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ArtinM lectin depends on CD14 to induce inflammatory response and IL-10 production

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ArtinM is a D-mannose binding lectin from *Artocarpus heterophyllus* that interacts with N-glycans present on TLR2 expressed on cell surface of antigen presenting cells. This association is responsible to induce IL-12 production leading to the development of Th1 response. This rise on IL-12 and IFN-γ levels is found in experimental infection to *Paracoccidiodes brasiliensis* and *Neospora Caninum.* Preliminary data showed that, when compared to unstimulated macrophages, ArtinM stimulus induced an increase in $TLR2^{+}/CD14^{+}$ cell population. Also, these stimulated macrophages produced higher amounts of cytokines like IFN-γ (6-fold increase), IL-12 (4-fold increase), and TNF-α (20-fold increase) characterizing the expected proinflammatory response. However IL-10 and IL-6 secretion showed a 115-fold and 1000-fold increase. This inflammatory response seems to be CD14 dependent, since macrophages from $CD14^{-/-}$ mice failed to produce these cytokines after ArtinM stimulation. These data suggest that ArtinM interference in the innate mechanism of defense is highly dependable on CD14 expression.

12969Assaying the immunomodulatory activities exerted by recombinant ArtinM: dependence on the protein quaternary structure

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ArtinM is an immunomodulatory lectin that induces Th1 balanced immunity, initiated by recognition of N-glycans of the TLR2 on the surface of APCs. ArtinM acts on macrophages and neutrophils, through the binding to N-glycans attached, not only to TLR2, but also to CXCR2, respectively. These abilities make ArtinM a good candidate for pharmaceutical applications, a fact that led us to obtain recombinant ArtinM in large scale. Recombinant ArtinM expressed in E. coli (bArtinM) and S. cerevisiae (yArtinM) were affinity purified on D-mannose columns and analyzed for several biological activities known to be exerted by the plant lectin (from jackfruit seeds) (jArtinM). Glycoarray analyses showed that all forms of ArtinM bind specifically to a similar set of glycans containing the trimannoside that constitutes the N-glycans core. In addition, jArtinM and yArtinM have induced macrophages to produce high levels of pro-inflammatory mediators, such as IL-6, IL-12, TNF-a and NO an effect that was reduced in TLR2-/- macrophages. However this inflammatory response was not observed with stimuli by bArtinM. Neutrophil haptotaxis was in vitro induced by yArtinM (129.2 ± 35.54 neutrophils/field), bArtinM (59 ± 21.45 neutrophils/field) and jArtinM (94 ± 32 neutrophils/field). Because the primary structures of the native and recombinant forms of ArtinM are coincident, we have investigated if the quaternary structure of these molecules could account for the observed differences in their biological activities. Preliminary data on gel filtration analysis has revealed molecular masses that suggest that bArtinM is a monomer and yArtinM is a homodimer, contrasting with the homotetrameric organization of the jArtinM molecule. The reported results support our postulation that the lectin oligomerization is required for part of the biological activities exerted by ArtinM. Further biological and structural characterization on ArtinM recombinant forms are in progress in order to validate, or not, the pharmaceutical application of this immunomodulatory lectin.

Title: EFFECT OF TRIATOMINE SALIVA ON ACTIVATION OF LPS-TREATED MURINE MACROPHAGES

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INTRODUCTION: Macrophages are essential cells of the innate immunity and may play critical roles in host defense, immune regulation and wound-healing. Triatomines are hematophagous Reduviid insects vectors of *Trypanosoma cruzi,* the causative agent of Chagas disease. To subvert the host immune system during feeding it is suggested that these insects may secrete, via saliva, molecules with antihemostatic, anti-inflammatory and immunomodulatory properties. Thus, the main purpose of this work was to investigate the role of the saliva from *Triatoma infestans, Rhodnius prolixus and R. neglectus* on the activation of murine macrophages. **METHODS AND RESULTS:** Saliva was obtained by dissection of salivary glands (Rhodnius sp) or collected directly from the mouthparts (*T. infestans*). The macrophages were obtained from C57BL/6 mice as previously described (PLoS ONE. 5: e15263, 2010). Macrophages (0.2 x 10^6 cells/well) were pre-incubated with saliva (diluted 1:30, 1:100, 1:200, 1:300 v/v) for one hour and then stimulated with Lipopolysaccharide (LPS – 500 ng/mL) for 18 hours. The production of TNF-α, IL-1 and IL-10 and the expression of surface markers (MHC-II, CD40, CD86) in the culture were determined by ELISA and flow cytometry, respectively. The saliva of the three species were capable of enhancing the production of IL-10 by LPS-treated macrophages ($p < 0.05$) but the production of the TNF-α and IL-1 was not affected. With regard to CD40 and CD86, regardless of species, saliva also did not produce any alteration in the expression of these molecules. On the other hand, the expression of MHC-II was toughly increased by the action of *T. infestans* saliva ($p < 0.05$), regardless of the presence or absence of LPS. **CONCLUSION:** An anti-inflammatory environment, with high concentrations of IL-10 and no induction of TNF-α, IL-1 and co-stimulatory molecules may be more suitable for hematophagy and transmission of *T. cruzi* which comes in contact with skin injured after triatomine biting. Data from the literature showing that increased IL-10 facilitates the transmission of pathogens by blood-sucking arthropods (Parasite Immunol. 28:131-41, 2006; J Immunol. 180:5771-5777, 2008) supports our hypothesis. The increase of MHC-II induced by *T. infestans* saliva is still open for discussion. Possibly, an increased MHC-II expression only is not sufficient to characterize the complete macrophage activation or much less to induce the activation of other immune cells in the feeding site.

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EVALUATION OF THE INFLUENCE OF INTERFERON-GAMMA DURING INFECTION BY *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* IN A MURINE MODEL.

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Introduction: Caseous lymphadenitis is a chronic infectious disease of worldwide occurrence that affects small ruminants, causing great economic losses. This disease has as etiologic agent the bacterium *Corynebacterium pseudotuberculosis* and is characterized by the formation of granulomas in the superficial lymph nodes, which can also affect internal organs and lymph nodes, as a response of the host immune system to the penetration of this agent that resists the bactericidal action of phagocytic cells. This pathogen is phylogenetically related to *Mycobacterium tuberculosis*. The evaluation of the immune response is an important tool to identify possible infected animals, in this way, preventing the dissemination of the disease. Studies have shown the importance of cytokines in the defense of facultative intracellular pathogens, including *C. pseudotuberculosis*, especially interferon-gamma (IFN-γ) cytokines characteristic of Th1 cells, which can be involved in protection against intracellular pathogens. The present study evaluated the aspects of the immune response of mice of the strain C57Black/6 wild and *knockout* IFN-gamma during infection with *C. pseudotuberculosis*.

Methods and Results: We used 28 mice, 14 wild-type (WT) and 14 IFN-gamma *knockout* (KO) (C57Black / 6), 20 infected experimentally animals with T1 strain of *C. pseudotuberculosis* and 08 uninfected control animals. Subsequently, 7 and 14 days of infection was sacrificed by cervical dislocation 05 infected animals from each experimental group and 02 controls animals. The groups were followed over 7 and 14 days after intraperitoneal infection with 10^7 CFU of the *C. pseudotuberculosis*, during which were evaluated bacterial dissemination, the frequency of abscess, the change in spleen weight, the

pattern of cell migration into the peritoneal cavity, and humoral immune response by the dose and subclasses of IgG and cellular immune response through dosage cytokine and immunophenotyping. IFN-gamma *knockout* mice were more susceptible to infection by *C. pseudotuberculosis* with higher bacterial dissemination in mesenteric lymph nodes, increased frequency of granulomas, significant increase in spleen weight, intense cell migration into the peritoneal cavity, mainly neutrophils and macrophages and expression of TCD8+ lymphocytes, and consequently lower expression of proinflammatory and regulatory cytokines. Were considered statistically significant results with P value < 0,05.

Conclusion: These findings highlight the importance of IFN-γ in the innate immune response to intracellular pathogens like *C. pseudotuberculosis*, demonstrating the poor response of the immune system of *knocked* out animals during the infection, as evidenced by the increased spread of bacteria and the frequency of granuloma in various organs of the IFN-gamma *knockout* mice, as well an increased weight of the spleen of these animals with severe migration into the peritoneal cavity cells typically involved in innate immune responses following infection for *C. pseudotuberculosis*, and especially greater expression of CD8 + T lymphocytes, and diminished expression of proinflammatory cytokines that suggests an attempt to overcome the infection in the absence of IFN-gamma.

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TOLL-LIKE RECEPTOR 9 IS REQUIRED FOR FULL MITOGEN-ACTIVATED PROTEIN KINASE (MAPK)/NF-κB ACTIVATION AND HOST RESISTANCE TO *BRUCELLA ABORTUS* IN MICE

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Introduction: *Brucella abortus* is a facultative intracellular bacterial pathogen that causes undulant fever in humans and abortion in domestic animals. Recent studies have revealed that Toll-like receptor (TLR)-initiated immune response to *B. abortus* depends on myeloid differentiation factor 88 (MyD88) and interleukin-1 receptor-associated kinase 4 (IRAK-4) signaling. These molecules are essential for the activation of the transcriptional factor NF-κB and MAPK cascade leading to production of cytokines that induce inflammatory responses. Therefore, herein we intended to study the role of TLR9 in host innate immune response against *B. abortus* and its relevance in activation of MAPK and NF-κB signaling pathway. **Methods and Results:** Fifteen C57BL/6 or TLR9$^{-/-}$ mice were infected i.p. with *B. abortus* strain S2308 and sacrificed at 1, 3 and 6 weeks after infection. Residual *Brucella* colony-forming units (CFU) were then count in harvested spleen from each infected animal. It was observed that the number of CFU in TLR9$^{-/-}$ mice spleen was higher compared to wild type animals at 1 and 3 week post-infection. At 6 weeks post-infection, TLR9$^{-/-}$ mice were able to control the infection as the C57BL/6 animals. To evaluate the *in vitro* production of cytokines of TLR9$^{-/-}$ mice, macrophages derived from bone marrow were stimulated with *B. abortus* or its genomic purified DNA. The production of IL-12p40 and TNF-α from mice genetically deficient inTLR9 was partially reduced at 24 h after these stimuli. Finally, to investigate the role of TLR9 in MAPK and NF-κB signaling pathways, macrophages were stimulated with *B. abortus* and the signaling components were analyzed by Western Blot detecting protein phosphorylation. The MAPK proteins (ERK1/2, p38 and JNK) as well as p65 NF-κB phosphorylation was partially impaired in TLR9$^{-/-}$ macrophages activated by *B. abortus* or its DNA. However, with these same stimuli, the activation of these proteins was completed abrogated in MyD88$^{-/-}$ macrophages. **Conclusion:** In summary, the results shown in this study demonstrated that TLR9 is critical to trigger the initial immune response against *B. abortus* but not at latter phases of infection. In addition, the results suggest an alternative pathway of *B. abortus* DNA activating MAPK and NF-κB signalization promoting proinflammatory cytokine production partially independent of TLR9 but dependent of MyD88.

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TOLL-LIKE RECEPTOR 4 SIGNALING VIA MYD88 IS ACTIVE IN PANCREATIC β CELLS AND MODIFIES INSULIN SECRETION

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Introduction: Toll-like receptor 4 is a pattern recognition receptor that recognizes LPS produced by Gram-negative bacteria. There are two TLR4 signaling pathways, typically in antigen-presenting cells: one is MyD88-dependent, activating NF-kB transcription factor and triggering inflammatory cytokine production and the other is TRIF-dependent, leading to activation of IRF3 and IRF-7 and production of α and β interferon, involved in antiviral and antibacterial immune responses. Non-immune cells in several tissues also express TLR4, including human and murine pancreatic β cells. Our previous study identified TLR4 as a molecule which activates inflammatory signals and induces changes in β cell homeostasis. In this study, we investigated which TLR4 signaling pathways are activated by LPS in β cells and whether LPS-mediated signaling interacts with the effects of glucose levels on β cell viability and insulin production in a mouse insulinoma cell line.

Methods and Results: MIN6 cells were maintained in low (2,8mM), normal (5,6mM) and high (11,2mM) glucose levels for 4 days, and then incubated with LPS (50 ng/mL) for 48 hours. Three independent experiments were done in triplicate for each condition. Analyses were done by real-time PCR, Western Blot, ELISA and flow cytometry. Analysis confirmed increase in TLR4 gene expression in hyperglycemic conditions and showed that the signaling pathway activated by LPS is MyD88-dependent. The interferon induction pathway is absent in these cells. Furthermore, upon activation by LPS, TLR4 impacts on insulin secretion in response to glucose, without triggering cell death.

Conclusion: We conclude that in the presence of LPS, TLR4 expression in mouse pancreatic β cells is induced in response to increased glucose levels and modifies insulin secretion, and may constitute a new link in the chain of events that occurs in response to hyperglycemia.

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The adjuvant effect of the killed-*Propionibacterium acnes* on B1 and phagocyte-derived from B1 cells is mediated by TLR-2

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Introduction:B1 lymphocytes, like macrophages, are predominant in the peritoneal cavity. These cells are responsible for natural IgM secretion and they are considered as one of the cells involved in innate immunity. They simultaneously express macrophages and lymphocytes markers and are classified according to surface molecules as B1a (CD19/CD11b/CD5) and B1b (CD19/CD11b). Enriched B1b cell cultures can be obtained from mouse peritoneal adherent cells and, when re-cultivated, differentiate into a phagocyte derived from B1 cells (B1CDP) that express (F4/80/CD11b/CD19), also showing phagocytic activity. Recently we demonstrated the ability of heat- killed-*Propionibacterium acnes* suspension and their soluble polysaccharide (PS) compound to increase all B1 subtypes absolute number in BALB/c mice peritoneal cavity, including those expressing TLR2, 4, 9 and co-stimulatory molecules. The aim of this study was to verify the *in vivo* effect of the *P. acnes* and its PS on B1CDP and on its phagocytic function. We also verified if TLR2 and TLR4 mediated bacteria effects.
Methods and Results:Wild type C57Bl/6 (WT), $TLR2^{-/-}$ and $TLR4^{-/-}$ mice were treated with an intraperitoneal injection of *P. acnes* (140 μg), PS (25 μg) or saline (control). After 24 h were determined the absolute numbers of B1 lymphocytes and B1CDP. To phagocytosis assay *in vivo* were injected yeast labeled with CFSE. Internalized yeasts by cells were detected by flow cytometry. In WT we detected decreased B1 $TLR2^{+}$ and no difference in B1 $TLR4^{+}$ absolute numbers, but elevated $TLR4^{+}$ absolute number and TLR2 expression/cell in B1CDP after bacterial stimulus. PS rose only B1b $TLR2^{+}$. *P. acnes* also elevated B1b number and the phagocyte differentiation, and both effects were mediated by TLR2, once in $TLR2^{-/-}$ mice we did not detect these effects. The analysis of the phagocytic function in $TLR2^{-/-}$ and $TLR4^{-/-}$ mice revealed rise in B1b absolute number with internalized yeasts in *P. acnes* group; in contrast, this effect was observed in B1a subtype in WT. Bacteria stimulus decreased B1CDP and also their phagocytic function on $TLR2^{-/-}$ group. Any effect was observed in PS treated groups.
Conclusion:The adjuvant effects induced by *P. acnes* on B1 cells, mediated undoubtedly by TLR2, such as increasing absolute number, differentiation into phagocytes and phagocytic activity, reinforce the importance of this adjuvant and also the involvement of these cells in the innate immune response.

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EFFECTS OF OLEIC AND LINOLEIC ACIDS ON WOUND HEALING IN DIABETIC RATS

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Introduction: Healing of wounds involves resident and infiltrating cells and mediators. Diabetes is a well know cause of foot ulcer, a hard to heal wound. Since, fatty acids have important roles in immune and inflammatory responses, it can help diabetic wound to heal. So, the aim of the present study was to investigate the effects of oral supplementation of oleic (OLA) and linoleic (LNA) acids on diabetic wound healing. **Methods:** Diabetes was induced by intravenous injection of streptozotocin (65mg/Kg of body weight). After diabetes confirmation (glucose > 250 mg/dL), rats received oral supplementation (0.22 g/body weight/day) of OLA or LNA acids during 5 days. Subsequently, a 20 mm^2 skin area was removed and the supplementation continued until wound closure. Wound closure was measured by digital images at days: 0, 1, 3, 7, 10, 14, 16 and 18. At different time-points (0, 1, 3, 7 and 14) wound tissue was removed to analysis of H_2O_2, cytokines content (IL-1, CINC-2, IL-6, MIP, TNF-α, VEGF and TGF-β), at protein and mRNA levels and eicosanoids production (LTB_4 and 15(S)-HETE). **Results:** OLA not altered the rate of wound closure. However, LNA restored the healing rate as in control group. OLA restored the concentrations of H_2O_2 at 1 hour, and both fatty acids reduced it at 24 hours after wound. At 1 day, diabetic groups diminished mRNA expression of CINC-2 and IL-1β. Diabetic (D) and OLA, also reduced TNF-α mRNA, but LNA restored it. OLA augmented the expression of CINC-2, IL-1β and MCP-1 at 3 days. All the diabetic groups reduced TGF-β mRNA expression at this time-point. On 14th day, diabetic groups inhibited the expression of CINC-2 and IL-1β. OLA and LNA restored TGF-β expression at this time-point. On the protein levels, LNA elevated the concentrations of IL-6. CINC and IL-1β levels were elevated 3 days after wound in diabetic groups. On 7th day, D and OLA diminished TNF-α concentrations and LNA restored it. There were no differences eicosanoids concentrations. **Conclusion:** Even though, OLA did not alter the wound closure rate, it elevated inflammatory cytokines and restored the expression of growth factors. LNA was more potent, since it improved the wound healing in diabetic rats.

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ROS PRODUCTION THROUGH DECTIN-1 IS IMPORTANT IN THE CONTROL OF INFECTION BY *Neospora caninum*

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Introduction: *Neospora caninum* has been associated with cattle abortions since early 1990's. The mechanisms underlying host resistance against this pathogen remains unclear and it has been the subject of intense study by our group. To unravel the initial host-parasite interactions, we recently demonstrated the importance of pathogen recognition receptors (PRRs) in *N. caninum*innate immune recognition. However, the signaling programs induced by the interaction between *N. caninum* and PRRs and the consequences of such interaction remains unclear. Here, we investigated the role of Reactive Oxygen Species (ROS) following stimulation by *N. caninum* in different cell types. **Methods and Results:** We observed that soluble antigens of *N. caninum* (NLA) induces ROS production in peripheral blood mononuclear cells (PBMC) of naïve calves and murine splenocytes. The relative importance of ROS in controlling protozoan infection was further verified in NADPH oxidase enzyme (NOX2-/-) deficient mice. To determine the specific receptor involved in ROS generation during *N. caninum* infection, we evaluated the role of Dectin-1 receptor in this context. We found that Dectin-1 downregulates ROS production by spleen cells exposed to live parasites and NLA. Furthermore, treatment of WT mice with Laminarin (selective Dectin-1 antagonist) increased ROS production and limited parasite replication during acute phase of infection, along with lower brain parasitism and inflammation during chronic (latent) infection. **Conclusion:** Together, our results demonstrate that *N. caninum* limits oxidative stress during infection through Dectin-1 recognition. These findings suggest that PRR-mediating ROS inhibition is a potential target for the development of therapeutic and prophylactic measures against neosporosis.

