ABSTRACTS

INFLAMMA III

Third International Symposium on Inflammatory Diseases

21 - 23, June - 2017 | Ribeirão Preto, Brazil

CRID
CENTER FOR RESEARCH IN INFLAMMATORY DISEASES

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POSTER

PROGRAMME SCHEDULE

JUNE 21st 2017

19:00 to 19:15 Welcome Greetings
Fernando de Queiroz Cunha e Paulo Louzada Junior

19:15 to 20:15 Opening Conference
Chair: José Carlos Alves Filho (FMRP - USP)
Ari Waisman (Institute for Molecular Medicine Mainz - Germany) | How does the tissue respond to IL-1 and IL-17 during CNS inflammation

JUNE 22nd 2017

08:30 to 10:10 SYMPOSIUM # 1 - INNATE IMMUNITY AS A TARGET FOR INFLAMMATORY DISEASES

Chair: Dario S. Zamboni (FMRP - USP)

(8:30 - 8:55) **Marcelo Bozza (UFRJ)** | IRONies of ROS on macrophage function

(8:55 - 9:20) **Sergio Costa Oliveira (UFMG)** | Brucella abortus triggers a cGAS-independent STING pathway to induce host protection that involves guanylate-binding proteins and inflammasome activation

(9:20 - 9:45) **Bernardo Franklin (University of Bonn - Germany)** | The pathophysiology of platelet-derived interleukin-1 in sterile inflammation and infection

(9:45 - 10:10) **Karina Bortoluci (UNIFESP)** | Multiple pathways involved in the activation of NLRC4 inflammasome

10:10 to 10:40 COFFEE BREAK

10:40 to 12:30 SYMPOSIUM # 2 - CHRONIC INFLAMMATORY DISEASES

Chair: Flavio Amaral (UFMG)

(10:40 - 11:05) **Marcellus Souza (UFC)** | Gastroesophageal reflux disease: from inflammation to impairment in mucosal integrity

(11:05 - 11:30) **Simone Appenzeller (UNICAMP)** | Neuropsychiatric lupus: role of inflammation and autoantibodies

(11:30 - 11:55) **Wilson Savino (FIOCRUZ)** | Inflammation in the skeletal muscle: from pathophysiology to cell therapy

(12:00 - 12:15) Short Talk 1 - To be selected from abstracts

(12:15 - 12:30) Short Talk 2 - To be selected from abstracts

12:30 to 13:30 Sponsored Symposium (PensaBio)
Nanobiotechnology as an investigative tool for inflammatory processes.
Geisi Rojas Barreto – Product Specialist

13:30 to 14:30 LUNCH/POSTER SETUP

14:30 to 16:10 SYMPOSIUM # 3 - VIRUS INFECTION TRIGGERING INFLAMMATION

Chair: Mauro M. Teixeira (UFMG)

(14:30 - 14:55) **Stephan Ludwig (University Muenster - Germany)** | New aspects of influenza virus interference with the innate immune response

(14:55 - 15:20) **Jean Pierre Peron (ICB - USP)** | Zika Virus and Congenital Syndrome in Experimental Models

(15:20 - 15:45) **Danielle da Glória de Souza (UFMG)** | Treating the host in Dengue: identification of novel anti-inflammatory and pro-resolving mediators as a new therapeutic paradigm

(15:45 - 16:10) **François Trottein (Institut Pasteur - France)** | The lung/gut axis during influenza A virus infection

16:10 to 16:40 COFFEE BREAK

16:40 to 17:30 **Conference**
Chair: Joao Santana Silva (FMRP - USP)
Savvas Savvides (Ghent University - Belgium) | Structure, mechanism, and antagonism of cytokine-receptor complexes pivotal to inflammation and allergy

17:30 to 19:30 POSTER SESSION

JUNE 23rd 2017

08:30 to 10:10 SYMPOSIUM # 4 - REGULATION OF INFLAMMATION

Chair: Paulo Louzada Jr. (FMRP - USP)

(8:30 - 8:55) **Patricia Silva Martins (FIOCRUZ)** - Searching for effective therapies for chronic pulmonary diseases

(8:55 - 9:20) **Fernando Spiller (UFSC)** - Immunomodulatory Effects of Siglecs on Neutrophil Functions

