, we examined the correlation between the L1+ cells and the parasite load of spleens and livers of dogs naturally infected with *Leishmania chagasi*. Thirty dogs naturally infected with *Leishmania chagasi* were sacrificed with lethal dose of Thionembutal and T61. Samples of tissues were collected and fixed in 10% neutral buffered formalin and other fragments were collected and frozen for immunohistochemical studies. A quantitative study for parasite tissue load and L1 expression was carried out under optical microscope. We observed more parasites in the spleen than in liver of all naturally infected dogs. Ours previous results have showed there was a positive correlation between the parasite load and the L1 expression in the spleen. However, this correlation was not observed in the liver. These results could be in according to the literature that report to have a distinct immune response against *Leishmania chagasi* in target organs, in the visceral leishmaniasis canine.

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IM10 - CR3 (CD11b/CD18) CELL EXPRESSION IN SPLEENS OF DOGS NATURALLY INFECTED WITH *Leishmania (Leishmania) chagasi* AND ITS CORRELATION WITH THE PARASITE LOAD

LIMA, W.G.^{1,2}, *OLIVEIRA, P.S.*², *MELO, F.A.*², *CALIARI, M.V.*³, *MICHALICK, M.S.M.*⁴, *TAFURI, W.L.*³, *TAFURI, Wg.L.*³

1 – Departamento de Ciências Biológicas – Instituto de Ciências Exatas e Biológicas / UFOP;
2 – Departamento de Anatomia Patológica e Medicina Legal – Faculdade de Medicina/ UFMG;
3 – Departamento de Patologia Geral – Instituto de Ciências Biológicas/ UFMG; 4 – Departamento de Parasitologia – Instituto de Ciências Biológicas/ UFMG.

Leishmania is an intracellular protozoan parasite that is delivered to its vertebrate host by the bite of an infected sandfly. Following injection into the skin, the flagellated promastigotes forms of the parasite must rapidly enter its host cell, the macrophage, and later transform into amastigotes forms. CD11b and CD18 proteins compose the Complement Receptors 3 (CR3) and its expression appears to make a quantitatively greater contribution in interaction between parasite and mammalian cells contributing to survive of protozoan inside mononuclear vertebrate host cells. This work aim to correlate the parasite load and the CR3 expression of spleens of dogs naturally infected with *Leishmania chagasi*. Thirty mongrel dogs were obtained from municipality of Sabará/MG (Belo Horizonte metropolitan area). Dogs were divided in: (NI) ten uninfected dogs; (INP) twenty infected animals with serological and parasitological positive exams for *Leishmania* infection. All animals were sacrificed with lethal dose i.v.(1ml/kg) of sodic Thiopental (2,5%) and T-61 (0,3ml/kg). Spleen fragments were collected for tissue touch preparations (LDU indices) for amastigotes detection. The slides were stained with Giemsa 10%. Others fragments were fixed in formaldehyde 10% solution for histological and immunohistochemistry studies. Others fragments were included in Tissue Teck medium freezing and cut in cryostat for CR3 characterization. The parasite load results by LDU indices and immunohistochemistry analysis showed ten infected animals without amastigotes. These animals were separated in a distinct group of other ten animals with positive parasite load in spleens. Our results has been indicating a higher CD11b and CD18 expression in infected dogs than non

infected ones ($p=0.0263$). Moreover, CD11b spleen expression was higher in infected dogs with positive parasite load in spleens ($p=0.0453$) than the infected ones without spleen parasite load. Likewise CD18 spleen expression was higher in the formers ($p=0.0263$). These results showed a parallel between the number of parasites and CD11b/CD18 positive spleen cells. It could have pointed out evidence that primary lymphoid organs are able to maintain the infection by *Leishmania* in dogs.

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IM11 - DETECTION OF IGG ANTIBODIES IN MEAT EXTRACT FROM ANIMALS EXPERIMENTALLY INFECTED WITH *TOXOPLASMA GONDII*

*MECCA, J.N.*¹; *MEIRELES, L.R.*¹; *de ANDRADE JR., H.F.*¹

¹ Instituto de Medicina Tropical da Universidade de São Paulo, São Paulo, Brazil.

Toxoplasma gondii is a cosmopolitan obligate intracellular parasite that infects warm-blooded animals, causing serious problems in immunosuppressed individuals or congenital infection during acute infection of pregnant women. This agent causes also several economic losses in animal production, as the causal agent of abortion. The main human transmission is the ingestion of raw or undercooked meat containing cysts, being toxoplasmosis one of the main meat-transmitted zoonotic diseases. Currently, meat inspection in Brazil is devised to gross macroscopic lesions or parasites, as tuberculosis or teniasis, without any *Toxoplasma* search. Several laboratory methods are used for detection of this infection, mainly by serology, but detection in slaughter meat needs PCR or cumbersome biological tests in mice. We devised to study exuding extracts from frozen meat, composed mainly by blood retained in capillary vessels, looking for its possible use as a source from "serum" of the source animal for *Toxoplasma* serology. We standardize an Enzyme-Linked Immunosorbent Assay for detection of IgG anti-*T. gondii* in meat extracts from experimentally infected rabbits, correcting the amount of blood in those meat extracts by hemoglobin determination. We used frozen blood from those animals as standard of hemoglobin, using a nitrite solution for 540 nm O.D. stabilization. Clear discrimination of infection was obtained in those extracts, allowing

both the assumption of infected or *T.gondii* free animals, with adequate cut-offs and serological indexes, which was not possible without hemoglobin determination blood content extrapolation. This extract is easily obtained after freezing, without any destruction or sampling in the meat, without affecting its commercial value. This diagnostic approach is very promising and important for meat safety quality, allowing control at end distributors or users in meat prepared for marketing, contributing directly to the prevention of human infection.

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IM12 - INFLUENCE OF pH DISSOCIATION OF IMMUNE COMPLEXES IN IgG SEROLOGY DURING *Leishmania chagasi* INFECTION IN HAMSTERS

CARVALHO, C. A.¹, MEIRELES, L. R.¹,
BORBOREMA, S. E. T.², ANDRADE Jr, H. F.¹

¹Instituto de Medicina Tropical da Universidade de São Paulo, São Paulo, Brazil; ²Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil.

Leishmaniasis, a zoonosis caused by several species of Kinetoplastidae protozoa, is endemic in South America tropical areas. Usually presented as a cutaneous or visceral disease, leishmaniasis is caused by several species, affecting at least 2 million people yearly, with half a million with systemic visceral disease or kala-azar. In Brazil, visceral leishmaniasis is caused by *Leishmania (L.) chagasi*, transmitted by sandflies and with dogs as reservoirs, as typical zoonosis. Despite useful in cutaneous disease, serology in humans and dogs had several constrains in visceral disease, despite chronic disease with high levels of serum immunoglobulins, with diagnosis performed by invasive parasitology. IgG serology in those cases are frequently erratic with negative serum samples in parasitological proof patients. This problem is usually ascribed to low affinity antibodies and immune complexes. Here, we devised to test an in plate dissociation of immune complexes in the serology of *L. (L.) chagasi* infected hamsters. Experimental model consisted in groups of 10⁶ amastigotes i.v. challenged hamsters, killed sequentially at monthly intervals both for serology and parasitology. Immune complexes in serum were dissociated by a pH gradient, by an in-well reagent protocol. Serums from infected hamsters

were positive for specific IgG in the first month after infection, but after this, consistently negative in usual ELISA, despite high parasite load. After dissociation, those samples provide high levels of specific IgG, showing that the specific IgG were blocked by circulating antigens, inducing false negative results. This ELISA test with pH gradient could be an elegant and cheaper improvement in visceral leishmaniasis serology, especially in serum from patients with high parasite load, which had high levels of circulating immune complexes, allowing diagnosis without invasive parasitology, risky by bleeding due to thrombocytopenia, frequently seen in those patients. Financial support: LIMHCFMUSP-49 and CNPq.

IM13 - THE ROLE OF Nod1 AND Nod2 RECEPTORS IN THE EXPERIMENTAL INFECTION BY *L. amazonensis*

LIMA JUNIOR, D. S.¹, Costa, D. L.¹, GUTIERREZ, F. R.¹, CARREGARO, V.¹, AFONSO, L. C. C.²,
Zamboni, D. S.¹, Silva, J. S.¹

¹School of Medicine Ribeirao Preto, University of Sao Paulo, São Paulo, Brazil; ²Federal University of Ouro Preto, Minas Gerais, Brazil

NOD1 and NOD2 are innate immune receptors that sense microbial molecules produced during the synthesis and/or degradation of peptidoglycan. The parasite *Leishmania sp.* secretes a family of heavily glycosylated proteins and proteoglycans as well as glycans that are similar in structure to those found on LPG. Together, these molecules are important for parasite's virulence. The activation of NOD receptors result in the degradation of NFκB inhibitor IκBα and the subsequent translocation of NF-κB to the nucleus, where transcription of NF-κB-dependent target genes occurs. In addition to the NF-κB pathway, NOD1 or NOD2 stimulation results in the activation of the MAP kinases p38, ERK, and JNK. In this context, we investigated the participation of NOD1 and NOD2 during the experimental infection by *L. amazonensis*. Thus, C57BL/6, NOD1^{-/-} and NOD2^{-/-} mice were infected with *L. amazonensis* promastigotes in the ear and development of skin lesions was evaluated weekly. At the 8th week, p.i., parasite load was also evaluated. Experiments *in vitro* were performed using BMMΦ from Nod1^{-/-}, Nod2^{-/-} and C57BL/6 mice infected with promastigotes of *L. amazonensis* expressing GFP, in order to evaluate parasite uptake, killing and costimulation and cell activation. Lesions were higher in NOD1^{-/-} and NOD2^{-/-} animals infected with *L. amazonensis* comparing with controls. Moreover, Nod1^{-/-} mice

displayed higher parasitism in the ear, lymph nodes and spleen than the WT littermates. Conversely, Nod2^{-/-} mice showed higher parasitism only in lymph node when compared with control group. BMM Φ from NOD1^{-/-} and NOD2^{-/-} mice presented lower leishmanicidal activity and lower expression of CD40, CD80, CD86, MHC-II and CD1d, than do macrophages from C57BL/6 mice. Our results suggest that NOD1 and NOD2 receptors contribute to the control of the infection by *L. amazonensis*.

Financial Support: CNPq, FAPESP, FMRP/USP

IM14 - CHARACTERIZATION OF EXPERIMENTAL MODEL FOR *LEISHMANIA (VIANNIA) SHAWI* INFECTION

Passero, L.F.D., Sacomori, J.V.; Bordon, M.L.A.C., Tomokane, T.Y., Corbett, C.E.P., Laurenti, M.D

São Paulo University, Medicine School, Laboratory of Pathology of Infectious Diseases.

Leishmania (Viannia) shawi was identified by Lainson and cols in 1989, and there are few studies concerning this parasite specie until now. The aim of this work was characterize the experimental infection caused by *L.(V.)shawi*. BALB/c and C57BL/6 mice were infected into hind footpad with 10⁶ parasite/animal. Weekly the footpad size were measured, and at 2th, 4th, 6th and 8th weeks pos infection (pi) the mice were bleeding and biopsies from skin and lymph nodes collected. The histopathological and humoral immune response changes were evaluated and quantitative analyses of the parasite loads were carried out using the Axion Vision 5.0 software. The BALB/c mice developed higher lesion size since 4th weeks pi. The BALB/c lesion size (2.40mm) was twice higher than C57BL/6 (1.18mm) at 8th week pi. BALB/c mice showed higher inflammatory infiltrate compared to C57BL/6 mice during all period of infection, and BALB/c mice presented focal area of necrosis since 6th week pi, while C57BL/6 presented light necrosis since 8th weeks pi. The parasite density of BALB/c and C57BL/6 mice increased with the time of infection and it was always bigger in BALB/c compared to C57BL/6. In lymph nodes the parasite was detected since 6th week pi in both mice strain and their densities showed no statistical significance. The humoral immune response was firstly detected in BALB/c mice since 4th week pi, increasing four times until 8th week pi, it was characterized by mainly IgG1

isotype, which is related to Th-2 response. In C57BL/6, the humoral immune response was detected since 6th week pi and there was a tendency of IgG2b isotype increase. The data showed that BALB/c mice had the classical behavior of susceptibility while C57BL/6 in spite of non-healing lesion showed lower lesion size, parasite burden, inflammation, and higher IgG2b levels. Studies concerning to cellular immunity are been developed.

Supported by FAPESP and LIM-50.

IM15 - IMMUNE EFFECTS OF ENALAPRIL DURING EXPERIMENTAL CHAGAS CARDIOMYOPATHY

COSTA, G.P.¹, SILVA, R.R.¹, FIGUEIREDO, V.¹, PEDROSA, M.C.¹, PINHO, V.², TEIXEIRA, M.M.², BAHIA, M.T.¹, and TALVANI, A.¹

¹Universidade Federal de Ouro Preto, Minas Gerais, Brazil; ²Universidade Federal de Minas Gerais, Minas Gerais, Brazil

Chagas heart disease (CHD), caused by *Trypanosoma cruzi* infection, is a significant cause of morbidity and mortality in South and Central America. Enalapril, an Angiotensin Converting Enzyme (ACE) inhibitor is an important drug used to ameliorate heart functional capacity and its remodeling in individuals presenting CHD. By interfering with the angiotensin II and bradykinin pathways, Enalapril reduces systemic arterial pressure, peripheral vascular resistance and increases cardiac output. ACE inhibitors appear to act as anti-inflammatory agents reducing inflammation and fibrosis and improving cardiac function. In this work, we are investigating the interference of Enalapril on immune response and on cardiac remodeling during experimental CHD. Our previously *in vivo* results indicate an elevation of circulating leukocytes and heart mast cells in C57BL/6 mice treated with Enalapril (25 mg/Kg), in association or not with infection with 50 trypomastigotes forms of *T. cruzi* (Colombiana strain). Enalapril treatment reduced drastically the peak of blood parasitism and avoided mortality among all animals during 60 days. Serum levels of IFN-gamma and CCL5/RANTES were lower in those infected animals treated with ACE inhibitor while IL-10 presented high serum levels only in those infected but not-treated mice. Interestingly, this reduced pattern of proinflammatory cytokines was associated with a low index of inflammatory infiltrate on heart tissue. Also, our data showed

that Enalapril enhances nitric oxide induced by tripomastigote forms of *T. cruzi*, in peritoneal macrophage cultures. Our preliminary data suggest that ACE inhibitor exert an important role on inflammatory response and on the exacerbation of events associated to experimental CHD. However, further studies are still necessary to clarify if this mechanism is based only on ACE inhibition or other secondary pathway. **Supported by CNPq, FAPEMIG and FIP-PUC MG.**

IM16 - Quantitative study of Peyer's patches during experimental infection with *Trypanosoma cruzi*

Jacob, V. V^{1**}, Caires, M.^{1*} Ferracioli, P.¹, Tatakihara, V.L.H.¹, Panis, C.¹, Araújo, P.M.F.², Rizzo, L.V.R.³, Pinge-Filho, P.¹

¹ Laboratório de Imunopatologia Experimental, Departamento de Patologia Geral, CCB, Universidade Estadual de Londrina (UEL). ² Departamento de Microbiologia e Imunologia, Universidade Estadual de Campinas (UNICAMP). ³ Departamento de Imunologia, Universidade de São Paulo (USP).

** Bolsista PIBIC/UEL, * Bolsista IC/UEL

The Peyer's patches (PP) are structural components of the gut-associated lymphoid tissues and provide structural organization for efficient cellular interactions and the initiation of immune responses against infection. It is well established that *Trypanosoma cruzi* infection elicits nitric oxide, eicosanoids and pro-inflammatory cytokines essentials in host defense. In the present work, attention was focused on the sequential quantitative changes caused by *T. cruzi* infection in PP and possible contribution of nitric oxide (NO) and eicosanoids in that process. Wild type C57BL/6, 129sv mice treated or not with NDGA (nordihydroguaiaretic acid, 5-lipoxygenase inhibitor) and knockout mice to 5-lipoxygenase (5-LO) were infected with Y strain of *T. cruzi*. Entire small intestines were removed from normal mice and infected and the number of PP was determined by macroscopic observation. PP were collected, fixed in formalin 10%, cut in 5 µm sections, mounted in glass slides, stained with haematoxylin-eosin and analyzed with bright-field microscopy. Our data indicated that *T. cruzi* infection provoked alterations in PP, as reduction in the number and disturbance in the architecture of germinal center. These alterations occurs during the acute phase of infection and were considered

to be critically dependent of the amount of parasites used in the infection and does not depend on NO and eicosanoids produced during *T. cruzi* infection in mice. Supported by Fundação Araucária and CNPq.

IM17 - SMALL INTESTINE HISTOPATHOLOGICAL EVALUATION OF BEAGLE DOGS DURING EXPERIMENTAL ACUTE CHAGAS DISEASE

FONSECA, K.S.¹, BITENCOURT, F.C.O.¹, NOGUEIRA, C.¹, VIEIRA, P.M.A.¹, BAHIA, M.T.², CHIARI, E.³, LANA, M.², CARNEIRO, C.M.¹, TAFURI, W.L.¹, VELOSO, V.M.²

¹ Laboratório de Imunopatologia, Universidade Federal de Ouro Preto, Ouro Preto, Brazil;

² Laboratório de Doença de Chagas, Universidade Federal de Ouro Preto, Ouro Preto, Brazil;

³ Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

The commitment of the digestive organs observed in patients with Chagas disease is mainly attributed to neuronal damage induced by immune and inflammatory processes elicited by the presence of *Trypanosoma cruzi*. Whereas megaesophagus and megacolon are the most notable and most extensively studied expressions of the digestive form of Chagas disease, involvement of the small intestine (Chagasic enteropathy) is less frequent and less studied than involvement of the two above mentioned entities. The histopathological evaluation, number of mast cells and the measurement of tissue parasitism were analyzed in histological sections stained by HE, Giemsa or submitted to immunohistochemistry, respectively. It was observed significant increase of the inflammatory process in animals infected with the Be-78 strain in both layers analyzed, muscular and submucous, when compared to all the others groups. Also, we observed a significantly increase of mast cells in Be-78 and ABC groups when compared to Control and Y groups. Morphometric analysis of tissue parasitism showed that animals infected with Be-78 strain had significantly greater parasitism when compared with all the other groups. There have been no significant differences in the morphometric analysis of the size of plexus and in the area of fibrosis. When comparing with the histopathological evaluation of the esophagus, obtained by our group, we observed that the animals infected with Be-78 strain presented more inflammation in the muscular layer and greater

tissue parasitism in the esophagus compared with the small intestine. However, the esophagus of these animals had lower numbers of mast cells in relation to the small intestine. These results suggested that esophagus is more affected than the small intestine in dogs infected with the Be-78 strain. We further observed that the numbers of inflammatory cells in the plexus region inversely correlate with the number of neurons.

Supported by FAPEMIG, CNPq and UFOP

IM18 - ESOPHAGUS HISTOPATHOLOGICAL EVALUATION OF BEAGLE DOGS DURING EXPERIMENTAL ACUTE CHAGAS DISEASE

NOGUEIRA, C.¹, CARVALHO, T. V.¹, FONSECA, K.S.¹, VIEIRA, P.M.A.¹, BAHIA, M.T.², CHIARI, E.³, LANA, M.², CARNEIRO, C.M.¹, TAFURI, W.L.¹, VELOSO, V.M.²

¹ Laboratório de Imunopatologia, Universidade Federal de Ouro Preto, Ouro Preto, Brazil;

² Laboratório de Doença de Chagas, Universidade Federal de Ouro Preto, Ouro Preto, Brazil;

³ Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Trypanosoma cruzi, the etiological agent of Chagas disease, presents a high degree of intraspecific genetic variability, with possible implications for the clinical forms of the disease, like the development of cardiopathy, megaesophagus, and megacolon, alone or in combination. The basic mechanism of megas is destruction of neurons of the enteric nervous system. The aim of this work was to investigate the impact of infection with different *T. cruzi* strains (Be-78, Y or ABC) on esophagus alterations during acute experimental infection in Beagle dogs. The histopathological evaluation, number of mast cells and tissue parasitism were analyzed in histological sections stained by HE, Giemsa or submitted to immunohistochemistry, respectively. It was observed significant increase of the inflammatory process in animals infected with the Be-78 strain in both layers analyzed, muscular and submucous, when compared to all the others groups. Also, the mast cell count in the animals infected with Be-78 strain presented a significantly increase in the number of this cells when compared to the Control group. Fifty percent of animals infected with Be-78 strain (eight nests) and twenty five percent of animals infected with Y strain (one nest) showed tissue parasitism. Although there have been no

significant difference in the morphometric analysis in the size of plexus, there was a tendency to animals infected with Be-78 strain showed greater inflammatory infiltrated in this region when compared to Control group. There have been no significant differences in the analysis of fibrosis area. These data strongly suggest that the Be-78 strain may induce greater inflammatory infiltrated in the esophagus from Beagle dogs, similarly in both layers analyzed, muscular and submucous, that may be related to higher parasitism observed in this group.