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TNFα PLAYS IMPORTANT ROLE IN PATHOGENESIS AND IMMUNE RESPONSE DURING INFECTION WITH *Neospora caninum*

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Introduction: The protozoan parasite *Neospora caninum* has been associated to abortions in cattle since the early 1990's, and the infection leads to major economic impact to the segment. TNFα is a cytokine belonging to the family of cytokines that stimulate the reactions of the acute phase. It has proinflammatory character and acts in the induction of systemic inflammation. It is produced chiefly by activated macrophages, although it can be produced by other cell types as well. Given the importance of this cytokine during acute infectious processes, its role becomes important target for understanding the pathologies arising from clinical neosporosis. **Methods and Results:** Aiming to determine the role of TNFα mechanisms for the pathogenesis of acute infection by *Neospora caninum*, wild type (WT) and genetically disabled receptor I (p55) TNFα (TNFR-/-) C57BL/6 mice, were infected intraperitoneally with 3x107 tachyzoites of *N. caninum*, considered lethal dose of infection. The animals were monitored for their body weights and survival for 30 days. It was found that TNFR-/- animals show increased survival and less weight loss than WT mice during the acute phase of infection. Furthermore, it has found the role of this cytokine in the production of immunoglobulins during chronic infection by protozoa. To this end, animals were infected with sub-lethal dose (1x107 viable *N. caninum* tachyzoites), and followed for 45 days. We observed that, regardless of the presence of TNFα, mice show production of IgM specific to soluble antigens of the parasite. However the production of total IgG, IgG1 and IgG2a specific TNFR-/- mice showed impaired. **Conclusion:** These results demonstrate that TNFα participates in the mechanisms of pathogenesis-induced acute infection by *N. caninum* and suggest that TNFα is important to assure that the switch antibody class specific IgM / IgG.

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MYD88 AND NF-KB ARE REQUIRED FOR IL-12p40 PRODUCTION DURING MACROPHAGES ACTIVATION BY *Bordetella pertussis* AND *B. parapertussis*

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Introduction: Macrophages initiate the innate immune response by recognizing pathogens, phagocytosing them and producing inflammatory mediators. IL-12 is generated especially by dendritic cells and macrophages as a heterodimeric cytokine (70kDa) comprising two disulphide-linked subunits designated p35 and p40. Their production can be differentially regulated by several signaling components through multiple pathways. MyD88 is critical for the signaling from TLRs. This pathway activates NF-kB and the induction of proinflammatory cytokines. IL-12 is critical for host defense against a variety of pathogens acting directly on the development of Th1 response. Whooping cough is a respiratory disease of humans caused by *B. pertussis* and *B. parapertussis*, which express differences in LPS and *B. parapertussis* is a mutant in pertussis toxin gene expression. Studies have demonstrated a dominant role for IFN-g secreted by Th1 cells in the protection against *B. pertussis*. This study was conducted to analyze signals pathways responsible for the control of IL-12p40 synthesis, specifically, MyD88 and NFkB, during the murine bone marrow-derived macrophage (BMDMO) activation by *B. pertussis* and *B. parapertussis.*
Methods and Results: BMDMO obtained from femur and tibiae of C57BL/6 and MyD88$^{-/-}$ mice were differentiated in complete RPMI medium supplemented with supernatant of L929 cell culture plus 10% of FBS. On the 7th day the adherent cells were pretreated or not with NFkB inhibitor (BAY 11-7082, 10mM) for 1hr prior stimulation with soluble protein from *B. pertussis* or *B. parapertussis* (30mg/mL). IL-12p40 was quantified by ELISA after 20 hours of incubation. Our results showed that macrophages from MyD88$^{-/-}$ mice activated with the lysate of *B. pertussis* or *B.parapertussis* significantly reduced the production of IL-12p40 as well as inhibition of NFkB. **Conclusion:** MyD88 and NFkB are involved in the regulatory pathway of IL-12p40 during the activation of macrophages by soluble protein from *B. pertussis* and *B. parapertussis*. These data suggest that TLRs play role in the host response to both bacterias.

Financial suport: FAPESP, CAPES

ANALYSIS OF OXIDATIVE BURST AND PHAGOCYTIC ACTIVITY IN APECED PACIENT

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INTRODUCTION: The autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome presents as autoimmunity and *Candida albicans* susceptibility. Although the autoimmunity can be explained by defects in lymphocytes development in thymus, candidiasis has not been fully clarified. Recently, our group noted that AIRE protein interacts with the main molecules on Dectin-1 signaling pathway, that is important to the response against *C. albicans* and induces cytokines and chemokines production, oxidative burst and phagocytosis. Thus, we investigated how the absence of AIRE can influence on specific events for the recognition and elimination of pathogens via Dectin-1.

METHODS AND RESULTS: We evaluated the phagocytic activity, production of reactive oxygen species and expression of NADPH oxidase system molecules on an APECED patient cells. The analysis of phagocytic activity against *C. albicans* found to be increased compared to the control. Thus, we observed the expression of NADPH oxidase system molecules on monocytes and found that flavocytochrome b_{558} showed a difference in population profile with a slight reduction of its expression in patient monocytes compared to the control. In parallel, we analyzed the production of reactive oxygen species by dihydrorhodamine assay and found that the patient’s monocytes showed a decrease in hydrogen peroxide production when stimulated with PMA, CURDLAN and depleted Zymosan. (at least 10% less than control).

CONCLUSION: We observed a partial deficiency in oxidative burst in patient cells that can impair *C. albicans* elimination.

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HYDROETHANOLIC EXTRACT OF EUGENIA SPP SHOWS PRO- AND ANTI-INFLAMMATORY EFFECTS

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Introduction: Plant extracts are important tools to investigate various cellular and molecular mechanisms involved in pharmacological and inflammatory process. The family Myrtaceae has been studied because its potential pharmacological and different biological activities have been attributed it, such as, regulating menstruation, pain, intestinal disorders, abortion and infertility, antifungal, antitumor and others. Among its members, the species of *Eugenia spp* seem to present pro and anti-inflammatory activities when tested *in vivo* and *in vitro* biological systems.

Here, our aim was to study the effects of hydroethanolic extract of *Eugenia spp* on neutrophil migration when injected by different pathways (subcutaneous and intraperitoneal). **Methods:** For this purpose, two methods were adopted: (i) female Swiss mice were injected i.p. with different concentrations of the hydroethanolic extract (EtOH:H_2O 70% v/v) of *Eugenia spp,* or (ii) the mice receive a s.c. injection of extract of *Eugenia spp* in different concentrations (0.8-20mg/mL) one hour before to be injected i.p. with thioglicolate 3%. After 6 hours, peritoneal cells migrating were collected and accounted. **Results:** Total and differential counting of peritoneal cells suggests that the extract of *Eugenia spp* is able to induce or inhibit neutrophil migration on pathway-dependent. In the first group, the i.p. injection of extract of *Eugenia spp* induced a dose-dependent neutrophil migration where 0.8mg/mL induced maximum cellular recruitment ($4x10^6$ neutrophils/mL ± $1.5x10^6$; n=5). On the other hand, s.c. injection of *Eugenia spp* previously to inflammatory stimuli was efficient to diminish the inflammatory influx when compared to mice that received PBS in place of the vegetal extract. In addition, the inhibitory activity showed by different doses of *Eugenia spp* was similar to one effect of dexamethasone (by 60%; n=5). **Conclusion:** Together, our preliminary results suggest that the hydroethanolic extract of *Eugenia spp* when administrated by different pathways, subcutaneous and intraperitoneal, can show different effects on neutrophil migration. Further studies, can open perspectives about new

biopharmacologic strategies and products especially in inflammatory process control.

Financial support: PROPE-UNESP

EVALUATION OF THE IMMUNOSTIMULATORY ACTIVITY OF *Brucella abortus* NUCLEOTIDE SEQUENCES

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Introduction: *B. abortus*, the etiologic agent of brucellosis, is a Gram-negative intracellular bacterium that causes a zoonosis of worldwide occurrence leading extensive economic losses. The *B. abortus* DNA is a target for the pattern recognition receptors, which initiate the host defense against this bacterium. It has been demonstrated that *B. abortus* DNA is recognized by TLR9 resulting in a Th1-like cytokine response and this receptor plays a prominent role during *Brucella* infection. Moreover, the *B. abortus* DNA has been shown to induce type I IFN production. Strikingly, type I IFN signaling was demonstrated to be detrimental to host control of *Brucella*. Given this apparent dual role of the *Brucella* DNA during the infection, we decided to identify and to evaluate the immunostimulatory properties of different nucleotide sequences derived from *Brucella* genome.

Methods and Results: The *B. abortus* strain 2308 genome was searched for the CpG-B class and for the AT ODNs containing the consensus motif 5'-NNNNNNATTTTTACNNNNNN-3' using the fuzznuc algorithm (EMBOSS package). The AT-rich motif and the CpG-B class were found 65 times and 12 times in *B. abortus*, respectively. We have selected 3 CpG motifs and 5 AT-rich motifs and the corresponding ODNs to be synthesized in order to analyze its immunostimulatory properties. Bone marrow derived macrophages (BMDM) were obtained from C57BL/6 mice and the cells were stimulated with the constructed ODNs at three different concentrations. The production of pro-inflammatory cytokines (IL-12 and TNF-α) were analyzed in macrophages supernatants by ELISA. The ODNs containing AT-motif were not able to induce such cytokines, while two of the three tested ODNs containing CpG motifs induced high levels of IL-12 and TNF-α. To analyze the contribution of the TLR9 and the adaptor molecule MyD88 in the induction of pro-inflammatory cytokines in response to the CpG ODNS, we took advantage of BMDM from mice lacking

TLR9 or MyD88. The induction of IL-12 and TNF-α was completely abolished on absence of either TLR9 and MyD88.

Conclusion: Taken together, these data suggest that CpG motifs derived from *B. abortus* genome, but not AT ODNs, have great pro-inflammatory activity, which is dependent on TLR9 and MyD88. These molecules may serve as potential vaccine adjuvants.

Financial support: CAPES e CNPq

PRESENTING ANTIGEN CELLS AND CARDIOMYOCYTES FUNCTION DURING *Trypanosoma cruzi* INFECTION IS MODULATED BY SOCS2

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Background. In the course of *Trypanosoma cruzi* infection, many cells types participate in the innate immune response, which is crucial to control pathogen growth and avoid the development of pathology. Suppressor of cytokine signaling (SOCS)2 modulates immune response and its expression is partially dependent of Lipoxin $(LXA)_4$ generation. Here we investigated the effects of LXA_4 and SOCS2 in innate immune cells and cardiomyocytes function during *T. cruzi* infection. ***Methods and results.*** Peritoneal macrophages (MO) and splenic dendritic cells (DCs) were harvested, purified from WT and/or SOCS2 KO mice, exposed to LXA_4 (15h) and than stimulated with tripomastigote forms of *T. cruzi* in presence or absence of IFN-g *in vitro*. The mRNA expression of TNF-α, IL-6 and IL-12 p40 was increased during *T. cruzi* infection and almost abolished when WT dendritic cells was exposed to LXA_4. Also, LXA_4 induced SOCS2 expression and inhibited the IL-12 p40 mRNA expression in WT MO infected with trypomastigote *in vitro*. Absence of SOCS2 results in elevated levels of TNF, IL-12, IL-6, IL-10, SOCS1 and SOCS3 expression by macrophages when compared with WT cells. Our results demonstrate that deficiency of SOCS2 results in reduction of AhR expression when stimulated with *T. cruzi* and IFN-g when compared with WT MO. The down modulation of

AhR expression was detected in *T. cruzi*-infected SOCS2 KO cardiomyocytes. In addition, upon *T. cruzi* infection, the SOCS2 deficiency *in vivo* resulted in altered cardiac remodeling by increasing cardiac ventricular mass and reducing calcium/potassium levels in ventricular myocytes. ***Conclusions.*** The results indicate LXA/AhR/SOCS2 role in the regulation of innate immunity and cardiomyocytes function during experimental *T. cruzi* infection. ***Keywords:*** SOCS2, *Trypanosoma cruzi*. Supported by: CNPq and FAPEMIG, Belo Horizonte, MG, Brazil.

PPARγ CONTRIBUTES TO INFLAMMATION DURING OBESITY AND FOR SEVERITY OF SEPSIS

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Introduction: Obesity is a complex disorder, affecting individuals of all ages and is characterized by a moderate state of chronic inflammation, with increased levels of several pro-inflammatory cytokines and acute phase proteins that maintain this inflammatory state. Obesity has also been shown to be a risk factor "dose dependent" for morbidity and mortality in sepsis; although, little is known about the specific role of obesity in innate immunity activation, cellular and tissue dysfunction that are observed in sepsis. Our goal is to study the relationship between obesity and the regulation of immune response in sepsis, in order to contribute for the understanding of the mechanisms involved in immune regulation of the inflammatory response of sepsis in obese individuals. **Materials and Methods:** We used a model of obesity induced by high fat diet in C57BL/6 and MyD88 knockout (KO) mice for 60 days, and then they were submitted to sepsis by caecal ligation and puncture (CLP) with two perforations using 23G needles and sacrificed 24h after sepsis induction. Fat and kidney tissues and serum were collected for analyses. Microbiota was also analyzed. **Results**: MyD88 KO mice showed higher weight gain and increased adipocyte size and reduced inflammation as compared to wild type mice. After sepsis, these mice were protected with longer survival, decreased of systemic inflammation, less macrophage infiltration in white adipose tissue and decreased gene and protein expression of pro-inflammatory molecules IL-1β, TNF-α, IL-6 and KC. In addition, we observed a significant increase in gene expression of arginase-1 and decreased of MCP1, suggesting a change in macrophage profile. The inflammation in these mice after sepsis appears to be reduced by a significant increase in gene expression of PPAR-γ and its targets, such as Glut4, LPL and FABP4. Up regulation of PPAR- γ seems to inhibit NF-κB pathway and thus inflammation. Microbiota analyses showed that obese

MyD88 mice presented decreased of Firmicutes compared with obese WT mice. **Conclusions**: Here, we concluded that the innate immunity mainly through MyD88 that plays an important role in obesity and inflammatory status in adipose tissue and after a severe inflammatory stimulus. Support: FAPESP 2012/02270-2 and 2011/15682-4; CNPq/Inserm and CNPq/FAPESP/INCT (Complex Fluids INCT) and CEPID-FAPESP (CLEAR).

THE SPIDER ACYLPOLYAMINE MYGALIN IS A POTENT MODULATOR OF INNATE IMMUNE RESPONSES

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Introduction: Cellular polyamines have multiple functions, and their analogues have been explored in anti-proliferative function to tumors and parasites. Mygalin is an antibacterial molecule isolated from the hemocytes of the spider *Acanthoscurria gomesiana* and identified as bis-acylpolyamine spermidine. We evaluated the modulator effects of synthetic Mygalin in the innate immune response. **Methods and Results:** Splenocytes cultures were stimulated with or without Mygalin (5, 10 or 20 μg/mL) and treated or not with 2 μg/mL Con A for 72 hours. Proliferation was measured on day 3 of stimulation after cells were pulsed with 1μCi [3H]-thymidine. Cytokines or nitrite secretions were assessed after 24 or 72 hours of stimulation. The cytotoxicity was examined by MTT assay and nitrite level using the Griess reaction. Bone marrow macrophages differentiated for 7 days was stimulated or not with Mygalin (5-40μg/mL) for 20 hours. Some cultures were treated with iNOS inhibitor (L-NIL) before challenging with Mygalin. We demonstrate that Mygalin induces IFN-γ synthesis by splenocytes increasing the nitrite secretion by splenocytes and macrophages. A specific inhibitor of iNOS abrogated Mygalin-induced nitrite production in macrophages independent of IFN-γ activation. In addition, Mygalin-activated macrophages produced TNF-α but not IL-1β, demonstrating that Mygalin does not act directly on the inflammasome. Furthermore, this compound did not affect spontaneous or Concanavalin A-induced proliferative responses by murine splenocytes and did not induce IL-5 or apoptosis of splenocytes or bone marrow-derived macrophages. **Conclusion:** These data provide evidence that Mygalin modulate the innate immune response inducing IFN-γ and NO synthesis that associated with its antibacterial activity could be explored associated or not with other molecules to enhance immune responses to infection.

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FLAGELLIN-DEPENDENT AND INDEPENDENT INNATE IMMUNE RESTRICTION OF *LEGIONELLA PNEUMOPHILA INFECTION IN VITRO AND IN VIVO*

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Legionella pneumophila is a Gram-negative, facultative intracellular bacterium causative agent of Legionaries' disease in humans. Recognition of bacterial flagellin by Naip5-Nlrc4 inflammasome is required for innate immune restriction of *Legionella pneumophila* (WT Lp) infection in C57BL/6 mice (WT) macrophages. Although these macrophages are resistant to wild type bacteria, the bacteria deficient for flagellin can multiply normally. Thus *flaA*$^{-}$ mutant bacteria is a useful tool to investigate additional immune molecules important for flagellin-dependent growth restriction. In this study, we use bacteria deficient for flagellin to infect mice deficient for: 1) molecules important for effectors mechanisms of immune response (IFN-g $^{-/-}$, IL-6, 10, 12, 17, 18, 23, MIP-1a and TNFRp55$^{-/-}$); 2) cells of immune response (CD4KO-MHCII$^{-/-}$, CD8KO-b2m$^{-/-}$, BKO and neutrophils-anti-LY6G); 3) transmembrane innate immune receptors (CCR2$^{-/-}$, CCR4$^{-/-}$, Fas/Fas-L$^{-/-}$, ST2$^{-/-}$, TLRs$^{-/-}$); and 4) intracellular signaling molecules (PI3k$^{-/-}$, MyD88$^{-/-}$, Nod1$^{-/-}$, Nod2$^{-/-}$ and Rip2$^{-/-}$) to dissect the involvement of the immune molecules responsible for restriction of *Legionella pneumophila* infection in a C57BL/6 mice background. Bacterial replication was analyzed *in vivo* and *ex vivo.* Mice were infected intranasally with $1x10^5$ and the lungs were collected after 48 h for CFU determination. In parallel, bone marrow derived macrophages were infected with *L. pneumophilla flaA*$^{-}$ for 24, 48 and 72 hours and growth curve were assessed by CFU. Overall our data shows that mechanisms related with innate immune response, but not adaptive response are critical bacterial growth restriction when compared with C57BL/6 mice (WT). *In vivo* and *in vitro* experiments shows that activation of these pathways are required for restriction of *flaA*$^{-}$ bacteria and resolution of pulmonary infection in a murine model of Legionnaires' disease.

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RESPIRATORY SYNCYTIAL VIRUS FUSION PROTEIN STIMULATES NEUTROPHIL EXTRACELLULAR TRAPS FORMATION

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INTRODUCTION: Respiratory Syncytial Virus (RSV)-induced acute bronchiolitis is the most prevalent disease in children under 2 years old, which causes a huge impact in hospitalizations and costs to the health system. RSV is a single stranded RNA virus, whose genome encodes up to 11 proteins. The Fusion (F) protein is present at the virion surface and mediates the virus-target cell fusion. It has been shown that RSV F protein activates the recognition receptor TLR-4, inducing cytokine secretion. There is a growing body of evidence showing that neutrophils and its products are present in the airways of infants and animals infected with RSV. Neutrophils activated by a wide range of microorganisms form neutrophil extracellular traps (NETs), which are composed of decondensed DNA and antimicrobial proteins. Our hypothesis is that RSV F protein binds to and activates TLR-4 on neutrophils, inducing NETs formation. The massive recruitment of neutrophils to the lungs of children infected with RSV may cause an excessive production of NETs, which can worse the lung function and pathology in these children.

METHODS AND RESULTS: Human neutrophils (2×10^5/300 µL) were stimulated with F protein (1µg/mL), LPS (100ng/mL), PMA (25nM) or medium alone for 3 h at 37°C with 5% CO_2. After that, cells were fixed with 4% paraformaldehyde and stained with Hoechst 33342 (1:2000). Images were taken in an Olympus IX51 fluorescence microscope. Alternatively, NETs were quantified in the culture supernatants using Quant-iT dsDNA kit according to the manufacturer's instructions. RSV F protein was able to induce NETs, as visualized by fluorescence microscopy, similarly to LPS and PMA. Also, F protein induced a 2-fold increase in NET release compared to control. As expected, LPS and PMA also induced a 2-fold increase in NET production. We are currently investigating the effect of DNase on F protein-induced NET formation and the role of TLR-4 in this process.

CONCLUSION: RSV F protein is able to stimulate NET formation, and the excess of NET production in the lungs of children with bronchiolitis cause by RSV may worse the pathology, since these DNA traps induce endothelial injury and impair lung function.

FINANCIAL SUPPORT: CNPq, FAPERGS, CAPES.