(9:20 - 9:45) **Alexandre Steiner (ICB - USP)** - Leptin and immunomodulation: back to the drawing board

(9:45 - 10:10) **Geraldo Castelar Pinheiro (UERJ)** - The role of pentraxin 3 (PTX3) on the acute gout attack

10:10 to 10:40 COFFEE BREAK

10:40 to 11:30 **Conference**
Chair: Fernando Cunha (FMRP - USP)
John Wallace (University of Calgary - Canada) - Exploiting the Anti-Inflammatory Potential of H2S: Human Studies

11:30 to 12:30 **SBIn General Assembly**

12:30 to 14:30 LUNCH

14:30 to 16:20 SYMPOSIUM # 5 - METABOLISM AND INFLAMMATION

Chair: Niels O. Câmara (ICB - USP)

(14:30 - 14:55) **Emiliano Medei (UFRJ)** - IL-1b-TLR2 axis induce heart arrhythmias through CaMKII oxidation in type 1 diabetes

(14:55 - 15:20) **Sandra Y. Fukada (FCFRP - USP)** - The adipokine chemerin links metabolic dyslipidemia and bone loss

(15:20 - 15:45) **Luciana Berod (TWINCORE - Germany)** - Metabolic programs controlling immune cell function

(15:50 - 16:05) Short Talk 3 - To be selected from abstracts

(16:05 - 16:20) Short Talk 4 - To be selected from abstracts

16:20 to 16:50 COFFEE BREAK

16:50 to 17:40 **Closing Conference**
Chair: Thiago M. Cunha (FMRP - USP)
Tim Sparwasser (TWINCORE - Germany) | Influencing immune cell activation and differentiation by microbe-associated immunomodulatory metabolites

17:40 CLOSING REMARKS
Fernando de Queiroz Cunha e Mauro Martins Teixeira

CONFERENCE

How does the tissue respond to IL-1 and IL-17 during central nervous system (CNS) inflammation

Ari Waisman

Institute for Molecular Medicine, University Medical Center Mainz.

Experimental autoimmune encephalomyelitis (EAE) is a common animal model for multiple sclerosis. Following disease induction, primed T cells enter the central nervous system (CNS) and initiate a process that lead to tissue damage. During this process, IL-17 produced by T cells and IL-1 of myeloid cells are realty involved. In order to understand the direct effect of these cytokines on the tissue, we generated a series of mouse mutants that lack the expression of the receptors that respond to IL-1 or IL-17. We found that deletion of either the receptor of IL-1 or that of IL-17 in the cells of the blood brain barrier (BBB) greatly reduced disease severity. Further analysis of cell infiltrates in the mutant mice and gene expression profile of the BBB suggested that IL-1 and IL-17 have different affect on the tissue. These results will be discussed during the talk.

Structure, mechanism, and antagonism of cytokine-receptor complexes pivotal to inflammation and allergy

Savvas N. Savvides

VIB-Ghent University Center for Inflammation Research, Ghent, Belgium.

Mammalian cellular development and regulation of the immune system crucially depend on appropriate signalling pathways initiated by dedicated cytokine-receptor assemblies at the cell-surface. The downside of such a key physiological activity is that wild type and mutant forms of cytokines and their receptors are often pivotal to the initiation and progression of inflammatory disorders, cellular malignancies and cancer. The interleukin 12 (IL-12) family cytokines are produced by activated antigen-presenting cells, such as dendritic cells and macrophages, and crucially coordinate innate and adaptive immune responses through regulation of T-cell populations. Despite the plethora of studies on the cellular and (patho)physiological role of signaling mediated by IL-12 family cytokines and their cognate receptors, the field is characterized by a paucity of structural and mechanistic insights. Such information has become essential in understanding the functional dichotomies displayed by IL-12 family cytokines, the intriguing sharing of protein domains both at the level of cytokines and their receptors, and in facilitating specific therapeutic targeting of IL-12 family members against widespread inflammatory diseases. My presentation will focus on our latest work on unraveling the structural and molecular basis of signalling assemblies mediated by pro-inflammatory IL-23, currently the best studied and most intensely targeted member of the IL-12 family to treat plaque psoriasis and Crohn's disease. Finally, I will attempt to link our findings to undertakings in translational science towards the design of novel protein-based scaffolds with antagonistic properties.