Supported by FAPEMIG, CNPq and UFOP

IM19 - CCR2 RECEPTOR IS ESSENTIAL TO ACTIVATE MICROBICIDAL MECHANISMS TO CONTROL *TOXOPLASMA GONDII* INFECTION IN THE CENTRAL NERVOUS SYSTEM

BENEVIDES, L.¹, MILANEZI, C.M.¹, YAMAUCHI, L.M.², BENJAMIM, C.F.³, SILVA, J.S.¹, SILVA, N.M.⁴

1- Departamento de Bioquímica e Imunologia da Faculdade de Medicina de Ribeirão Preto-USP

2- Departamento de Microbiologia da Universidade Estadual de Londrina - UEL

3- Departamento de Farmacologia Básica e Clínica da Universidade Federal do Rio de Janeiro-UFRJ

4- Departamento de Morfologia da Universidade Federal de Uberlândia - UFU

e-mail: lubenevides@usp.br

Chemokines are structurally related family of cytokines that regulate leukocyte trafficking. Because infection with *Toxoplasma gondii* induces an important inflammatory reaction if uncontrolled can lead to the death of the animals, we investigated the role of CCR2 in the progression of infection with *T. gondii*. To address this question, we infected CCR2^{-/-} mice with five ME-49 *T. gondii* cysts orally and the morbidity, survival and immune response were monitored. The CCR2^{-/-} mice displayed higher susceptibility to infection being all of mice died on day 28 post-infection. Although develop a Th1 cytokine profile such as C57BL/6, the CCR2^{-/-} presented an anti-inflammatory response more evident than WT mice in the peripheral organs. CCR2^{-/-} mice presented greater parasitism and a milder inflammatory reaction in the peripheral organs with a smaller CD4⁺ and MAC-1⁺ cell and a greater CD8⁺ cell migration. The parasite load decreased in these organs from CCR2^{-/-} mice but remained uncontrolled in the

Central Nervous System (CNS). Additionally, it was observed a down regulated iNOS expression in the organs from CCR2^{-/-} mice associated with a small nitric oxide production by spleen macrophages. In conclusion, in the absence of CCR2 another mechanism is activated to control tissue parasitism in the peripheral organs. On the other hand, CCR2 is essential to activate the microbicidal mediators to control *T. gondii* replication in the CNS.
Financial support: CAPES.

IM20 - Immune responses against resistant and susceptible to nitric oxide and antimony treatment *Leishmania braziliensis* isolates

Costa, D.L.¹, Carregaro, V.¹, Lima-Júnior, D.S.¹, Silva, N.M.², Milanezi, C.M.¹, Giudice, A.³, Carvalho, E.M.³, Almeida, R.P.⁴, Silva, J.S.¹

1- Department of Biochemistry and Immunology, Ribeirão Preto School of Medicine – University of São Paulo

2- Biomedical Sciences Institute – Federal University of Uberlândia

3- Immunology Service of the University Hospital Professor Edgar Santos – Federal University of Bahia.

4- Department of Internal Medicine and Pathology – Federal University of Sergipe
E-mail: dlcosta@usp.br

Leishmania braziliensis is the main causative agent of cutaneous leishmaniasis in Brazil. In the *L. major* mouse model of infection, protection is related to Th1 responses, resulting in nitric oxide production by macrophages and parasite elimination, while Th2 responses, IL-10 and TGF- β production favors parasite survival. The susceptibility mechanisms to *L. braziliensis* infection are not so clear, once different mouse lineages develop strong Th1 responses and control the infection. In order to understand susceptibility factors involved in human infections with resistant or susceptible to antimony and NO *L. braziliensis*, we developed an experimental model using BALB/c mice, which were challenged intradermally in the ear, with 1×10^6 promastigotes of LTCP393 (resistant) or LTCP15171 (susceptible) isolates of *L. braziliensis*. The ear lesion size, parasite burden, inflammatory infiltrate, and cytokine production were evaluated in different time points of infection. All animals healed the lesions spontaneously, however the infection severity was isolate-dependent, and reflected the human disease. Mice infected with

LTCP393 had larger lesions, which took longer time to heal and contained higher parasite burden. In both groups, the cellular infiltrate contained mainly CD4⁺T lymphocytes, neutrophils, macrophages and dendritic cells. No role for any single cell population could be related to the disease outcomes. Also, no difference was found in the IFN- γ production, and IL-10 did not seem to contribute to this aspect either. Interestingly, animals infected with LTCP393 isolate produced more IL-4, presenting production over twenty times higher in some time-points. In conclusion, we show that isolates of *L. braziliensis* may induce different disease patterns in mice, as in humans; that the induction of Th2 responses is related to the higher severity of the resistant isolate, and that this kind of immune response may be considered as a susceptibility factor in the murine infection with *L. braziliensis*.

Financial support: FAPESP.

IM21 - PROTEIN-ENERGY MALNUTRITION DECREASES IMMUNE RESPONSE TO LEISHMANIA CHAGASI INFECTION

MARIM, F. M.¹, MALAFAIA, G.¹, SILVA, M.E.², ARANTES, R.M.³, LIMA, W.G.⁴, PEDROSA, M.L.⁴, REZENDE, S.A.^{1,5}.

Laboratório de Imunoparasitologia/NUPEB¹, Dep. de Nutrição Experimental, ENUT², Dep. de Patologia Geral, UFMG³, Dep. Ciências Biológicas, ICEB⁴, Dep. de Análises Clínicas, Escola de Farmácia⁵, Universidade Federal de Ouro Preto, Ouro Preto.

Protein-energy malnutrition (PEM) and visceral leishmaniasis (VL) are important problems of public health affecting millions of people worldwide. PEM is responsible, directly or indirectly, for 54% of the 10.8 million deaths per year in children under five. VL is the most devastating type of leishmaniasis and human infection with *Leishmania (Leishmania) chagasi/infantum* can range from sub-clinical infection to progressive fatal disease. In our murine model, we evaluated the effect of PEM in the immune response induced by infection with *L. (L.) chagasi*. Mice were divided into two groups receiving either the control (14% casein) or the low protein (3% casein) diet and water *ad libitum*. These diets had sufficient iron and zinc. Total body weight was analyzed weekly. After malnutrition was established, mice were inoculated intravenously with promastigotes of *L. (L.) chagasi*. Four or six weeks later, mice were

sacrificed and liver and spleen parasite load was evaluated. Our data show that malnourished mice presented a significant reduction in the following parameters: body weight and total protein, albumin, glucose and globulin serum concentration. Six weeks post infection, the parasite load in liver was increased in infected malnourished group comparatively to infected control group. In the spleen, an increased parasite load in malnourished group was detected four and six weeks post infection comparatively to control group. Our data demonstrate a positive correlation between nutritional status and immune response to infection with *L. (L.) chagasi* indicating that PEM alters immune response of BALB/c mice to *L. (L.) chagasi*.

This research was sponsored by: PIP/UFOP, Rede Mineira de Bioterismo, PPM/FAPEMIG.

IM22 - CYTOKINE PROFILE IN LYMPH NODES ASSOCIATE WITH PARASITE LOAD AND CLINICAL FORM OF DISEASE IN CANINE VISCERAL LEISHMANIASIS

*Alves C.F.*¹, *De Amorim I.F.G.*¹, *Moura E.P.*¹, *Ribeiro R.R.*¹, *Alves C.F.*², *Michalick M.S.*¹, *Bruna-Romero O.*¹, *Tafuri W.L.*¹, *Teixeira M.M.*¹, *Melo M.N.*¹.

¹Universidade Federal de Minas Gerais, Minas Gerais, Brazil, ²Laboratório Tafuri de Patologia, Minas Gerais, Brazil.

American visceral leishmaniasis is a zoonosis of the New World. Dog's role as the main reservoir of the disease has led to an increasing interest in the mechanisms implied in protective cell immunity against canine infection. Nevertheless, the factors that account for the progression of canine visceral leishmaniasis have not been well established, since most immune studies do not reflect cell responses to organs that are targets of infection. This work is the first report that determines the profile of cytokines and quantifies parasitic burdens in the lymph nodes, important target organs in visceral leishmaniasis, in dogs naturally infected with *Leishmania chagasi*, as determined by real-time PCR. With this purpose, 18 mongrel dogs were divided in three groups: control non-infected dogs (n=6) and dogs naturally infected with *L. chagasi*, asymptomatic (n=6) and symptomatic (n=6). In order to better define these groups, the presence of clinical signs compatible with the disease was assessed systematically. Infection was confirmed by serological (IIF and ELISA) and

parasitological tests (direct examination and cultivation of material from bone marrow aspirates). Results show that prescapular lymph nodes present the highest expression of type Th1 cytokines (IFN- γ and TNF- α) in asymptomatic dogs and with low parasite burdens, indicating that these cytokines play a role in protection against infection. Highest expression of regulatory cytokines (IL-10 and TGF- β) was observed in symptomatic dogs and with high parasite burdens, suggesting a role in the progression of this disease. In this study, we have identified that anti-inflammatory cytokines, produced by lymph nodes of naturally-infected dogs, play a role in the progression of the disease and also in the parasite proliferation. Blocking these cytokines in infected dogs, may represent an important adjuvant treatment to control the canine disease. This work was supported by grants from CNPq and UFMG.

IM23 - ANALYSIS OF CYTOKINE GENE EXPRESSION AND PARASITE LOAD IN SKIN OF EAR OF DOGS NATURALLY INFECTED WITH LEISHMANIA CHAGASI

*Alves C.F.*¹, *De Amorim I.F.G.*¹, *Moura E.P.*¹, *Ribeiro R.R.*¹, *Alves C.F.*², *Michalick M.S.*¹, *Bruna-Romero O.*¹, *Tafuri W.L.*¹, *Teixeira M.M.*¹, *Melo M.N.*¹.

¹Universidade Federal de Minas Gerais, Minas Gerais, Brazil, ²Laboratório Tafuri de Patologia, Minas Gerais, Brazil.

The leishmaniasis includes a spectrum of diseases due to great variability and biological complexity of the parasite, hosts and vectors. The American visceral leishmaniasis is considered the most severe form of the disease. It is a zoonosis in the New World with the dog considered the main reservoir of the parasite. However, studies on the immunology of canine visceral leishmaniasis are scarce and the knowledge about immune aspects in dogs infected with *L. chagasi* is essential for interventions in the mechanism to eliminate the parasite. The main objectives of this work went to determine the cytokines profile and the parasite load in the skin of ear of dogs naturally infected by *L. chagasi*. Eighteen dogs were subdivided in no infected dogs (n =6) and dogs naturally infected by *L. chagasi*, asymptomatic (AD, n =6) and symptomatic (SD, n =6). Fragments of the skin of ear were collected for the immunohistochemical studies, and for the determination of cytokines profile (IFN- γ , IL-12, IL-4, IL-10, TGF- β and TNF- α)

and quantification of parasite load, both using real time PCR. The quantification of the parasite showed the presence of the parasite in all dogs (AD and SD) both by immunohistochemistry and real-time PCR, and the average of the values found in the group SD were significantly higher than the AD. The levels of expression of IFN- γ , IL-4, IL-10, TGF- β , and TNF- α were significantly higher in the group SD on the other. A balance in the Th1 and regulatory cytokines production was observed in the skin of ear in the SD animals, revealing that the mechanisms involved in the immunology of canine visceral leishmaniasis are more complex than those observed in murine model and different of that observed in the human disease. This work was supported by grants from CNPq and UFMG.

IM24 - CYTOKINES PROFILE IN DOGS NATURALLY INFECTED WITH *L. (L.) chagasi*

Laurenti, M.D.; Matta, V.L.R.; Pernichelli, T.; Tomokane, T.Y.; Corbett, C.E.P.

Laboratory of Pathology of Infectious Disease, Medical School of São Paulo University, São Paulo (SP), Brazil.

Introduction and Aim: We have been studied aspects of the humoral and cellular immunity in dogs naturally infected with *L.(L.)chagasi* correlating to the clinical forms and tissue parasitism. Previous results showed that the parasitism but no clinical signs are an important factor to determine the cellular immunity. In order to better study the cellular immune response, cytokines profile was evaluated in the sera of symptomatic and asymptomatic dogs naturally infected with *L.(L.)chagasi*.

Material and Methods: Animals referred to the Center of Zoonosis Control of Araçatuba city (SP), Brazil, were submitted to euthanasia and biopsies of skin and viscera were collected for parasitological diagnosis by immunohistochemistry, as well as sera for TNF- α , IFN- γ , TGF- β and IL-10 determination by Capture-ELISA (R&D Systems, USA). According to the clinical signs, the dogs were classified as symptomatic (n=57) and asymptomatic (n=25). Sera of dogs from non-endemic area were used as control (n=28).

Results: Positive parasitological diagnosis was confirmed in all animals of symptomatic and asymptomatic group. Compared to the control, animals from non-endemic area, increase on TGF- β and decrease on TNF- α levels were observed in

the sera of dogs from endemic area ($p<0.05$) without difference between the clinical groups. Increase on IFN- γ level was observed in symptomatic dogs ($p<0.05$). IL-10 did not show difference between the groups.

Conclusion: The results showed the Th1 and Th2 cytokines production on *L.(L.)chagasi* infection in dogs independent to the clinical groups. The balance of these cytokines production, probably, is responsible for the parasite spread leading to the susceptibility.

Supported by FAPESP and LIM50 HC-FMUSP.

IM25 - Dendritic Langerhans cells profiles in American Cutaneous Leishmaniasis due to *Leishmania (Viannia) braziliensis* and *L. (Leishmania) amazonensis*

Rodrigues, F.M.D.¹, Marthos, L.¹; Fernezlian, S.M.¹, Corbett, C.E.P.¹, Silveira, F.T.^{1,2}, Gomes, C.M.C.¹

1Laboratório de Patologia de Moléstias Infecciosas (LIM-50), Departamento de Patologia, Faculdade de Medicina da USP, 2 Instituto Evandro Chagas-Pará

e-mail: ferodrigues@hotmail.com

In Brazil, despite many species of *Leishmania* may cause American cutaneous leishmaniasis (ACL), *Leishmania (V.) braziliensis* and *Leishmania (L.) amazonensis* are the principal pathogenic species to humans.

Considering the functional role of dendritic Langerhans cells (LCs) known as crucial antigen-presenting cells (APC) in stimulating specific T-cell response in *Leishmania* infection, the present study aims to determine the density of dendritic Langerhans cells in the lesions of patients with different clinical forms of ACL caused by *L. (V.) braziliensis* and *L. (L.) amazonensis*.

Paraffin-embedded biopsies of 25 patients with different clinical forms of ACL caused by *L. (V.) braziliensis* (10 patients) and *L. (L.) amazonensis* (15 patients) were submitted to immunohistochemistry using monoclonal antibodies for CD1a (clone 010¹/DAKO). The immunolabelled cells were counted with the help of image analysis system (Zeiss). Cell densities were calculated and the mean for each cell population across patients was derived.

The density of CD1a+ cells was higher in patients with anergic diffuse cutaneous leishmaniasis (ADCL) and borderline disseminated cutaneous leishmaniasis (BDCL) by *L.(L.) amazonensis*,

769,6 and 622,6 cells/mm², respectively, when compared with the groups of localized cutaneous leishmaniasis (LCL) patients by *L. (L.) amazonensis* (369,5 cells/mm²) and *L. (V.) braziliensis* (346,8 cells/mm²). These findings suggest the conclusion that LCs might be contributing to the cellular immune suppression found in the ADCL and BDCL forms caused by *L. (L.) amazonensis*.

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IM26 - QUANTIFICATION OF TGF- β mRNA EXPRESSION IN THE GLOMERULONEPHRITIS IN EXPERIMENTAL VISCERAL LEISHMANIASIS BY REAL-TIME PCR

MIKULSKI-ALI, T.^{1,2}; RAMOS-SANCHEZ, E. M.²;
CASTRO L.R.¹; GOTO, H.^{1,3}; PRIANTI, M.G.¹;

¹Instituto de Medicina Tropical de São Paulo,

²Instituto de Ciências Biomédicas, ³Dep. Patologia and Dep. Med. Preventiva da Faculdade de Medicina, da Universidade de São Paulo, São Paulo, Brazil - e-mail: gprianti@usp.br

Introduction: In visceral leishmaniasis (VL), renal involvement is very frequent but the pathogenesis is unclear yet. The glomerular changes are mainly of proliferative type and infiltrate of inflammatory cells is present, consisting by mononuclear cells. (Costa et al. Braz J Med Biol Res. 33:1455, 2000; Mathias et al., Braz J Med Biol Res. 34(4):539-43, 2001; Prianti et al., Braz J Med Biol Res. 40(6):819-23, 2007). It is known that cytokines are important mediators of the immune system, they are involved in renal disease and that TGF- β plays an important role in the pathogenesis of glomerulonephritis (GN). **Aim:** the present work aims to quantify and detect the participation of TGF- β cytokine, in pathogenesis of GN in VL, using *Leishmania (L.) chagasi*-infected mice in different periods of time by Real-Time PCR method. **Methods:** BALB/c mice were infected through intraperitoneal route with 2x10⁷ purified *Leishmania (L.) chagasi* (MHOM/BR/72/strain 46) amastigotes. We evaluated by morphometry the expression of CD11⁺ and F4/80⁺ cells by immunohistochemistry and analyzed the mRNA expression of TGF- β cytokine in renal cells of these animals by Real-Time PCR method. The analysis was in different time periods. **Results:** We observed in infected animals CD11⁺ and F4/80⁺ cells in greater amount from 7 days PI, reaching a peak at 15 days PI. The analysis showed that the presence of TGF- β was significant

in 15 days post infection and that occurred higher expression of TGF- β cytokine in renal cells in *Leishmania (L.) chagasi*-infected mice at 15 days of infection. **Conclusion:** The data suggest important participation of CD11⁺ cell and F4/80⁺ macrophage infiltration in glomerulonephritis in murine visceral leishmaniasis, and the TGF- β contributes for this process.

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IM27 - DECREASE OF PARASITE BURDEN INDUCED BY NEUTROPHILS IN *LEISHMANIA (L.) AMAZONENSIS*-INFECTED MACROPHAGES

CARMO, E.V.S.¹; KATZ, S.¹; BARBIÉRI, C.L.¹
¹Universidade Federal de São Paulo, São Paulo, Brazil.

Previous results from our laboratory showed the resistance of C3H/HePas mice to *L. (L.) amazonensis* and a predominance of neutrophils in the site of infection, suggesting that these cells play an important role in the resistance of this mouse strain to *L. (L.) amazonensis*. In contrast, BALB/c mice infected with *L. (L.) amazonensis* display an infiltrate of macrophages harboring a large number of amastigotes and very few neutrophils in foot lesions. The present work focuses on the possible role of neutrophils in leishmanicidal activity on macrophages infected with *Leishmania (L.) amazonensis*. Peritoneal macrophages cocultured with inflammatory neutrophils isolated from BALB/c and C3H/HePas mice are able to kill *L. (L.) amazonensis* amastigotes, and no significant difference in the leishmanicidal activity was observed between the two mouse strains. ELISA assays in the supernatants from these cocultures showed that the leishmanicidal activity did not depend on IFN- γ or nitric oxide, but it was reverted when a monoclonal anti-TNF- α was added to the cocultures. It was also shown that aspirin could partially inhibit the leishmanicidal effect, and a significant kalikrein-like activity was also detected in the supernatants of these cocultures. Taken together, these results led us to propose the involvement of TNF- α , as well as a serino proteinase, and a cyclooxygenase product in the leishmanicidal effect induced by neutrophils on *L. (L.) amazonensis*-infected macrophages, and provide possible mechanisms for the resistance of

C3H/HePas mouse to *L. (L.) amazonensis* infection.

Supported by FAPESP.

IM28 - IMMUNOLOGICAL FUNCTION OF ALPHA/BETA CD4⁺CD8⁻, DOUBLE NEGATIVE (DN) T CELLS AND SEMI-INVARIANT NKT CELLS FROM CUTANEOUS LEISHMANIASIS PATIENTS

KEESEN, T.S.L.¹, VIEIRA, E.L.M.¹, ANTONELLI L.R.V.⁴, GUIMARÃES, L.H.³, BACELLAR, O.³, CARVALHO E.M.³, DUTRA, W.O.², GOLLOB, K.J.¹

¹ Department of Biochemistry and Immunology, and ² Department of Morphology, Institute of Biological Sciences-Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, and ³ Immunology Service, Hospital Edgar dos Santos, UFBA-Salvador, and ⁴ National Institutes of Health (NIH), USA.