TOLL-LIKE RECEPTOR (TLR)-INDUCIBLE RASGEF1B EXERTS NEGATIVE REGULATION IN THE NF-KAPPAB SIGNALING

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Introduction: Strict control of the NF-kappaB signaling pathway is critical to efficiently avoid uncontrolled host immune responses, yet the molecular mechanisms that negatively regulate this pathway are not entirely clear. Here, we show that RasGEF1b, a guanine nucleotide exchange factor, whose expression is induced upon stimulation by Toll-like receptor (TLR) agonists, and is localized at the early endosomes associated with Ras, negatively regulates the NF-kappaB activation.

Methods and Results: To determine whether RasGEF1b is involved in the TLR-mediated NF-kappaB activation, HEK293 cells were transfected with TLR2, TLR3 or TLR4 simultaneously with NF-kB-luc reporter DNA, with or without RasGEF1b and then treated with Pam3Cys, poly I:C or LPS. The results show that RasGEF1b exerts a negative regulatory effect in the activation of NF-kB mediated by TLRs agonists. Besides, RasGEF1b exhibits a negative regulatory effect in the activation of NF-kB mediated by signaling pathway components of TLRs including Mal/Tirap, TRAF6, TAK1-TAB1, IKK-α and IKK-β. In contrast, RasGEF1b does not inhibit p65/RelA-mediated NF-kB-luc activation, suggesting that it may target IKK complex signaling molecules upstream to p65/RelA. To test this, HEK293 cells were transfected with V5-tagged RasGEF1b together with Flag-tagged IKK-alpha plasmid construct. Co-immunoprecipitation and western blot analyses reveal that RasGEF1b physically associates with the IKK complex. In addition, RasGEF1b does not exert any effect on IRF-3 activation induced by TLR3. Functional studies carried out in murine macrophages RAW264.7 transfected with RasGEF1b short

hairpin RNAs show an increase in NF-kappaB activation after Pam3Cys or LPS treatments. **Conclusion:** Together, these findings indicate a function for TLR-inducible RasGEF1b by specifically controlling TLR-mediated NF-kappaB signaling.

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LC3-ASSOCIATED PHAGOCYTOSIS MEDIATES TYPE I INTERFERON PRODUCTION IN RESPONSE TO DNA-IMMUNE COMPLEXES

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INTRODUCTION: Compartmentalization of intracellular TLR9 is largely responsible for discriminating self from pathogenic DNA. However, association of host DNA with autoantibodies activates TLR9, inducing the secretion of large amounts of type I IFNs in plasmacytoid dendritic cells (pDC).

METHODS AND RESULTS: Here, we show that TLR9-mediated production of IFN-**α** depends on the convergence of the phagocytic and autophagic pathways in response to DNA-containing immune complexes. LC3-associated phagocytosis (LAP) was required for TLR9 trafficking into a specialized IRF7-signaling compartment by a mechanism that involved ATG7 but not the conventional autophagic pre-initiation complex.

CONCLUSIONS: Our findings unveil a new role for autophagy in inflammatory responses, and provide one of the mechanisms by which anti-DNA

autoantibodies, like those found in several autoimmune disorders, bypass the controls that normally restrict TLR9 activation to pathogenic DNA.

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The role of Dectin-1 receptor in *Paracoccidioides brasiliensis* infection

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Introduction: Dectin-1 is a C-type lectin receptor which binds to β1,3-glucan, the most abundant polysaccharide in fungal pathogens. This receptor collaborates with TLR signaling and is required for fungal resistance in mice and humans by promoting fungal phagocytosis and cytokines production. However, the immunological mechanisms that govern the interaction between innate immune receptors of hosts and the dimorphic fungus *Paracoccidioides brasiliensis* have been scarcely studied. **Objectives**: The aim of our study was to characterize *in vitro and in vivo* the role of Dectin-1 in murine paracoccidioidomycosis. **Methodology**: Wild-type (WT) and Dectin-1-KO C57BL/6 mice were used in our investigation. *In vitro*, nitric oxide (NO) and cytokines were measured after macrophage infection with *P.brasiliensis* yeasts. *In vivo*, after intra-tracheal infection with one million yeasts, fungal burdens, levels of cytokines, histopathology and phenotype of inflammatory leukocytes in the lungs were characterized. **Results:** First, we verified that Dectin-1-KO macrophages infected *in vitro* with *P. brasiliensis* presented increased fungal loads, IL-10 and MCP-1 associated with impaired synthesis of NO and IL-6. *In vivo*, Dectin-1-KO mice produced diminished levels of pulmonary Th1, Th2 and Th17 cytokines. In addition, infected Dectin-1-KO mice developed an impaired inflammatory immune response, as evidenced by the reduced presence of activated $CD8^+$ T cells and $CD4^+$ and $CD8^+IL\text{-}17^+$ cells. These events led to increased fungal loads in the lungs of Dectin-1KO mice and allowed a marked fungal dissemination to distant organs such as liver and spleen. These organs presented severe lesions composed by coalescent granulomas containing high numbers of fungal cells. As consequence, Dectin-1KO mice were unable to control fungal growth and presented a decreased survival time. **Conclusions**: Our findings demonstrate for the first time that Dectin-1 receptor plays a fundamental role in the activation of fungicidal mechanisms, and in the induction of protective innate and adaptive immunity against *P. brasiliensis* infection.

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ACTIVATION OF AIM2 OR NLRP3 INFLAMMASOME BY *BRUCELLA ABORTUS* IS REQUIRED FOR IL-1β PRODUCTION AND HOST RESISTANCE IN MICE

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Introduction: *Brucella abortus* is a gram-negative bacterium that causes brucellosis, a systemic infectious disease characterized by undulant fever in humans and abortion in domestic animals. The inflammasome is a large multiprotein complex whose assembly leads to the activation of caspase-1, which promotes the maturation of pro-IL-1β and pro-IL-18. These cytokines generate inflammatory response against diverse pathogens which might lead to a host resistance. In that context, herein we analyzed the inflammasome activation by *B. abortus* and its relevance during host infection.
Methods and Results: Bone-marrow derived macrophages (BMMO) from wild type C57BL/6 or genetically deficient Nlrp3, AIM2, Nlrc4, Casp-1 and Asc mice were stimulated *in vitro* with *B. abortus* virulent strain S2308, heat-killed *B. abortus* (HKBa) or the *virB* mutant which lack a functional type four secretion system (T4SS). Culture supernatants were harvested 17 h after stimulation to measure IL-1β and caspase-1processing by ELISA or Western Blot, respectively. It was observed that *B. abortus* induces caspase-1 activation, IL-1β maturation and secretion dependent of Nlrp3 and AIM2 but independent of Nlrc4. This effect was not observed when cells were stimulated with *virB* mutant or HKBa. In an attempt to identify a *B. abortus* component involved in activating these inflammasome receptors, BMMO were stimulated with purified prokaryotic RNA or genomic DNA. The results suggest that DNA and RNA elicited IL-1β production in an AIM2 and Nlrp3-dependent manner, respectively. In addition, wild type or Nlrp3, AIM2, Nlrc4, Casp-1 and Asc KO mice (5 from each group) were infected with *B. abortus* and 4 weeks thereafter bacterial load were analysed in spleen of each animal. The count of colony-forming units revealed that NIrp3, AIM2, Asc and Casp-1KO mice were more susceptible to *Brucella* infection than wild type. However, Nlrc4 KO mice controlled infection as the C57BL/6 mice.

Conclusion: In summary, Nlrp3 and AIM2 inflammasomes are critical for IL-1β production in macrophage induced by *B. abortus* and these receptors play an important role controlling *Brucella* infection. Furthermore, T4SS was required for full IL-1β secretion elicited by *B. abortus in vitro.*

Financial support: CAPES**,** CNPq and FAPEMIG**.**

TRIBUTYRIN ATTENUATES INFLAMMATION IN THE ADIPOSE TISSUE OF HIGH-FAT FED MICE.

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Introduction: The aim of this study was to investigate whether treatment with tributyrin (Tb, a butyrate pro-drug) results in protection against diet-induced obesity and associated insulin resistance.

Methods and Results: C57BL/6 male mice fed with a standard chow or high-fat diet were treated with Tb (2g/kg bw, 10 weeks) and evaluated for glucose homeostasis, plasma lipid profile and inflammatory status. Tb protected mice against obesity and obesity-associated insulin resistance and dyslipidemia without affecting food consumption. Tb attenuated the production of TNF-α and IL-1β by peritoneal macrophages and their expression in adipose tissue. Furthermore, in the adipose tissue Tb reduced the expression of MCP-1, infiltration by leukocytes and restored the production of adiponectin. These effects were associated with a partial reversion of hepatic steatosis, reduction in liver and skeletal muscle content of phosphorylated JNK and an improvement in muscle insulin-stimulated glucose uptake and Akt signaling. Although part of the beneficial effects of Tb are likely to be secondary to the reduction in body weight, we also found direct protective actions of butyrate reducing TNF-α production after LPS-injection and *in vitro* by LPS- or palmitic acid-stimulated macrophages. We also observed attenuation of lipolysis *in vitro* and *in vivo,* an effect that may be relevant for the reduction in macrophage accumulation in the adipose tissue as observed in the models of lipolysis induced leukocyte recruitment (intraperitoneal administration of isoproterenol).

Conclusion: The results, reported herein, suggest that Tb attenuate inflammation associated to weight gain and that this compound may be useful for the treatment and prevention of obesity-related metabolic disorders.

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IMPAIRED NEUTROPHIL EXTRACELLULAR TRAP (NET) GENERATION: A NEW PRIMARY IMMUNODEFICIENCY?

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Introduction: Recently, it has been described a different type of neutrophil response to infections, the neutrophil extracellular traps (NETs). These structures are composed of chromatin and microbicidal proteins, such as elastase and calprotectin. Since this is an important strategy, and in some cases decisive in resolution of infections, we analyzed NETs generation in patients suggestive of primary immunodeficiency and presenting severe fungal infection.

Methods and results: We conducted a study of 5 patients with severe chronic candidiasis and one with APECED (AIRE-1 deficiency). The NET generation assay was based on specific labeling of proteins, histone and elastase, and also genetic material (DNA). Then we analyzed the samples by fluorescence microscopy and fluorescence reading. We also evaluated the oxidative burst by flow cytometry (dihidrorodamina oxidation) and myeloperoxidase function (MPO) by chemiluminescence. We performed all assays *in vitro* with neutrophil treated or not with PMA (100nM), *C. albicans* (2:1), Curdlan and depleted Zymosan (both 100ug/mL). From the six patients analyzed, four have failed to generate NETs (< 5% of total area compared to NETs area from control). This failure was more evident when neutrophils from patients were stimulated specifically with C. albicans ($p < 0.001$, ANOVA). The APECED patient also showed failure in NETs generation when stimulated with agonists dectin-1 (AIRE-1 signalling pathway). All patients had NADPH oxidase and MPO normal function.

Conclusion: Our results starts a study of a new standard of primary immunodeficiency involving the specific failure NETs production associated with susceptibility to *C. albicans*. Furthermore, we confirmed that AIRE-1 is important in the neutrophils response to candida. This study brings an

opportunity to describe new primary immunodeficiencies, leading to the development of diagnostic methods and therapeutic strategies to improve the quality of life of these patients.

Financial support: CNPq, CAPES

INNATE ACTIVATION OF NK AND NK-LIKE T LYMPHOCYTES FROM OVARIAN CANCER PATIENTS.

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Introduction: NK lymphocytes are known by their ability to eliminate a variety of malignant cells in a process involving innate recognition by an array of stimulatory and inhibitory receptors. Similarly, the variant subset of NK-like T lymphocytes has been reported to eliminate tumor cells, but the targeting process might involve either innate or adaptive immune recognition. This study aimed to evaluate the functional activation of NK and NK-like T cells (pre, short- and long-term IL-2 stimulated), from blood and ascites of ovarian neoplasia patients.

Methods and Results: Blood was collected from 20 patients with pelvic neoplasias after signed consent: 9 benign (Bng, 6 ovarian neoplasia and 3 other pelvic neoplasia), 5 ovarian malignant without metastasis (Mlg) and 6 ovarian malignant with metastasis (MlgMt). Ascites (Asc) was collected from 6 patients with ovarian neoplasia. Mononuclear cells were separated by Ficoll-Paque gradient. NK and NK-like T cells activation (pre, short- and long-term stimulated) were evaluated against K562 (1:1 ratio) by the expression of CD107a, analyzed by flow cytometry. Short-term stimulation with IL-2 (1000UI/ml) was conducted overnight in RPMI-1640 medium supplemented with FBS (10%) and L-glutamine (2mM). Long-term stimulation was conducted by a 21 day culturing process with SCGM CellGro medium supplemented with anti-CD3 (10ng/ml, first 5 days), IL-2 (1000UI/ml) and FBS (10%). NK functional activation of pre stimulated cells, seemed to be impaired by the development of the disease as assessed by the MFI median/variation (Bng=72.90/ 0.54-134.09; Mlg=53.32/ 29.63-74.54; MlgMt=25.83/ 10.99-59.13; Asc=15.17/ 3.50-99.52). Short term stimulation increased significantly ($p<0.05$) NK cells activation (Bng=133.18/ 7.25-441.15; Mlg=239.53/ 122.62-367.17; MlgMt=127.46/ 59.24-241.40; Asc=156.77/ 11.41-241.35). NK-like T cells showed no activation and short-term IL-2 stimulation caused any effect. Interestingly, long-term stimulation elicited NK-like T functional activation. Statistical analysis inter groups was performed by Kruskal-Wallis test and intra groups by Mann Whitney test.

Conclusion: Results indicate that the functional integrity of NK cells is impaired as ovarian malignancies develop. Short-term stimulation restored functional activation of NK cells from patients of malignant groups. NK-like T lymphocytes were activated by innate recognition only after long-term stimulation.

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HUMAN PROPERDIN BINDS TO *LEPTOSPIRA* SPECIES AND PROMOTES ALTERNATIVE PATHWAY ACTIVATION

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Introduction: Leptospirosis is one of the most important worldwide zoonosis and is caused by pathogenic *Leptospira*. Activation of the alternative pathway (AP) of complement is crucial for killing non-pathogenic *L. biflexa* and properdin (P) acts effectively since this bacterium proliferates in P-depleted human serum. The aim of this work was to investigate if P binds equally to both non-pathogenic and pathogenic *Leptospira* and if this binding requires previous deposition of C3b. **Methods and results:** Several non-pathogenic and pathogenic serovar of *Leptospira* were incubated with different sources of properdin [normal human serum (NHS), unfractionated purified P, physiological dimers, trimers, tetramers of P (P2, P3, P4), or non-physiological aggregates (Pn)]. The binding was evaluated by ELISA and Western blot. All strains interacted with unfractionated purified P as well as P present in total NHS, but no significant binding differences were observed. However, non-pathogenic *Leptospira* bound significantly more to physiological forms P2 and P3 (0.79±0.2 and 0.64±0.2, respectively) than the pathogenic serovar (0.51±0.01 and 0.45±0.03, respectively); $p<0.05$. Also, the data show that the interaction of *Leptospira* and P occurs independently of previous deposition of C3b fragments on the bacteria surface. In addition, pathogenic, but not non-pathogenic, secreted leptospiral *proteases* were able to degrade purified properdin in 30 kDa fragment. Among several recombinant leptospiral membrane proteins tested, lipoprotein LIC11087, present only in pathogenic *Leptospira,* was the major ligand for P. **Conclusion:** In conclusion, the physiological forms of P bind most efficiently to *L. biflexa* and we propose that this may play a role in limiting

non-pathogenic *Leptospira* survival by potentially triggering activation of the AP. On the other hand, pathogenic *Leptospira* can degrade properdin, which may contribute to complement evasion and pathogen survival.

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NEUTROPHIL EXTRACELLULAR TRAPS (NETS) ARE INDUCED BY *PARACOCCIDIOIDES BRASILIENSIS*

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Introduction: *Paracoccidioides brasiliensis* is a dimorphic fungus that is the causative agent of paracoccidioidomycosis, a chronic, subacute or acute mycosis, with visceral and cutaneous involvement. This disease that is acquired through inhalation, primarily attacks the lungs but, can spread to other organs. Among the mechanisms of innate immunity against this fungus, phagocytic cells play an essential role. Recently, studies have focused on the role of neutrophils that are involved in primary response to the fungus. In addition to their ability to eliminate pathogens by phagocytosis and antimicrobial secretions, it has been shown recently that neutrophils can trap and kill microorganisms by release of extracellular structures composed by DNA and antimicrobial proteins, called neutrophil extracellular traps (NETs).The aim of this study was to demonstrate whether *P. brasiliensis* induces NETs release by human neutrophils. **Methods and Results:** Human neutrophils were isolated from peripheral blood of healthy donors and incubated with *P. brasiliensis* for 60, 90, 120 and 150 minutes. Some of these cultures were pré-treated with DNAse (to degrade NETs) or PMA (positive control of NETs release). The samples were analyzed by scanning electron microscopy and photomicrographs demonstrated that *P. brasiliensis* induces NETs 60, 90, 120 and 150 minutes after interaction with neutrophils. DNAse treatment degraded these structures, whereas PMA induced their formation. Moreover, by using confocal imunofluorescence microscopy the following NET constituents were identified: DNA (stained with DAPI, elastase (stained with FITC) and histones (stained with TEXAS RED). **Conclusion:** *Paracoccidioides brasiliensis* is able to induce NETs. These structures can be fungistatic and thus contribute to the effector mechanisms of neutrophils in early stages of infection with *P. brasiliensis*.

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PLASMINOGEN BINDS TO LEPTOSPIRAL IMMUNOGLOBULIN-LIKE PROTEINS AND INHIBITS COMPLEMENT ACTIVATION

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Introduction: The ability of *Leptospira* to evade the complement system is crucial for a successful infection. This assumption is supported by *in vitro* results demonstrating that saprophytic leptospiral strains are killed within a few minutes in the presence of normal human serum whereas pathogenic ones are able to survive for much longer periods of time. Different mechanisms have been shown to be involved in evasion of complement-mediated killing such as binding to negative complement regulators and the secretion of proteases. Another strategy employed by pathogens is the acquisition of plasminogen and its conversion to plasmin, which is able to cleave the complement components C3b and C5 on the pathogen's surface. Leptospiral immunoglobulin-like (Lig) proteins and LipL32, that are present only in pathogenic but not in saprophytic species, were shown to interact with complement regulators Factor H and C4BP and other components of the extracellular matrix. We therefore decided to evaluate the interaction of these proteins with plasminogen as a complement evasion mechanism.

Methods and Results: Both C- and N-terminal portions of LigA and LigB genes were cloned, expressed and purified as recombinant proteins in *E. coli*. We demonstrate that the Lig proteins and LipL32 were able to bind plasminogen, which was activated to plasmin by addition of human urokinase type plasminogen activator (uPA). We also show that plasminogen bound to LipL32 and Lig proteins was converted to active plasmin and this enzyme was able to degrade the C3b to produce 68, 46, 40, 30 and 17 kDa fragments. These

cleavages inactivate C3b, preventing progression of the complement cascade and blocking the three complement pathways. The identification of these leptospiral ligands is of great relevance since they may represent targets for immune interference.

Conclusion: Lig proteins are multifunctional molecules that contribute to leptospiral adhesion and immune evasion.