Exploiting the Anti-Inflammatory Potential of H2S: Human Studies

John L. Wallace

University of Calgary, Calgary, Alberta, Canada & Antibe Therapeutics, Toronto, Ontario, Canada.

There is a rapidly expanding body of evidence for important roles of hydrogen sulfide in protecting against tissue injury, reducing inflammation, and promoting repair. There is also growing evidence that H_2S can be successfully exploited in drug development. H_2S synthesis and degradation are regulated in circumstances of inflammation and injury so as to promote repair and re-establish homeostasis. In animal studies, several novel H_2S-releasing drugs exhibited enhanced anti-inflammatory, analgesic and pro-restorative effects, while having reduced adverse effects in many tissues. H_2S is a pleiotropic mediator, having effects on many elements in the inflammatory cascade and promoting the resolution of inflammation and injury. It also contributes significantly to mucosal defence in the gastrointestinal tract, and in host defence against infection. The gastrointestinal microbiota is both a significant source and target of H_2S. A better understanding of the physiological and pathophysiological roles of H_2S continues to be restrained by the lack of simple, reliable methods for measurement of H_2S synthesis, and the paucity of highly selective inhibitors of enzymes that participate in endogenous H_2S synthesis. On the other hand, there is emerging evidence that novel, H_2S-based therapeutics are safe and effective in humans. One such drug, ATB-346 (an H_2S-releasing derivative of naproxen) is now in Phase 2 clinical trials for arthritis.

Influencing immune cell activation and differentiation by microbe-associated immunomodulatory metabolites

L. Berod[1], L. Almeida[1], P. Stüve[1], M. Lochner[1], Mamareli, P.[1], B. Raud[1], P. Ghorbani[1], L. Minarrieta[1], T. Sparwasser[1]

[1]Institute of Infection Immunology, TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany; a joint venture between the Helmholtz Centre for Infection Research (HZI) Braunschweig and the Hannover Medical School (MHH).

Recent advances in the field of immunometabolism support the notion that essential processes in T cell biology, such as TCR-mediated activation and T helper lineage differentiation, are closely linked to changes in

the cellular metabolic programs. Although the main task of the intermediate metabolism is to provide the cell with a constant supply of energy and molecular precursors for the production of biomolecules, the dynamic regulation of metabolic pathways also plays an active role in shaping T cell responses. Key metabolic processes such as glycolysis, fatty acid and mitochondrial metabolism are now recognized as crucial players in T cell activation and differentiation, and their modulation can differentially affect the development of T helper cell lineages. We only begin to understand the diverse metabolic processes that T cells engage during their life cycle from naïve towards effector and memory T cells. Following activation, T cells switch from fatty acid oxidation to fatty acid synthesis, suggesting that de novo lipid synthesis actively supports T cell proliferation and differentiation. We could show recently that pharmacological or genetic ACC1 inhibition impairs T helper cell induction, with the strongest impact on Th17 development. Here we discuss the molecular mechanisms that link metabolic changes with the control of gene expression.

SYMPOSIUM

Symposium #1 - Innate immunity as a target for inflammatory diseases

Brucella abortus triggers a cGAS-independent STING pathway to induce host protection that involves guanylate-binding proteins and inflammasome activation

Sergio Costa Oliveira

Immunity against microbes depends on the recognition of pathogen-associated molecular patterns by innate receptors. Signaling pathways triggered by Brucella abortus DNA involves TLR9, AIM2 and STING. In this study, we observed by microarray analysis that several type I IFN associated genes, such as IFN-β and guanylate-binding proteins (GBPs), are down-regulated in STING knockout (KO) macrophages infected with Brucella or transfected with DNA. Additionally, we determined that STING and cGAS are important to engage the type I IFN pathway but STING is predominant to induce IL-1β secretion, caspase-1 activation and GBP2 and GBP3 expression. Further, we determined that STING but not cGAS is critical for host protection against Brucella infection in macrophages and in vivo. This study provides evidence of a cGAS-independent mechanism of STING-mediated protection against an intracellular bacterial infection. Additionally, infected IRF-1 and IFNAR KO macrophages had reduced GBP2 and GBP3expression and these cells were more permissive to Brucella replication compared to wild-type control macrophages. Since GBPs are critical to target vacuolar bacteria, we determined whether GBP2 and GBP^{chr3} affect Brucella control in vivo. GBP^{chr3} but not GBP2 KO mice were more susceptible to bacterial infection, and siRNA treated macrophages showed reduction in IL-1β secretion and caspase-1 activation. Finally, we also demonstrated that Brucella DNA co-localizes with AIM2, and AIM2 KO mice are less resistant to B. abortus infection. In conclusion, these findings suggest that the STING-dependent type I IFN pathway is critical for the GBP-mediated release of Brucella DNA into the cytosol and subsequent activation of AIM2.