Leishmaniasis is a disease caused by different species of *Leishmania* and affects millions of individuals worldwide. Whereas the acquired T cell mediated protection against intracellular pathogens such *Leishmania* has been well studied, the cells and mechanisms involved in their innate control are still poorly understood. Our group has examined T cell responses in human CL caused by *L. braziliensis* and has determined that alpha/beta CD4⁺CD8⁻, double negative (DN) T cells, are the second most prevalent cellular source of Th1 type cytokine producing cells. Our goal is to study this unique, alpha/beta DN T cell subpopulation and its important role in the development of protective and/or pathogenic immune responses in human CL disease. Within this sub-population we studied semi-invariant double negative natural killer T (NKT) cells which constitute a distinct lymphocyte lineage at the interface between innate and adaptive immunity. Whilst NKT cells share features with other conventional T lymphocytes, they are unique in their rapid, concomitant production of T helper type 1 (Th1) and Th2 cytokines upon T-cell receptor (TCR) ligation. Recent studies have identified multiple ways in which NKT cells can become activated during microbial infection. To clarify the role of these heterogeneous populations in the immune response against *Leishmania*, a detailed study characterizing the subsets bearing alpha-beta T cell receptors was performed. In this work we demonstrated that within the alpha-beta DN T cells, the semi-invariant NKT cells from

cutaneous leishmaniasis patients express a profile compatible with previous antigen exposure and recent activation with a pro-inflammatory cytokine profile, suggesting that this T cell subpopulation contributes importantly to the immunoregulatory environment in human CL. Our data provide new insights into the functional competence of NKT cells and DN T cells which will lead to a better understanding of their protective or pathogenic role during immune responses against *L. braziliensis*.

Financial support from UNICEF/UNDP/WHO-TDR, NIH-TMRC, FINEP CT-Infra, and CNPq for fellowships.

IM29 - IMMUNOPHENOTYPICAL CHARACTERIZATION OF PERIPHERAL BLOOD DERIVED-MONOCYTES IN CANINE MODEL: A PRELIMINARY STUDY

MUDADO, A.M.¹; MOURA, E.P.¹; AMORIM, I.F.G.¹; BIASIZZO, R.L.¹; FIGUEIREDO, M.M.¹; MELO, F.A.¹; SILVA, L.C.¹; MICHALICK, M.S.M.¹; TAFURI, W.L.¹

¹ Universidade Federal de Minas Gerais, Belo Horizonte-MG, BRAZIL.

Monocytes act as antigen presenting cells and therefore, are capable of expressing different surface molecules such CD11b, CD11c and Toll-like receptors (TLR's). All play an important role in the host's immune response. The CD11b and CD11c were integrins which plays an important role for the adhesion of leukocytes. Under normal circumstances, the integrins are nonadhesive, and become adhesive for their cell surface ligands, the intercellular adhesion molecules (ICAMs), or soluble ligands such as fibrinogen and iC3b, when leukocytes are activated. The differential involvement of CD11c and CD11b in adhesion and subsequent cytoskeleton changes in monocytes exposed to different conditions indicates the importance of each integrin in distinct responses during inflammation. Membrane-bound Toll-like receptors (TLRs) are frontline guardians in the mammalian innate immune system. They primarily function to recognize pathogen-associated molecular patterns (PAMPs) of invading microorganisms and on activation mount rapid, nonspecific innate responses and trigger sequential delayed specific adaptive cellular responses, which are mediated by complex signal transduction pathways involving adaptor molecules, costimulatory ligands and receptors,

kinases, transcription factors, and modulated gene expression. Toll-like receptors (TLRs) play a crucial role in the recognition of invading pathogens and the activation of subsequent immune responses against them. The objective of this study was to examine peripheral monocytes isolated from dogs and to characterize CD14, CD11b, CD11c, TLR-2 and TLR-4 expression. Peripheral blood (20 ml) was collected of seven dogs. Density gradient separation (Histopaque 1070 - Sigma) was used to enrich for peripheral blood lymphocytes from canine blood. CD11b, CD11c, TLR-2 and TLR-4 were quantified by flow cytometric analysis. Evidence is now accumulating showing that the most of CD14⁺ canine cells to express CD11b, CD11c, RT-2 and RT-4 surface molecules. A study concerning recognition of the role of these surface molecules in the host-parasite relationship would be an interesting challenge for future study.

Financial Support: FAPEMIG, CNPq, UFMG

IM30 - EFFECT OF THE NITRIC OXIDE DONOR, S-NITROSOGLUTATHIONE (GSNO) ON *LEISHMANIA MAJOR* INFECTION

COSTA, I.F.S.¹, SOUZA, G.F.², de OLIVEIRA, M.G.², ABRAHAMSOHN, I.A.¹

¹ Departamento de Imunologia, Instituto de Ciências Biomédicas da Universidade de São Paulo, USP, São Paulo, Brazil; ² Departamento de Físico-Química, Instituto de Química, Universidade Estadual de Campinas, UNICAMP, Campinas, SP, Brazil

Protozoa of the genus *Leishmania*, the etiological agents of leishmaniasis, reside and multiply inside macrophages; intracellular parasites are killed mainly by reactive oxygen (ROI) or nitrogen intermediates (RNI) stimulated by the innate and adaptive immunity. Among RNI, nitric oxide (NO) is a potent effector molecule. Several molecules known as NO donors are currently being tested for cytotoxicity against *Leishmania*. Among these, the compounds known as S-nitrosothiols are relatively more stable. It has been shown recently that S-nitroso-glutathione (GSNO) is toxic to *Leishmania* cultivated in axenic cultures (de Souza et al. Nitric Oxide. 2006.15: 209). In this project we investigate the effect of GSNO treatment on *Leishmania major*-infected macrophages and on the lesion and parasite load in Balb/c mice presenting a single ulcerated lesion at the site of infection performed in the dorsum 6 to 8 weeks before. The mice were

topically treated with GSNO in PBS or incorporated in the Gel F127 developed by one of us (de Oliveira, M.G.); control groups received treatments with Gel F127 or PBS, amphotericin B or were left untreated. The lesion size and the number of parasites in the draining LN and in the lesion were quantified. Preliminary results show that daily application of GSNO + PBS for a period of 30 days to the ulcer led to its reduction in size and eventual healing. Gel F127-treated mice also had some reduction of ulcer size. Decreased parasite load in the lesion and in the draining LN were seen in GSNO + Gel F127-treated mice in comparison with Gel F127-treated mice. The *in vitro* studies showed reduction in the number of intracellular amastigotes in GSNO-treated THP-1 macrophages. Additional experiments are being carried out in mice, in THP-1 cells and in human monocyte-derived macrophages in order to identify the mechanisms involved.

Supported by CNPq. Inez S.F. Costa receives a FAPESP MSc fellowship.

IM31 - THE ROLE OF SUPEROXIDE IN INFECTION WITH *LEISHMANIA AMAZONENSIS*

Macedo, J.P.¹, Gonzalez, C.Z.L.¹, Radi, R.², Vieira, L.Q.¹

¹ Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Brazil
² Facultad de Medicina, Universidad de La Republica, Uruguay

The production of superoxide is an essential process in microorganisms killing by mammalian host cells; however few works addressed the role of this molecule in cutaneous leishmaniasis. In the other hand, nitric oxide is known as a key radical for the resistance against *Leishmania* ssp. Taking such information into account we decided to evaluate the role of superoxide in C57BL/6 animals infected with *Leishmania amazonensis*, using both NADPH oxidase knockout mice (phox^{-/-}) and inducible nitric oxide synthase (iNOS) knockout mice. We found that iNOS^{-/-} animals developed the largest lesion and highest parasite burden when compared to wild type (wt) and phox^{-/-}. Both wt and phox^{-/-} displayed similar parasite burden at 10 weeks post-infection in the lesion site and in the draining lymph node although phox^{-/-} showed a slightly faster progressive lesion reaching the peak at week seven while wt partners reached the peak at 9 weeks of infection. No difference between phox^{-/-} and wt was found in the production of IFN-

g at lesion site and draining lymph node, neither in the presence of serum nitrite. Histopathological analysis showed a more disorganized tissue with many inflammatory cells in *phox-/-*. More importante, when *phox-/-* mice were treated with aminoguanidine the growth of lesion and parasite burden increased significantly and reached higher levels when compared to treated wt as controls, demonstrating that superoxide, in absence of NO, acts as a critical mechanism in host resistance to *L. amazonensis* infection.

Support: CNPq, Capes and FAPEMIG

Keywords: *Leishmania amazonensis*, superoxide, NADPH oxidase, iNOS, nitric oxide.

IM32 - Role of 5-LO, COX-2 and cNOS on NO production in control of *Trypanosoma cruzi* infection.

Panis, C.¹, Cechini, R.¹, Victorino, V. J.; Tatakihara, V.L.H.¹, Borges C.², Martins-Pinge, MC.³, Rizzo, LV.⁴, Pinge-Filho, P.¹

¹ Laboratório de Imunopatologia Experimental, Departamento de Patologia Geral, CCB, Universidade Estadual de Londrina (UEL). ² Departamento de Análises Clínicas, Universidade Estadual de Ponta Grossa (UEPG). ³ Departamento de Ciências Fisiológicas, CCB, Universidade Estadual de Londrina (UEL). ⁴ Departamento de Imunologia, Universidade de São Paulo (USP).

Trypanosoma cruzi, the causative agent of Chagas' disease, is known to be susceptible to nitric oxide (NO)-dependent killing by gamma interferon and leukotrienes-activated macrophages. Mice deficient for inducible nitric oxide synthase (iNOS) are highly susceptible to *T. cruzi*, and inhibition of iNOS from the beginning of infection was reported to lead to an increase in trypomastigotes in the blood and to high mortality. In the present study, we investigated whether 5-lipoxygenase (5-LO) and cicloxygenase-2 (COX-2) metabolites are essential for NO production during acute infection with *T. cruzi*. Wild type C57BL/6, 129sv mice and knockout mice to 5-lipoxygenase (5-LO^{-/-}) were treated at different time intervals after *T. cruzi* infection with an iNOS inhibitor, aminoguanidine, cNOS inhibitor, L-Name or celecoxib, COX-2 inhibitor. Treatment initiated after 4 h of the infection resulted in 100% mortality by day 23 postinfection (p.i.). All the normal mice treated survived. Parasitic load in the blood peaked 9 days post-infection was significantly

higher in 5-LO^{-/-} compared to C57BL/6 and WT 129sv mice and declined progressively thereafter. There were large and statistically significant differences ($P < 0.001$) in survival between all two strains of mice studied. We observed that C57BL/6, WT 129 sv infected mice (all on 12th day of infection) treated with NDGA or celecoxib significantly inhibited the production of NO in the plasma. These data suggest that 5-LO as well as COX-2-metabolites activate NO release and is essential for *T. cruzi* control in the early phase of acute infection. Interestingly, our data suggest an important role of cNOS in NO synthesis in 5-LO^{-/-} mice infected with *T. cruzi*. Supported by Fundação Araucária (Edital Universal, Processo 8427).

IM33 - Absence of CD43 modulates immune response and compromises T cells homing to hearth during *Trypanosoma cruzi* infection

Alisson-Silva, F.¹; Freire de Lima, L.¹; Carvalho, ST.¹; Takiya, CM.²; Previato, JO.¹; Todeschini AR.¹ and Mendonça-Previato, L.¹.

- 1- Laboratório de Glicobiologia, Instituto de Biofísica Carlos Chagas Filho UFRJ
- 2- Laboratório de Patologia Celular, Instituto de Ciências Biomédicas UFRJ

One characteristic of infection by *Trypanosoma cruzi* is the pronounced inflammatory process in the myocardium at the early stage of infection. Thus, elucidation of molecules involved in the recruitment of leukocytes to hearth of infected hosts is an important step to elaborate new strategies for therapeutic intervention in the chagasic cardiopathy. CD43 leukosialin is a large sialoglycoprotein abundantly expressed on CD4 and CD8 T cell surface and is involved in CD4 T cell activation during *T. cruzi* infection. Several studies have demonstrated that CD43 has a positive role in T cell homing to peripheral tissues. In this work we investigated the involvement of CD43 on immune response triggered by *T. cruzi* infection. C57Bl6 and CD43 knockout (CD43KO) mice were infected intraperitoneally with trypomastigotes (10⁴) of Y strain, parasitemia were evaluated at days 6 to 10 post infection (pi) and spleen, heart and blood were harvested at day 15 pi. Splenic T lymphocytes of CD43KO mice displayed a Th2 profile, with high percent of IL-4 producing CD4 cells and high amounts of IL-4 in supernatants, while no variation was observed in IFN- γ levels. Despite a Th2 biased immune

response, CD43KO mice showed no difference in number of blood parasites and parasite load on the cardiac tissue when compared to WT, most likely by an enhanced cytotoxic activity of antigen specific CD8 T cells of CD43KO mice compared with their wild-type littermates. However, histopathology analysis showed a decreased inflammatory infiltrate in heart from CD43KO mice, corroborating with a reduced creatine kinase (CK) activity in serum of KO mice. These results demonstrate that CD43 plays an important role in T cell homing to heart of *T. cruzi* infected mice representing an interesting target to control local inflammatory damage triggered by *T. cruzi*.

Supported by: CAPES, CNPq, FAPERJ

IM34 - DOUBLE-NEGATIVE T CELLS FROM CARDIAC PATIENTS DISPLAY AN ACTIVATED PROFILE AND ARE A MAJOR SOURCE OF IFN-GAMMA

Villani, F.N.A.¹; Santos, J.S.C.¹; Antonelli, L.R.²;
Rocha, M.O.C.³; Gollob, K.J.⁴; Dutra, W.O.¹

¹Department of Morphology, ICB, ²FIOCRUZ-MG,
³School of Medicine, ⁴Department of Biochemistry-
Immunology, ICB, Federal University of Minas
Gerais.

waldutra@gmail.com

Keywords: DN T cells, Chagas disease, cytokines

Chagas disease is caused by the protozoan *Trypanosoma cruzi* and the cardiomyopathy associated with this disease is the main cause of its high morbidity. The lifelong nature of human Chagas disease suggests the concomitant occurrence of inflammatory and anti-inflammatory responses in patients. We have previously shown that CD4-CD8- DN T cells expressing alpha-beta ($\alpha\beta$ TCR) or gamma-delta T-cell receptors ($\gamma\delta$ TCR) display inflammatory or anti-inflammatory profiles, respectively, in patients with cutaneous leishmaniasis. In this work, we analyzed the expression of IL-10, IFN- γ , TNF- α and of the co-stimulatory molecules, CD28 and CTLA-4, by $\alpha\beta$ or $\gamma\delta$ DN T cells freshly isolated from cardiac chagasic patients and non-chagasic individuals. We found a higher frequency of $\alpha\beta$ and $\gamma\delta$ DN T cells expressing TNF- α or IL-10 in cardiac patients, as compared to non-infected individuals, suggesting *in vivo* activation. IFN- γ expression was higher in $\alpha\beta$, but not $\gamma\delta$, DN T cells from cardiac patients, as compared to controls. Interestingly, DN

T cells contribute to 55% of the overall IFN-gamma expression by cells from cardiac patients, as compared to 16% in controls. While CTLA-4 expression was higher in $\alpha\beta$, but not $\gamma\delta$, DN T cells from cardiac patients, as compared to controls, CD28 expression did not differ among groups. However, within the cardiac group, we observed higher frequencies of $\gamma\delta$ +CD28- than $\alpha\beta$ +CD28-cells. These results show that DN T cells from cardiac patients are highly activated *in vivo*, accounting for a major source of IFN-gamma, a cytokine shown to be important in the development of cardiac injury.

Financial support: CAPES, CNPq, TDR/WHO

IM35 - NON-ADHERENT PHENOTYPE OF INFECTED RED BLOOD CELL IMPAIRS PARASITE CLEARANCE IN ACUTE INFECTION OF C57BL/6 MICE BY *Plasmodium chabaudi*.

RODRIGUEZ-MÁLAGA S.¹, MUXEL S.¹,
FREITAS A.P.¹, BORGES H.¹, BORRELI P.¹,
D'IMPERIO LIMA M. R.¹

¹ Universidade de São Paulo, São Paulo, Brasil

In the erythrocytic phase of malaria infection, red blood cells parasited by some species of *Plasmodium sp.* have the property of adherer to endothelial cells, phenomenon known as sequestration, and related with pathogenecity in *P. falciparum* infection. In murine model was demonstrated that erythrocytes infected by *P. chabaudi* have the capacity of adhere to CD36 receptor preferentially in the liver vasculature, and that this interaction promote the uptake of parasited cells by macrophages. In this study we used two strains of *P. chabaudi* with different lethality levels, AS strain, a non lethal parasite in C57BL/6 mice, and AJ strain, highly lethal in these animals. We have demonstrated that in an acute infection with both strains, there is an increase in the number of dendritic cells, lymphocytes T and B, displaying a similar activation phenotype. *In vitro* assays confirmed that dendritic cells stimulated with equal number of AS or AJ infected erythrocytes, produced similar amounts of cytokines. As well, parasites of both strains promote similar lymphocyte proliferation and cytokine production. The differences found in acute infections were related with three aspects, i) decrease of adhesion of mature form of AJ strain to hepatic vasculature during schizont formation and re-invasion period; ii) decrease of

phagocytosis of erythrocytes infected with AJ strain by macrophages during *in vitro* assays; iii) a significant decrease of erythrocytes precursors in spleen of mice infected with AJ strain. These results suggest that in an acute infection by non lethal AS strain, the adherent phenotype promote the uptake of infected erythrocytes by macrophages allowing the control of the infection. On the other hand, in an infection by AJ strain, the non adherent phenotype decrease the phagocytosis of parasited cells, avoiding its control; this fact promote a severe anemia that can't be counteracted by the production of new erythroid cells.

FAPESP, CNPq

IM37 - ORAL TOXOPLASMA GONDII INFECTION OUTCOMES IN SEVERE GUT INFLAMMATION IN GENETICALLY SELECTED MICE FOR EXTREME ORAL TOLERANCE PHENOTYPES

DIAS, R.R.F.¹; LEITE, C.C.S.¹; DA SILVA, A.C.²; DAMATTA, R.A.¹; DA SILVA, M.F.S.¹

¹Universidade Estadual do Norte Fluminense; Campos dos Goytacazes; RJ; Brasil,

²Universidade do Estado do Rio de Janeiro; Rio de Janeiro; RJ; Brasil

Introducion: The induction of oral tolerance is a key feature of the intestinal mucosal immunity, which generates a systemic non-responsiveness to ingested antigens. Two strains of mice were obtained by bidirectional genetic selection for extreme phenotypes of oral tolerance, one strain resistant (TR), and the other susceptible (TS) to induction of tolerance. The TR strain shows stronger inflammatory response to different challenges than the TS. The *T. gondii* natural route of infection is the ingestion of cysts. **Objectives:** The objective of this work was the characterization of the oral infection with *T. gondii* in the TS and TR mice strains concentrating on the immunopathology of the infection. **Methods:** Cysts of ME 49 strain of *T. gondii* were inoculated by gavage in TR and TS mice. Organs were harvested in five different time points after infection for histopathological analysis and parasite load evaluation. **Results:** During the acute stage of infection, the mortality was higher for TR mice. Histopathological analysis shows more severe infection in the TR mice, with intestinal erosion, hepatic dystrophy and uveitis-like patterns, but the

parasite load was lower than in the TS. The mortality of TS mice was higher in the chronic stage, and their brain presented gliosis and perivascular clogging. A higher parasite number was observed in the TS macrophages cultured *in vitro*, corroborating with the *in vivo* results of parasite load evaluation. In the TS mice brains, during the initial stages of the chronic stage, was observed a higher number of cysts, and the size of this cysts was also bigger, comparing with the TR strain. **Conclusions:** The TR and TS strains are promising new models to investigate the features of the toxoplasmosis in both acute and chronic stages.

Keywords: oral infection, oral tolerance, inflammation, *Toxoplasma gondii*, mice

Support: CNPq, CAPES, FAPERJ

IM38 - Toxoplasma gondii infection in dendritic cells and macrophages bone- marrow and spleen-derived from mice with extreme phenotypes of Oral Tolerance

Leite, C.C.S., daMatta, R.A., Kipnis T., da Silva, A.C., da Silva, M.F.S.

The intestinal mucosal immunity, physiologically, operates both responding to pathogenic organisms, through effective immune reactions, as responding to the antigens food and flora through induction of a specific state of low or "no" systemic reactivity, known as oral tolerance. Because the regulatory role of the oral tolerance, two strains of mice were obtained by bidirectional selection for extreme phenotypes of oral tolerance - resistant (TR) and sensitive (TS). The intracellular and extracellular parasitic infections carried out in this animal model showed divergent interstrain immune responses. The results of *Toxoplasma gondii* infection in TS and TR mice, as the difference in the mortality rate, parasite load and the pathology severity degree, indicated the need to deepen the cellular study of parasite-host interaction. The *T. gondii* is an intracellular protozoan, causative agent of toxoplasmosis in humans and animals that infects several vertebrates, presenting a high prevalence in the human population. Naturally, through contaminated food and water, *T. gondii* reaches the mucosal epithelium and in that environment becomes tachyzoite infecting neighboring cells as macrophages and dendritic cells. These antigen-presenting cells, play other roles that lead the course of infection, such as inflammatory or anti-inflammatory action. The

dendritic cells and macrophages depending on the environment and interactions with parasites show distinct profiles and distinct functions. The method to derive cells from progenitors in the blood or bone marrow has been used in searches with these cells. Results obtained with marrow and spleen cells harvest from TR and TS mice showed differences, during macrophages and dendritic cells differentiation, in the number and size of the cells as well as in the phagocytic rate and the percentage of infectivity, which were always higher in TR. These results show consistency with the high- and low-inflammatory profile of TR and TS mice respectively.