Financial Support: FAPESP and CAPES

THE ROLE OF MAP KINASES IN ANTIGEN PRESENTATION OF *NEOSPORA CANINUM*

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Introduction: Due to the high prevalence and economic importance of neosporosis, the development of safe and effective vaccines against this parasite has been a priority in the field and it is crucial to limit vertical and horizontal transmission in natural hosts. In that sense, the major aim of this study was to determine the role of mitogen-activated protein kinases (MAPK) signaling after contact of bone marrow-derived macrophages (BMDMs) with *Neospora caninum*. **Methods and Results:** Immune responses elicited in BMDMs infected with *N. caninum* tachyzoites or stimulated with its soluble antigen (NLA) was determined by IL-12 and IL-10 production in BMDMs culture supernatants, as well as by the expression of B7 co-stimulatory molecules and MHC. In order to evaluate the role of MAPK in these processes, BMDMs were pretreated with specific inhibitors to Erk 1 and 2 (PD98059), JNK (SP600125), and p38 (SB203580) BMDMs treated , with SB203580 upregulated IL-12 production, B7 and MHC expression, while downregulating IL-10 production after exposure to live tachyzoites and parasite soluble antigens. In order to check if that phenomena could be associated with protection *in vivo*, BMDMs were adoptively transfered to naïve C57BL/6 mice after in vitro stimulation with NLA, NLA+SB203580, or left untreated. Mice transferred with p38-inhibited BMDMs demonstrated increased survival to lethal parasite challenge. **Conclusion:** These results showed that *N. caninum* manipulates the p38 pathway in its favor, in order to downregulate the host's innate immune responses. Therefore, this piece of information can be useful for the development of a cellular vaccine against neosporosis.

Keywords: *Neospora caninum*; immune response; vaccine.

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Ts2 or Ts6 of *Tityus serrulatus* venom induce leukocyte recruitment and inflammatory mediators

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Introduction: Scorpion envenomation has become a public health problem in many regions of the world due to the increasing number of accidents. *Tityus serrulatus* venom (TsV) induces a systemic inflammatory response characterized by increase of cytokines and chemokines. There are many studies regarding venom actions, however little is known about the interactions of the fractions IX (Ts2) or X (Ts6) with the cells of immune system. Our aim was to characterize the local inflammatory response induced by intraperitoneal injection (i.p.) of Ts2 or Ts6 isolated from *T. serrulatus* venom. We analyzed influx and the profile of leukocytes into the peritoneal cavity, concentration of cytokines and oxide nitric (NO) in the cell-free peritoneal fluid. In addition, total proteins were quantitated.

Methods and Results: Ts2 or Ts6 (250 µg/kg) or PBS were injected in the peritoneal cavity of mice and after 4, 24 and 48 hours the peritoneal fluids were collected. The kinetics of cell recruitment induced by either Ts2 or Ts6 revealed an increase in neutrophils in the peritoneal cavity in all periods with peak at 4 hours. The cell-free peritoneal fluid was used to measure cytokines by ELISA, nitric oxide (NO) by Greiss method and total protein by Coomassie reagent. Ts2 or Ts6 were not able to induce NO production in peritoneal cavity. However, IL-6, TNF-α, IL-10, IL-1β and IFN-γ were increased as well as the amount of total protein.

Conclusion: Our results showed that Ts2 and Ts6 induced an inflammatory response with edema formation, neutrophils recruitment and inflammatory cytokines release. We suggest that the physiopathological manifestations of *T. serrulatus* envenomation may be mediated by the release of inflammatory mediators. These finding could be important in the development of specific drugs in the treatment for the envenomation scorpion.

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Evaluation of the biological impact of galectin-1 on the oxidative stress of neutrophils.

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Introduction - Galectin-1 (Gal-1) is a multifunctional glycan-binding protein that participates in modulation of the inflammation. It is known that Gal-1 is involved in some aspects of the neutrophil biology, as migration, phagocytic recognition, and production of reactive oxygen species (ROS). In the literature, there are few data on the role of Gal-1 in the oxidative metabolism of these phagocytes. This study evaluated the impact of exogenous and endogenous Gal-1 on the ROS production by neutrophils. **Methods and Results:** Through chemiluminescence (CL) assay it was possible to analyze the ability of recombinant human Gal-1 to induce and to inhibit ROS production in naïve and fMLP (formyl-Met-Leu-Phe) activated human neutrophils. Gal-1 induces ROS production in a dose-dependent way only in fMLP preactivated cells (10^{-7}M fMLP + 10μM Gal-1 CL area: $3x10^{7}$±$1,9x10^{7}$), similarly as J Immunol. 168(8):4034-41, 2002. Curiously, the oxidative stress induced by fMLP on naïve neutrophils is inhibited (49%) by pretreatment of the cells with Gal-1(10^{-6}M fMLP CL area: $3,6x10^{8}$±$1,3x10^{8}$; 10μM Gal-1 + 10^{-6}M fMLP CL area: $1,6x10^{8}$±$9,5x10^{7}$). The effects of Gal-1 on ROS production in both non-activated cells and activated cells are partially dependent of their lectin property. This lectin did not affect the reduction of ROS production in neutrophils stimulated sequentially with fMLP. The cell viability was similar in all experimental situations. Interestingly, peritoneal neutrophils from Gal-$1^{-/-}$ mice (n=3) release more ROS in response to 10^{-7}M fMLP (CL area: $1,9x10^{7}$±$5,4x10^{6}$) and exogenous 10μM Gal-1 (CL area: $8,4x10^{5}$±$1,0x10^{5}$) when compared to neutrophils from wild type animals (10^{-7}M fMLP CL area:$1,3x10^{7}$±$5x10^{6}$ and 10μM Gal-1 CL area: $5x10^{5}$±$2,1x10^{5}$). **Conclusion:** Therefore, this set of results suggests that Gal-1 could modulate, negative or positively, ROS production by neutrophils and it is associated with the cell activation level and by either the presence or absence of endogenous Gal-1. Finally, these results may help to elucidate the participation of Gal-1 in the regulation of inflammatory/infectious disorders.

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LIPID DROPLETS FORMATION AND LIPID MEDIATORS PRODUCTION BY ALVEOLAR MACROPHAGES INFECTED *IN VITRO* WITH ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS* FROM HUMANS

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Introduction: *Mycobacterium tuberculosis* (Mtb) is a virulent intracellular pathogen that infects and could persist in host macrophages. During the infection cell-mediated response protects the immunocompetent host by arresting, killing, and removing the multiplying bacteria. Leukotrienes (LT) are a lipid mediator from arachidonic acid (AA) metabolism that plays an important role in host defense against microorganisms. Lipid droplets (LD) also have importance by working like multifunctional organelles in the cellular signalization, activation, synthesis and secretion of inflammatory mediators. In this study, we investigate the formation of LD, LTB_4, PGE_2 production, cytokines and nitrite (NO) production by alveolar macrophages (AM) infected *in vitro* with 3 different isolates of *Mtb* in humans. **Methods and Results:** AM were obtained from Wistar rats, plated (2 x 10^5/well in round coverslip) and incubated at 37°C, 5% CO_2/O_2, 18 hours. AM were infected with pulmonary (P), extra pulmonary (EP) and non-cavitary (Ncav) isolates of *Mtb* (MOI 1:1) during 24 hours. After this period, the supernatants of AM were collected and nitric oxide (NO), cytokines, LTB_4 and PGE_2 production were determined. The round coverslips were fixed in formaldehyde 3.7% HBBS buffer for 48 hours and stained with Oil Red® for the counting of the LD (objective 100x). Dead by heat/formaldehyde or alive, P and EP isolates induce significant formation of LD and LTB_4 production in AM when compared to the medium and Ncav stimulation. They also induce IL-6 and IL-10, whereas low of NO, TNF-α, PGE_2, IL-1β and IL-4 were observed. However, in AM infected with the Ncav infection these mediators were increased. **Conclusion:** The P and EP did not induce in the AM a pro-inflammatory profile. However, the productions of LD and LTB_4 are important in the activation of the macrophages. Interestingly, the Ncav infection induces a different response with the increase of NO and cytokines. These differences in the activation of AM by the isolates can be an associated virulence factor and/or PRRs recognition.

Financial Support: FAPESP, CNPq.

THE INTERACTION OF *L. braziliensis* WITH TLR2 AND TLR4 PARTICIPATE IN THE PATHOGENESIS OF CUTANEOUS LEISHMANIASIS

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Introduction: Cutaneous Leishmaniasis (CL) caused by *L. braziliensis* is characterized by a strong Th1 immune response that is important for parasite elimination, but also is associated with the development of lesions. Although immunological studies have increased our understanding of the adaptive response in CL, several aspects of the initial stages of *Leishmania* infection in humans remain unknown. Macrophages act as the principal host cells for *Leishmania,* playing a central role in the development of the immune response against this parasite. The Toll Like Receptors (TLR) recognize pathogen-associated molecular patterns (PAMPs) which are characteristic of various groups of pathogens. Activation of these receptors initiates signaling pathways resulting in the production of proinflammatory cytokines by macrophages. Several studies have suggested the involvement of TLRs in the initial interaction of *Leishmania* with the immune system; however the exact role of these receptors in CL caused by *L. braziliensis* is still unknown. Aim of this study was to evaluate the expression of TLR 2 and TLR4 in monocyte subsets after exposition with *L. braziliensis* from patients with CL and from healthy controls (HC).

Methods and Results: The expression of TLRs was evaluated in monocytes subsets from 21 patients with CL and 8 HC infected with *L. braziliensis* stained with CFSE, upon exposition to soluble *Leishmania* antigen (SLA) and to LPS by flow cytometry. The expression of TLR2 and TLR4 after infection (15 ± 13% and 9 ± 9%, respectively) was decreased compared to LPS (50 ± 23% and 26 ± 22%), SLA (51 ± 26% and 24 ± 23%) and no stimuli (58 ± 20% and 34 ± 27%)

($p<0.0001$). The expression of TLR2 and TLR4 was higher in the intermediate monocyte subset *ex-vivo* (7 ± 5% and 11 ± 9%, respectively) and in the infected group (54 ± 21% and 40 ± 22%) compared to the classical and nonclassical subset ($p<0.001$). The expression of these receptors was also lower in monocytes infected with *L. braziliensis* from CL patients (36 ± 21% and 26 ± 17%) as compared to monocytes from HC (65 ± 14% and 47 ± 21%) ($p<0.001$ and $p<0.05$, respectively). Decreased expression of TLRs 2 and 4 was associated with increased intracellular expression of TNF-α.

Conclusion: The interaction of *L. braziliensis* with TLR2 and TLR4, in intermediate monocytes, triggers TNF-α production, one important cytokine involved in the pathogenesis of CL.

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EICOSANOIDS AND INFLAMMATORY MEDIATORS PRODUCTION IN BONE-MARROW DERIVED MACROPHAGES ACTIVATED BY ALTERNATIVE PATHWAY.

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Introduction: The innate immune response against microorganisms is dependent on the interaction of cells with certain molecules of pathogens and stimulation by cytokines of the pattern of response of T lymphocytes (Th1 or Th2). Classical macrophage activation (M1) is fundamental in the immune response against intracellular microorganisms and tumors. However, alternative macrophage activation (M2) is more varied, but generally plays a role in immune response by inhibiting Th1 type inflammation, and promoting tissue repair and remodeling. Thus, here we correlate the alternative activation of bone-marrow derived macrophage (BMDM) with the production of inflammatory mediators (including eicosanoids) after stimulation *in vitro* with microbial particles.
Methods and Results: We used C57BL/6 mice. Cells isolated from bone marrow (10^6/ml) were grown for 10 days (37 °C and 5% CO2) with Dulbecco's medium (DMEM) supplemented with 10 mM L-glutamine, antibiotics, with the addition of 20% horse serum and 30% from conditioned culture medium of L929 cells (M-CSF). BMDM were incubated or not with IL-4 and /or IL-13 (10 ng / ml) for 24 and 48 hours at 37°C in 5% CO_2. After this period the BMDM cultures were stimulated with LPS or β-glucan by 24 hours. The dosages of cytokines were performed by ELISA. The production of NO was indirectly assessed by the Greiss method. The lipid mediators (LTB_4 and PGE_2) were quantified by competitive enzyme immunoassay (Cayman). The BMDM activated by IL-4, IL-13 and IL-4/IL-13 (M2) for 24 and 48 hours showed no significant spontaneous production of NO and cytokines (IL-6, IL-10, TNF -α and KC). After stimulation with LPS or β-glucan, we observed down-modulation of proinflammatory mediators (NO, TNF-α and KC) on M2 in correlation with non-activated macrophage (M0) and an increase in the IL- 10 production in the M2 supernatant. The cytokine IL-6 was not modulated in these stimulated macrophages. For lipid mediators, we observed a natural tendency for more LTB_4 production than PGE_2 in M2 phenotypes. However, after stimulation with LPS or β-glucan the PGE_2 production were increased.
Conclusion: The plasticity of macrophage in M1 and M2 from cytokines not restricted only to the change in the physiology and metabolism but the immune

response in the microenvironment, providing useful information about the pathological mechanisms in which these cells participate.

Financial support: FAPESP and CNPq.

NF-kB MEDIATES CYTOSOLIC FLAGELLIN-INDUCED CASPASE-1-DEPENDENT iNOS ACTIVATION

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Introduction: We have recently described a pathway for iNOS activation in response to cytosolic flagellin through NAIP5/NLRC4 inflammasomes. Cytosolic flagellin-induced iNOS activation does not require MYD88, IL-1β and IL-18 but it is abrogated in the absence of caspase-1. Despite these relevant observations, it remains to be elucidated how caspase-1 mediates iNOS activation.

Methods and Results: First, we observed that cytosolic flagellin is able to induce NF-kB activation, as observed by western blot evaluation of IkB-a degradation. Moreover, iNOS induction in response to cytosolic flagellin is abrogated in the presence of PDTC, a selective IkB-a degradation inhibitor. Thus, cytosolic flagellin-induced caspase-1-dependent iNOS activation seems to be mediated by NF-kB. Infection of macrophages with flagellin-suficient *S. typhimurium* but not with flagellin-deficient *S. typhimurium* leads to caspase-1-dependent nitric oxide (NO) production. The inhibition of iNOS with aminoguanidine (AG) renders wild-type macrophages as susceptible as casp1$^{-/-}$ macrophages, suggesting that flagellin-induced caspase-1-dependent NO production during *S. typhimurium* infection strongly correlates to the control of infection by macrophages.

Conclusion: Our results indicate that NF-kB is required for caspase-1-mediated iNOS activation in response to cytosolic flagellin and that this pathway contributes to the control of *S. typhimurium* infection by macrophages.

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IDENTIFICATION OF NOD LIKE RECEPTORS (NLRs) IN HUMAN ATHEROMA LESIONS

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Introduction: Activation of innate immune receptors in atheroma lesions is involved in initiation and disease progression. Recently, a new group of innate receptors, called Nod Like Receptors (NLRs), was described and associated with recognition of several PAMPs and DAMPs. In addition, several members of the NLRs can assemble multimolecular complexes, termed inflammasomes, promoting activation of inflammatory caspases and maturation of pro-inflammatory cytokines IL-1b and IL-18. The aim of this study was to investigate whether components of the NLRs family are present in atheroma lesions and activation of inflammasomes by atheroma related antigens.

Methods and Results: 44 patients with critical stenosis of carotid submitted to endarterectomy were evaluated. Carotid specimens were analyzed for mRNA and protein expression by real time RT-PCR and immunohistochemistry, respectively. Peripheral blood monocytes of 4 healthy donors were differentiated in macrophages cultured in the presence or absence of LPS or cholesterol crystal or LPS + cholesterol crystal and production of IL-1b and TNF-a was analyzed by ELISA. Our data showed expression of mRNA for NLRP1, NLRP2, NLRP3, NLRC1, NLRC2, NLRC3, NLRC4, NLRC5, Naip, ASC, caspase-1, AIM2 and IL-1b in all carotid lesions. We also detected immunoreactivity for NLRP1, NLRP3, NLRC2, NLRC3, NLRC5 and caspase-1 in macrophages and foam cells. Stimulation of macrophages derived from blood monocytes with LPS induced small production of IL-1b while the concomitant stimulation with cholesterol crystals promoted high levels of IL-1b production. In addition, production of TNF-a was unaffected by concomitant stimulation with cholesterol crystals.

Conclusion: Altogether our results showed expression of several components of NLRs family in macrophages and foam cells of human atherosclerotic

lesions. In addition, the production of IL-1b, but not TNF-a, by macrophages after stimulation with cholesterol crystals suggests inflammasome activation.

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CYTOSOLIC FLAGELLIN INDUCES CASPASE-1/11-INDEPENDENT LYSOSOMAL CATHEPSINS-MEDIATED INFLAMMATORY CELL DEATH THAT PARTICIPATES IN THE CONTROL OF *S. TYPHIMURIUM*

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Introduction: Cytosolic flagellin is sensed by NAIP5/NLRC4 inflammasomes leading to caspase-1 activation. Caspase-1 is responsible for IL-1β/IL-18 processing and secretion and inflammatory cell death, named pyroptosis. Regardless of the well-established role of pyroptosis as an effector mechanism to clear intracellular infections, its molecular regulation remains to be solved.

Methods and Results: We demonstrate that purified flagellin from *Bacillus subtilis* delivered directly into macrophages cytosol by transfection lipid vesicles leads to NLRC4, ASC and caspase-1/11-dependent IL-1β secretion but cell death still occurs in the absence of either of these molecules as assessed by fluorescence microscopy according to ethidium bromide (EtBr) incorporation and loss of vital acridine orange (AO) staining. Also, flagellin-induced cell death contributes to the control of *S. typhimurium* replication by wild type and caspase-1$^{-/-}$ macrophages. Besides cytosolic flagellin-induced caspase-1/11-independent cell death display some apoptotic features, this cell death does not require apoptotic caspases activation and results in loss of membrane integrity and release of the known damage signal IL-1a, unlike classic apoptosis. Finally, cell death induced by cytosolic flagellin seems to rely of lysosomal pathways in which cathepsins B and D have a synergistic role.

Conclusion: These results demonstrate that cytosolic flagellin induces a novel pro-inflammatory caspase-independent lysosomal-dependent form of cell death that could be considered an additional effector mechanism to restrict flagellated bacterial infections.

Financial support: CAPES; CNPq, FAPESP.

INVOLVEMENT OF NLRP3 IN THE CONTROL OF *TRYPANOSOMA CRUZI* BY MACROPHAGES

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Introduction: *Trypanosoma cruzi*, the agent that causes Chagas disease, is a flagellated intracellular protozoan whose control depends on both innate and adaptive immunity. Although the role of Toll-like receptors has been described as important for the control of the infection, there is no data regarding the involvement of inflammasomes. It is known that these receptors induce the activation of caspase-1 that, in its turn, is responsible for the secretion of IL-1 β and IL18 and the induction of a type of cell death called pyroptosis. In addition, our group recently described a pathway for the activation of the enzyme nitric oxide synthase (iNOS) that occurs via inflammasomes by the action of caspase-1. Thus, the aim of this work was to access the involvement of NLRP3 in the control of *T.cruzi* by macrophages.

Methods and Results: Macrophages from NLRP3$^{-/-}$ and caspase-1$^{-/-}$ are more permissive to *T. cruzi* replication than the WT and the highly susceptible MyD88$^{-/-}$ macrophages. Evaluation of lactate dehydrogenase (LDH) release and ethidium bromide and acridine orange staining revealed a similar frequency of dead *T. cruzi*-infected macrophages from WT and caspase-1$^{-/-}$ mice, ruling out the participation of pyroptosis in the control of *T.*cruzi by NLRP3. Interestingly, NLRP3$^{-/-}$ and caspase-1$^{-/-}$ macrophages are fully defective in the production of nitric oxide (NO). Moreover, the inhibition of caspase-1 but not IL-1R in MyD88$^{-/-}$ macrophages abrogates the little NO production by these cells and renders MyD88$^{-/-}$ macrophages as susceptible as NLRP3$^{-/-}$ and caspase-1$^{-/-}$ macrophages to *T. cruzi* replication.

Conclusions: Taken together our results suggest that NLRP3 inflammasomes are involved in the control of *T. cruzi* by macrophages through a mechanism dependent on caspase-1-mediated NO production.

Financial support: CAPES; CNPq, FAPESP.