Heme modulates innate immune signaling through SYK and ROS

Marcelo T. Bozza

MD, PhD, Laboratório de Inflamação e Imunidade, Departamento de Imunologia, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, 21941-902. Rio de Janeiro, Brazil.

In the past years our group made the original discovery that heme activates innate immune receptors, including TLR4 and NLRP3, and potentiates the cytokine production induced by microbial molecules dependently of Syk. Interestingly, the mechanisms and consequences of heme-induced activation differ from the prototypic activators of these receptors but the reasons are not well understood. Our results implicate the generation of reactive oxygen species (ROS) by heme as an essential step to macrophage activation. Heme-induced TNF and ROS cause macrophage necroptosis dependent of RIP1, RIP3 and MLKL. Although ROS affect these signaling pathways, generation of ROS, including mitochondrial ROS, by heme-stimulated macrophages occurs independently of TLR4 or NLRP3. Syk activation occurs early after stimulation with heme and is essential to the activation of TLR4 and NLRP3 signaling pathways through modulation of ROS production. Lack of TLR4/TNFR1, NLRP3/IL1R or the use of a mitochondrial-target antioxidant are protective in a model of hemolysis. Finally, heme causes the formation of Aggresome-Like Induced Structures (ALIS) through a mechanism dependent of oxidative stress and iron release by HO-1. In vivo, hemolysis promotes the accumulation of these structures in spleen, liver and kidney. These observations support the contention that targeting heme-triggered pathways might be beneficial in the treatment of hemolytic disorders.

Inflammasomes: Cell death, cytokines and beyond

Karina Ramalho Bortoluci

Departamento de Ciências Biológicas e Centro de Terapia Celular e Molecular (CTC-Mol), Universidade Federal de São Paulo, São Paulo, Brasil.

Inflammasomes are caspase-1 and caspase-11-activating platforms responsible for the control of several infections

and involved in metabolic syndromes, neurological disorders and autoinflammatory diseases. The major effector mechanisms mediated by inflammasomes are the secretion of IL-1® and IL-18 and the induction of pyroptosis, a peculiar form of programmed cell death. In the last years, our group has been focusing in the study of unconventional effector responses performed by inflammasomes, including epigenetic regulation of inflammatory genes and activation of macrophage microbicidal activity, which culminate in the better control of intracellular pathogens. Here, I will talk about the role of NLRP3 inflammasomes in the regulation of autophagy and its impact to the trypanocidal capacity of macrophages.

The pathophysiology of platelet-derived interleukin-1 in sterile inflammation and infection

Verena Rolfes, Lisa Böttcher, Salie Maasewerd, Lucas S. Ribeiro, Eicke Latz, Bernardo S Franklin

Institute of Innate Immunity, University of Bonn.

Platelets are small blood non-nucleated cells primarily known for their role in coagulation. However, in recent years it has become increasingly clear that platelets also play an important roles in innate immunity, especially by interacting with other immune cells. Platelets can secrete hundreds of molecules including cytokines, chemokine and lipid mediators. In this study, we focus on the platelet interactions with immune cells in the context of inflammasome activation. We found that platelets amplify Interleukin-1b (IL-1b) response of both murine and human macrophages, neutrophils and monocytes. Importantly, this phenomenon was independent of expression of IL-1 cytokines, or inflammasomes in platelets. Using highly sensitive methods and an inflammasome reporter mouse, we demonstrate that platelets do not secrete IL-1b after inflammasome activation and do not express the inflammasome components NLRP3, ASC and Caspase-1. Additionally, platelet-mediated IL-1b amplification was independent of the expression of the IL-1 receptor in macrophages. We are currently investigating the mechanisms by which platelet-macrophage interaction boost IL-1b-driven inflammation.