Supported by: PIBIC/FAPERJ, CAPES.

IM39 - RECOMBINANT ANTI-TRYPANOSOMA CRUZI ANTIBODIES FROM A HUMAN ANTIBODY LIBRARY

NIBORSKI, L.L.¹, GRIPPO, V.¹, RAATS, J.²,
LEVIN, M.J.¹

¹Lab. de Biología Molecular de la Enfermedad de Chagas (LaBMECh), INGEBI-CONICET, Buenos Aires Argentina; ²Nijmegen University, Nijmegen, Holland. niborski@dna.uba.ar

Analysis of the repertoire of VH genes of patients with Chronic Chagas Heart Disease (cChHD) demonstrates that neither autoimmune-prone VH segments nor polyclonal B cell activation evidences were present in these patients. On the contrary, the repertoire showed a high level of hypermutations in CDR regions suggesting a *Trypanosoma cruzi* driven selection. To further characterize the human antibody response against the parasite, we constructed single chain variable fragment (scFv) libraries derived from cChHD patients. Total RNA was isolated from bone marrow and peripheral blood, and DNA was synthesized. Variable chains genes (V_H, V_k y V_λ) were amplified by PCR and cloned into a phagemid vector. ScFv were expressed on the surface of M13 viral particles and phage-displayed scFv were affinity-selected by using immobilized *T. cruzi* epimastigote lysate. To determine the number of different human recombinant antibodies (hu-rAbs) that recognized the parasite lysate, ELISA-positive clones were characterized by DNA fingerprinting and DNA sequencing. Soluble hu-rAbs bearing (his)₆ tag at C-terminus were produced in *E. coli* and purified from periplasmic extracts. A hu-rAb set that belong to the VH 3-30*01 family recognized a 175 kDa protein and another hu-rAb that belongs to the VH

3-73*01 family recognized two proteins of about 47.5 kDa in *T. cruzi* Western blot. Comparisons of *T. cruzi* recognition pattern of the different bone marrow donors allowed us to identify the origin of hu-rAb. Immunofluorescence assays confirmed the binding of these hu-rAb to the parasite. These results showed that phage display technology was an effective method for selection of hu-rAbs against parasite antigens. This approach may allow us to progress in the understanding of pathogenesis of Chagas disease and in the development of an effective immunoprophylaxis.

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IM40 - EARLY POLYMORPHONUCLEAR GRANULOCYTE MIGRATION TO THE SITE OF INFECTION WITH LEISHMANIA AMAZONENSIS

SOUSA, L.M.A.¹, RESENDE, M.E.¹, ROMERO, M.N.¹, VIEIRA, A.T.¹, TAFURI, W.L.¹, TEIXEIRA, M.M.¹, VIEIRA, L.Q.¹

¹Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Minas Gerais, Brazil.

Neutrophils provide the first line of defense against infection and also contribute to the initiation of inflammation. The role of neutrophils in infections with *Leishmania* has been mainly studied using *in vitro* approaches and murine model of cutaneous leishmaniasis induced by subcutaneous injections with *L. major*. *In vitro* studies have suggested that *L. major* uses granulocytes to enter macrophages silently. Data concerning *in vivo* studies are conflicting. Since there is a profound impairment of inflammatory cytokine production at early stages of infection with *L. amazonensis*, when compared with *L. major*, the aim of this study is to investigate the migration of neutrophils to the site of the infection with *L. amazonensis*. C57BL/6 and BALB/c mice were inoculated intradermally with 1x10⁶ metacyclic promastigotes of *L. amazonensis* or *L. major* in the ear in order to determine the kinetics of migration of neutrophils to the site of infection. Neutrophils were detected in all groups following the first six hours of infection, and the highest mieloperoxidase (MPO) activity was detected between 12 and 24 h after infection. BALB/c mice infected with *L. amazonensis* had a more sustained MPO activity over time. BALB/c mice infected with either parasite presented higher

MPO activity than C57BL/6 mice. Histopathological analyses confirmed the MPO data. Assessment of cytokines and chemokines at the site of infection demonstrated the production KC, MIP-2 and TNF- α during the first 24 h following infection, which correlates with the most intense infiltrate of neutrophils. In conclusion, we found that neutrophils migrated to the site of infection with *L. amazonensis* and that this migration correlated with KC and MIP-2 levels. In addition, in BALB/c mice the presence of neutrophils at the site of infection was sustained for at least one week after infection, contrary to C57BL/6 mice. Support: CNPq, CAPES, FAPEMIG

IM41 - Relationship to clinical status and bone marrow parasite load with leucopoiesis and erythropoiesis in canine visceral leishmaniasis (CVL)

Raquel Trópia de Abreu¹; Henrique Gama Ker¹; Rodolfo Cordeiro Giunchetti^{1,2}; Cláudia Martins Carneiro¹; Rodrigo Dian de Oliveira Aguiar¹; Maria das Graças Carvalho³; Rodrigo Corrêa Oliveira²; Olindo Assis Martins-Filho⁴; Alexandre Barbosa Reis^{1,2}.

1-Laboratório de Imunopatologia, NUPEB/UFOP

2-Laboratório de Imunologia Celular e Molecular, IRR/FIOCRUZ

3-Laboratório de Hematologia Clínica, Faculdade de Farmácia/UFMG

4-Laboratório de Biomarcadores de Diagnóstico e Monitoração, IRR/FIOCRUZ

The bone marrow is considered an important reservoir of parasites in sick *Leishmania*-infected dogs. The aim of the study was to evaluate leucopoiesis and erythropoiesis alterations in dogs naturally infected by *L. chagasi* presented a distinct bone marrow parasite load and different clinical status. Herein, bone marrow smears stained by *Giemsa* were evaluated considering three clinical groups: asymptomatic (AD, $n=50$), oligosymptomatic (OD, $n=44$) and symptomatic (SD, $n=65$) compared with non-infected dogs (NID, $n=28$). Parasite density was performed in bone marrow and the results expressed as "Leishman Donovan Units" (LDU index), and classified into tertiles as low (LP, $n=51$), medium (MP, $n=51$) or high (HP, $n=48$) parasitism. Our major results indicated significant differences in relation to erythropoiesis resulting in the erythroid hypoplasia mainly in AD and SD groups. Leucopoiesis presented alterations in infected dogs. For

example, eosinophilic lineage cells number showed a significant decrease in the different clinical groups compared to NID group. However, neutrophilic lineage cells number showed alterations, such as a significant increase in the different clinical groups. Related to mononuclear cells, it was observed for lymphocytes number an increase in OD and SD groups when compared with NID group. Similar results were found for plasma cells number. Monocytes cell number had significantly increase in OD compared to AD group. Differential cell counts of bone marrow demonstrated an increase in the myeloid: erythroid (M:E) ratio. Assessment of the impact of parasite density on bone marrow showed similar results when the dogs were evaluated in different clinical status and highlighting the high parasitism dogs group with the most significant results. Our study showed that the progression of the disease from asymptomatic to symptomatic clinical status was accompanied by intense parasitism in the bone marrow. Therefore, these results indicate that clinical status of CVL is related to bone marrow parasite load.

Financial support: CNPq, FAPEMIG, UFOP/UFMG.

IM42 - PERFORIN-EXPRESSING CYTOTOXIC CELLS CONTRIBUTE TO CHRONIC CARDIOMYOPATHY IN *Trypanosoma cruzi* INFECTION

Silverio, J.C.¹, de-Oliveira-Pinto, L.M.¹, Silva, A.A.^{1,2}, Oliveira, G.M.³, Terra, M.A.B.L.¹, Marino, A.P.M.P.¹, Lannes-Vieira, J.^{1,4}

Laboratory of Autoimmunity and Immunoregulation, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil¹, Department of Pathology, UFF, Niterói, Brazil², Laboratory of Cell Biology, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil³.

Introduction and objectives: The comprehension of the dualistic participation of the immune response controlling the invader and leading to heart tissue damage might contribute to design effective vaccines and new therapies in Chagas disease. Perforin, a cytolytic protein employed by killer cells, is critically involved in resistance to acute infection with *Trypanosoma cruzi* virulent strains. Increased numbers of perforin⁺ cells were detected in the heart tissue of C57BL/6 mice infected with the low virulence Colombian strain. In

the present study, we approached the contribution of perforin in parasite control and chronic cardiomyopathy using perforin-deficient ($\text{pfp}^{-/-}$) mice. **Material and Methods and Results:** We showed that high inoculum (5×10^3 or 10^3 parasites) resulted in higher parasitemia and precocious mortality in $\text{pfp}^{-/-}$ than C57BL/6 mice, while low inoculum (10^2 parasites) led to acute phase survival in both wild-type and $\text{pfp}^{-/-}$ mice strains. During the chronic infection, parasitism and inducible nitric oxide synthase expression were more elevated in the heart tissue of $\text{pfp}^{-/-}$ mice. Higher levels of circulating nitric oxide and anti-parasite IgG2c and IgG3 as well as more prominent frequencies of *T. cruzi* responsive IFN- γ^+ splenocytes were evidenced in $\text{pfp}^{-/-}$ infected mice. Thus, although the perforin-dependent pathway plays a role, it is not crucial for anti-parasite immunity and acute phase survival. Importantly, perforin deficiency resulted in lower activity of CK-MB isoenzyme in serum and a more restricted loss of connexin 43, markers of cardiomyocyte lesion. Moreover, perforin deficiency hampered the development of severe electrocardiographic abnormalities. **Conclusion:** Our results corroborate that perforin-bearing cytotoxic cells might contribute to cardiomyocyte lesion and heart dysfunction during chronic *T. cruzi* infection.

Support: Universal-2006/CNPq, IC/CNPq, Bolsa de Produtividade/CNPq, FAPERJ.

Key-Words: Trypanosoma cruzi; CD8 T cell; Perforin

IM43 - DETERMINATION OF THE ACTIVATION STATE AND TOLL LIKE RECEPTOR EXPRESSION BY PERIPHERAL BLOOD MONOCYTES FROM CUTANEOUS LEISHMANIASIS PATIENTS

VIEIRA, E.L.M.¹, KEESEN, T.S.L.¹, GUIMARÃES, L.H.³, BACELLAR, O.³, CARVALHO, E.M.³, DUTRA, W.O.², GOLLOB, K.J.¹

¹ Department of Biochemistry and Immunology, and, ² Department of Morphology, Institute of Biological Sciences-Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, and ³ Immunology Service, Hospital Edgar dos Santos, UFBA-Salvador, Brazil.

Leishmaniasis, caused by infection with the protozoan parasite *Leishmania*, affects millions of

individuals worldwide, causing serious morbidity and mortality. Activated monocytes and macrophages play an important role in protection against intracellular infections due to their ability to internalize pathogens and produce toxic molecules such as nitric oxide that lead to killing of intracellular pathogens. Moreover, these cells are key in orchestrating subsequent T cell differentiation and aid in shaping the immunoregulatory environment following infection. Toll like receptors (TLRs) play an essential role in the initial immune response against pathogens, but little is known about their role in *Leishmania braziliensis* infection. In this work, we evaluated peripheral blood mononuclear cells (PBMC), expressing co-stimulatory molecules and TLR 2, 4 and 9 in CD14⁺ monocytes from cutaneous leishmaniasis patients infected with *Leishmania (Viannia) braziliensis*, using flow cytometry. Our results showed that: 1) cutaneous leishmaniasis patients demonstrated an increase of CD14⁺ monocytes expressing the co-stimulatory molecule CD80 after stimulus with soluble *Leishmania* antigen (SLA), when compared with ex vivo and medium alone. CD14⁺ monocytes expressing co-stimulatory molecules CD86 and CD40 did not present changes in their frequency between different stimuli; 2) evaluating the expression of TLRs; CD14⁺ monocytes displayed a higher expression of TLR 2 and 4 after culture with medium or SLA, as compared with cells ex vivo. There was no difference in the expression of TLR9 amongst different conditions. In conclusion, these findings suggest components of *L. braziliensis* may act to regulate the expression of co-stimulatory molecules and TLRs in cutaneous leishmaniasis, which could influence the subsequent immune response.

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IM44 - TOLL-LIKE RECEPTORS EXPRESSION IN PERIPHERAL BLOOD-MONOCYTES OF DOGS NATURALLY INFECTED WITH LEISHMANIA (LEISHMANIA) CHAGASI: A PRELIMINARY STUDY

AMORIM, I.F.G.¹, MOURA, E.P.¹, MUDADO, M.A.¹, BIASIZZO, R.L.¹, SILVA, L.C.¹, SOUZA, C.C.¹, FIGUEIREDO, M.M.¹, ALVES, C.F.¹, MICHALICK, M.S.M.^{1,2}, TAFURI, W.L.¹

¹ Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; ² Universidade FUMEC,

Faculdade de Ciências da Saúde, Belo Horizonte, Brazil.

Innate immunity coordinates the inflammatory response to pathogens, and the contribution of Toll-like receptors (TLRs) to this response is becoming widely recognized. TLRs are triggered by pathogen-associated molecular pattern (PAMPs) which are characteristic of various groups of pathogens. Activation of TLRs on antigen presenting cells of the innate immune system initiates amplifies and directs the antigen-specific acquired immune response. In according to the literature, a specific adaptor cytoplasmatic molecule such as MyD88 is involved in the healing of *L. major* infections. However there are no reports about TLR engagement by the intracellular parasite *Leishmania*, and the contribution of TLRs to innate leishmanicidal responses is unknown. Many researches have suggested a role for TLR-type 2 (TLR2) and TLR4 in the phagocytosis and control of *Leishmania* infections. The aim of the present study was to evaluate the TLR2 and TLR4 expression in monocytes proceeding from peripheral blood of dogs naturally infected with *Leishmania (Leishmania) chagasi*. Peripheral blood (20 ml) was collected of ten dogs naturally infected with *L. chagasi* and seven dogs uninfected. Density gradient (Histopaque 1070) separation was used to enrich for peripheral blood mononuclear cells from canine blood. TLR2 and TLR4 expressions were quantified by flow cytometric analysis. So far, these previous results have shown that the *Leishmania* infection not change the TLRs expression in peripheral blood monocytes. Additional studies with monocytes-derived macrophages from canine peripheral blood and bone-marrow under different experimental conditions *in vitro* have been done for further analysis.

Financial Support: CNPq and FAPEMIG.

IM45 - EVALUATION OF TRACE ELEMENTS IN SERUM OF DOGS WITH VISCERAL LEISHMANIOSIS: A PRELIMINARY STUDY

SOUZA, C. C.¹, FABRINO, H. J. F.¹, BORGES NETO, W.¹, PINTO, A. J. R. W.¹, AMORIM, I. F. G.¹, MOURA, E. P.¹, FIGUEIREDO, M. M.¹, SILVA, J. B. B.¹, TAFURI, W. L.¹.

¹Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Visceral Leishmaniosis (VL) is a zoonotic infection that leads to chronic and systemic disease that affects many organs and tissues of the dog, the domestic reservoir. Infections can be influenced by the trace elements zinc (Zn), iron (Fe), copper (Cu) and selenium (Se). These elements are present in the chemical structure or in the active sites of metalloenzymes that participate in many biological processes. Acute infections may alter the serum level and the metabolism of these chemical elements. In another side, the deficiency of these elements has been related to a lower resistance to diseases associated to alterations of the leukocyte functions. A method for elements trace detection using graphite furnace atomic-absorption spectroscopy (GF AAS) was standardized to analyze the Cu levels in control and mongrel dogs naturally infected with *Leishmania chagasi*. Twelve canine sera samples (7 non-infected and 5 infected) were collected and the Cu concentration levels ranged from 341.3 to 914.9. Values of infected dog samples were compared with control samples. Our preliminary data did not show any statistical difference between the infected and uninfected dogs, but Cu serum levels were higher in serum of infected ones. In Human Visceral Leishmaniosis there are reports showing a strict correlation between higher concentrations of serum Cu and the severity of the disease. Thus, we are looking forward that the Cu serum increasing levels could be related to the progression of the canine visceral leishmaniasis. Clinical, histological and immunological investigations have been carried out to elucidate this hypothesis.

Support: UFMG, Cnpq, Capes e Fapemig

IM46 - *Leishmania (L.) amazonensis* cysteine-proteinase B COOH-terminal region epitopes: interaction with MHC I molecule from CBA mice and *in vitro/in vivo* effects.

PEREIRA, B.A.S.¹, SILVA, F.S.², BENÉVOLO-DE-ANDRADE, T.C.³, BERTHO, A.L.⁴, CAFFARENA, E.R.², ALVES, C.R.¹

¹Laboratório de Biologia Molecular e Doenças Endêmicas, IOC; ²Programa de Computação Científica, Presidência; ³Laboratório de Imunologia Clínica, IOC; ⁴Laboratório de Imunoparasitologia, IOC; Fiocruz, Rio de Janeiro, Brasil.

Leishmania (Leishmania) amazonensis is one of the causative agents of leishmaniasis, a disease that, despite its prevalence of 12 million cases

worldwide, has been neglected, due its association with poverty. Thus, studies that may lead to the understanding of infection mechanisms and, this way, reveal novel possible targets for infection control are utmost necessary. In the present work, we have selected potential MHC-I-binding peptides derived from *L. (L.) amazonensis* cysteine-proteinase B COOH-terminal sequence and studied their interaction with MHC I molecules by molecular docking assays and their effects on lymph node cells from infected mice in culture and on mice in experimental infections. We used CBA mice as the selected peptides were designed for its specific MHC-I haplotype (k). Our results showed that two out of the four selected peptides for haplotype k were able to induce blastogenesis in the cell culture, as well as one out of the six peptides designed for another mice haplotype (d). However, despite this ability in inducing cell proliferation, no peptides had any effects on the development of lesions in experimental infection assays. The molecular docking assays showed that the two peptides with capacity to induce blastogenesis had similar docking energies, but induced distinct patterns of charges distribution on the H-2K^k molecule. Furthermore, although one of the peptides without *in vitro* effects had a better docking energy, it also was not properly anchored into the MHC cleft, thus, the peptides with proliferation effects had the better combination of docking energy and interactions with the MHC molecule. Our perspectives are to define which T-cell type responds to the peptides in the culture and also carry on similar assays with BALB/c mice, a lineage susceptible to the infection, to assess the effects of the peptides designed for its specific haplotype. Financial support: CAPES and PAPES IV.

IM47 - EVALUATION OF EXPERIMENTAL CHAGAS CARDIOVASCULAR REMODELING IN 2K1C GOLDBLATT RENOVASCULAR HYPERTENSION MODEL

SILVA, M.C.¹, AZEVEDO, M.A.¹, MOURA Júnior, M.R.¹, MACHADO, R.P.¹, CHIANCA Júnior, D.A.¹, ALZAMORA, A.C.¹, BAHIA, M.A.¹, TALVANI, A.¹

¹Universidade Federal de Ouro Preto, Ouro Preto, Brazil

Angiotensin II (Angio II), the effector hormone of the renin-angiotensin system, has been implicated in the pathophysiology and progression of hypertension and, consequently, cardiovascular

failure. In parallel, the Chagas heart disease, a secondary cause of *Trypanosoma cruzi* infection, is accompanied by an intense inflammatory response driving the cardiovascular system to a deleterious autonomic and functional dysfunction. Starting from the idea that Angio II influences leukocyte activation directly or by cytokines or nitric oxide generation mechanisms, we propose here to investigate the cardiovascular inflammatory aspects concerning the association of experimental Chagas disease and hypertension. Wistar rats (200g) were infected, or not, with 1.2×10^6 trypomastigote forms of Y strain of *T. cruzi* and grouped into (i) two-kidney one-clip (2K1C) Goldblatt hypertensive group (Goldblatt et al. 1943) and (ii) normotensive group (SHAM). Animals were accompanied (i) daily to parasitism evaluated by tail blood and (ii) weekly to systolic hypertension level, by tail plethysmography using RTBP 2000 – Kent Scientific. Morphological and inflammatory parameters will be investigated after the peak of hypertension in serum and *in situ* (heart and endothelium vessel). Preliminary results shown a peak of hypertension of 2K1C rats on third week post surgery, which did not interfere with sanguine parasitism. Vascular endothelial integrity was assessed by Evans' blue dye extraction and the most pronounced change in vascular permeability was observed in heart and lung tissues from SHAM and non-infected animals in comparison with 2K1C and non-infected rats. No alterations were observed among the groups concerning histological analysis in this experimental phase. Our findings suggest that, apparently, during the acute phase of infection, the hypertension does not contribute to the heart damage, but the prominent cardiovascular events are expected in association with chronic phase of Chagas disease.

Financial support: UFOP (PIP), FAPEMIG.

IM48 - EXPERIMENTAL MENINGOENCEPHALITIS INDUCED BY TRYPANOSOMA CRUZI: MOLECULAR MECHANISM INVOLVED IN THE FORMATION/RESOLUTION OF INFLAMMATION AND PARASITISM

DOS SANTOS, A.L.¹, SILVA, A.A.^{1,2}, LANNES-VIEIRA, J.¹

¹Laboratory of Autoimmunity and Immunoregulation, IOC/Fiocruz, Rio de Janeiro, RJ, Brazil; ² Fluminense Federal University, Niterói, RJ, Brazil.