ERYTHROPOIETIN TREATMENT ALTERS THE EVOLUTION OF MURINE EXPERIMENTAL INFECTION CAUSED BY *H. capsulatum*

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Introduction: Histoplasmosis is a infectious disease caused by the fungus *Histoplasma capsulatum* (*H. capsulatum*), which is widely distributed around the world, but mainly in the Americas and parts of Africa and Asia. Infection control is associated with the effective dominance of the Th1 immune response that leads to classical activation of macrophages, key cells involved in containing the fungal proliferation. Erythropoietin (EPO) is a glycoprotein hormone classically known to regulate the erythrocytes production, but there is evidence of its effects in non-erythroid cells, such as macrophages. **Objective:** To investigate whether treatment of *H.capsulatum* infected mice with EPO alters the course of infection. **Material and Methods**: C57BL / 6 mice were infected or not with $1x10^6$ viable yeast *H.capsulatum* and after treated 3 times a week subcutaneously with EPO (45 or 180U/animal). Animals survival was evaluated until 35 days after infection. After 2, 7 and 14 days of infection the animals were evaluated for the number of colony forming units (CFU) in the lung and spleen, cells recruitment to bronchoalveolar space and total number of circulating erythrocytes. **Results**: Treatment with EPO increased the mortality of infected animals compared with those not treated. At 7 days post infection, in animals treated with 180U of EPO, we observed increase in CFU number either lungs and spleen, but at 14 days post infection this phenomenon is reversed. In animals treated with the dose of 45U of EPO, we observed, 2 days after infection, reduction in number of neutrophils in bronchoalveolar space. Regarding the number of erythrocytes, treatment with 180U of EPO, reduced the number of these cells compared to untreated infected controls. **Conclusion:** Our preliminaries results suggest that EPO administration may facilite the spread of fungi promoting the increased in mortality of infected animals.The mechanism are been investigating.

Financial Suport: FAPESP, CNPq.

LIPID DROPLET BIOGENESIS IS TRIGGERED BY THE PATHOGENIC FUNGI *Paracoccidioides brasiliensis* AND THEIR CELL WALL LIPIDS

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Introduction: The dimorphic fungus *Paracoccidioides brasiliensis* is the etiological agent of Paracoccidioidomycosis, a human systemic granulomatous disease that affects mainly the rural population of South America. The initial inflammatory and innate immune response caused by this fungus is still poorly understood. The present work aimed to investigate whether the inflammatory activation marker lipid droplets are involved in the immune response triggered by *P. brasiliensis.* In addition, we analyzed the immunomodulatory properties of lipids from *P. brasiliensis* cell wall. **Methods and Results**: *P. brasiliensis* lipids were isolated, purified and characterized by Thin Layer Chromatography (TLC). In order to verify whether two different strains of *P. brasiliensis* and its respective lipids fraction are capable of inducing lipid droplets formation, peritoneal macrophages from BALB/C and C57BL/6 mice were infected with *P. brasiliensis* (ratio 1:1) and lipid droplets formation was accessed by confocal microscopy, transmission electron microscopy and flow cytometry. Lipid droplets were stained with Bodipy, Osmium tetroxide and Oil Red O. To characterize the cellular and molecular mechanisms involved in lipid droplets formation induced by *P. brasiliensis*, macrophages were previously treated or not with different pharmacological inhibitors: GW9662 ($PPAR_\gamma$ antagonist), JSH-23 (Inhibitor of NFκB), C75 (inhibitor of fatty acid synthase), Metil-b-ciclodextrin (disruptor of lipid-rafts), Piceatanol (tyrosine kinase Syk inhibitor). *P. brasiliensis,* as well as their cell wall lipids, triggered lipid droplet formation in mouse macrophage after 24h of infection/stimulation. Lipid droplet biogenesis was dependent of: (I) the integrity of lipid rafts, (II) fatty acid synthase, (III) tyrosine kinase Syk, (IV) $PPAR_\gamma$. However, it was independent of the translocation of the transcription factor NFkB towards the nucleus. **Conclusion**: Taken together our data suggests an immunomodulatory property for *P. brasiliensis* cell wall lipids. Furthermore, we showed that *P. brasiliensis* triggered lipid droplets formation in a specific pathway, which may be a potential

target of pharmacological intervention against infection by this pathogenic fungus.

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5-LIPOXYGENASE REGULATES THE PRODUCTION OF PRO-INFLAMMATORY MEDIATORS BY BONE-MARROW DERIVED MACROPHAGES IN THE PRESENCE OF LIPOPOLYSACCHARIDE

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Introduction: Gram-negative bacteria activates the host innate immune system, inducing the release of pro-inflammatory molecules such as chemokines, cytokines and reactive oxygen species. These inflammatory mediators are related to various signs and symptoms seen during bacterial infections. 5-Lipoxygenase (5-LO) metabolites have been suggested to participate in inflammatory response in the presence of pathogen-associated molecular pattern, such as LPS. Furthermore, leukotriene B_4 (LTB_4) has also been implicated in antimicrobial defense promoted by immune system. Here we addressed whether 5-LO-metabolites are required for the production of pro-inflammatory mediators by bone-marrow derived macrophages (BMDM) stimulated with LPS. **Methods and Results:** BMDM from wild type (WT) and 5LO-knockout mice (5-LO$^{-/-}$) were primed or not with IFN-γ during 2 hours and then stimulated 24 hours with TLR4 agonist, LPS. The culture supernatants were used to measure pro-inflammatory mediators. Cytokine and chemokine quantification were determined by ELISA. NO was determined using the Griess reaction. The LPS stimulated the release of IL-6, TNF-α, KC and NO in BMDM and the primming with IFN-γ potentiated this effect, except in the case of KC production, which decreased after IFN-γ-priming. Compared with WT cells, 5-LO$^{-/-}$ BMDM exhibited a marked reduction in IL-6, KC and NO either on primed and non-primed BMDM. On the other hand, TNF-α production by 5-LO$^{-/-}$ BMDM was higher compared with WT cells. **Conclusion:** There is a divergent production of TNF-α by WT and 5-LO$^{-/-}$BMDM. Since the transcription of IL-6, TNF-α and iNOS genes are under the control of NF-κB transcription factor, this divergence production of TNF-α can evidence a different molecular regulation mechanism by which LTB4 amplifies NF-κB activation.

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REGULATION OF INFLAMMASOME ACTIVATION BY *Paracoccidioides brasiliensis*

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Introduction: Fungal infections represent a serious threat, especially in immunocompromised patients. Paracoccidioidomicosis is a granulomatous disease caused by *Paracoccidioides brasiliensis* and has relevant impact to tropical medicine. Interleukin-1beta (IL-1b) is a crucial key pro-inflammatory factor in innate antifungal immunity regulated by inflammasome. The mechanism by which the mammalian immune system regulates IL-1b production after fungal recognition is still not fully understood. Here we evaluated the regulation inflammasome activation by *P. brasiliensis* in murine macrophages in vitro. **Methods and Results**: In order to verify whether *P. brasiliensis* is capable of activating the inflammasome complex, we infected bone marrow derived macrophages (BMM) or peritoneal macrophage and analyzed: (I) the cytokine profile by ELISA and qPCR, (II) the gene expression of inflammasome componentes by qPCR-Array, (III) ATP release by chemiluminescent luciferease assy, (IV) caspase-1 activation by Flow Cytometry and (V) the cellular and molecular mechanism involved in this process by using different pharmacological inhibitors. Our results indicated that *P. brasiliensis* induced ATP release, caspase-1 activation and IL-1b, TNF and IL-6 secretion. We also verified that IL-1b secretion induced by *P. brasiliensis* was dependent of caspase-1 activation, ROS release, lysossomal damage, tyrosine kinase SyK and potassium efflux. In addition *P. brasiliensis* triggered up regulation of: (I) NOD-like receptors mRNA *Nlrc*5, *Nlrp*3 e *Nlrp*4e; (II) Caspases gene mRNA *Casp*1*, Casp 8, Casp 12;* (III) adaptor protein ASC, and (IV) Cytokines gene mRNA *IL-1β, IL-18, IL-33, IL-6* and *TNF-α.* However *P. brasiliensis* induced inhibition of *Nlrp*1 mRNA gene expression. Moreover, in order to better understand the role of NLRC4, Caspase-1/11 and adaptor protein ASC in the host ability to kill *P. brasiliensis,* BMM cells from WT, NLRC4-/-, Caspase-1/11-/- and ACS -/- were infected with *P. brasiliensis* and CFU assay was performed. Our results showed that ASC and Caspase-1/11, but not NLRC4, played an important role in the killing of *P. brasiliensis*. **Conclusion:** Taken together our data demonstrated that *P. brasiliensis* is capable of inducing inflammasome activation in vitro and characterized how this

pathogenic fungus modulates the cellular and molecular mechanism involved in this process.

Support: CAPES and CNPq.

A ROLE FOR DIET, SHORT CHAIN FATTY ACIDS AND GPR43 IN INFLAMMASOME ACTIVATION AND GUT HOMEOSTASIS

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Introduction: Diet and gut microbiota represent new avenues to understand the basis of human diseases. Intake of fibre has beneficial effects on numerous conditions including inflammatory bowel disease. The gut microbiota break down complex polysaccharides from dietary fibres into Short chain fatty acids (SCFAs), mostly acetate, playing a role in immune regulation by signalling through the G protein-coupled receptor GPR43. The gut epithelium plays a critical role in the control of inflammatory diseases. One pathway that regulates epithelial integrity is activation of inflammasome and production of the cytokine IL-18, which enhances epithelial cell-cell adhesion. Thus, our aim is investigate the role of fibre diet and GPR43 on gut integrity and inflammasome activation, in colitis. **Methods and Results:** To explore the beneficial effects of fibre diet on gut homeostasis we performed the DSS colitis model (2%w/v) in C57/bl6 mice on high fibre and low fibre. Diet affected clinical score and colon length in wild type mice in the DSS model, with high fibre providing the greatest benefit. However, when *Gpr43*$^{-/-}$ mice were fed with the same diets, and subjected to DSS, then the protective effect of high fibre was not observed. Serum concentration of FITC-dextran

after gavage was used by measure gut epithelial integrity, *Gpr43*$^{-/-}$ mice showed higher fluorescence when compared to WT mice. Western blot analysis of inflammasome related proteins (cleaved caspase-1 and IL-18) were increased on colonic epithelium cells isolated from DSS-fed mice and that received high fibre. Surprisingly, absence of GPR43 resulted in a marked reduction of cleaved caspase 1 in isolated colonic epithelial cells as well as reduced level of serum cytokine IL-18 during treatment with DSS colitis indicating an impairment of inflammasome activation. Interestingly, germ-free mice, which are devoid bacteria and don’t produce SCFAs, also showed compromised epithelial integrity, which was reversed when germ-free mice were treated with acetate. **Conclusion:** High fibre diet protects against DSS colitis in mice, in a process dependent in part of GPR43. SCFAs binding GPR43 were necessary for proper activation of the inflammasome pathway in gut epithelial cells, which is important for epithelial integrity and tissue repair. Thus, this work suggest a close relationship between diet, microbiota and gut homeostasis.

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COMPARATIVE FECAL MICROBIOME OF HOLSTEIN AND NELORE BOVINES: DIFFERENTIAL BACTERIAL COMPOSITION CAN BE RELATED TO SUSCEPTIBILITY TO ECTOPARASITES

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Introduction: The microbial composition of gastrointestinal tract plays important role in immunological status in animals. Bovine breeds present different levels of immunity to tick infestations; zebu are resistant and taurine are susceptible. Since microbial metabolites can be associated with the innate immunity of hosts, we evaluated the fecal microbiome from a zebu and taurine breed, for analyzing if the diversity found could be related to traits such as ectoparasites susceptibility. **Methods and Results:** We extracted genomic DNA of fecal samples collected from rectum of 6 Nelores (zebu) and 6 Holsteins (taurine), under same feed and animal management system. The 16S rRNA genes were amplified using primers for V5-V6 region and pyrosequencing of samples was performed using Roche FLX high-thoughput sequencing. Microbial biodiversity and richness were analyzed based on OTUs. The 12 bovine fecal samples yielded 131,737 of filtered high-quality reads with median read length of 287 nucleotides. The taxonomic assignment was achieved at phylum, family and genus level. As expected, the most abundant phyla in fecal microbiome were Firmicutes (66.4% in Hol; 68.8% in Nel) and Bacteroidetes (30.4% in Hol; 26.6% in Nel). Some genera presented differential prevalence according to the breed (compared with test-t). The genera with frequency significantly higher in Nelore are: Streptococcus, Alistipes and Methanobrevibacter; whereas in Holstein are: Acetivibrio, Bulleidia and Oscillibacter and Bacteroides. Among them, we highlight the Bacteroides genus, significantly more frequent in Holstein (50.4% vs 31.1% in Nel, $p=0.02$). *Bacteroides* bacteria generate metabolites from aromatic aminoacids. Noteworthy, the skatole metabolite (3-methylindole, derived from tryptophan) is considered one of the more malodorous compounds in swine manure odor, also implicated as an off-flavor component of pig meat. Moreover, it has been related with acute pulmonary edema and emphysema bovines. **Conclusion:** As skatole is attractive to insects, we believe that the major prevalence of *Bacteroides* in feces of Holsteins might be related to attraction of ectoparasites, such as myiasis-causing flies and ticks. Thus, the odor generated in feces would be one among several factors that contribute for susceptibility to tick infestations in Holstein breed.

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EXPRESSION OF PENTRAXIN-3 IN CHORIOAMNIOTIC MEMBRANES FROM PREGNANCIES COMPLICATED BY PRETERM PREMATURE RUPTURE OF MEMBRANES OR PRETERM LABOR

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Introduction: Pentraxin 3 (PTX3) is an essential component of the innate immune response and it acts as a pattern recognition receptor (PRR). It recognizes microbial products and opsonizes fungi, Gram-positive and Gram-negative bacteria. In addition, it has been shown to have the capacity to activate the complement system. Few studies investigated the PTX3 expression in gestational complications associated with microbial invasion of the amniotic cavity, and conflictive results have been described. This study aimed to evaluate the expression of PTX3 in chorioamniotic membranes of pregnancies complicated by the Preterm Premature Rupture of Membranes (PPROM) or Preterm Labor (PTL) in the presence of histologic chorioamnionitis (CA). **Methods and Results:** Thirty membranes from pregnancies complicated by PPROM (15 with and 15 without chorioamnionitis) and twenty-five membranes from pregnancies complicated by PTL (15 with and 10 without chorioamnionitis) which had preterm delivery as gestational outcomes were included in this study. All membranes were evaluated histopathologically and fragments were subjected to total RNA extraction. RNA samples were reverse transcribed and the quantification of PTX3 expression measured by the real-time PCR technique. Quantification of PTX-3 among the groups was performed by Kruskal-Wallis test. The level of significance adopted was 5%. Of the chorioamniotic membranes samples included, 92.7 % expressed PTX3 and no statistically significant difference was observed in the relative concentrations of PTX3 mRNAs ($p = 0.35$) among the studied groups: PPROM with CA (Md: 0.15; 0.02-0.88), PPROM without CA (Md: 0.15; 0.00-0.36), PTL with CA (Md: 0.25; 0.04-2.14) and PTL without CA (Md: 0.40; 0.12-1.85). **Conclusion:** The chorioamniotic membranes from pregnancies complicated by PPROM and PTL express PTX3 and such expression is not increased in the presence of histologic chorioamnionitis.

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PENTRAXIN-3 LEVELS IN AMNIOTIC FLUID AND IMMUNOLOCALIZATON IN THE CHORIOAMNIOTIC MEMBRANES FROM TERM AND PRETERM PREGNANCIES

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Introduction: Pentraxin 3 (PTX3) is an important protein that has an essential role in the regulation of innate immune response, and is rapidly produced and released by several cell types in response to proinflammatory signals. Few studies in the literature have investigated PTX3 expression during normal pregnancy and in gestational complications associated with prematurity and conflictive results have been described. This study aimed to evaluate the PTX3 levels in the amniotic fluid (AF) and to describe the immunolocalization of this mediator in chorioamniotic membranes during the last weeks of term and preterm pregnancies complicated or not by premature labor (PL). **Methods and Results:** Ninety five health pregnant women submitted to pregnancy resolution by caesarean section [37 weeks (n=15); 38 weeks (n=34), 39 weeks (n=31) and 40 weeks (n=15)] and 26 pregnant women who delivered prematurely (PTD), [in the presence (n=15), or absence of PL (n=11)] were included in the study. Measurement of PTX3 levels in the AF was performed by immunoenzimatic assay (ELISA) and the immunolocalization was performed by immunohistochemistry in the chorioamniotic membranes. The statistical analysis for comparing the PTX3 levels in the groups was performed by Kruskal-Wallis test followed by Dunn's Method, and the level of significance adopted was of 5%. Patients with PTD and PL (Md: 1280.4 pg/mL; 567.9-3091.5), had a significantly higher median AF PTX3 levels than patients with PTD without PL (Md: 128.9 pg/mL; 87.5-152.7) (p=0,001). Patients who delivered at the 40th week of gestation (Md: 355.7 pg/mL; 255.6-644.6) had a

significantly higher median AF levels of PTX3 compared with patients who presented PTD without PL (Md: 128.9 pg/mL; 87.6-152.7) (p=0,0007). No statistically significant difference of PTX3 levels was found in term pregnancy regardless of the gestational age. Immnostaining for PTX3 was localized in the amnion, corion and decidua of all the chorioamniotic membranes. **Conclusion:** The chorioamniotic membranes are sources of PTX3 and its levels can be detected in AF in term pregnancies throughout gestation age. PTX3 levels are statistically significantly higher during the labor in PTD. This finding suggests that the increased PTX3 levels are part of the physiologic activation of the proinflammatory maternal response during the process of labor.

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ROLE OF NEUTROPHILS IN THE LIVER STERILE INJURY

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Introduction: Acetaminophen (APAP) is a safe analgesic and antipyretic drug. However, at high doses, APAP causes acute liver failure (ALF), a disorder associated with massive hepatocyte necrosis. In this context, spilled intracellular contents, cytokines and chemokines would induce neutrophil recruitment and activation, which could lead to additional liver injury. Therefore, our objective was to investigate the role of neutrophils during APAP-induced sterile liver injury.

Methods and Results: ALF was induced by APAP overdose (500mg/kg) in C57BL/6 and TLR9$^{-/-}$ mice (n≥5 for all experiments). Liver injury was quantified by serum alanine aminotransferase levels (U/L) and histopathology; lung injury was assessed by bronchial-alveolar lavage and histopathology. Neutrophil influx was measured by myeloperoxidase activity (neutrophils x10^4) and intravital microscopy. Mitochondrial DNA was quantified by Real-Time PCR. Cytokines and chemokines were measured by ELISA in liver, lung and serum (pg/mL). Also, co-culture of human neutrophils and HepG2 cells was performed (% viability). Data is shown as (mean±SEM).

During APAP overdose, mice developed liver injury (6961±1223) and hepatic neutrophil accumulation (77.6±15.4). Blockage of neutrophil infiltration by anti-GR1 depletion (RB6-8C5; 200µg/mouse) or combined CXCR2-FPR1 antagonism (DF2156a, 30mg/kg; BOC-1, 2mg/kg) significantly prevented liver injury (aprox. 50% reduction). Isolated human neutrophils were cytotoxic to HepG2 cells when co-cultured (56.72±2.4), and the mechanism of neutrophil killing was dependent on direct contact with HepG2 cells (90% inhibition) and the CXCR2-FPR1 signaling pathway (30% inhibition). Also, in mice and humans, serum levels of both mitochondrial DNA and CXCR2-chemokines were increased during acute liver injury (1000-fold and 2-fold, respectively). Accordingly, APAP-treated mice presented a marked systemic inflammation, shown by higher levels of serum cytokines / chemokines (aprox.3 fold increase)

and lung injury, which were prevented by CXCR2-FPR1 blockage and TLR9 absence.

Conclusion: Chemokines and mitochondrial products collaborate in neutrophil-mediated injury and systemic inflammation during acute liver failure.

Ethical Approval: Human (CEP-FIOCRUZ 22/03); Animal (CETEA UFMG (051/11)).

Financial Support: CAPES, CNPq, FAPEMIG.

DYSFUNCTIONAL INNATE ANTIVIRAL FACTORS EXPRESSION IN MOTHERS INFECTED BY HIV-1 AND NEWBORN

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Introduction: A high percentage (65-80%) of infants from mothers infected by HIV-1 infection was uninfected even in the absence of antiretroviral therapy. This finding emphasizes the importance of evaluating the immunological characteristics in the maternal-fetal interface, mainly related to the innate immune response. The purpose of this study is to evaluate the expression of antiviral factors in HIV-infected mothers and umbilical cord blood (CB), compared with uninfected mothers-CB.