Symposium #2 - Chronic inflammatory diseases

Gastroesophageal reflux disease: from inflammation to impairment in mucosal integrity.

Marcellus H L P Souza

Associated professor, Division of Gastroenterology and Hepatology, Federal University of Ceará

Gastroesophageal reflux disease (GERD) is one of the most prevalent disorders, affecting about 10–20% of adults in the Western world. Classically, patients with GERD are classified in to 3 sub-groups: patients with erosive esophagitis (EE), Barrett`s esophagus (BE), and non-erosive reflux disease (NERD). It was described that patients categorized with NERD exhibit absence of macroscopic lesion in esophageal mucosa in routine endoscopy; however, their mucosa is not entirely normal. NERD patients show inflammatory responses in the esophageal epithelia mediated by pro-inflammatory cytokines such as interleukin (IL)-8 and IL-1β. In addition, they have an impaired mucosal integrity with an increase in esophageal epithelial permeability. Despite the fact that PPI, such us omeprazole, is effective for GERD patients. There are at least 30% of these patients that have not response to PPI. Among these, NERD patients are more refractory for PPI then erosive esophagitis. It is important to understand more about the patophysiology of NERD to development new treatments. In this conference, we will discuss about a novel murine surgical model of NERD development in our group, with microscopic inflammation and impairment in esophageal epithelial integrity. This experimental model was important for our group to define the role of TRPV1 receptors in acid induced esophageal inflammation and impairment of esophageal epithelial integrity in NERD. In addition, it will be discuss new topical treatment by Cashew gum, a natural polymer obtained from a Brazilian northeastern tree (Anacardium occidentale L.), on human oesophageal mucosa for NERD patients.

Neuropsychiatric lupus: role of inflammation and autoantibodies

Simone Appenzeller

UNICAMP.

Neuropsychiatric manifestations are frequently observed in systemic lupus erythematosus (SLE). Ninteen central and peripheral neurological manifestations have been described occuring in SLE, however the low individual frequency and diversity of symptoms is a challange to identify fisiopathology involved. Most studies show that inflammation in SLE has a peripheric origin and the break of the blood brain barrier occurs in a subset of patients leading to CNS inflammation. Studdieng the role of antibodies and inflammation in addition to neuroimmaging can help to identify biomarkers for these manifestations associated with increased morbi-mortality.

Inflammation in the skeletal muscle: from pathophysiology to cell therapy

Wilson Savino

Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz, Rio de Janeiro, Brazil

Duchenne muscular dystrophy (DMD) affects 1:3,500

to 1:5,000 male births, and is caused by X-linked mutations in the dystrophin gene. The disease is manifested by progressive muscle weakness and wasting due to the absence of dystrophin protein. This leads to degeneration of skeletal muscle. DMD patients are clinically heterogeneous and the functional phenotype often cannot be correlated with the genotype. Therefore, defining reliable noninvasive biomarkers aiming at predicting if a given DMD child will progress more or less rapidly will be instrumental to better design inclusion of defined patients for future therapeutic assays. We recently showed that CD49d membrane expression levels in blood-derived $CD4^+$ and $CD8^+$ T-cell subsets can predict disease progression in DMD patients. Moreover, T cells expressing this integrin were found within the inflammatory infiltrates seen in affected muscles. Moreover, we showed that this molecule can be placed as a potential target for therapeutics in DMD, since we could inhibit both transendothelial and trans-extracellular matrix migration of T cells derived from DMD patients. **Funding:** Fiocruz, CNPq, Faperj, Capes (Brazil), Focem (Mercosur)

Symposium #3 - Virus infection triggering inflammation

Treating the host in Dengue: mediators and pathways of resolution as a new therapeutic paradigm.

Daniele Glória Souza

Departamento de Microbiologia - UFMG.