Introduction and objectives: The experimental *T. cruzi* infection leads to intense inflammation in the central nervous system (CNS) in C3H/He mice. In this model, we showed that the CD8-enriched meningoencephalitis is restricted to the acute infection (Silva et al., 1999) and that the VLA-4/VCAM-1 interaction pathway plays a pivotal role in the formation of *T. cruzi*-elicited meningoencephalitis (Roffê et al., 2003). During the chronic phase of infection the meningoencephalitis is absent paralleling low parasitemia and CNS parasitism. Thus, the molecular mechanism involved in formation/resolution of *T. cruzi*-elicited meningoencephalitis is unclear. **Material and Methods and Results:** We investigated the participation of the inflammatory cytokine tumor necrosis factor (TNF) in susceptible mice to develop *T. cruzi*-induced meningoencephalitis injecting 100 trypomastigotes of the Colombiana strain in C3H/He mice. *T. cruzi*-infected mice present increased frequencies of TNFR1/p55-bearing and TNF-expressing splenocytes during the acute infection. In the CNS increased expression of TNF mRNA is restricted to the acute infection, whereas high levels of circulating TNF levels were detected during the acute and chronic phases. Further, the presence of parasite antigen in the CNS was detected, by immunofluorescence, in MHC-II+, F4/80+ and GFAP+ cells during the acute phase of infection. *In vitro*, astrocyte primary cultures of newborn C3H/He mice are infected by *T. cruzi*. **Conclusion:** Our findings suggest that TNF is produced into the brain. The source of TNF is unclear. Further, CNS inflammation formation/resolution is independent of peripheral TNF levels. Moreover, the glial cells are target for *T. cruzi* infection and may contribute to resolution of the *T. cruzi*-elicited meningoencephalitis. **Financial support:** IOC, CNPq, FAPERJ

**IM49 - TRYPANOSOMA CRUZI:
HISTOPATHOLOGICAL ALTERATIONS IN
SPLEEN AND CERVICAL LYMPHNODE OF
DOGS IN THE ACUTE PHASE OF INFECTION
WITH METACYCLIC OR BLOOD
TRYPOMASTIGOTES**

VIEIRA, P.M.A.¹, RIBEIRO, K.L.S.¹, FRANCISCO, A.F.¹, VELOSO, V.M.¹, GIUNCHETTI, R.C.^{1,2}, LIMA, W.G.¹, REIS, A.B.^{1,2}, TAFURI, W.L.¹, CARNEIRO, C.M.¹

¹ Laboratório de Imunopatologia, Núcleo de Pesquisas em Ciências Biológicas/NUPEB, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brazil

² Laboratório de Imunologia Celular e Molecular, Instituto de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil

Experimental infection of dogs with *Trypanosoma cruzi* leads to disturbances in the peripheral immune system, such as polyclonal lymphocyte activation and host cardiac tissue damage. In secondary lymphoid organs, splenomegaly and lymphadenopathy are reported, with persistent T and B polyclonal activation. This study aimed to evaluate the histopathological alterations of spleen and lymphnode during the acute phase of dogs infected with blood (BT group, n=4) or metacyclic forms (MT group, n=4) of the Berenice-78 *T. cruzi* strain in relation to the Control (C group, n=4). Morphometric analysis of cervical lymphnode demonstrated that MT group presented a significantly increase on the area of follicles when compared to BT and C group, also the group BT demonstrated a significantly reduction of macrophages in the medullary area when compared to the other groups. This fact can be related to the decrease in the marked area for iNOS when compared to the C and MT groups. Although there have been no significant difference in the area of follicles in the spleen, there is a tendency to animals of the MT group make greater reactivity in this organ when compared to C and BT groups. Besides this, it was demonstrated a thickening of the capsule and an increase in the numbers of macrophages and lymphocytes in the MT group compared with the C group. These results can be correlated with greater area marked for iNOS found in this group. These facts are in agreement with our previous results in serum and in heart, where animals of MT group had increased serum production of nitric oxide and greater expression of iNOS in inflammatory infiltrate. Taken together, these results show that infection by metacyclic forms leads to greater activation of the spleen and lymphnode, with consequent lymphocytes and macrophages increase, as well as greater expression of iNOS.

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**IM50 - BLOOD AND METACYCLIC
TRYPANOSOMA CRUZI TRYPOMASTIGOTES
TRIGGERED SYSTEMIC ALTERATIONS ON
CD4⁺ AND CD8⁺ T-CELLS WITH
COMPARTMENTALIZED CHANGES IN B-CELLS
AND IMMUNOGLOBULIN PATTERNS**

VIEIRA, P.M.A.¹, SOUZA, S.M.¹, NOGUEIRA, C.¹,
FONSECA, K.S.¹, VELOSO, V. M.¹, REIS, A. B.^{1,2},
CORREA-OLIVEIRA, R.², TAFURI, W. L.¹,
MARTINS-FILHO, O. A.³, CARNEIRO, C. M.¹

¹ Núcleo de Pesquisas em Ciências Biológicas,
NUPEB, Universidade Federal de Ouro Preto,
Ouro Preto, MG, Brasil;

² Laboratório de Imunologia Celular e Molecular,
Instituto René Rachou, Fiocruz, Belo Horizonte,
MG, Brasil;

³ Laboratório de Biomarcadores e Diagnóstico
Molecular, Instituto René Rachou, Fiocruz, Belo
Horizonte, MG, Brasil

We have previously demonstrated that dogs infected with blood-BT or metacyclic-MT *Trypanosoma cruzi* trigger distinct immunological features which may be related with particular disease outcome. Herein we have further focus on the cell phenotypic profile immunity at different host compartments (peripheral blood-PB, spleen-SP and lymphnode-LN) and the seric humoral immune response following infection with BT or MT. Our findings demonstrated that despite the inoculum source lower percentage of CD4⁺T-cells and higher frequency of CD8⁺T-cell in PB and SP are the hallmark of acute Be-78 *T. cruzi* infection. Although positive correlation between PB and SP-CD8⁺T-cell frequency observed despite the inoculum source, outstanding frequency of PB-CD8⁺T-cell was observed in MT infection. Reverse profile of CD21⁺B-cells was observed in PB in comparison to SP and LN, suggesting active B-cell compartmentalization following BT and MT infection, respectively. Negative correlation between PB versus SP and LN-CD21⁺B-cell frequency was observed despite the inoculum source. However, outstanding frequency of SP-CD21⁺B-cell and LN-CD21⁺B-cell was the biomarker of BT and MT infection, respectively. Anti-*T. cruzi* IgM synthesis was detectable in both inoculums, whereas an overall lower IgG profile triggered by BT acute infection. Negative association was observed between anti-*T. cruzi* IgG and IgG2 with PB-CD4⁺T-cell or PB-CD21⁺B-cell in contrast with a positive association with PB-CD8⁺T-cells, mainly due to MT infection. These results re-emphasize the importance of the

inoculum source triggering distinct aspects of the immune response, with systemic changes in CD4⁺ and CD8⁺T-cell frequency and whereas the expansion of B-cells may represent a compartmentalized phenomenon restricted to SP or LN, depending on the inoculum source.

Supported by FAPEMIG, CNPq and UFOP

**IM51 - PREVIOUS INFECTION WITH GENETIC
DISTINCT STRAINS OF TOXOPLASMA GONDII
INTERFERE BUT NOT PREVENT SUBSEQUENT
REINFECTION IN EXPERIMENTAL MOUSE
MODEL**

MEIRELES, L. R., KAGAJO, G. M., PEREIRA, M.
A., ANDRADE Jr, H. F.

*Instituto de Medicina Tropical de São Paulo,
Universidade de São Paulo, Brazil.*

Toxoplasmosis is one of the more common parasitic zoonoses worldwide. The causative agent *Toxoplasma gondii* infects most warm-blooded animals including man, with significant veterinary and medical importance. Several strains of *T. gondii* have been described in recent years, with diverse area distribution, with variable virulence that could be associated with human disease severity, causing a risk of reinfection of a previous immune host. We tested those reinfection effects in experimental mouse models, using two prototypic cystogenic avirulent strains of *T. gondii*, type II ME-49 strain and type III VEG strain. The infected host was analyzed both by IgG response during reinfection but also for parasite burden by cerebral histology. Groups of mice were infected orally with cysts of each strain, alone, together or challenged after 4 weeks of first infection with another strain. Specific IgG were determined by ELISA, using type I strain antigens in solid phase, and brains analyzed by histology and total cyst counts at the end (8 weeks) of the experiment in each group. Type II strains induces more brains cysts with similar levels of specific IgG, as compared to type III infected mice. When mouse infected with type II strains were challenged with type III strains, brain cysts numbers were similar without interfering in humoral response. When type III strain infected mice were challenged with type II strain, higher numbers of cysts were found in brains, suggesting an effective reinfection, despite some protection as the numbers were lower than isolated type II strain infection. Our data supports that infection with one strain of *T. gondii* induces adequate humoral response that diminishes the burden of reinfection

with another strain, but do not prevent cyst development of the new strain in the brain, with implications in the comprehension of human disease, especially in immune compromised hosts. Financial support: CNPq and LIMHCFMUSP-49.

IM52 - PREVENTION OF TOXOPLASMOSIS IN SCHOOLCHILDREN: IMPACT OF INTERVENTION AND TOXOPLASMOSIS CONTACT DETECTION BY SALIVA SPECIFIC IgG

MACRE, M. S.¹, MEIRELES, L. R.², CARVALHO, B. F.², ANDRADE Jr, H. F.²

¹ Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil; ² Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, Brazil.

Toxoplasmosis affects large fractions of human population, with a high transmission rate in Brazilian children, usually with more than 40% with infection under 10 years. This population is difficult to access either by sample collection or educational intervention. We had detected toxoplasmosis history by saliva specific IgG students from 3rd and 4th series of fundamental public schools of East Zone of São Paulo County. The saliva tests consisted in modified ELISA and we also standardize a dot plot test for use in tooth brush support for easy sampling. We submitted this population to a one day educational intervention on toxoplasmosis. After one year, we revisited the same school population in order to evaluate the effect of educational intervention, by a illustrated questionnaire, both with figures and text questions, which was applied randomly between intervened (45) or naïve students (147), used as controls. Students who assist the intervention recall basic toxoplasmosis concepts much more frequently than control inmates. Transmission by cats was recalled in intervened students (87%, 39/45) as compared to controls (20%, 29/147). Food transmission had less efficiency but also significant with a larger fraction of analyzed students (62%, 28/45) as compared to population (28%, 41/147). Interestingly, the recall for cat transmission appears more effective in students without history of toxoplasmosis (100%, 21/21) as compared to those had history (75%, 18/24). Our data clearly shows that one day educational intervention could have longstanding effects in schoolchildren and must be implemented as a prevention tool. Saliva tests could be an alternative

for sampling in those populations especially if specific single use devices were devised to allow minimal physical sampling intervention in this age group. Financial support: CAPES and LIMHCFMUSP-49.

IM53 - SYSTEMIC AND MUCOSAL HUMORAL IMMUNE RESPONSE IN IFN- γ ^{-/-} MICE IMMUNIZED WITH GAMA-IRRADIATED *Toxoplasma gondii* TACHYZOITES

Zorgi, N.E.¹, Augusto, L.S.¹, Galisteo Jr., A.J.^{1,2} & de Andrade Jr., H.F.¹

¹Lab. de Protozoologia (IMTSP – Inst. de Medicina Tropical de São Paulo/USP); ²Centro de Biologia Molecular (IPEN- Inst. de Pesquisas Energéticas e Nucleares/CNEN SP)

Toxoplasmosis, worldwide most prevalent protozoan disease, is caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite that induces potent and long-lasting immune response in host. Ionizing radiation has been used as a tool in immunization models, by creating immunity without infection, with similar levels of protection as natural infection. IFN- γ ^{-/-} mice presented an increased susceptibility to challenge with cyst forming strains, with severe disease and high numbers of cysts but few studies were conducted looking for the effect of IFN- γ in the mucosal immunity in this disease. In this work, the immune response to irradiated tachyzoites was evaluated in IFN- γ ^{-/-} mice, using groups of mice immunized by oral or intraperitoneal routes with 3 biweekly doses of 10⁷ *T. gondii* gamma irradiated (255Gy) tachyzoites in a Cobalt-60 source, looking for specific IgA and IgG production and cerebral cysts counting after challenge with infecting agents. ELISA-specific assays to IgG and IgA detection was performed in serum and fecal suspensions. It was found that *knockout* IFN- γ ^{-/-} mice presented low levels of total antibodies production as compared to controls, with parenteral immunized mice with higher levels of IgG production in serum and with a small increase of IgA in oral immunized mice. Secretory IgG and IgA was presented in fecal samples of *knockout* mice immunized by both routes. Mortality was not observed during immunization period. After oral challenge with 10 cysts/animal (ME49 strain), immunized groups presented higher survival rates compared to non-immunized IFN- γ ^{-/-} mice groups. Surviving *knockout* immunized animals (20%) presented significant lower amounts of intra-

cerebral cysts than wild-type models. Our data suggests that IFN- γ activated immune response participate, despite not crucial, in the humoral response to this immunogen, but appears much more involved in the systemic parasite control. Mucosal immune response could be an effective barrier for host protection in this disease.

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IM54 - PROTECTIVE IMMUNITY AGAINST CHALLENGE WITH *LEISHMANIA CHAGASI* IN HIGHLY SUSCEPTIBLE BEAGLE DOGS VACCINATED WITH RECOMBINANT A2 PROTEIN

COSTA, M.M.S.¹, FERNANDES, A.P.², COELHO, E.A.F.³, MICHALICK, M.S.M.³, FREITAS, E.³, MELO, M.N.³, TAFURI, W.L.⁴, HERMONT, V.⁵, ABRANTES, C.F., GAZZINELLI, R.T.

Vaccines against canine visceral leishmaniasis (VL) constitute an alternative approach for interrupting the VL domestic cycle. Ideally, besides the induction of protective immune responses, vaccine formulations should allow the serological differentiation of vaccinated and infected dogs, in endemic areas where identification of seropositive dogs is required for control measures. A2, an amastigote specific antigen, has been shown to be protective against *L. donovani*, *L. chagasi* and *L. amazonensis* infections in mice. In this work, we aimed to investigate the immune responses induced by vaccination with A2, as recombinant protein (rA2). Beagles were vaccinated with rA2 and challenged with 5×10^7 *L. chagasi* promastigotes. Anti-A2 and anti-total parasite antigenic extract (LcPA) antibody levels (IgG1, IgG2 and total IgG) were measured by ELISA, before and after challenge. Levels of IFN- γ and IL-10 were assessed by sandwich ELISA. Vaccinated animals produced significant levels of total IgG and IgG2 anti-A2, but not IgG1 antibodies and remained negative for anti-LcPA antibodies. Significantly increased IFN- γ and low IL-10 levels were detected in vaccinated animals, comparing to the control group, before and after challenge. In contrast, in control infected animals increased IL-10 and anti-LcPA total IgG and IgG1 levels were detected. While the onset of symptoms appeared as early as three months after infection in control dogs, one year after challenged, 5 out of 7 vaccinated dogs remained asymptomatic.

Therefore, immunization with rA2 antigen induced type I protective immune responses and in addition, it allows the serological differentiation between vaccinated and infected animals, an important requirement for a canine VL vaccine in Brazil. Financial support: CNPq and FAPEMIG.

IM55 - Nucleoside hydrolase DNA vaccine against canine visceral leishmaniasis (CVL)

G.P. Borja-Cabrera; D. Nico, F. B. Santos, E. Picillo, A.E. Gravino, E., L Manna, C.B. Palatnik-de-Sousa*

*Federal University of Rio de Janeiro, Brasil immgcpa@micro.ufrj.br; Università di Napoli Federico II, Naples, Italy

Introduction: The *Leishmania donovani* Nucleoside hydrolase is the main antigen of the FML antigen of the Leishmune® vaccine against CVL. Objective: Six mongrel dogs were treated with 3 doses of VR1012-NH36 plasmid, 13 dogs with saline and all of them challenged with *L. chagasi* amastigotes. On day 93, 6/13 seropositive and symptomatic controls were treated with 3 doses of the VR1012NH36 vaccine (immunotherapy). The protective effect was monitored by the assay of anti-NH36 and anti FML antibodies, DTH against leishmanial lysate, the CD4+ and CD8+ *Leishmania* specific lymphocyte proportion increase, the score of symptoms, the microscopical parasite load in lymph nodes and the IFN gamma expression and Leishmanial DNA by the Real Time PCR. Results: the anti-FML antibodies increased in vaccinated and control dogs (ANOVA $p=0.000$) while anti-NH36 antibodies, in vaccinees only (day 170). Protection was evident by: higher DTH positivity ($p=0.001$) and larger skin test diameters in vaccinated dogs than in controls; increased average of CD4+ *Leishmania*-specific lymphocyte proportions in the prophylaxis (44.42%) and immunotherapy (43.97%) groups over the untreated controls (31.75%, CI95%; 24.8-38.7); decreased accumulated scores of clinical symptoms in prophylaxis group (mean=16.66) in relation to control dogs (mean=26.14, CI95%, 17.83-34.45) but not in the immunotherapy group (mean=23.83); increase of the ratio of parasites to lymph node cells in control group (ratio= 2.05) over the prophylaxis group (ratio= 0.64; CI95%, 0.65-1.93), but not over the immunotherapy group (ratio= 1.18); increased of over the control dog (158). At month 16, the parasite load of the control dog (638.05 parasites) felt outside the IC95% of

that of vaccinated dogs (32.02, IC95% 9.45-64.59) and expressed less IFN γ - β actin relative quantification (158) than the vaccinated dogs (221.2, IC 95% 172.97-269.43). Discussion: We conclude that the VR1012-NH36 vaccine induces strong prophylactic protection against a high dose canine infection with *Leishmania chagasi*.

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ); Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) Brazil

IM56 - LBSAP AND LEISHMUNE® VACCINES PROMOTE A DISTINCT CELL MIGRATION IN DERMIS OF HAMSTERS

MOREIRA, N.D.¹, BRAGA, S.L.¹, VITORIANO-SOUZA, J.¹, GIUNCHETTI, R.C.^{1,2}, CARNEIRO, C.M.¹, KER, H.G.¹, ROATT, B.M.¹, MALAQUIAS, L.C.C.³, CORRÊA-OLIVEIRA, R.², REIS A.B.^{1,2}

¹Laboratório de Imunopatologia, Núcleo de Pesquisas em Ciências Biológicas/NUPEB, Departamento de Análises Clínicas, Escola de Farmácia, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brazil

²Laboratório de Imunologia Celular e Molecular, Instituto de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil

³Laboratório de Imunologia, Núcleo de Pesquisa em Imunologia, Universidade Vale do Rio Doce, Governador Valadares, Minas Gerais, Brazil

Dogs represent the most important domestic reservoirs of *L. chagasi*, and a vaccine against canine visceral leishmaniasis (CVL) would be an important tool in the control of visceral leishmaniasis (VL). Although, an effective vaccine against human and CVL is not yet available, much effort has been expended in this area in recent years and several candidate vaccine antigens have been studied in dogs. In this context, hamsters have been used as a model for understanding the mechanisms of immunogenicity for vaccines against CVL. The present work describes the detailed analysis of the histological dynamics following inoculation of two vaccines (Leishmune® and LBSap) and their separated components into the dermis of Syrian golden hamsters. The kinetics of cell migration in dermal inflammatory infiltrate, and the presence of serum nitric oxide (NO) or induced nitric oxide synthase (iNOS) have been determined during the early (1-

24 h) and late (48-168 h) periods following inoculation of hamsters with antigenic components of anti-canine visceral leishmaniasis vaccines Leishmune® and *Leishmania braziliensis* antigen (LB) with and without saponin (Sap) adjuvant. LB caused an early reduction of lymphocytes in the dermis while Sap and LBSap triggered a late recruitment, suggesting the role of the adjuvant in the traffic of antigen-presenting cells and the induction of lymphocyte migration. Increases in NO levels with Leishmune® and LBSap suggested the involvement of type 1 cytokines that can be essential in the polarisation of the protective acquired immune response after vaccination. These results indicate that the kinetics of cell migration on hamster model may be of value in the selection of vaccine antigens prior the tests in dogs particularly in respect of the toxicity of the preparations.