Methods and Results: The antiviral factors, including antimicrobial peptides, as α-defensins, endogenous antiviral proteins, as APOBEC (apolipoprotein B)3G and 3F, TRIM (Tripartite Motif) -5α, TRIM22, factors induced by type I IFN, and MxA (Myxovirus resistance protein A) and teterin were evaluated in cord blood and peripheral blood mononuclear cells (PBMC) as well as placental tissue. The results show that expression of teterin, TRIM-5α, MxA and IFN-β are increased in PBMC from infected mothers (n =7) compared to control mothers (n =12). Moreover, the expression of these factors in CB from mothers infected with HIV were similar those found in CB from control mothers group. Higher α-defensin 1 expression was detected in newborn compared to the adult counterparts. In contrast, it was observed decreased expression of APOBEC3G protein in decidua and TRIM-5α in fetal face of HIV-infected mothers compared to control mothers.

Conclusion: This data shows that HIV infection alters the expression of APOBEC3G and TRIM-5α at the post-translational stage, in placental tissue, contrariwise to the enhanced expression in PBMC from HIV-infected mothers. Despite of the immunological immaturity in the neonatal period, the maternal

infection with HIV generated a similar profile of antiviral factors expression in newborn, probably due to a complex factor interaction related to the pregnancy and HIV-1 infection.

Financial support: FAPESP/CAPES/ LIM-56/HCFMUSP.

LIPID BODIES FORMATION, TNF-α AND EICOSANOIDS GENERATION IN MONOCYTES FROM HIV-1 INFECTED SUBJECTS STIMULATED WITH β-GLUCAN FROM *HISTOPLASMA CAPSULATUM* CELL WALL.

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Introduction: Infection with HIV-1 results in the development of AIDS by causing the depletion and dysfunction of $CD4^+$ T lymphocytes. One consequence of the depletion and dysfunction of $CD4^+$ T lymphocytes in AIDS progression is the decline of production of cytokines, marked impairment of Leukotrienes (LTs) synthesis, chemotaxis, phagocytosis, and the killing of microorganisms by macrophages, monocytes, and neutrophils, facilitating opportunistic infections such as by fungus. Infection with *Histoplasma capsulatum* (*Hc*) a facultative, intracellular fungal, produces a broad spectrum of diseases, and progress to life-threatening systemic disease in individuals with AIDS. However, the mechanisms that regulated intracellular lipid accumulation in the course of HIV-1 infection and eicosanoids production after *Hc* infection are not well understood.

Methods and Results: Human PBMC were isolated over gradients of Ficoll-Paque from patients infected or non with HIV-1. Adherent monocytes from HIV-infected and non-infected were stimulated *in vitro* with β-glucan or medium for 8 h. The HIV-infected subjects were divided in two groups according with $CD4^+$ T cells count: $CD4^+$ Low (CD4<500 cells/mm^3) and $CD4^+$ high (CD4>500 cells/mm^3). Lipid bodies (LB) were enumeration microscopically after Oil Red® staining, and the cells were stimulated with A23187 (0.5 μM) for LTB_4 and PGE_2 release measured by EIA. TNF-α levels release in supernatants were measured by ELISA. Monocytes from HIV-1-infected patients with <500 $CD4^+$ T cells/mm^3 were unable to elicit either LB formation induced by β-glucan or eicosanoid generation when compared with PBMC from healthy donors. The inability to form LBs was correlated to low production of LTB_4 and high production of TNF-α and PGE_2.

Conclusions: The imbalance between LTB_4 and PGE_2 production could increase virus proliferation and occurrences of opportunistic infections, such as histoplasmosis. Under these circumstances, LB could be pivotal for eicosanoids production and host defense during fungal infection in HIV-1 infected subjects.

Financial support: FAPESP and CNPQ.

EXPRESSION OF BETA DEFENSINS IN CHORIOAMNIOTIC MEMBRANES OF PREGNANCIES COMPLICATED BY PREMATURITY

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Introduction: Inflammatory events can be pronounced in the amniotic cavity in pregnancies complicated by prematurity. The chorioamniotic membranes play fundamental role in the local innate immunity and inhibit the microorganisms growth, partly by the expression of human beta defensins (HBDs). These molecules are natural antimicrobials that present antibacterial, antifungal and antiviral activities and are produced by epithelial cells. The objective of this study was to quantify the expression of HBDs 1, 2, 3 and 4 of mRNA and protein in chorioamniotic membranes from pregnancies complicated by prematurity. **Methods and Results:** Fragments of chorioamniotic membranes were collected from pregnant women admitted at the Obstetrics Unit of the Clinical Hospital of the Botucatu Medical School, Botucatu, São Paulo. The study group consisted of 20 chorioamniotic membranes samples from pregnant women with preterm labor, in the presence or not of Preterm Premature Rupture of Membranes (PPROM), who delivery prematurely. In the control group, 19 chorioamniotic membranes from pregnancies at term were analysed. Samples of the membranes were fixed in 10% formalin, embedded in paraffin and sectioned for immunolocalization of HBD 1, 2, 3 and 4 by immunohistochemistry technique. Other samples were collected in liquid nitrogen and total RNA was extracted to quantify mRNA expression of HBD 1, 2, 3 and 4 using Real-time quantitative PCR. Statistical analyses were performed using Mann Whitney test in SigmaStat Software and the level of significance adopted was of 5%. HBD-2 mRNA was not detectable in chorioamniotic membranes samples included in the study. The relative expression of HBD1, HBD3, and HBD4 were not significantly different when compared preterm HBD1(fold-change 0.62 (0.0-105.0)); HBD3 (fold-change

0.18 (0.0-5.2)); HBD4 (fold-change 0.13 (0.0-140.5)) and term deliveries (HBD1 (fold-change 1.38 (0.02-25.0)); HBD3 (fold-change 0.19 (0.0-43.2)); HBD4 (fold-change 0.0 (0.0-972.1)). HBD 1, 2, 3 and 4 were immunolocalized to amnio, chorion and decidua cells. **Conclusion:** mRNA expression analysis and immunolization of HBD 1, 2, 3 and 4 revealed that chorioamniotic membranes are sources these defensins and their mRNA expressions are not associated with presence of preterm labor.

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ROLE OF NLRP3 INFLAMMASOME IN GM-CSF PRIMED BONE-MARROW DERIVED MACROPHAGES RESPONSE TO GLUCANS FRACTIONS FROM *HISTOPLASMA CAPSULATUM* CELL WALL

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Introduction: Innate immune detection of microbial pathogens such as viruses, bacteria, and fungi is critical for their elimination as well as to trigger Ag-specific adaptive immunity. Macrophages recognize conserved microbial components (PAMPs) through pattern-recognition receptors (PRRs), and initiate inflammatory responses. These ligants include membrane-bound toll-like receptors (TLRs) and intracellular proteins such as NOD-like receptors (NLR). Among these NLRs, NLRP3 responds to multiple stimuli and forms a multiprotein complex with ASC and caspase-1(NLRP3 inflammasome), which triggers secretion of an endogenous pyrogen IL-1β. β-glucans are the major cell wall components of fungi and their recognition is mediated through PRRs that plays an important role in antifungal immune response and production of proinflammatory mediators. Here we addressed the correlation with inflammasome components and inflammatory mediators production after GM-CSF primed bone-marrow derived macrophage (BMDM) stimulated with glucans fractions from *Histoplasma capsulatum (Hc)* cell wall. **Methods and Results:** Bone marrow cells were isolated from femurs from mice deficient of Caspase-1 (Casp1$^{-/-}$), NLRP3 (Nlrp3$^{-/-}$), or ASC (ASC$^{-/-}$) and the wild type (WT) C57BL/6 mice and cultivated (10^6/ml) in a cell-conditioned medium as a source of macrophage colony-stimulating factor. After 10 days, BMDM (10^5 cells/well) were primed with GM-CSF and stimulated *in vitro* with different concentrations of β-glucan (F1) and α-glucan (F2) from *Hc* cell wall during 24h in 5% CO_2 at 37ºC. GM-CSF priming increased Dectin-1, TLR2 and MR expression by qRT-PCR method. Indeed, TRAM mRNA expression is up-regulated, which was associated with elevated MyD88 and TRIF mRNA expression after GM-CSF treatment. Our results also showed that β-glucan (F1) elicited few release of mature IL-1β. However, the transcribed immature form of IL-1β, pro–IL-1β (31 kDa form of IL-1β) was induced in β-glucan stimulated BMDM by Western Blot analysis from cell lysates. On the order hand IL-6, IL-10, TNF-α and PGE_2 had different mechanism of regulation by the NLRP3 inflammasome components. **Conclusion:** These data suggest that β-glucan (F1) triggers the transcription of IL-1β but not the secretion. The NLRP3 inflammasome could regulate the production of other inflammatory mediators in a non-classical pathway in our model.

Finnancial Support: FAPESP and CNPq.

NITRIC OXIDE PRODUCTION BY PERITONEAL MACROPHAGES OF MICE FED A DIET SUPPLEMENTED WITH *Perilla frutescens* DURING EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS

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Introduction: *Perilla frutescens* has 43.9-55% of omega-3, which is beneficial to human health. The perilla has several properties, such as immunomodulating effects. There is evidence that animals fed with diets rich in n-3 fatty acids tend to decrease the proliferative response of lymphocytes, natural killer cell activity and impair phagocytosis. The study had the goal to assess the role of perilla in levels of nitric oxide (NO) by peritoneal macrophages during paracoccidioidomycosis.
Methods and Results: Groups of male Swiss mice was formed: 1) Icom, infected with $2x10^6$ yeast cells of *Paracoccidioides barsiliensis* (Pb18) in 0.2ml i.p., fed with commercial food; 2) Iper, infected with $2x10^6$ cells of Pb in 0.2ml i.p., fed with a diet with 10% of perilla; 3) Uicom, control fed with commercial food; and 4) Uiper, control fed with a diet with 10% of perilla. At the end of 1st, 4th and 8th weeks, the animals were sacrificed. The culture of peritoneal macrophages was obtained by peritoneal washings, which were centrifuged and the cells resuspended. The concentration of NO was measured in the macrophages culture supernatants, using the standard reaction of Griess. In the 1st week, perilla did not influence NO production. In the 4th week there was no increase in NO synthesis between the groups fed with commercial food, but there was a tendency to raise the synthesis of infected groups. When comparing the groups fed with commercial diet with those fed with perilla diet, an increase in the NO synthesis was observed. When comparing the two groups fed with perilla, infected and uninfected, there was no difference in NO production. In the transition from acute to chronic (4th week) phases, an increased NO production was observed in Iper group when compared to Icom (P=0.0002) and UIcom (P=0.001). The same was observed when comparing the Uiper group with the Icom (P=0.013) and Uicom (P=0.001) groups. These results suggest that NO production may be due to perilla and not to Pb. In the chronic phase, 8th week, the fungus stimulated an increase in the NO synthesis (IcomxUicom, P=0.001), indicating that the perilla maintains the NO production as in the 4th week.
Conclusion: Diets with 10% of perilla, stimulated NO synthesis in the 4th week and it remained in the chronic phase of paracoccidioidomycosis.

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INHIBITION OF INFLAMMASOME ACTIVATION BY THE EFFECTOR PROTEIN IcaA OF *COXIELLA BURNETII*

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Introduction. The inflammasome is a molecular platform involved in caspase-1 activation, secretion of cytokines, pyroptosis and infection control in response to intracellular pathogens. *Coxiella burnetii*, the causative agent of Q fever, is an intracellular pathogen highly adapted to subvert host cell functions for the establishment of a replicative vacuole. Secretion of several effector proteins through a type IV secretion system, homologous to the Dot/Icm system of *Legionella pneumophila*, may account for host cell subversion by *C. burnetii*. Data from our laboratory indicate that *C. burnetii* inhibits the activation of caspase-1 in response to *L. pneumophila*. Herein, we investigate the mechanisms mediating inhibition of caspase-1 activation by *C. burnetii* and by the effector protein IcaA, which is secreted into the host cell by the Dot/Icm complex.

Methods and Results. Since *C. burnetii* is a yet genetically intractable bacterium, the role of secreted effectors can be investigated using adoptive secretion by the homologous Dot/Icm of *L. pneumophila*. We confirmed by western blot that caspase-1 activation in bone marrow-derived macrophages (BMMs) from C57BL/6 mice infected with Δ*flaA L. pneumophila* expressing IcaA was inhibited in comparison to bacteria expressing an empty vector. Once caspase-11 is responsible for a non-canonical activation of caspase-1, we used BMMs from 129S6 mice (a natural mutant for caspase-11) to test a possible role of caspase-11 in the inhibition of caspase-1 by *C. burnetii* and IcaA. As verified by western blot, *C. burnetii* inhibited caspase-1 activation in response to Δ*flaA L. pneumophila* in C57BL/6 and 129S6 BMMs. Differently from what was observed in C57BL/6 BMMs, the expression of IcaA did not inhibit caspase-1 activation in response to Δ*flaA L. pneumophila* in 129S BMMs.

Conclusions. We demonstrate that the effector protein IcaA of *C. burnetii* inhibits host caspase-1 activation mediated by caspase-11, thus suggesting a

novel mechanism of inflammasome inhibition by a pathogen effector. We also found that *C. burnetii* inhibits caspase-11-independent NLRC4-independent activaito of caspase-1, thus indicating that other effectors of *C. burnetii* might be involved in inflammasome subversion by the pathogen.

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Study of microbicide activity of murine macrophages infected in vitro with Salmonella Enteritidis

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Introduction: Salmonella nontyphoidal is a major cause of foodborne illness in the world. The Enteritidis has being identified as the most common serovar and is responsible for gastroenteritis and even systemic infections. In developing countries, Enteritidis and Typhimurium Serovar cause invasive infections leading to death of young children with underlying diseases and also, death of adults infected with HIV. These bacteria can proliferate within epithelial cells and non-activated macrophages, but by using specific mechanisms of pathogenicity island SPI2, they may persist also in activated macrophages, showing cytotoxic effects on these cells.

Objective: To evaluate the function and activity of murine macrophage cell line J774A.1 after infection with Salmonella Enteritidis.

Materiais e Métodos: J774A.1 macrophages were cultured on 96 well plates for 24 hours, and then were infected with Salmonella Enteritidis in a MOI (Multiplicity of infection) 50. The phagocytosis capacity and killing activity were evaluated by Alamar Blue metabolization. Cell viability was evaluated by the MTT (brometo de tiazolil-(3-[4,5-Dimetiltiazol-2-il]-2,5-difeniltetrazólio) reduction assay. We also determined nitric oxide and hydrogen peroxide liberation by macrophages using the Greiss reaction and phenol red, respectively.

Results: The bacteria were efficiently phagocytized by macrophages that had been able to kill the intracellular pathogen, as shown by Alamar Blue-based assay. We also have shown that Salmonella Enteritidis had no cytotoxic effect on macrophages. Therefore, the production of nitric oxide and hydrogen peroxide was not significant in macrophages infected with Salmonella Enteritidis.

Conclusion: The phagocytosed-bacteria were efficiently killed by macrophages in our experimental model. However, the mechanism involved in the microbicide effect seems to be independent of nitric oxide and hydrogen peroxide release by macrophages.

Keywords: Salmonella Enteritidis, macrophages, phagocytosis, killing

Financial support: CAPES, FAPESP

SEPSIS-INDUCED ACUTE LUNG INJURY (ALI) IS MILDER IN DIABETIC RATS AND CORRELATES WITH IMPAIRED NFKB ACTIVATION

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Introduction: Acute lung injury (ALI) develops in response to a direct insult to the lung or secondarily to a systemic inflammatory response, such as sepsis. There is clinical evidence that the incidence and severity of ALI induced by direct insult are lower in diabetics. In the present study we investigated whether the same occurs in ALI secondarily to sepsis and the molecular mechanisms involved. **Methods:** Diabetes was induced in male Wistar rats by alloxan and sepsis by caecal ligation and puncture surgery (CLP). Six hours later, the lungs were examined for oedema and cell infiltration in bronchoalveolar lavage. Alveolar macrophages (AMs) were cultured in vitro for analysis of IkB and p65 subunit of NFkB phosphorylation and MyD88 and SOCS-1 mRNA. **Results:** Diabetic rats were more susceptible to sepsis than non-diabetics. In non-diabetic rats, the lung presented oedema, leukocyte infiltration and increased COX2 expression. In diabetic rats these inflammatory events were significantly less intense. To understand why diabetic rats despite being more susceptible to sepsis develop milder ALI, we examined the NFkB activation in AMs of animals with sepsis. Whereas in non-diabetic rats the phosphorylation of IkB and p65 subunit occurred after 6h of sepsis induction, this did not occur in diabetics. Moreover, in AMs from diabetic rats the expression of MyD88 mRNA was lower and that of SOCS-1 mRNA was increased compared with AMs from non-diabetic rats. **Conclusion:** These results show that ALI secondary to sepsis is milder in diabetic rats and this correlates with impaired activation of NFkB, increased SOCS-1 and decreased MyD88 mRNA.

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SIGNALING PATHWAYS ASSOCIATED WITH NETOSIS INDUCTION BY *LEISHMANIA AMAZONENSIS* PROMASTIGOTES

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Introduction: Leishmaniasis affects over 800.000 people worldwide (PLoS ONE 7(5): e35671, 2012). After *Leishmania* inoculation in a pool of blood (Exp. Parasit. 79: 215-18, 1994), neutrophils are rapidly recruited to the inflammatory site (Science 321:970–74, 2008). Neutrophils (NØ) can die releasing traps composed of chromatin associated to granule and cytoplasmic proteins named neutrophil extracellular traps (NETs). This novel cell death mechanism, netosis, is dependent on reactive oxygen species (ROS), but still poorly understood. Since *Leishmania amazonensis* promastigotes (La) induce netosis (PNAS 106: 6748–53, 2009), here, we aimed to elucidate the signaling pathways behind netosis upon La stimulation. **Methods and Results:** Healthy donors NØs were pretreated with inhibitors of protein kinase C (BIS VIII or I), phosphoinositide 3-kinase (PI3K, LY-294002, AS605240 and Wortmannin), ERK (PD98059) or Ca^{2+} chelators (BAPTA and EGTA), then activated by La or PMA. NETs were measured as DNA (shown as n fold control) in the culture supernatants using PicoGreen® Assay for dsDNA and ROS generation with specific probes by flow cytometry. Our results revealed that La induced NET (14 ± 5.1; n=6) was inhibited 32% by BIS VIII (9.5 ± 3.0; n=6). Similarly, netosis induced by La (8.5 ± 4.7; n=4) was inhibited 50% by BIS-I (4.2 ± 1.8; n=4). LY294002 (6.5 ± 1.9; n=8) showed a slight effect over NETs release induced by the parasite (7.9 ± 2.0; n=8). Also La induced netosis (17.6 ± 5.5; n=6/ 3.1 ± 0.6; n=6, respectively) was decreased 54% and 52% by Wortmannin (8.1 ± 3.2, n=6) and AS-605240 (1.5 ± 0.1, n=6), respectively. AS-605240 (0.2 ± 0.04, n=4) treatment abrogated 97% of ROS generation induced by La (7.0 ± 1.2; n=4). PD98059 (5.1 ± 1.4, n=6) decreased 41% NET release stimulated by La (8.7 ± 1.9; n=6). ERK phosphorylation was evidenced after 15 min of NØ-La interaction and PD98059 (2.9 ± 1.0, n=5) inhibited 60% ROS generation induced by La (7.3 ± 1.0; n=5). Finally, netosis stimulated by La (8.8 ± 2.5; n=4) was decreased 37.5% by BAPTA (5.5 ± 1.3, n=4). EGTA (1.3 ± 0.3, n=8) inhibited 88% La-induced netosis (11.2 ± 2.9; n=8). **Conclusion:** In totum, we showed that PKC, ERK,

PI3K and Ca^{2+} participate of the La induced netosis. Additionally, ROS production induced by La is dependent on PI3K and ERK.