Supported by: FAPEMIG, CNPq, FIOCRUZ and UFOP

IM57 - MOBILISATION OF INFLAMMATORY CELLS AND INOS EXPRESSION IN THE SKIN OF DOGS INOCULATED WITH ANTIGENIC COMPOUNDS OF LBSap vaccine

VITORIANO-SOUZA, J.¹, MATHIAS, F.A.S.¹, MOREIRA, N.D.¹, BRAGA, S.L.¹, REIS, L.E.S.¹, KER, H.G.¹, GIUNCHETTI, R.C.^{1,3}, CARNEIRO, C.M.¹, CORRÊA-OLIVEIRA, R.³, REIS A.B.^{1,2}

¹Laboratório de Imunopatologia, Núcleo de Pesquisas em Ciências Biológicas/NUPEB, Departamento de Análises Clínicas, Escola de Farmácia, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brasil

²Laboratório de Imunologia Celular e Molecular, Instituto de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brasil

Vaccines, one the most effective strategies of immunization, are usually associated with the adjuvant to amplify the protective immune response. On the basis that immunization against infectious agents requires the participation of innate and adaptive immune responses. In this context, the determination of cell migration kinetics in the inoculation area is extremely relevant since the number and types of cells recruited immediately after inoculation will stimulate the innate-immune system and will influence the development of specific acquired immunity. In the present study, were evaluated the kinetics of the

inflammatory reaction and the expression of inducible nitric oxide synthase induced (iNOS) in the inoculation area by the antigen and adjuvant present in LBSap vaccine at different times (1, 12, 24, 48, 96 hours). The results demonstrate that the LBSap vaccine and the isolated saponin adjuvant were able to induce intense cell migration in the skin (dermis and hypodermis) of inoculated dogs, thus triggering the initial immunogenic events. Moreover, the components of the vaccine were shown to be safe since no ulcerated lesions were observed at the inoculation sites during the study period, except for local oedemas in individuals within the Sap group at 12 h after inoculation. Moreover, we observed the correlation between the number of inflammatory cells and iNOS expression in the dermis and hypodermis of animals of the Sap and LBSap groups may be explained by the great number of NO-producing cells recruited to the inoculation area, activation of which would contribute to the eradication of the parasite. Thus, we can conclude that dogs immunized by LBSap and the saponin adjuvant elicited a potential innate-immune activation status compatible with effective control of the resistance to infection by *Leishmania* and contributing to a better understanding of the innate-immunity events induced by the LBSap vaccine. Supported by PRONEX - FAPEMIG/CNPq and UFOP.

IM58 - A KILLED LEISHMANIA VACCINE WITH SAND FLY SALIVA EXTRACT AND SAPONIN ADJUVANT DISPLAYS STRONG IMMUNOGENICITY IN DOGS

Giunchetti, R.C.^{1,2}, Braga, S.L.¹, Martins-Filho, O.A.³,
Teixeira-Carvalho, A.³, Malaquias, L.C.C.¹,
Gontijo, N.F.⁴, Brodskyn, C.⁵, Lana, M.^{6,7}, Corrêa-
Oliveira, R.², Reis, A.B.^{1,2,7}

1. Laboratório Imunopatologia, Núcleo de Pesquisas em Ciências Biológicas/NUPEB, Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brasil;
2. Laboratório de Imunologia Celular e Molecular, Instituto de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brasil;
3. Laboratório de Biomarcadores de Diagnóstico e Monitoração, Instituto de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brasil;

4. Laboratório de Fisiologia de Insetos Hematófagos, Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.
5. Laboratório de Imunoparasitologia, Centro de Pesquisas Gonçalo Moniz Fundação Oswaldo Cruz, Salvador, Bahia, Brasil;
6. Laboratório de Doença de Chagas, Núcleo de Pesquisas em Ciências Biológicas, Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais
7. Departamento de Análises Clínicas, Escola de Farmácia, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, Brasil;

Dogs represent the most important domestic reservoirs of *L. chagasi*/*L. infantum*, and a vaccine against canine visceral leishmaniasis (CVL) would be an important tool in the control of visceral leishmaniasis (VL). Saliva of *Lutzomyia longipalpis* contains important proteins that contribute to the success of the infection throughout of immune response modulation on the local of parasite inoculum. In this context, the sand fly saliva proteins were considered as potential antigens to constitute a new vaccine candidate against leishmaniasis. Aiming to perform a detailed analysis of the antigenicity/immunogenicity in dogs a new CVL vaccine composed of *Leishmania braziliensis* antigen, sand fly gland extract (SGE) and saponin adjuvant (LBSapSal vaccine) was studied. In this context, we evaluate the humoral (IgG, IgG1, IgG2) and cellular immune response by immunophenotyping, lymphoproliferation and nitric oxide levels. LBSapSal vaccine elicited strong antigenicity in respect of both anti-SGE and anti-*Leishmania* IgG isotypes. The western blot experiments showed that the major saliva proteins recognized by serum from immunized dogs exhibited molecular weights of 35 and 45 kDa, and were related to the resistance pattern against *Leishmania* infection. Immunophenotypic analysis revealed increased circulating CD21⁺ B-cells and CD5⁺ T-cells, reflected by higher counts of CD4⁺ and CD8⁺ T-cells. The higher frequency in *L. chagasi* antigen-specific CD8⁺ T-lymphocytes, and their positive association with intense cell proliferation, in addition to the progressively higher production of serum nitric oxide levels, suggested that the potential resistance profile elicited by the candidate vaccine was compatible with effective control of the etiological agent of CVL.

Supported by: FAPEMIG, CNPQ, PAPES IIIb/FIOCRUZ, CAPES, UFOP.

IM59 - EFFICACY DURATION OF THE INTRANASAL NAKED LACK-DNA VACCINE AGAINST MURINE VISCERAL LEISHMANIASIS

Schwedersky R.P., Gomes D.C.O., de Mello, L.D.B., Pinto, E.F., Lopes, U.G and Rossi-Bergmann B.

Instituto de Biofísica Carlos Chagas Filho-
UFRJ
danielc@biof.ufrj.br

LACK (*Leishmania* analogue of the receptor kinase C) is a conserved protein of all *Leishmania* species. Previously, we demonstrated that intranasal immunization with a plasmid carrying the LACK gene of *Leishmania infantum* (LACK-DNA) promotes protective immunity against *Leishmania amazonensis*. In the present study, we investigated the systemic expression of intranasally administered LACK-DNA and its ability to induce protection against murine visceral leishmaniasis. By using RT-PCR, we found that BALB/c mice doubly vaccinated intranasally with 30 µg of LACK-DNA expressed LACK mRNA in the spleen, brain, cervical and popliteal lymph nodes by up to 3 months after immunization. Mice vaccinated with i.n. LACK-DNA and challenged i.v. with 10⁷ *L. chagasi* promastigotes 7 days and 3 months, but not 6 months after the second vaccine dose displayed significantly lower parasite loads in the liver and spleen one month after infection. Animals infected 7 days after the booster produced higher amounts of IFN-γ and reduced levels of IL-10 during recombinant LACK recall response in the spleen as compared with infected controls. Spleen cells of animals infected 3 months after booster also showed significant antigen-specific proliferative response and reduced Jones-Mote hypersensitive response as compared with non-vaccinated controls. However, animals infected 6 months after booster were unable to build a significant proliferative response, and responded to in vivo antigen challenge with a TH2-type Jones-Mote hypersensitivity. Together, these data show that the protective intranasal vaccination with LACK-DNA promote systemic expression of the antigen. Studies on the duration of the protective immunity showed that in mice this lasts at least 3 months but is not detectable after 6 months, suggesting that adjuvants should be used to improve the duration of the naked vaccine.

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IM60 - POLYMERIZED PEPTIDES SELECIONED BY PHAGE DISPLAY INDUCE PROTECTION AGAINST *LEISHMANIA AMAZONENSIS*

LIMA, M.P.¹, FUMAGALLI, M.A.², MACHADO DE ÁVILA, R.A.¹, MOREIRA, R.S.M.¹, OLIVEIRA, D.M.¹, ALVES, D.C.R.¹, CAMPOS, A.C.F.A.¹, RIBEIRO, C.C.¹, COSTA, L.E.¹, COELHO, V.T.S.¹, PFEIFFER, V.N.¹, MACHADO, C.M.², TAVARES, C.A.P.¹, CHÁVEZ-OLORTEGI, C.¹, MOTA-SANTOS, T.A.¹, COELHO, E.A.F.^{1,3}

1. Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.
2. Departamento de Farmacologia Bioquímica e Molecular, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.
3. Setor de Patologia Clínica, Coltec, UFMG, Minas Gerais, Brazil.

Leishmania amazonensis is one of the major etiologic agents of a broad spectrum of clinical forms of Leishmaniasis and has a wide geographical distribution in Americas. Control of leishmaniasis in the Americas is a difficult task, because of the zoonotic features of transmission and the sylvatic nature of reservoirs and vectors. In this context, the development of a prophylactic vaccine is strongly desirable. Two peptides (11H and 12A) selected by phage display technology that presented high specificity and affinity by IgGs antibodies purified from sera of dogs with visceral leishmaniasis were synthesized by spot synthesis and purified by affinity chromatography. Peptides were polymerized with glutaraldehyde and BALB/c mice were immunized subcutaneously with three doses, 2-weeks-interval, from polymerized peptides (100 µg per dose) plus Freuds'adjuvant. Thirty days after, mice were challenged with 1x10⁶ promastigotes of *L. amazonensis*. Groups of mice received PBS or Freuds'adjuvant, as control. The parasite load, cellular (IFN-γ, IL-4 and IL-10) and humoral (IgG total, IgG1, IgG2a) immune response were evaluated. Results demonstrated that polymerized peptides induced a Th1 immune response, with high production of IFN-gamma and low levels of IL-4, IL-10 and anti-*L. amazonensis* antibodies. Polymerized peptides showed to be good candidates to compose an effective vaccine against *L. amazonensis*.

Support: FAPEMIG, CNPq, PRPq/UFMG

**IM61 - INVESTIGATION OF ORAL LaAg
VACCINE AGAINST CUTANEOUS
LEISHMANIASIS IN GERM FREE MICE**

diBarros, C.B.A.¹, Bezerra, I.P.S.¹, Carneiro,
M.B.H.², Vieira, L.Q.², Rossi-Bergmann, B.¹.

¹Universidade Federal do Rio Janeiro, Rio de Janeiro, Brazil; ²Universidade Federal de Minas Gerais, Minas Gerais, Brazil.

We demonstrated that oral immunization with 100 µg of total antigen from *Leishmania amazonensis* (LaAg) promastigotes induce protection in BALB/c mice against cutaneous leishmaniasis. We have observed that BALB/c mice decontaminated by antibiotic are equally protected against infection. In this work, we deepen the studies of intestinal microbiota immunological modulation in the LaAg vaccine efficacy using germ-free mice. Germ-free and conventional Swiss/NIH mice were given sterile drink water containing LaAg at 10 µg/ml in the first 2 weeks followed by 40 µg/ml in the last 2 weeks of vaccination, *ad libitum*. Mice were then s.c. infected in the footpad with 2×10^6 GFP-transfected *L. amazonensis* promastigotes and kept under sterile conditions for 6 weeks. Lesion development was measured during 20 weeks. Germ-free mice were naturally more susceptible to leishmanial infection than conventional. This was confirmed by the greater parasite burden, as indicated by the increased fluorescence intensity of the infected tissues. LaAg was effective in both groups. However, protectiveness was detected earlier in conventional mice (week 12), whereas in germ-free mice it was apparent only after week 14, likely due to the greater severity of the illness. Germ free mice responded strongly to the vaccine, and managing to reduce the parasite burden by 45,2%, whereas burden reduction in conventional mice was 20,8%, as compared to the respective non-vaccinated mice. These results corroborate with our previous findings using antibiotic-decontaminated BALB/c mice and demonstrate that the presence of the natural microbiota confers enhanced resistance to *L. amazonensis* infection, but is irrelevant for the effectiveness of the oral LaAg vaccine.

**IM62 - INFLUENCE OF RETINOL ON THE
EFFECTIVENESS OF THE ORAL LaAg
VACCINE AGAINST CUTANEOUS
LEISHMANIASIS**

Bezerra, I.P.S.; diBarros, C.B.A.; Rossi-
Bergmann, B.

Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Retinoic acid is an immunomodulator factor, which in the presence of TGF-beta activates differentiation of naive T cells in mesenteric lymph nodes to Foxp3+ regulatory T cells, which display tolerance induction in the intestine. We have shown that oral immunization with 100 µg of *Leishmania amazonensis* promastigotes total antigen (LaAg) induces protection in BALB/c mice against cutaneous leishmaniasis. Influence of dietary retinol on the effectiveness of the oral vaccine was investigated. Four groups of BALB/c mice were fed with pelleted food without vitamins and water supplemented with the following vitamins: D₃ (40 UI/mL), E (10 µg / mL), B₁ (40 µg/ml), B₂ (15 µg/ml), B₆ (20 µg/ml), B₁₂ (0,048 µg/ml), B₃ (100 µg/ml), C (2,5 µg/ml) throughout the experiment. Two of the groups (Vit A+) also received vitamin A (450 UI/mL). Ten days after starting the diet, Vit A+ and Vit A- animals were vaccinated with two doses of LaAg (100 µg) orally. Controls received PBS. Seven days after the last dose, animals were infected subcutaneously in the footpad with 2×10^6 *L. amazonensis* promastigotes. Non-vaccinated Vit A+ animals were shown to be slightly more susceptible to infection than Vit A-. LaAg vaccine effectively controlled lesion growth in Vit A+ but not in Vit A-. Parasite burden on day 90 of infection corroborated the lesion sizes. These results suggest that the presence of retinol in the diet is important for the effectiveness of the oral LaAg vaccine. It is feasible that retinol-sensitized Foxp3+ regulatory T cells mediated oral immune tolerance against parasite deleterious antigens, preventing the development of unwanted peripheral Th2 responses and resulting in a more effective response against infection.

**IM63 - PROTECTION INDUCED AGAINST
LEISHMANIA CHAGASI BY RECOMBINANT
BACTERIOPHAGES-BASED ANTIGENS
SELECTED BY PHAGE DISPLAY
TECHNOLOGY**

COELHO, V.T.S.¹, MARTINS, V.T.¹, MOREIRA, R.S.M.¹, OLIVEIRA, D.M.¹, LIMA, M.P.¹, ALVES, D.C.R.¹, FERREIRA, A.A.C.¹, COSTA, L.E.¹, PFEIFFER, V.N.¹, FUMAGALLI, M.A.², MACHADO, C.M.², TAVARES, C.A.P.¹, RIBEIRO, C.C.¹, MACHADO DE ÁVILA, R.A.¹, CHÁVEZ-OLORTEGI, C.¹, MOTA-SANTOS, T.A.¹, COELHO, E.A.F.^{1,3}

1. Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.
2. Departamento de Farmacologia Bioquímica e Molecular, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.
3. Setor de Patologia Clínica, Coltec, UFMG, Minas Gerais, Brazil.

Visceral leishmaniasis (VL) is a progressive fatal disease considered a worldwide health problem. Infected domestic dogs are the most important reservoir hosts of the parasites from which the disease can be transmitted to humans. Vaccines are considered an important prophylactic measure from control to canine VL. Phage display technology was used for selecting phage clones expressing peptides that present high affinity with specific IgG antibodies purified from sera of dogs infected with *Leishmania chagasi*. Several bio-pannings cycles were performed to elevate the specificity and affinity from these phage clones. Five of them were selected for the best specificity to anti-*Leishmania* antibodies. BALB/c mice were immunized subcutaneously with three doses, 2-weeks-interval, from each phage clone (1×10^{12} transducing units per mL) or a mix of them (1×10^{12} TU/mL each one) and 30 days later mice were challenged with 1×10^7 promastigotes of *L. chagasi*. Groups received PBS or sylvester phage clone, as control. The parasite load and cellular (IFN- γ , IL-4 and IL-10) and humoral (IgG total, IgG1, IgG2a) immune response were evaluated. Results demonstrated that phage clones induced an elevated production of IFN-gamma and low levels of IL-4, IL-10 and antibodies anti-*L. chagasi*. The selected phages clones might be candidates to compose an effective vaccine against *L. chagasi* infection.

SUPPORT: FAPEMIG, CNPq, PRPq/UFMG

**IM64 - IMMUNIZATION OF DOGS IN AN
ENDEMIC AREA OF CANINE VISCERAL
LEISHMANIASIS WITH A RECOMBINANT
CYSTEINE PROTEINASE FROM LEISHMANIA
(LEISHMANIA) CHAGASI**

PINHEIRO, P.H.C.^{1,2}; FERREIRA, J.H.L.³; DIAS, S.S.⁴; KATZ, S.⁴; BARBIÉRI, C.L.⁴

- ¹Faculdade NOVAFAPI, Teresina, Piauí, Brazil;
²Faculdade de Ciências Médicas-FACIME/UESPI, Teresina, Piauí, Brazil; ³Universidade Federal do Piauí, Teresina, Piauí, Brazil; ⁴Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil.

In a previous study we demonstrated that a recombinant cysteine proteinase from *Leishmania (L.) chagasi*, rLdccys1, is a suitable immunological marker for several stages of visceral leishmaniasis (VL) in humans and dogs, as well as an appropriate antigen for serodiagnosis of human visceral leishmaniasis by ELISA assay. We also showed by use of rLdccys1 in ELISA assays and DTH reactions the inverse correlation between humoral and cellular responses during the development of canine visceral leishmaniasis. In the present study, the rLdccys1 antigen was used for immunization of dogs in Teresina, Piauí, an important endemic area of VL in Brazil. The immunization of non-infected dogs with three subcutaneous doses of rLdccys1 plus *Propionibacterium acnes* as adjuvant resulted in secretion of IFN- γ (1.620 pg/ml), whereas IL-10 could not be detected in the sera from immunized dogs. The rLdccys1-immunized dogs were challenged with 1×10^4 *Leishmania (L.) chagasi* amastigotes by endovenous route and showed a significant protection in comparison with controls immunized with either saline or the adjuvant alone. These results encouraged us to extend the immunization protocols with rLdccys1 and the next step involved the immunization of dogs with rLdccys1 and challenge by bite of the VL vector. Colonies of *Lutzomyia longipalpis* were infected by feeding them with dog blood containing *L. (L.) chagasi* promastigotes. A proportion of 25 bloodfed sandflies per dog were used for challenge. The evaluation of parasite load and clinical signs of VL in rLdccys1-immunized dogs are currently in progress. Supported by FAPESP, Faculdade NOVAFAPI and FACIME/UESPI.

IM65 - Heterologous prime-boost vaccination using plasmid DNA and recombinant adenovirus 5 elicits protective immunity against a human parasite through a mechanism dependent on perforin.

DE ALENCAR, B. C.^{1,2}, PERSECHINI, P.³, BRUNA-ROMERO, O.⁴,
GAZZINELLI, R. T.⁵ AND RODRIGUES, M. M.^{1,2}

¹ Centro Interdisciplinar de Terapia Gênica (CINTERGEN) and

² Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo-Escola Paulista de Medicina, São Paulo, SP, Brazil.

³ Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ, Brazil.

⁴ Departamento de Microbiologia, and

⁵ Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais e Centro de Pesquisas René Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil.

Introduction and Objectives: Heterologous prime-boost strategies using plasmid DNA followed by replication-defective recombinant Adenoviruses 5 are being proposed as potent prophylactic vaccines against infectious diseases. The success of these strategies is credited to the fact that strong CD8 Tc1 immune response can be generated. In spite of the efficiency described, the protective mechanisms mediated by specific CD8+ T cells are still largely debatable. Because immunity to *T. cruzi* infection is largely dependent on the activation of specific CD8+, we considered this model interesting to determine whether perforin was a critical mediator of immunity generated by vaccination. **Results:** Here we describe that, as expected, heterologous prime-boost strategy using plasmid DNA and adenovirus 5 vectors expressing the gene encoding the Amastigote Surface Protein-2 can be successfully used to generate protective immunity against experimental infection with a human protozoan parasite, *Trypanosoma cruzi*. Indeed, we observed that following immunization with recombinant adenovirus alone or after DNA prime adenovirus-boost, CD8 epitope-specific *in vivo* cytotoxicity was largely dependent on perforin. In contrast, the absence of perforin did not significantly change the number of epitope-specific, interferon- γ -producing CD8+ cells detected *ex vivo* by ELISPOT. Following challenge with *T. cruzi*, vaccinated perforin-deficient mice controlled the acute parasitemia, however were unable to survive. **Conclusion:** We concluded that after heterologous-prime boost

vaccination, mouse survival was critically dependent on perforin. This result provides the first evidence of a CD8+ T cell-mediated anti-parasitic mechanism generated by a recombinant viral vaccine and may have important implications for the evaluation of immunological status against this parasitic infection. **Financial support:** FAPESP and CNPq.

IM66 - ESSENCIAL ROLE OF NOD-LIKE RECEPTORS FOR DETECTION AND CONTROL OF TRYPANOSOMA CRUZI.

SILVA, G.K.^{1,2}, GUTIERREZ, F. R.², GUEDES, P. M. M.², SANTIAGO-SILVA, J.², SILVA, J. S.², ZAMBONI, D. S.¹

¹ Department of Cell Biology and Microbial Pathogenesis and ² Department of Immunology and Biochemistry, University of São Paulo, Medical School Ribeirão Preto. FMRP/USP.

Chagas disease is induced by the intracellular parasite *Trypanosoma cruzi*. Previous studies have demonstrated the Toll-like receptors are essential for parasite recognition via its adaptor molecule MyD88. However, MyD88-independent responses were also observed. Recently, it was described a new family of pattern recognition receptors, called Nod-like receptors (NLRs). Among NLRs are Nod1 and Nod2, which operates in the host cell cytoplasm and trigger NF- κ B activation pathway. Because previous studies demonstrated a MyD88-independent response upon *T. cruzi* infection we aimed to determine the contribution of Nod1 and Nod2 in the immune response against this parasite. We found that macrophages deficient for Nod1 and Nod2 but not wild type (WT), fails to induce expression of co-stimulatory molecules, such as CD80, which expression is dependent of NF- κ B pathway. Although Nod1-/- macrophages were as susceptible as Nod2-/- and WT, they fail to eliminate intracellular parasites in the presence of IFN- γ . To test the susceptibility of these animals *in vivo*, WT, Nod1-/-, or Nod2-/- animals were infected with 1000 forms of *T. cruzi* Y strain. Parasites in bloodstream, heart inflammation and survival were measured. The results show that Nod1-/- mice present higher amounts of parasites in the bloodstream when compared with WT and Nod2-/. Accordingly, Nod1-/- animals show 100% mortality by day 25, whereas WT and Nod2-/- did not die. The inflammatory infiltrate in myocardium of Nod1-/- mice was reduced compared with that

of WT and Nod2 *-/-*. Together, these results suggest that Nod1 is an important innate immune receptor, which operates in the signaling responses against *T. cruzi* infection. This seems to be the first report of the role of NLRs in the recognition and control of a parasite infection. Financial Support: FAPESP, WHO/TDR, CNPq and PEW Latin America.

IM67 - RECOMBINANT ADENOVIRUSES EXPRESSING TRANS-SIALIDASE (TS) ANTIGEN AND AMASTIGOTE SURFACE PROTEIN 2 (ASP2) INDUCED PROTECTIVE IMMUNITY AGAINST CHALLENGE WITH *Trypanosoma cruzi* MYOTROPIC STRAINS

¹Marques, V.F., ¹Terra, M.A.B.L., ^{2,3}Caetano, B.C., ^{1,4}Silva, A.A., ¹Silvério, J.C., ²Machado, A.V., ⁵Bruña-Romero, O., ⁶Rodrigues, M.M., ^{2,3}Gazzinelli, R.T., ¹Lannes-Vieira, J.

1-Laboratory of Autoimmunity and Immunoregulation, IOC, Fiocruz, Rio de Janeiro, Brazil

2-Laboratory of Immunopathology, CPqRR, FIOCRUZ, Belo Horizonte, Brazil

3-Department of Biochemistry and Immunology, UFMG, Belo Horizonte, Brazil

4-Department of Pathology, UFF, Niterói, Brazil

5-Department of Microbiology, UFMG, Belo Horizonte, Brazil

6-Department of Microbiology, Immunology and Parasitology, UNIFESP, São Paulo, Brazil

Introduction and Objectives: Chagas disease affects 16-18 million people in Latin America, being one of the main causes of death in this region. The illness is mainly characterized by intense chronic myocarditis associated with heart dysfunction. Currently, efficient drugs for treatment of chronic form of the disease are not available. Two antigens of *T. cruzi*, trans-sialidase (TS) antigen and amastigote surface protein 2 (ASP2), when associated, are highly protective, reducing parasitemia, parasitism and mortality in mice strains with differential susceptibilities to *T. cruzi* infection when challenged with the highly virulent Y strain. These studies led us to construct TS and ASP2-expressing recombinant adenoviruses, that present high capacity of cellular infection, are highly immunogenic and serve as vehicles for vaccine. In the present study, we evaluated the cellular and humoral immunity induced by vaccination with both TS and ASP2-expressing

recombinant adenoviruses and analyzed the protective capacity of this vaccine against challenge with CL-Brener and Colombiana *T. cruzi* strains. **Methods and Results:** C57BL/6 mice were immunized with rAdASP2+rAdTS in prime-boost protocol, control groups were vaccinated with adenovirus expressing an irrelevant protein (rAdLacZ) or injected with saline. rAdASP2+rAdTS vaccination induced CD8⁺ T cells with CTL activity and IFN- γ production. When mice were challenged with the myotropic CL-Brener strain a protective effect of rAdASP2+rAdTS vaccination, verified by reduction of parasitemia, cardiac parasitism and CK-MB activity (marker of cardiomyocyte lesion) was observed. After challenging with the low virulence Colombiana strain of *T. cruzi* no difference in parasitemia and CK-MB levels in serum. However, a trend to decreased cardiac parasite burden, associated to reduction of cardiac electrical changes (ECG) in chronic phase of infection, was detected in vaccinated *T. cruzi*-challenged mice. **Conclusion:** Our results show that prime-boost protocol using rAdASP2+rAdTS is promising for further studies using prophylactic and even therapeutic protocol against Chagas disease.

Support: IMTEV-CNPq, CNPq, FIOCRUZ, PIBIC CNPq/Fiocruz.

Key-Words: *Trypanosoma cruzi*; Chagas Disease; Vaccine; Adenoviruses.

IM68 - PROTECTIVE IMMUNITY AND LATE STAGE CHRONIC SYMPTOMS IN GENETICALLY VACCINATED HIGHLY SUSCEPTIBLE MICE CHALLENGED WITH COLOMBIA STRAIN (*T. cruzi* II) OF *TRYPANOSOMA CRUZI*

HAOLLA, F.A.B.¹, CLASER, C.¹, DE ALENCAR, B.C.G.¹, SILVEIRA, E.L.V.¹, DE OLIVEIRA, G.M.², SILVÉRIO, J.C.², MACHADO, A.V.², LANNES-VIEIRA, J.², GAZZINELLI, R.T.^{2,3}, BRUNA-ROMERO, O.³, AND RODRIGUES, M.M.¹

¹ Centro Interdisciplinar de Terapia Gênica (CINTERGEN), UNIFESP-EPM,

² Instituto Oswaldo Cruz and Instituto Renée Rachou, FIOCRUZ,

³ Departamento de Microbiologia, and Departamento de Bioquímica e Imunologia, UFMG.

Introduction and objectives: In earlier studies, we demonstrated protective immunity against

experimental infection with *T. cruzi* parasites of the Y strain by the genetic immunization of highly susceptible mice with *asp-2* and *TS* genes (Hum Gene Ther. 2004. 15:878-86). The aim of the present study was to evaluate, using this same mouse model, whether protective immunity could also be elicited against a challenge with parasites of the Colombia strain, a *T. cruzi* II strain isolated from a Colombian triatomine. **Methods and Results:** Highly susceptible A/Sn mice were vaccinated with different protocols using recombinant plasmids and/or adenoviruses expressing the *asp-2* and *ts* genes. Vaccinated and control mice were challenged i.p. with a lethal dose of bloodstream trypomastigotes (250/mouse). The vast majority (80-100%) of mice vaccinated with *T. cruzi* genes survived the lethal infection. On the other hand, 100% of control mice injected with irrelevant genes died. After 220 days of infection (late stage chronic phase), we evaluated the health status of the vaccinated mice by ECG. We found that many animals developed cardiac alterations compatible with the symptoms described for individuals with chronic chagasic cardiomyopathy, such as partial atrioventricular block and sinus bradycardia. **Conclusion:** Our results confirm and extend our previous published observations that vaccination with *T. cruzi* genes provides strong protective immunity against a lethal challenge in highly susceptible mice. However, in contrast to our previous observation, mice infected with the Colombia strain still developed late stage chronic chagasic cardiomyopathy symptoms. These results suggest that in highly susceptible hosts, a higher or broader degree of protective immunity will have to be elicited in order to avoid the development of late stage cardiologic symptoms following infection with any strain. **Financial support:** Supported by FAPESP and CNPq (Millennium Institutes).

IM69 - IL-17 REGULATES *Trypanosoma cruzi*-INDUCED MYOCARDITIS IN MICE

GUEDES, P.M.M.; GUTIERREZ, F.R.S.; MAIA, F.L.; PAVANELLI, W.R.; SILVA, J.S.*

Department of Biochemistry and immunology,
School of Medicine at Ribeirão Preto, University of
São Paulo, Ribeirão Preto, São Paulo, Brazil
(*e-mail for contact: jsdsilva@fmp.usp.br)

IL-17 is involved in the pathogenesis of several autoimmune, inflammatory and infectious diseases. As the infection with *T. cruzi* results in a heart inflammatory disease with possible

autoimmune environment, this study aimed to evaluate the role of IL-17 in the chagasic cardiomyopathy. *T. cruzi* infected mice were treated with anti-mouse IL-17 MAb or rat IgG. Animals infected and treated with anti IL-17 showed premature mortality when compared to IgG-treated controls. Moreover, it was observed an enhancement of inflammatory cytokines (TNF- α and IFN- γ) production and cardiac inflammatory process of anti-IL-17 treated animals, and 40% of the animals suffered from cardiomegaly. IL-17 expression was detected in the CD4⁺, CD8⁺ and NK cells of the cardiac inflammatory infiltrate and splenocytes, at 14 days post-infection with *T. cruzi* Y strain. Our results indicate that IL-17 is important for the control of cardiac inflammation by negative feedback of TNF- α and IFN- γ production during experimentally *T. cruzi* infection, modulating heart immunopathological process of Chagas disease.

Financial support: CNPq, CAPES, FAPESP and USP.

IM70 - DEVELOPMENT OF AN EXPERIMENTAL VACCINE BASED ON M2-MAEBL ANTIGEN AGAINST *Plasmodium yoelii* EXPERIMENTAL INFECTION

LEITE, J. A.¹, BARGIERI, Y. D.², LOPES, S. C. P.¹, RODRIGUES, M. M.^{2,3}, SOARES, I. S.⁴, COSTA F. T. M.^{1,5}

¹ Department of Microbiology & Immunology, State University of Campinas – UNICAMP, Campinas, SP, P.O. Box 6109, 13083-862, Brazil

² Interdisciplinary Center of Gene Therapy - CINTERGEN, Federal University of São Paulo, São Paulo, SP., Brazil.

³ Department of Parasitology, State University of São Paulo – USP, São Paulo, SP, Brazil.

⁴ Department of Toxic Clinic Analysis, State University of São Paulo - USP, São Paulo, SP, Brazil.

⁵ Department of Parasitology, UNICAMP.

Malaria continues to be a major health problem in many parts of the world, causing 300–500 million new infections and 1-2 million deaths each year. One of the principal reasons for the high morbidity and mortality is the widespread presence of drug-resistant strains of the parasite. Moreover, immunoprotection achieved by the most successful malaria vaccine was partial and short lived. MAEBL is a chimeric molecule expressed in infected erythrocytes (IE) displaying features of

AMA-1 (apical membrane antigen) and EBP (erythrocyte binding proteins) antigens. MAEBL possesses an amino terminal cysteine-rich, a transmembrane and a cytoplasmatic domain and two other domains (M1 and M2) involved in parasite attachment. Of note, MAEBL has a high homology with its correspondent in *P.falciparum* and its M2 domain, essential for merozoite invasion, displays adhesive capacity higher than the M1. Recently, it was shown that MAEBL is also expressed in salivary glands sporozoites and in infected hepatocytes. MAEBL unique features open perspectives for the development of an experimental vaccine targeting erythrocytic and pre-erythrocytic stages of the parasite. Here we amplified, cloned and expressed a recombinant protein corresponding to the MAEBL M2 domain of *Plasmodium yoelii*. C57BL/6 mouse immunized with four doses of rM2-MAEBL (5 µg / animal) in complete/incomplete Freund adjuvant induced higher levels of anti-recombinant antibodies in ELISA. Moreover, in immunofluorescence assays (IFA) anti-rM2-MAEBL recognized the native protein on *P. yoelii* and, at lower levels, in *P. falciparum* merozoites. After challenge, with 10⁶ IE of *P. yoelii* YM (lethal strain), we observed 30% protection and a significant reduction on parasitemia levels. Collectively, these data demonstrate that rM2-MAEBL display similarities with its homologue and heterologue native antigen. Thus, this recombinant protein can be used to achieve higher levels of immunoprotection upon a challenge with salivary glands sporozoites. This project is supported by Capes, CNPq and Fapesp.

IM71 - TLR-5 AGONIST FLAGELLIN CAN SUCCESSFULLY ELICIT PROTECTIVE IMMUNITY WHEN ADMINISTERED WITH THE MALARIA VACCINE CANDIDATE MSP1(19).

Bargieri, D.Y.¹, Rosa, D.S.¹, Braga, C.J.M.², Carvalho, B.O.³, Costa, F.T.M.³, Soares, I.S.², Ferreira, L.C.S.² and Rodrigues M.M.¹

¹Cintergen, UNIFESP-EPM, São Paulo-SP, Brazil.

²Universidade de São Paulo, São Paulo-SP, Brazil.

³Universidade Estadual de Campinas, Campinas-SP, Brazil.

Objectives: We evaluated the immune response and protective immunity provided by vaccination of mice with recombinant proteins derived from *Plasmodium vivax* or *Plasmodium yoelii* 19kDa C-terminal region of the merozoite surface protein 1 (MSP1(19)) administered with the TLR-5 activator

FliC (flagellin) of *S. Typhimurium*. The adjuvant FliC was molecularly fused or not to the malaria antigen. **Methods:** C57BL/6 mice were immunized i.n. or s.c. with different doses of the purified recombinant proteins His(6)PVMSP1(19) linked or not to the Pan-allelic DR epitope (PADRE), or His(6)PYMSP1(19), in the presence or not of FliC, or with the recombinant fusion proteins His(6)FliC-PVMSP1(19)-PADRE or His(6)FliC-PYMSP1(19). Antibody immune responses were detected in sera by ELISA. Cellular immune responses were determined by secretion of IFN-γ after *in vitro* stimulation of spleen cells. Mice immunized with the recombinant proteins derived from *P. yoelii* were challenged and the course of the infection was followed by regular blood smears. **Results:** We observed strong antibody responses in mice immunized with the recombinant proteins either fused or mixed with FliC when given in high doses (up to 25 µg). In doses as low as 0.1 µg, the fused protein was more efficient in eliciting specific humoral immune response. Antibody titers of mice immunized with recombinant protein His(6)PVMSP1(19) alone were negligible. Immunization with the fusion protein His(6)FliC-PVMSP1(19)-PADRE induced cellular immune response after stimulation with His(6)PVMSP1(19), whereas the non-fused proteins did not. Immunization with His(6)FliC-PYMSP1(19) protected 40% of mice after challenge. Mice immunized with His(6)PYMSP1(19) in the presence of FliC were not protected. **Conclusions:** We show that the TLR-5 agonist FliC of *S. Typhimurium* can be used as carrier/adjuvant to induce humoral and cellular immune responses against recombinant proteins derived from *P. vivax*. This new approach can be useful to improve the immunogenicity of current and future malaria vaccine candidates. **Support:** FAPESP and CNPq.

IM72 - PRE-CLINICAL IMMUNIZATIONS AGAINST MALARIA USING A RECOMBINANT PROTEIN BASED ON DOMAIN II OF PLASMODIUM VIVAX APICAL MEMBRANE ANTIGEN 1 (PvAMA-1)

GENTIL, F.¹, PATRÍCIO, M.B.M.¹, BARGIERI, D.Y.², PALATNIK-DE-SOUSA, C.B.³, RODRIGUES, M.M.², SOARES, I.S.¹

¹Universidade de São Paulo, São Paulo, Brazil;

²Universidade Federal de São Paulo, São Paulo,

Brazil;³Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Introduction: Earlier we described the antibody immune responses in individuals from malaria endemic areas of Brazil against recombinant proteins based on different domains of *Plasmodium vivax* AMA-1 (PvAMA-1). Our results indicated that proteins containing domain II (DII) were particularly immunogenic during *P. vivax* infection. Aimed at using the DII of PvAMA-1 as part of a sub-unit vaccine against malaria, here we compared the immunogenicity of this recombinant protein following immunization in the presence of different adjuvant formulations.

Methods and Results: Six to eight-week-old female BALB/c mice were immunized, two weeks apart, via the s.c. route with a recombinant protein (10 µg/animal) containing DII of PvAMA-1 in the presence of the adjuvants CFA/IFA, Alum, Quil A, TiterMax Gold, QS-21 or CpG ODN 1826. After three doses, IgG antibodies titers against PvAMA-1 ectodomain were estimated in the sera of immunized mice by ELISA. We found that animals developed high specific IgG1, IgG2a and IgG2b levels when the recombinant antigen was administered in the presence of CFA/IFA, Quil A, TiterMax Gold, QS-21 or CPG ODN 1826. However, when administered in presence of Alum, the antibody titers to PvAMA-1 were significantly lower than the titer obtained with other formulations. **Conclusion:** The results described here indicated that the recombinant protein PvAMA-1 (DII) is highly immunogenic in mice when administered in different adjuvant formulations. We will use this study as a guideline for future pre-clinical trials in non-human primates and the development of a recombinant protein that can be used as a sub-unit vaccine against malaria.

Financial support: FAPESP, CNPq (Millennium Institute-IMTEV) and FAPERJ.

IM73 - EVALUATION OF THE EFFECT OF INTESTINAL MICROBIOTA IN MICROBICIDE ACTIVITY IN *LEISHMANIA MAJOR*-INFECTED MACROPHAGES

CARNEIRO, M.B.H.¹, MACEDO, J.P.¹, VIEIRA, L.Q.¹

¹Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Minas Gerais, Brasil.

Animals are colonized with their indigenous microbiota from the first day of life. The estimate

number of associated bacterial cells in a human being is around of 10^{14} , most of them in the gut. Several studies have investigated the microbiota-host relationship and the use of germ-free animals has been an important tool in these studies. These animals, when infected with a pathogen, have shown to be sometimes more resistant and other times more susceptible to infection. Previous studies showed that, during infection with *Leishmania major*, Swiss/NIH germ-free mice developed a typical Th1 response, but fail to heal lesions, while conventional mice developed the same response and controlled the infection. A Th1 response is clearly related with healing and parasite clearance in this infection. Thus, our aim in this study is to evaluate a possible adjuvant effect of the microbiota in the microbicide activity of macrophages. Therefore, we infected resident peritoneal macrophages from germ-free and conventional Swiss/NIH mice *in vitro* with *L. major* amastigotes, and measured NO and TNF-α as markers of a pro-inflammatory profile. We also assayed IL-10 production and the arginase activity, as markers of an anti-inflammatory profile. Our results showed that macrophages from germ-free animals surprisingly produced more NO and TNF-α than cells from conventional mice. However, germ-free macrophages infected with *L. major* also produced more IL-10 and showed higher arginase activity. When we counted the number of parasites within the macrophages, we found that, in the presence of IFN-γ, conventional macrophages were more efficient to kill the parasite. These data suggest that, although germ-free animals can produce large amounts of NO and TNF-α, they would be less efficiently to eliminate the parasites because of their higher production of IL-10 and higher arginase activity. Support: CNPq, CAPES, FAPEMIG

IM74 - ANTI-TUMOR NECROSIS FACTOR THERAPY IN ACUTE EXPERIMENTAL CHAGAS DISEASE

Pereira, I. R.¹, Vinagre, N. F.¹, Lannes-Vieira, J.¹

¹Laboratório de Pesquisas em Auto-imunidade e Imuno-regulação, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ, Brasil.

Trypanosoma cruzi molecules induce activation of the transcription factor NFκ-B leading to TNF production. Acting on TNFR1/p55, TNF induces the production of chemokines and nitric

oxide (NO), showing the participation of TNF in parasite growth control, revealing also the potential role of this cytokine in CD8-enriched myocarditis formation and heart dysfunction. In this context, TNF plasma levels are directly related to decrease in ejection fraction of the left ventricle in patients with Chagas disease, suggesting that unbalance in TNF production is directly related to the progression of chronic *T. cruzi*-elicited myocarditis. Our main goal is to contribute to the understanding of the molecular mechanisms involved in the generation and progression of Chagas cardiomyopathy, as well as to design strategies for rational intervention in heart inflammation, potentially involved in the generation of fibrosis and heart dysfunction, without interfering in parasite growth control. In the present work, we investigated the participation of TNF in the pathogenesis and immunoregulation of chagasic myocarditis, the molecular mechanisms by which TNF control the migration of inflammatory cells for cardiac tissue, using immunocompetent murine models of experimental *T. cruzi* infection and strategies for TNF production blockade. Our data indicate that, as we predicted (Kroll-Palhares et al., 2008), TNF blocking therapy reduced the influx of CD8+ T cells into the heart tissue, but increased the parasitism during the acute *T. cruzi* infection. Further, TNF blockade also aggravates heart dysfunction. Thus, our data suggest that TNF blockade during the acute infection is not beneficial.

Support: Universal-2006/CNPq, IC/CNPq, Bolsa de Produtividade/CNPq, FAPERJ.

IM75 - IMMUNOTHERAPY WITH RECOMBINANT BACTERIOPHAGES-BASED EPITOPES AGAINST *LEISHMANIA AMAZONENSIS* INFECTION

COSTA, L.E.¹, FERREIRA, A..A.C.¹, OLIVEIRA, D.M.¹, MOREIRA, R.S.M.¹, LIMA, M.P.¹, ALVES, D.C.R.¹, PFEIFFER, V.N.¹, COELHO, V.T.S.¹, FUMAGALLI, M.A.², MACHADO, C.M.², TAVARES, C.A.P.¹, CHÁVEZ-OLORTEGI, C.¹, MOTA-SANTOS, T.A.¹, COELHO, E.A.F.^{1,3}

1. Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.