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MODULATION OF DENDRITIC CELL BIOLOGY BY P.BRASILIENSIS FRACTIONS

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Introduction Dendritic cells (DCs) are antigen presenting cells that connect innate and adaptative immunity. They are able to capture, process and present antigens to T lymphocytes. Immature DCs containing pathogens and its antigens migrate from the periphery to lymphoid organs, pass through an important maturation process essential for the development of an effective adaptative immune response. Some pathogens, as the fungi *P. brasiliensis* (Pb) are able to develop immune escape mechanisms interfering in DCs biology. The aim of this study is to evaluate the effect of three distincts fractions from Pb (F1, F2 and MP) on human DC differentiation from monocytes. **Methods** Peripheral blood mononuclear cells (PBMC) were obtained from healthy donors (n=12) through a ficoll gradient. Monocytes were obtained by magnetic selection with anti-CD14-micro beads. Cells were cultivated for seven days in medium supplemented with IL-4 and GM-CSF in the presence of F1, F2 and MP from Pb18. Dendritic cell differentiation was evaluated by CD1a, DC-SIGN, HLA-DR , CD80 and CD83 cell surface expression by flow cytometry. Cell culture supernants were harvested and citokines measured by Cytokine Bead Array (CBA). **Results** When monocytes were cultured in F1 and MP presence we observed a normal DC development but these DCs CD1a+ expressed less HLA-DR, CD80 and CD83. On the other hand, F2 fraction impaired CD1a (22,16 +/- to 5,78 +/- 2,2%), DC-SIGN (23,3+/-6,7% to 7,02 +/- 2,1 %) and HLA-DR (4,23+/- 0,89 to 0,77+/-0,21%) expression. The impaired DCs differentiation observed in F2 presence was accompanied by a significant lower TNF-a (2575+/- 901,3 to 60,68+/- 35,62 pg/ml) and IL-8 (18203+/- 2785 to 1342+/-541,5 pg/ml) production, and more IL-1 beta production (37,33+/- 4,2 to 59,33+/-26,58 pg/ml). **Conclusion** In this way, we concluded that F1 and MP fractions had a relevant effect on co-stimulatory cell surface expression on

CD1a+ DCs, probably interfering in T cell activation on lymphoid organs. On the other hand, F2 Pb18 fraction have an even more relevant effect, impairing DCs CD1a+ differentiation from monocytes. These events observed in innate immune response are a possible immune escape mechanism developed by the fungi to avoid acquired immune responses from the human host.

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POLYMORPHISM OF CD11B ON CR3: A NOVEL GENETIC VARIANT ASSOCIATED WITH SUSCEPTIBILITY TO SYSTEMIC LUPUS ERYTHEMATOSUS AND REDUCED OXIDATIVE BURST IN NEUTROPHILS

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Introduction: Complement receptors (CR) play an important synergistic action with FcγR in mediating effect functions involved in immune complex (IC) clearance and microbicidal mechanisms in neutrophils such as production of reactive oxygen species (ROS). These events have been impaired in patients with systemic lupus erythematosus (SLE) as reflected by IC deposition and their susceptibility to infections. Recent data have provided evidence that genetic polymorphisms of FcγR and CR are associated with abnormalities and risk to development of SLE. Regarding CR3 (CD11b/CD18), the human neutrophil antigen (HNA)-4a has been described and represents a polymorphism of CD11b subunit of CR3, which frequency and functional significance are unknown in SLE. We identified the distribution of HNA-4a genotypes in Brazilian SLE patients compared to healthy subjects. We also analyzed whether the HNA-4a polymorphism in CR3 might affect the neutrophil oxidative burst. **Methods:** SLE patients (n=157) and control individuals (n=147) were included in this study. Genotype frequencies of HNA-4a were determined using PCR with sequence-specific primers and agarose gel 2%. Neutrophils ($5x10^5$/500µL) were stimulated with 30µg of IC, opsonized or not by complement from normal human serum (NHS), IC treated with heat-inactivated NHS or PMA 10^{-7}M and the oxidative burst was measured by chemiluminescence in the presence of luminol 10^{-4}M and lucigenin 10^{-4}M. CR3 expression was analyzed by flow

cytometry. **Results:** There was an association of the HNA-4a positive allele with protection and the HNA-4a negative allele with susceptibility to SLE. In addition, the HNA-4a negative allele was associated with a reduced neutrophil oxidative burst when in homozygosis and healthy individuals and heterozygosis in SLE active compared to inactive SLE patients. There was also a negative correlation between the neutrophil oxidative burst and SLE disease activity index in HNA-4a heterozygosis, which was not observed in the HNA-4a positive homozygosis. **Conclusions:** These results have implications for the pathophysiology of SLE and support the hypothesis that polymorphism of HNA-4a in CR3 modulates the neutrophil oxidative burst in healthy and SLE. This is the first work that describes the association of the HNA-4a polymorphism with susceptibility to SLE and it may contribute to the understanding of abnormalities in neutrophil functions in SLE.

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OXIDIZED LDL INDUCES CD36-PAFR COMPLEX FORMATION IN LIPID RAFTS AND CONTRIBUTES TO MACROPHAGE ACTIVATION

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Introduction: CD36 is a scavenger receptor responsible for oxidized **L**ow **D**ensity **L**ipoprotein (oxLDL) uptake by macrophages, contributing to foam cell formation and atherosclerosis development. In previous study we showed that Platelet-activating factor (PAFR) is also involved in oxLDL uptake by increasing CD36 expression and that the signaling pathways elicited by oxLDL also involves both receptors. In the present study we investigated whether co-localization of these receptors in membrane microdomains is required for oxLDL effects.

Methods: LDL was obtained from human plasma and oxidized by $CuSO_4$. Bone marrow-derived macrophages were obtained by culture in L929-conditioned medium. FITC-oxLDL uptake was determined by FACS. Co-localization and co-immunoprecipitation of PAFR and CD36 were evaluated by confocal microscopy and western blot, respectively. HEK293T cells were co-transfected with plasmids encoding PAFR and/or CD36. Gene expression was evaluated by real-time PCR.

Results: In macrophages, pre-treatment with PAFR antagonists alone or in combination with anti-CD36 blocking antibody significantly reduced oxLDL uptake. Lipid rafts disruption by previous treatment with mβCD similarly affected oxLDL uptake. Only double transfected HEK293T cells with PAFR and CD36 produced IL-10 mRNA upon oxLDL activation. By confocal microscopy, we observed that oxLDL induces the co-localization of CD36 and PAFR in the plasma membrane. This was confirmed by co-immunoprecipitation. By triple staining experiments for CD36, PAFR and GM1 we observed that oxLDL induces the recruitment of CD36 and PAFR to lipid rafts microdomains.

Conclusion: It is shown that oxLDL induces the recruitment of PAFR and CD36 to lipid raft microdomains and this receptor assembly is necessary for oxLDL uptake and induction of gene transcription.

Financial support: FAPESP, CNPq

THE USE OF *Legionella pneumophila* AND *L. gratiana* TO IDENTIFY A NOVEL PATHWAY DEPENDENT ON NLRC4 AND INDEPENDENT OF CASPASE-1 FOR RESTRICTION OF THE INFECTION BY FLAGELLATED BACTERIA

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Introduction: *Legionella pneumophila* is a Gram negative bacterium that replicates in human macrophages and causes a severe pneumonia called Legionnaires' disease. We have shown that the restriction of *L. pneumophila* replication in macrophages occurs through the flagellin recognition by cytoplasmic receptors like NLRC4 and NAIP5, which leads to activation of caspase-1 and cell death. Additionally, our group has showed that the control of *L. pneumophila* occurs through a novel NLRC4-dependent pathway which is independent of caspase-1. Data generated in our lab indicated that wild-type (WT) or bacteria deficient for motility (*fliI*) had a higher replication in macrophages from NLRC4$^{-/-}$ mice as compared to Caspase-1$^{-/-}$. A similar feature was observed *in vivo*. Moreover, bacteria deficient for flagellin (*flaA*) multiplied better in Caspase-1$^{-/-}$ mice as compared to *fliI* or WT. The aim of this work is to determine the mechanisms involved in the control of *L. pneumophila* infection by this new pathway.
Methods and Results: Bone marrow-derived macrophages (BMDMs) were obtained from C57BL/6 (WT), NLRC4$^{-/-}$ and Caspase-1$^{-/-}$ animals were infected with bacteria WT, *fliI* and *flaA* (MOI10) and the bacterial growth was quantified by CFU and counting of bacteria on LCV by immunofluorescence. We confirmed the increased growth of *flaA* when compared to WT bacteria in Caspase-1$^{-/-}$ macrophages, and the greater replication of WT and *fliI* bacteria in NLRC4$^{-/-}$ macrophages when compared with Caspase-1$^{-/-}$ by both methods. Besides, this pathway was investigated to other flagellated species of *Legionella*. We found that *L. gratiana* exhibited the same pattern than *L. pneumophila*.
Conclusion: *L. gratiana*, the less pathogenic species of the genus, will function as a toll to identify this novel pathway by which the immune system triggers restriction of bacterial replication. Pharmacological inhibition of specific process and shRNA will be used to identify cellular pathways engaged by this novel pathway that is dependent on NLRC4 and independent of caspase-1.

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CARBON NANOTUBES-INDUCED LUNG INFLAMMATION INVOLVES ACTIVATION OF THE INFLAMASSOME PATHWAY

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Introduction: Carbon nanotubes (CN) are carbon allotropes that belong to the fullerene family. Since their discovery in the 90's, they have been applied in various fields including biology. Questions on the safety of nanocompounds have been raised but little information about their toxicity is available. So, the aim of this work was to study the possible toxic effects of multiwall carbon nanotubes in lungs of mice and to assess whether *in vitro* experiments can predict *in vivo* toxicity**.**

Methods and results: For *in* vivo experiments, female C57BL/6 mice were instilled intranasally with 50 µg of CN. 1, 3 and 30 days after instillation (dpi) the broncoalveolar lavage (BAL) and the lungs were collected for total and differential cell count, ELISA, western blotting, MPO, NAG and hidroxiproline assays and tissue histology. We show that CN exposure induces acute neutrophil and mononuclear cells transmigration to the airways by the 1st dpi. Moreover, CN is able to induce chronic accumulation of neutrophils in lung parenchyma, by the 30th dpi. CNs were also able to induce IL-1β production in the BAL and lung parenchyma and promote pro-caspase 1 conversion to active caspase-1 by the 3rd dpi. 30 days after the exposure, CNs could induce little lung fibrosis, reaching only the perivascular and peribronchiolar regions. For *in vitro* experiments, RAW 264.7 and THP-1 stimulated with CN (1, 10 and 100 µg/mL). 24 hours after stimulation the cell supernatant was collected to evaluate NO production and IL-1β levels. In another experiment, intracellular ROS production was measured for 2 hours right after the stimulation. These experiments demonstrated that CNs induce NO production after 24 hours of stimulation even in very low concentrations. Furthermore, they were also able to induce a rapid and robust intracellular ROS production. CNs also induced the production of IL-1β by THP-1 cells after 24 hours of stimulation.

Conclusion: Summarizing, this specific CN structure is able to interact with cells in the mice lungs, causing acute and chronic inflammation, probably by the inflammasome pathway, but mild fibrosis. Moreover, *in vitro* experiments seem to be reliable to predict lung toxicity although current effort is to standardize these *in vitro* experiments. Altogether, these results indicate that CNs are a potential inhaled biohazard for those who are directly exposed to them as it may cause lung inflammation.

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Vaccine adjuvant Alum triggers CD1a expression in human monocytes: a new adjuvanticity mechanism

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Introduction: It has been reported that an important role of Alum's adjuvanticity is played by the $NLRP_3$ inflammasome, which induces type 2 immune responses at the site of injection. However, studies show that the $NLRP_3$ inflammasome is not required for antibody production in response to vaccination by the particulated adjuvant. Thus immunostimulatory properties of Alum are still open for further investigation. In this study, we investigate the role of CD1 molecules, which are responsible for the lipid antigens presentation to T cells, in Alum adjuvanticity properties. **Methods and results:** Human monocytes were isolated and purified from peripheral human blood and stimulated with different Alum concentrations. Our results showed that Alum triggered the expression of group 1 CD1 molecules, mostly CD1a, in human monocytes *in vitro,* as accessed by flow cytometry and confocal microscopy. In order to characterize the cellular mechanisms involved in the regulation of CD1a expression induced by the adjuvant alum, human monocytes were previously treated or not with different pharmacological inhibitors. Our results showed that CD1a expression induced by Alum occurred in a manner dependent on the: (I) integrity of lipid rafts, (II) adaptor protein Syk tyrosine kinase, (III) transcription factor NFkB, (IV) inflammasome IL-1b converting enzyme caspase-1 and (IV) IL-1b receptor IL-1R. However, it was independent of the action of fatty acid synthase. **Conclusion:** Taken together our data illustrated another pathway involved in Alum adjuvanticity. We showed for the first time that lipid presenting molecule CD1a could play an important role in the adjuvanticity of Alum. Moreover, understanding the cellular and molecular level mechanism for CD1 induction and its relation with immune resposnse triggered by vaccine adjuvant alum will provide important information for new adjuvants discovery for vaccine application.

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THE ROLE OF INFLAMMASSOME IN MACROPHAGE SUSCEPTIBILITY TO *COXIELLA BURNETII*

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Introduction: Nod-like receptors (NLRs) are intracellular receptors that sense molecular signatures of microbes within host cell cytosol. Certain NLRs trigger the formation of the inflammasome, a molecular plataform responsible for caspase-1 activation, culminating in cytokine secretion, pyroptosis and infection control. Caspase-11 is involved in inflammasome activation in response to intracellular pathogens, controlling responses dependent and independent of caspase-1. *Coxiella burnetii* is a bona-fide intracellular pathogen that primarily infects alveolar macrophages and that is able to evade immune responses in mammalian hosts, avoiding recognition by Toll-like receptors and by the complement system. However, a competent host can restrict the infection, which suggests that effective immune responses do occur. Therefore, we aimed to evaluate if the inflammasome is involved in infection control of *C. burnetii* by macrophages.
Methods and results: Bone marrow-derived macrophages (BMDMs) or alveolar macrophages (AMs) were obtained from C57BL/6 and A/J mice, either wild-type or deficient for Casp-1$^{-/-}$/Casp-11^{mutant}. To quantify *C. burnetii*, macrophages were lysated at different times and combined with the supernatant of each culture. Bacterial DNA was extracted and quantified by real-time quantitative PCR. We verified that Casp-1$^{-/-}$/Casp-11^{mutant} BMDMs derived from C57BL/6 strain control the infection by *C. burnetii*. This restriction was also observed in AMs, which are the primary target cells for infection by *C. burnetii*. We further tested the role of caspase-1 and caspase-11 in the susceptibility to *C. burnetii* using macrophages derived from A/J mice, which are significantly more susceptible to *C. burnetii.* We verified that in a less restrictive murine background, wild-type BMDMs and AMs are susceptible to *C. burnetii* infection, whereas Casp-1$^{-/-}$/Casp-11^{mutant} macrophages control infection by the pathogen.
Conclusion: The results obtained so far suggest that responses dependent on caspase-1 and caspase-11, pivotal molecules in inflammasome activation, may facilitate *C. burnetii* multiplication within infected macrophages.

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INTERACTION OF ESTERASE C1 INHIBITOR WITH SURFACE OF *L. INTERROGANS* AND *L. BIFLEXA*

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Introduction: Leptospirosis is one of the most important zoonosis caused by spirochete bacteria from the genus *Leptospira*. Leptospirosis is prevalent in developing countries and it is transmitted mainly by contaminated urine from rodents when in contact with injured skin or mucosa. Complement system is important to eliminate *Leptospira*. Non-pathogenic *Leptospira biflexa* is rapidly eliminated by complement while pathogenic *Leptospira interrogans* is able to avoid complement activation by acquisition of host complement inhibitors such as Factor H and C4b binding protein on the bacterial outer membrane. Since the activation of complement classical pathway is important to eliminate *Leptospira biflexa* we decided to investigate if pathogenic and non-pathogenic leptospires would interact differently with another complement regulator C1 esterase inhibitor (C1INH), one of the main inhibitors of the classical and lectin pathways. This regulatory complement protein is a single-chain protein that belongs to the family of serine proteinase inhibitors (serpins). C1INH (105 kDa) interacts covalently with C1r (55.3 kDa for A chain and 31.2 kDa for B chain) and C1s (52.2 kDa for A chain and 27.7 B chain) enzymes preventing the cleavage of C4 and C2 protein by this latter serine protease. **Methods and Results:** The interaction between C1INH and leptospires was analyzed by Western blot. Suspensions of $8x10^8$ *L. biflexa or L. interrogans* were incubated either with purified C1INH (10 µg) or normal human serum (NHS, 40%). After several washes, the pellets were suspended in PBS, proteins were separated by SDS-PAGE and analyzed by Western blot using polyclonal anti-human C1 INH. The presence of approximately 105 kDa and 130 kDa (only with NHS) bands indicated that both strains of leptospira bind to C1 INH in similar intensity. **Conclusions:** Our results showed that both pathogenic and non-pathogenic *Leptospira* are able to bind free C1 INH or C1s/C1 INH complex. In this context, the ability of pathogenic leptospire to bind to C4b binding protein seems to be relatively more important than C1 INH to avoid the activation of the classical and lectin pathways on the surface of this pathogen.

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NOD-LIKE RECEPTOR 12-INDUCED CASPASE-1 ACTIVATION AND SYSTEMIC INFLAMMATION IN MALARIA

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Introduction: Cyclic paroxysm is a hallmark of malaria, being associated with high levels of the pyrogenic cytokine, including IL-1β. While activation of Nod-Like Receptor (NLRP) 3 is implicated on *in vitro* induction of active IL-1β by *Plasmodium* hemozoin, the *in vivo* relevance of this process is unknown. **Methods and Results:** Here we report a signature for expression of inflammasome-related genes in rodent malaria. In addition to NLRP3 and ASC, *in vivo* release of IL-1β was highly dependent on NLRP12-induced caspase-1 activation. On the other hand expression of pro-caspase-1 was dependent on activation of MyD88, and IFNγ-priming. Consistently, inflammasomes in febrile malaria patients were pre-formed and characterized by ASC oligomers associated with NLRP12 and NLRP3. Finally, treatment with IL-1R antagonist interfered with inflammatory priming, preventing hyper-susceptibility of *Plasmodium*-infected mouse to septic-shock. **Conclusion:** Collectively, our results reveal that *Plasmodium* infection triggers NLRP12/NLRP3 to promote inflammasome formation and caspase-1 activation priming monoctytic cells to produce bulk amounts of IL-1β.

Key words: malaria, NLRP3, NLRP12, inflammasome, caspase-1 and IL-1β

MMP-9 AND TIMP-1 IN CHORIOAMNIOTIC MEMBRANES OF PREGNANCIES COMPLICATED BY RUPTURE PREMATURE MEMBRANES AND CHORIOAMNIONITIS.