2. Departamento de Farmacologia Bioquímica e Molecular, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.

3. Setor de Patologia Clínica, Coltec, UFMG, Minas Gerais, Brazil.

Leishmaniasis caused by *Leishmania amazonensis* is a severe disease and it can be fatal if untreated. This work was developed to assess the immunotherapeutic potential of the vaccine based in epitopes exposed in recombinant bacteriophages against *L. amazonensis* infection. Five bacteriophage clones selected by phage display, displayed peptides specific and reactive to anti-*Leishmania* antibodies, were used to immunize BALB/c mice infected with *L. amazonensis*. The vaccine was injected on month 2 after infection, when animals presented lesions development in the footpad swellings about 2-3 mm. Animals received five doses of the vaccine, 2-weeks interval, using 5×10^{12} TU (transducing units) per mL of each phage clone. Mice that received clones vaccine showed significant reduction of lesion size when compared to control groups (saline or sylvester bacteriophage M13 which not expressed peptides). An elevated IFN-gamma production, low levels of IL-4, IL-10 and anti-SLA IgG antibodies was detected in these mice. These results confirmed the immunotherapeutic potential of the recombinant bacteriophage M13-based epitopes vaccine, its Th1-mediated immune response and its potential use for the control of leishmaniasis caused by *L. amazonensis*.

SUPPORT: FAPEMIG, CNPq, PRPq/UFMG

IM76 - COLLOIDAL GOLD PARTICLES ADSORBED WITH *TOXOPLASMA GONDII* ANTIGEN FOR USE IN RAPID IMMUNOCHROMATOGRAPHIC TEST

MIORANZA, S. L.^{1,2}, MEIRELES, L. R.³, ANDRADE Jr, H. F.³

¹ Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil; ² Universidade Estadual do Oeste do Paraná, Paraná, Brazil; ³ Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, São Paulo, Brazil.

Toxoplasmosis, caused by a protozoan, *Toxoplasma gondii*, is probably the most widespread human parasitic infection. Mostly unapparent infections, toxoplasmosis can develop severe systemic illness, as in fetal infection, in which the mother, when infected for the first time during pregnancy, can present parasitemia with focal lesions within the placenta, infecting the

fetus. The treatment of acute infected pregnant women is obligatory and development of rapid immunochromatographic test with colloidal gold particles could provide in office diagnosis during prenatal care, with early intervention. Here, we standardize the use of colloidal gold particles adsorbed with antigen of *Toxoplasma gondii* for use in that quick test. Colloidal gold suspensions were produced, using sodium citrate and/or tannic acid for specific size production, with transmission electron microscopy control. Efficiency of flow trough and antigen binding was analyzed by Dot-Blot in nitrocellulose membrane, adsorbed with two dots, one with anti-*T.gondi* IgG, as index of particle specificity, and another with staphilococcal Protein A, for binding of particles recovered with IgG present in serum. Colloidal 5, 10, 16, 20 and 24 nm gold particles revealed size quality similar to the commercial sources. The adsorption of antigen was higher in pH 8.0 and 9.0 with better stability for 16 and 20 nm antigen recovered gold particles, with 5-10 µg ag / ml of gold particles. The particles adsorbed to the antigen presented appropriate visual discrimination after reaction of Dot Blot with positive (two stained dots) and negative serum (one stained dot). These conjugates allow appropriate staining for application in the development of rapid immunochromatographic test for detection of toxoplasmosis, using specific devices for in office quick diagnostic serology in mothers at risk of congenital toxoplasmosis. Financial support: Capes and LIMHCFMUSP-49.

IM77 - Evaluation of excreted-secreted antigens of *L.(L.)chagasi*, *L.(L.)amazonensis* and *L.(V.)braziliensis* in serodiagnosis of canine visceral leishmaniasis.

*PINEDO-CANCINO VIVIANA*¹, *LAURENTI MÁRCIA DALASTRA*², *KESPER NORIVAL JUNIOR*¹, *UMEZAWA EUFROSINA SETSU*¹

¹ Instituto de Medicina Tropical de São Paulo da Universidade de São Paulo, Laboratório de Protozoologia, Av. Dr. Eneas de Carvalho Aguiar 470. São Pulo (SP), Brasil.

² Faculdade de Medicina da Universidade de São Paulo, Laboratório de Patologia de Moléstias Infeciosas (LIM-50)

Leishmaniasis cause significant morbidity and mortality to hosts. Dogs which are infected with *Leishmania* serve as major reservoir hosts for zoonotic visceral leishmaniasis in endemic areas. The incidence of zoonotic visceral leishmaniasis is

rising in Brazil, and we need urgently for a specific and sensitive screening tool for identification of infected host. We present here an evaluation of serological performance of ELISAs for detection of IgG antibodies in dogs with canine visceral leishmaniasis. The assays were based on the utilization of excreted-secreted (exoantigens) antigens of promastigote forms of *L.(L.)chagasi*, *L.(L.)amazonensis* and *L.(V.)braziliensis*. They were obtained in a protein-free medium, used without purification. The results were compared with those obtained from total extract of promastigote forms. In this preliminary study we used samples from 89 dogs with visceral leishmaniasis, from Araçatuba-Brazil. Among them, 53 dogs were parasite-positive by immunohistochemistry in viscera and/or skin (37 were classified as symptomatic and 16 as asymptomatic); and 36 dogs were parasite-negative. Twenty healthy sera dogs and 23 dogs with other infections (*T. cruzi*, *T. evansi*, *Babesia* sp, *Ehrlichia* sp, *Neospora* sp, *Toxoplasma* sp) were used to calculate the specificity index. ELISA-exoantigens showed better sensitivity and specificity than ELISA-extract. Exoantigens from *L.(L.)chagasi* showed the best indices of sensitivity and specificity, which correlated 100% with parasitological diagnosis. Based on the indices of sensitivity and specificity the ELISA-exoantigens may prove useful for screening dogs with visceral leishmaniasis in different geographical regions including those living in areas endemic for *T. cruzi*.

IM78 - PHAGE DISPLAY AND SPOT SYNTHESIS USED TO SELECT PEPTIDES IN ORDER TO DEVELOPING AN IMMUNODIAGNOSTIC KIT FOR CANINE VISCERAL LEISHMANIASIS

*PFEIFFER, V.N.*¹, *MACHADO, C.M.*², *ALVES, D.C.R.*¹, *MOREIRA, R.S.M.*¹, *OLIVEIRA, D.M.*¹, *LIMA, M.P.*¹, *CAMPUS, A.C.F.A.*¹, *COSTA, L.E.*¹, *COELHO, V.T.S.*¹, *FUMAGALLI, M.A.*², *RIBEIRO, C.C.*¹, *MACHADO de ÁVILA, R.A.*¹, *TAVARES, C.A.P.*¹, *CHÁVEZ-OLORTEGI, C.*¹, *MOTA-SANTOS, T.A.*¹, *COELHO, E.A.F.*^{1,3}

1. Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.
2. Departamento de Farmacologia Bioquímica e Molecular, Instituto de Ciências Biológicas, UFMG, Minas Gerais, Brazil.
3. Setor de Patologia Clínica, Coltec, UFMG, Minas Gerais, Brazil.

Leishmaniasis is currently one of the most common infectious in tropical countries. Domestic dogs are considered important reservoir hosts for the *Leishmania* parasite. Diagnosis to canine visceral leishmaniasis (CVL) is particularly difficult to mainly due to low specificity of the tests available on the market. Therefore, the development of more specific and sensitive tests are important for a better disease control. In this way, this work used the phage display and spot synthesis techniques to select peptides expressed in phages surface that present high affinity to IgG antibodies purified of dogs with visceral leishmaniasis. Three clones were selected and its sequences were determined. The peptides were synthesized in cellulose membranes and tested in serological tests by ELISA. Two peptides (11H and 12A) showed high reactivity with serum samples of dogs with active visceral leishmaniasis and low reactivity with serum samples of healthy dogs or dogs with Chagas' disease. In conclusion, our results demonstrated that these peptides are good candidates to compose a kit for serological diagnosis of CVL.

SUPPORT: FAPEMIG, CNPq, PRPq/UFMG

IM79 - SPECIFIC IgG1 AND IgG2 ANTIBODY RESPONSE OF DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) CHAGASI*: A NEW TECHNICAL APPROACH

AMORIM, I.F.G.¹; ALVES, C.F.¹, MOURA, E.P.¹, RIBEIRO, R.R.¹, FREITAS, E.¹, SAMPAIO, W.M.¹, COSTA VAL, A.P.¹, TAFURI, W.L.¹, MELO, M.N.¹, MICHALICK, M.S.M.^{1,2}

¹Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; ²Universidade FUMEC, Faculdade de Ciências da Saúde, Belo Horizonte, Brazil.

Visceral leishmaniasis is a serious illness, being fatal when not treated and dogs are the major reservoir. Dogs naturally infected (63) with *Leishmania chagasi*, seropositive by RIFI (titre \geq 1:40) from Belo Horizonte, were evaluated clinical, serological and parasitologically. Thirty dogs composed the asymptomatic group (AD) and 33 the symptomatic (SD), when presented at least one clinical signs such as: lymphadenopathy, alopecia, hiperkeratosis, cachexia, dermatitis, skin ulceration, onychogriphosis and or ocular lesions. For titration by ELISA, the serum was diluted in the ration of 1 to 2 from the dilution of 1/100 to

1/819200. The titre, a greater dilution of each serum that showed reactivity above cut off, was selected and the values of IgG1 and IgG2 were compared. Parasitological diagnosis was performed by observations of parasite forms in cultures of bone marrow aspirates. All dogs had promastigote forms of *Leishmania* for up to three times, confirming the infection. Clinical signs with became evident in SD group were: generalized lymphadenopathy (100%), followed by alopecia (75%), hyperkeratosis (63%), expoliative dermatitis (17%), cachexia (38%), ulcers in the skin (17%), onychogriphosis (25%) and ocular lesions (17%). All dogs of the two experimental groups, showed titres of IgG2 higher or equal to 1/100. However, when assessing the IgG1 13 (43.3%) of AD and eight (24.2%) of SD showed no reactivity in dilution 1/100 and are considered negative for this subclass of immunoglobulin. The medians verified for IgG1 and IgG2 were compared within each experimental group. Both groups have to IgG2 significantly higher values than those of IgG1, respectively $p < 0.0001$ (AS) and $p < 0.005$ (SD). When the two clinical categories were compared to the values of IgG1 and IgG2 no significant difference was observed, $p < 0.05$, and wouldn't be possible to establish an association between canine visceral leishmaniasis clinical status and IgG subclasses.

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IM80 - MALNUTRITION IN *LEISHMANIA CHAGASI*/INFECTION MODEL: EVALUATION OF HISTOLOGICAL SPLEEN INJURIES

MARIM, FM.¹, MALAFAIA, G.¹, SILVA, M.E.²; RANTES, R.M.³, LIMA, W.G.⁴, PEDROSA, M.L.⁴, REZENDE, S.A.^{1,5}

Laboratório de Imunoparasitologia/NUPEB¹, Dep. de Nutrição Experimental, ENUT², Dep. de Patologia Geral, UFMG³, Dep. Ciências Biológicas, ICEB⁴, Dep. de Análises Clínicas, Escola de Farmácia⁵, Universidade Federal de Ouro Preto, Ouro Preto.

Leishmaniasis is an important parasitic infection of humans and produces a wide clinical spectrum of cutaneous, muco-cutaneous and visceral injuries. Protein-energy malnutrition (PEM), a potential aggravate of tissue injury for a variety of diseases, represents a public health problem mainly in poor and developing countries. The aim of this study was to evaluate the spleen histological alterations

observed in mice infected with *Leishmania (Leishmania) chagasi* in association to PEM. Initially, female BALB/c mice were divided in two groups: one group was fed with a low protein diet (3% casein) and the other one was fed with normal protein diet (14% casein). After malnutrition was established, half of the mice were inoculated endovenously with *Leishmania (L.) chagasi* promastigotes and euthanized four weeks later. In necropsy, spleen fragments were collected, fixed in buffer formalin and processed for histopathological analyses. Tissue alterations were evaluated using digital morphometric methods. Our previous results indicated that PEM mice spleen area was smaller than control group. Moreover, PEM promotes a decrease in the number of lymphoid follicles in the white pulp. In another hand, *Leishmania* infection caused an increase in reactive spleen area in well-nourished mice when compared to non-infected well-nourished and malnourished mice (infected or non-infected). Parasitological and immunological analyzes will be conducted at this moment in order to correlate to histopathological alterations in infected malnourished and well-nourished animals and to clarify the effects of malnutrition in parasitic tissue lesions.

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IM81 - A CCR1/CCR5 INDEPENDENT CD8-ENRICHED MENINGOENCEPHALITIS IS INDUCED BY *TRYPANOSOMA CRUZI* INFECTION

^{1,2}SILVA, AA, ³ROFFÊ, E., ¹MARINO, A.P.M.P., ³SANTIAGO H.C., ¹KROLL-PALHARES, K., ³TEIXEIRA, M.M., ³GAZZINELLI, R.T. ¹LANNES-VEIRA, J. ¹

IOC/Fiocruz, Rio de Janeiro, RJ, Brazil;
²HUAP/UFF, Niterói, RJ, Brazil; ³UFMG, Belo Horizonte, MG, Brazil.

Introduction and objectives: CD8-enriched meningoencephalitis restricted to the acute phase is induced in susceptible C3H/He mice by *Trypanosoma cruzi* infection. During the chronic phase the meningoencephalitis is absent paralleling low parasitemia and central nervous system (CNS) parasitism. Previously, we showed that VLA-4/VCAM-1 interaction pathway plays a pivotal role in the formation of *T. cruzi*-elicited meningoencephalitis (Roffê et al., 2003). *T. cruzi*-infected mice present increased frequencies of CCR5 expressing peripheral blood cells and

splenocytes. These results led us to test whether CC-chemokines participate in progression/resolution of *T. cruzi*-elicited meningoencephalitis. **Results:** We investigated the participation CC-chemokines, mainly CCL5/RANTES and CCL3/MIP-1 α , and their receptor CCR5 in *T. cruzi*-infected mice with the Colombian strain. Enhanced levels of CCL3/MIP-1 α , CCL4/MIP-1 β and CCL5/RANTES mRNA in the CNS were restricted to the acute infection. Interestingly, the elevated expression of CCR5, particularly by circulating CD8⁺ T cells, was associated with VLA-4 expression, in special, with the activated form of the β 1 integrin chain, showing their migration potential. In fact, peripheral blood mononuclear cells of infected mice selectively migrate *in vitro* towards CCL4/MIP-1 β and CCL5/RANTES, being this migration partially inhibited by Met-RANTES. In contrast, *in vivo* treatment of C3H/He infected mice with Met-RANTES, which resulted in partial blockade of *T. cruzi*-induced myocarditis, altered neither parasitism nor CNS inflammation. **Conclusion:** Our findings suggest that CC-chemokines play a role in inflammatory cell migration to the CNS in experimental *T. cruzi* infection. Lastly, contrasting with myocarditis, the results of Met-RANTES treatment indicate that parasitism control and inflammation in the CNS of *T. cruzi*-infected mice is a CCR1/CCR5 independent process.

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IM82 - CCR4 PARTICIPES IN THE MODULATION OF *TRYPANOSOMA CRUZI* INDUCED-MYOCARDITIS IN MICE

SANTIAGO-SILVA, J¹, SILVA, G. K.¹, GUTIERREZ, F.R.¹, SILVA, J. S.¹, GUEDES, P. M. M.¹

1- Faculdade de Medicina de Ribeirão Preto- USP, São Paulo, Brazil.

The chemokine receptor CCR4 is involved with migration of Th2 and CD4⁺CD25⁺ regulatory T cells. As the infection with *T. cruzi* results in inflammatory heart disease and this cellular profiles are important for the control of inflammation in patients and murine model, the aim of this study is to evaluate the role of CCR4 in the control of CD4⁺CD25⁺ T cells migration to the heart. Ten CCR4^{-/-} and WT (C57BL/6) mice were infected with 1 \times 10³ trypomastigotes of the Y *T. cruzi* strain, and the parasitemia, mortality and histopathological analysis were performed at day

14 p.i. Five not infected animals were euthanized in the same periods. The parasitemia levels are similar in both CCR4^{-/-} and WT mice and the parasitemia peak was observed on day 9 p.i. Additionally, WT mice survived until 26th day after infection, while not mortality was observed in the CCR4^{-/-} group. Regarding the heart inflammation, it was higher in CCR4^{-/-} mice than in WT mice. Our results indicate that the CCR4 are involved in the control of myocarditis during *T. cruzi* infection, and this chemokine receptor could play a role in the immunopathological mechanisms of Chagas disease. The cytokine profile and immune phenotype evaluation of cardiac cells will be performed.

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IM83 - ANTIBODIES AGAINST *TRYPANOSOMA CRUZI* RIBOSOMAL P PROTEINS INDUCE APOPTOSIS ON HL-1 CARDIAC CELLS.

LEVY, G.V.¹, TASSO L.M.¹, LEVIN M.J.¹, GOMEZ K.A.¹.

¹Laboratory of Molecular Biology of Chagas Disease, INGENI-CONICET, Buenos Aires, Argentina. e-mail: levy@dna.uba.ar

Chronic Chagas Heart Disease (cChHD) is characterized by high antibody (Ab) levels mainly toward parasite intracellular proteins. Among these, the acidic C-terminal region of the ribosomal P proteins (epitope R13 of TcP2 β protein) is considered to be highly immunogenic and bears similarity with the second extracellular loop of the β 1-adrenergic receptor (β 1-AR). Our previous results demonstrated that anti-R13 Abs from patients with cChHD recognize and activate β 1-AR and M2-cholinergic receptors (M2-ChR), as seen by immunocytochemistry and cAMP accumulation on stably transfected cells. The aim of this work was to evaluate the effect of anti-R13 Abs long-term stimulation on cardiac cells. Thus, adult murine cardiac HL-1 cells were treated with the adrenergic agonist Isoproterenol (ISO), mAb anti-R13, named 17.2, or IgG fractions from cChHD patients. The results showed that ISO produced cell cycle arrest, cellular senescence and apoptosis and mAb 17.2 induced apoptosis by β -AR stimulation on cardiac HL-1 cells as seen by TUNEL. Moreover, mAb 17.2 produced an increase in Bax/Bcl_{xL} ratio mRNA levels. Late apoptosis changes on cardiac cells induced by mAb 17.2 were further confirmed using annexin V-PI dual staining by flow cytometry. Likewise, IgG

fractions from cChHD patients produced apoptosis on these cells when compared to IgGs from healthy individuals. These results support the hypothesis that apoptosis caused by anti-R13 Ab chronic stimulation on β 1-adrenergic receptors could result in cardiotoxic effects similar to those known to be produced by long term exposure to agonists. This may justify the use of immunoadsorption approaches to avoid the cross-reactive immune response produced by anti-R13 Abs without interfering with anti-parasite immunity. This research was supported by grants from The National Agency of Scientific and Technological Promotion (FONCYT BID 1728/OC-AR 01-14389 and 25845).

IM84 - PRODUCTION AND PARTIAL CHARACTERIZATION OF MONOCLONAL ANTIBODIES SPECIFIC FOR THE SURFACE MEMBRANE ANTIGEN OF *Entamoeba histolytica*

Autores: Santos, H.L.C.¹, Gonçalves, J. L. S.¹, Monteiro, D. C.¹, Castro, M. J. C.¹, Peralta, R. H. S.², Peralta, J. M.¹

¹Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, RJ, Brasil

²Departamento de Patologia, Faculdade de Medicina, Universidade Federal Fluminense, RJ, Brasil

The identification of *Entamoeba histolytica* in fecal samples is still a problem in routine diagnostic of intestinal or invasive amebiasis. The use of monoclonal antibodies (MAbs) is one of the integral parts of a specific and sensitive diagnostic strategy. Here, we report the production and partial characterization of MAbs specific for *E. histolytica*. Total amoeba extract antigens of *E. histolytica* strain HM1:IMSS was used to immunize BALB/c mice for MAbs production. After fusion and selection of hybridomas, five clones secreting MAbs directed to *E. histolytica* antigens were obtained. One of them (006.07/1B4/1H5/1C8) was evaluated by enzyme-linked immunoassay (ELISA), indirect immunofluorescence test (IFAT) and immunoblotting. This MAb reacted with *E. histolytica* extract antigen in ELISA and exhibited bright fluorescence on the membrane of *E. histolytica* trophozoites using IFAT. No reactivity with *Giardia* trophozoites and cysts, *E. dispar* trophozoites, *E. moshkovskii* trophozoites, and *Cryptosporidium* oocyst was detected. The immunoblotting analysis showed a diffuse reactivity

without a define immunoreactivity band pattern. This reactivity was completely abolished when the antigen was treated with sodium metaperiodate. Thereby, this is an indication that the MAb is related to a carbohydrate epitope of the *E. histolytica*. Further studies were undertaken in order to clarify this finding. This MAb has a potential application for the specific diagnosis of intestinal and invasive amebiasis by detection of *E. histolytica* parasite in fecal samples using indirect immunofluorescence or/and ELISA tests.
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