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Introduction: Metalloproteinases (MMPs) are enzymes capable of degrading the extracellular matrix that play an important role in the remodeling process of the fetal membranes during pregnancy. MMP´s activity is regulated by Tissue Inhibitors of Metallopreoteinases (TIMPs).This study aimed to evaluate the expression of MMP-9 and TIMP-1and proteolytic activity of MMP-9 in chorioamniotic membranes from pregnancies complicated by Preterm Rupture of Membranes (PPROM) or Preterm Labor (PTL) in the presence of histologic chorioamnionitis (CAM) and in term pregnancies with intact membranes (TD) or Rupture premature of membranes (PROM). **Methods and Results:** Fourty membranes from PPROM (20 with and 20 without CAM), 37 from PTL (20 with and 17 without CAM), 20 TD and 27 PROM were included in study. All membranes were evaluated histhopatologically and fragments were subjected to total RNA and protein extraction. RNA samples were reverse transcribed and the expression of MMP-9 and TIMP-1 expression measured by real-time PCR. The proteolytic activity of MMP-9 was quantified by zimography. Expression of MMP-9 and TIMP-1 and proteolytic activity of MMP9 between the groups were compared by Kruskal-Wallis test and Mann Whitney test. A *p* value <.05 was considered significant. Expression of MMP-9 in the PTL group with CAM was significantly higher than in patients with PTL without CAm and PPROM without CA. There was no difference in the expression of TIMP-1 among the groups. Quantification of showed MMP-9 proteolytic activity quantification was significantly higher in pregnancies with PPROM with CAM than in PPROM and PTL without CA. This quantification in term pregnancies with PROM was

significantly higher than in TD. In PTL with CA patients the quantification was significantly higher than in patient without CA. Pregnancies complicated with PPROM had quantification significantly higher than PTL, regardless the inflammatory status. Among term pregnancies, the patients with PROM had a significantly higher quantification than TD. The MMP-9/ TIMP-1 imbalance was calculated by ratio between MMP-9 and TIMP-1 expression in each sample and a increased in MMP-9 was observed in the presence of chorioamnionitis. **Conclusion:** Chorioamniotic membranes express MMP-9 and TIMP-1 in the presence or absence of CAM. Histologic chorioamnionitis is associated with higher MMP-9 proteolytic activity in pregnancies complicated by PPROM and PTL and regarding term pregnancies, MMMP-9 proteolytic activity is related the mechanism of rupture of membrane and modulates the MMP-9/TIMP-1 imbalance in the PPROM and PTL.

ACTIVATION OF NLRC4 BY FLAGELLATED BACTERIA TRIGGERS CASPASE-1–DEPENDENT AND –INDEPENDENT RESPONSES TO RESTRICT LEGIONELLA PNEUMOPHILA REPLICATION IN MACROPHAGES AND IN VIVO.

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Introduction: Although NLRC4/IPAF activation by flagellin has been extensively investigated, the downstream signaling pathways and the mechanisms responsible for infection clearance remain unclear. **Methods and Results:** In this study, we used mice deficient for the inflammasome components in addition to wild-type (WT) *Legionella pneumophila* or bacteria deficient for flagellin (*flaA*) or motility (*fliI*) to assess the pathways responsible for NLRC4-dependent growth restriction in vivo and ex vivo. By comparing infections with WT *L. pneumophila*, *fliI*, and *flaA*, we found that flagellin and motility are important for the colonization of the protozoan host *Acanthamoeba castellanii*. However, in macrophages and mammalian lungs, flagellin expression abrogated bacterial replication. The flagellin-mediated growth restriction was dependent on NLRC4, and although it was recently demonstrated that NLRC4 is able to recognize bacteria independent of flagellin, we found that the NLRC4-dependent restriction of *L. pneumophila* multiplication was fully dependent on flagellin. By examining infected caspase-1-/- mice and macrophages with *flaA*, *fliI*, and WT *L. pneumophila*, we could detect greater replication of *flaA*, which suggests that caspase-1 only partially accounted for flagellin-dependent growth restriction. Conversely, WT *L. pneumophila* multiplied better in macrophages and mice deficient for NLRC4 compared with that in macrophages and mice deficient for caspase-1, supporting the existence of a novel caspase-1–independent response downstream of NLRC4. This response operated early after macrophage infection and accounted for the restriction of bacterial replication within bacteria-containing-vacuoles. **Conclusion:** Collectively, our data indicate that flagellin is required for NLRC4-dependent responses to *L. pneumophila* and that NLRC4 triggers caspase-1–

dependent and –independent responses for bacterial growth restriction in macrophages and in vivo**.**

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DISRUPTION OF NOD2 RESULTS IN IMPAIRED T_H1 RESPONSE DURING EXPERIMENTAL *N. caninum* INFECTION.

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Neospora caninum is an intracellular parasite that causes major economic impact on cattle raising farms, and infects a wide range of warm-blooded hosts worldwide. Cytosolic Nod-like receptors (NLRs) represent a important family of microbial sensor proteins that have been identified as key host molecules in innate immune recognition and the inflammatory response to microbial products. However, the role of NLR in the induction/regulation of adaptative immune response against intracellular protozoan infection is unclear. In this study, we evaluated the role of NLRs in host response to *N. caninum* infection. For that purpose,Nod2$^{-/-}$ and WT mice were infected with *N. caninum* tachyzoites to evaluation of acute phase parasitism, inflammatory cell migration and cytokine production. Nod2$^{-/-}$ mice exhibited higher parasite burden in the peritoneal exudate and lungs compared to WT mice. Inflammatory cell migration was impaired in both compartments, as Nod2$^{-/-}$ mice presented decreased migration of dendritic cells, B and T lymphocytes to the peritoneal cavity. Mononuclear cell infiltrates were also significantly reduced in the lungs of Nod2$^{-/-}$ mice, as compared to WT. In parallel, we observed that dendritic cells and macrophages from Nod2-/- mice presented lower MHCII expression, fact that was associated to lower IFN-γ production in spleen cell antigenic recall, and in lung and brain homogenates. However, no difference in IL-10 production was observed. Surprisingly, Nod2-/- mice demonstrated increased survival than WT mice. Based on the results herein presented we proposed that Nod2 has an important role in T_H1 programming initial immune responses to *N. caninum.* This additional activation of T_H1 response appears to be important to parasite clearance, but could contribute to pathogenesis and mortality during *N. caninum* infection.

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CHARACTERIZING INNATE ANTIVIRAL RESPONSES TO *DENGUE VIRUS* IN THE INVERTEBRATE VECTOR

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Introduction: Dengue fever, caused by *Dengue virus* (DENV), is one of the most important human infectious diseases. The World Health Organization estimates 100 million total cases per year worldwide including 500,000 cases of dengue hemorrhagic fever. *Aedes aegypti* mosquitoes are the main vectors for DENV. Targeting the mosquito is an important strategy to monitor and prevent virus transmission specially considering that are no specific vaccines nor effective treatments for dengue. Understanding how the virus interacts with the invertebrate host can help the development of strategies to control disease transmission. Although many antiviral pathways have been shown to help control DENV in the insect, the exact mechanisms remain unclear. In order to study virus-host interactions, we are currently establishing infection models in our laboratory.

Methods and Results: We infected *Ae. aegypti* mosquitoes and the fruit fly *Drosophila melanogaster* with DENV by intrathoracical injection. We also used

the natural route of infection by feeding *Ae. aegypti* mosquitoes with DENV-contaminated human blood. Infected animals were collected at different times post-infection, total RNA was extracted to analyze viral replication and host transcriptional responses by quantitative PCR (qPCR). We show that upon injection, almost 100% of mosquitoes become persistently infected with the virus. In contrast, upon feeding, infection rates varied greatly depending on the time post-infection analyzed. The rate of infection in *Drosophila* was below 50% and the viral loads were up to 10,000 times lower than in mosquitoes at the same time post-infection, suggesting flies are more resistant to the infection. Nevertheless, flies that were infected seemed to persistently carry the virus, without eliminating the infection. Using these infection models, we are analyzing expression patterns of genes involved in antiviral pathways in infected animals and functionally characterizing whether these genes are involved in DENV-resistance.

Conclusion: We are developing a model to study virus-host interactions in the invertebrate vector using the fruit fly as a host in order to take advantage of many tools that are available to study innate immunity in this animal model. This model could help lead to new ways to control transmission of DENV and other insect borne viruses.

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ARGINASE-1 EXPRESSION IN GRANULOMAS OF TUBERCULOSIS PATIENTS

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Introduction: Conclusion *Mycobacterium tuberculosis* (Mtb) is an intracellular pathogen able to survive and multiply within macrophages. Several mechanisms allow this bacteria to escape macrophage microbicidal activity. Mtb may interfere with the ability of mouse macrophages to produce anti-bactericidal nitric oxide, by inducing the expression of arginase 1 (Arg1). It remains unclear whether this pathway has a role in humans infected with Mtb. In this study, we investigated the expression of Arg1 in granulomas of human lung tissues from tuberculosis patients. **Methods and Results:** Histological analysis of HE stained lung tissues revealed the presence of granulomas, a classical feature of TB infection granulomas are distinct lesions represented by central necrotic area surrounded by inflammatory cells consisting of epithelioid macrophages, multinucleated giant cells, T cells and B cells and scattered foci fibroblasts. To determine whether Arg1 is expressed in the lungs of TB patients, staining of the same samples was performed. Arg1 protein expression was observed in infiltrating macrophages and giant cells in the inflammatory area of granulomas in all TB lungs tested. Arg1 expression was restricted to monocytic and giant cells, while lymphocytes were Arg1-negative. Type II pneumocytes also expressed Arg1 protein. The expression of Arg2 was detected in few macrophages within the inflammatory area of the granulomas. iNOS expression was also observed in inflammatory areas of the granulomas in all TB lungs tested. Interestingly, the number of Arg1-positive macrophages was higher than iNOS- (p=0.0048) or Arg2-positive (p=0.001) macrophages.Type II pneumocytes were negative for both Arg2 and iNOS. The presence of Mtb in granulomas was confirmed by a FITE staining. Mtb were detected in all TB patients sections analyzed. We show that Arg1 is expressed not only in granuloma-associated macrophages, but also in type II pneumocytes. **Conclusion:** Our results suggest that Arg1 expression by macrophages in human lungs of TB patients could play a role in the disease.

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INFLAMMASOME ACTIVATION AND IL-1 CONTRIBUTE TO INTRA-ABDOMINAL ABSCESS DEVELOPMENT INDUCED BY COMMENSAL BACTERIA *BACTEROIDES FRAGILIS*

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Introduction: *Bacteroides fragilis* is a strictly anaerobic Gram-negative bacterium frequently isolated from clinical infections, including intra-abdominal abscesses and bacteremia. The major source of these infections is the normal colonic microbiota where *Bacteroides* spp. outnumbers aerobic and facultative anaerobic bacteria. When intestinal integrity is disrupted (e.g. by surgery or trauma), *B. fragilis* and colonic contents escape into the peritoneum, featuring a virulent bacteria-host interaction. Proinflammatory cytokines (e.g. TNF-a and IL-1b) are critical factors in amplifying the host inflammatory response during sepsis and intra-abdominal abscess formation, contributing to the containment and elimination of these intestinal microorganisms. However, the innate recognition pathways that trigger this response are poorly understood. Inflammasomes are cytoplasmic multiprotein complexes that recognize a diverse set of inflammation-inducing stimuli that include PAMPs and DAMPs, and that control the production of potent pro-inflammatory cytokines, such as, IL-1β and IL-18. Since IL-1b is involved in abscess formation, our goal was to evaluate the participation of inflammasome pathway in the systemic response to *B. fragilis,* **Methods and Results:** Murine experimental model of abscess induction consists of intraperitoneal inoculum of *B. fragilis* combined with sterile cecal contents (SCC). In different time points after inoculation, the body weight (BW) was measured and at day 7 post infection mice were sacrificed and the inflammation score was determined based on several parameters (number, size and dissemination of abscess). Our results shown that C57BL/6 (WT) mice present an early decrease on body weight (5% loss at day 3), while the ASC-, Caspase-1-, and IL-1R-deficient mice showed a weight gain. Although abscess rates in WT and knockout mice did not differ significantly, deficiency in ASC, Caspase-1 and IL-1R resulted in a reduced inflammation score when compared to WT mice at day 7 after *B. fragilis* plus SCC challenge. Mortality rates, serum proinflammatory cytokine levels and peritoneal exudate cells are being analyzed. **Conclusions:** Taken together, our results strongly suggest that inflammasome pathway is activated in response to *B. fragilis* and SCC,

contributing to the IL-1b production. The involvement of IL-18 should be investigated as well as the source of the stimulus that activates the inflammasome pathway.

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IFN-β IS RESPONSIBLE FOR THE DIFFERENTIAL STAT-1 ACTIVATION AND NITRIC OXIDE PRODUCTION BY C57BL/6 AND BALB/c MACROPHAGES

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Introduction: C57BL/6 and BALB/c mice are, respectively, models of resistance and susceptibility to several infectious diseases. Macrophages from C57BL/6 (M-1) innately produce higher levels of NO than macrophages from BALB/c (M-2) when stimulated with LPS or LPS plus IFN-γ. LPS induces the expression of cytokines, among which IFN-β that induces pSTAT-1 and contributes to *iNOS* expression. In this study, we sought to investigate whether M-1 and M-2 differentially expressed IFN-β and its impact in NO production.
Methods and Results: Thioglycolate-elicited peritoneal M-1 and M-2 were cultured in the presence of 1 µg/ml LPS or Poly (I:C). Quantification of NO was assayed using the Griess reaction and proteins levels of p-STAT-1 was verified by Western blot analysis. When macrophages are stimulated by LPS, the relative amount of IFN-β mRNA (as assessed by Real-time PCR) in M-1 was approximately 2.5- and 3-fold (in other experiments, up to 10-fold) higher than in M-2 by 1 and 5 h, respectively. At 15 h, both cell types return to basal levels, but at 24 h, a smaller second wave of IFN-β mRNA is detectable. Poly (I:C) also induced more NO in peritoneal and bone-marrow derived M-1 as compared to M-2. Anti IFN-β neutralizing antibodies added to the LPS-stimulated macrophages abolishes pSTAT-1 and reduces NO in M-1 to levels as low as in M-2, indicating that IFN-β is determinant in their differential production of NO. To investigate whether M-2 have the machinery to produce NO, we stimulated cells with LPS for 8h, 10h and 24h and exchanged the supernatants of M-1 and M-2, maintaining the cultures for 18 – 72h. The supernatants of M-1 stimulated M-2 to produce the same levels of NO as M-1, suggesting that M-2 have the mechanisms to produce NO via LPS/IFN- β, but do not synthesize as much as M-1 due to a failure in IFN-β expression.
Conclusion: The increment of NO in M-1 as compared to M-2 is due to a more efficient activation of STAT-1, mediated by an augmented secretion of IFN-β.

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UNDERSTANDING INNATE IMMUNE RESPONSES TO DNA VIRUSES USING THE *DROSOPHILA MELANOGASTER* MODEL

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Introduction: The fruit fly, *Drosophila melanogaster*, is a well-characterized animal model to study innate immunity to diverse pathogens, including viruses. In addition, the fly genome is very well annotated which is essential to study differential gene expression and transcriptional responses. Thus, the fruit fly is a good model to characterize the interaction between the host innate immune system and a DNA virus in order to understand diverse aspects of the antiviral response.

Methods and Results: In order to understand the host response to virus infection, we infected adult Drosophila with Invertebrate iridescent virus 6 (IIV6), which has a double stranded DNA genome. We extracted RNA from individuals after 6, 12, 24 and 72 hours post infection and constructed libraries that were deep sequenced using the SOLiD platform. Here, we focused our RNA-seq analysis on the reads that mapped to the host genome. Around 8622 *Drosophila* genes were present at a significant level of expression (>10 FKPM) and 1618 were differentially expressed between the four libraries in pairwise comparisons. These genes showed GO (gene ontology) enrichment for 14 biological processes, out of which, 13 are related to immune response, suggesting that IIV6 infection is a powerful stimulus to the immune system. Considering the expression pattern, these 1618 genes were separated in 16 clusters, which were analyzed separately for specific GO-enrichment. 8 out of

the 14 clusters showed enrichment for genes involved in immune responses and host defense. In order to understand the co-regulation of gene within each cluster, we extracted the promoter region of all genes in the 8 immune process-enriched clusters and identified the presence of 25 commons TF binding sites. The presence of those TFs can help explain the co-expression of those genes in response to IIV6 infection. We are currently investigating the role of pathways and TFs that we identified to determine whether they are involved in the antiviral response against IIV6. We are also comparing our results to gene expression signatures induced in response to other pathogens *Drosophila* in order to define specific signatures activated by a DNA virus.

Conclusion: We have successfully validated our *Drosophila* model to understand how the innate immune system responds to infection by a DNA virus. Ultimately, this model could also help elucidate how the mammalian immune system responds.

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BACTERIAL CLEARANCE IN SEPTIC MICE IS MODULATED BY CCL2 AND NITRIC OXIDE

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INTRODUCTION: Survival during sepsis requires both swift control of infectious organisms and tight regulation of the associated inflammatory response. The aim of this study was investigate the role of CCL2 and NO in clearance of bacteria after CLP sepsis model.

METHODS AND RESULTS: Our results show that CCL2 and NO levels are increased in the peritoneal cavity of mice 6 hours after CLP sepsis model. To investigate the role of CCL2 in clearance of bacteria, the animals were treated with anti-CCL2 and 6 hours after CLP the peritoneal fluid were collected. The numbers of CFU were significant increased in anti-CCL2 group. Peritoneal macrophages treated with CCL2 or anti-CCL2 and stimulated with *E. coli* presented the significant increased in numbers of CFU after anti-CCL2 treatment and decreased in numbers of bacteria after CCL2 treatment. To investigate the involvement of NO in more bacterial elimination, we submitted the deficient mice of iNOS to CLP. We observed the significant increased in numbers of CFU in iNOS-/- group. In order to examine the relationship between CCL2 and NO in clearance of bacteria, peritoneal macrophages of CCL2-/- and WT mice were stimulated with LPS, IFN-γ or LPS+IFN-γ, and the NO levels were determined. We observed the significant decreased in NO levels into CCL2-/- group after all treatments when compared to WT group. In order to investigate the signaling pathway involved in NO production by macrophages treated with CCL2, we analyze the ERK1/2 pathway by immunolocalization technique. After CCL2 treatment we observed the increase in fluorescence of pERK and iNOS.

CONCLUSION: We conclude that the CCL2 and NO are important mediators involved in better elimination of bacteria in models of sepsis. We observed that CCL2 promotes the release of NO by a mechanism associated with ERK1/2 pathway signaling.

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STRESS RESPONSE DURING THE RECOVERY OF DSS-INDUCED COLITIS DECREASES THE NEUTROPHIL ACTIVITY AND WORSENS THE COLITIS

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Introduction: The etiology of the inflammatory bowel diseases (IBD) is not completely understood and the role of environmental factors in the pathogenesis of IBD has been neglected in the past years. However it is well established a positive correlation between environmental factors, such as physical and/or psychological stressors, and the relapse of the disease. Therefore, the aim of our work was investigate the effect of stress in the neutrophil activity in the recovery phase of mice subjected to a model of DSS-induced intestinal inflammation. **Methods and results:** C57Bl/6 male mice (n=6/group) (20-25g) were divided randomly in 4 groups: Control (C), DSS (D), Stress (S) and DSS+Stress (D+S). Colitis was induced by the addition of DSS to autoclaved drinking water for 5 days. Mice of the S and D+S groups were submitted to restraint stress protocol for 2 hours on the experimental day 14, while the mice of C and D groups were maintained in their home cage during the entire procedure. Blood, colon and bone marrow were collected in experimental day 15. The data (mean±sem) was analyzed by one-way ANOVA followed by Tukey test, $P<0.05$ was considered significant. We observed a decrease of the colon length (C:8.0±0.2, S:7.3±0.1, D:6.3±0.1, D+S:5.3±0.1) associated to an increase in the colon length/weight ratio (C:0.024±0.0009, S:0.021±0.001, D:0.034±0.002, D+S:0.043±0.003) in the groups D and D+S when compared to groups C and S ($P < 0.05$); we also observed in the group D+S a stronger response in these parameters when compared to group D ($P < 0.05$). We found that stress decreases the basal (C:28.7±1.7 S:19.6±1.4, D:33.9±3.1, D+S:19.8±1.2) and *S. aureus* (C:221.0±12.1 S:215.7±6.2, D:232±4.0, D+S:193.6±11.6) and PMA-induced oxidative burst of blood neutrophils (C:58.9±5.7 S:36.8±2.1, D:93.4±11.7, D+S:60.5±3.3) in the group

D+S when we compared to group D (*P* < 0.05). Additionally, we observed in the group D+S a decrease of the phagocytosis intensity when compared to the group D (C:53.1±3.5 S:53.8±3.2, D:68.1±4.2, D+S:65.2±3.7). **Conclusion:** Altogether these results suggest that a stressful stimulus is able to induce a relapse of the recovery phase in a model of DSS-induced colitis. We hypothesize that the organism fails to cope with the damage caused by DSS because the neutrophil activity is reduced, which in turn leads to the worsening of the colitis. Currently more studies are in progress in order to understand this phenomenon.

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