Dengue is characterized by an acute flu-like syndrome whose major initial symptoms are fever and pain; these represent the most frequent reason why patients seek medical attention, usually within 2-3 days after onset of symptoms. This phase is characterised by the presence of high levels of cytokines in plasma. Within Day 5-6 post onset of symptoms, the immune response starts to control infection, as seen by the presence of anti-dengue antibodies and active CD4+ and CD8+ T cells that produce large amounts of inflammatory mediators. Consequently, viral load drops and fever tends to subside. In most patients, resolution of infection will occur and patients will return to normality. However, in some patients, this 'resolving phase' is inadequate and onset of severe disease is manifested. Then, we propose evaluate the relevance and effects of Annexin-A1, an important pro-resolving mediator, in the context of dengue infection. Our results demonstrate that the concentration of Annexin-A1 decreases in patients with acute dengue infection, as compared to an irrelevant infection. Using Annexin-1 deficient mice we demonstrated that the absence of this molecule became the disease more severe and longer lasting in both primary and secondary infection. The absence of FPR2, the annexin-1 receptor, also was associated to more severe disease after dengue infection. Furthermore, the treatment with Ac2-26, an annexin A1-derived peptide, was able to decrease the disease severity. These data suggest that regulatory and pro-resolving processes may play a role in excessive inflammation associated with dengue, i.e. while mounting an inflammatory response is important for infection control, its containment is equally important for preventing over exuberant inflammation.

The lung/gut axis during influenza A virus infection

François Trottein

Institut Pasteur de Lille, Centre d'Infection et d'Immunité de Lille, CNRS UMR 8204, Inserm, U1019, Univ. Lille, France

Secondary bacterial (pneumococcal) infections post-influenza constitute an important public health issue and are associated with a considerable socio-economic burden. Understanding the upstream mechanisms leading to (or controlling) bacterial superinfection would be of great value in the design of novel therapeutics. Defective pulmonary innate immunity is one of the key factors favoring susceptibility to bacterial superinfections. In this presentation, I will describe the role of "innate-like" T cells in bacterial superinfection post-influenza, with a particular focus on invariant natural killer T cells. The potential role of the lung/gut axis during influenza will be also discussed. In particular, I will discuss recent data suggesting that gut disorders during severe influenza may influence the outcomes of the disease.

Zika Virus and Congenital Syndrome in Experimental Models

Jean Pierre Schatzmann Peron

Brazil has recently gone through an unprecedented public health crisis due to the Zika virus epidemics. As many other flaviviruses it has never been correlated with human morbidity or mortality. Unfortunately it has changed dramatically as the virus is now responsible for more than 2300 babies born with microcephaly. The so-called Zika Congenital Syndrome has, besides microcephaly, many other relevant features, as retinal damage, intra-uterine growth restriction and arthrogryposis. In fact, babies born without microcephaly but with significant neuronal and retinal lesions were already reported. In this context, the development of an experimental model is of great relevance for the studies on the pathogenesis of microcephaly. We demonstrated that the infection of pregnant SJL mice with ZIKV results in severe damage of the pups. Most prominently was the significant reduction in size and weight, associated high viral titers in the brains.

This was accompanied by high level of apoptotic death, probably of neuronal precursor cells (NPCs). To achieve that we infected NPCs and human brain organoids with ZIKV. Further, we observed a significant reduction on the number of NPCs, corroborating the apoptotic cell death hypothesis. Recently we are focused on several aspects of the brain inflammation during microcephaly. Interestingly, many pro-inflammatory cytokines are down-regulated, as well viral receptors and signaling transduction molecules. Interestingly, this is consistent with clinical findings, showing no sign of inflammation, at least through liquor protein and cellular analysis. This may indicate that the virus controls inflammation in situ. The elucidation of such mechanism would greatly contribute for the understanding of the viral biology in the central nervous system and also for the pathogenesis of microcephaly.

New aspects of influenza virus interference with the innate immune response

Stephan Ludwig

Institute of Molecular Virology (IMV), Center of Molecular Biology of Inflammation (ZMBE), Westfaelian-Wilhelms-University, Muenster, Germany.

The type I interferon system is a powerful first line of defense against viral infections. The interferon-inducing cascade is activated by RNA viruses such as influenza viruses that are sensed by pathogen pattern receptors such as RIG-I. Activated RIG-I is than recruited to the scaffold protein mitochondrial antiviral-signaling protein (MAVS), where a signal transduction complex is assembled to signal via activation of kinases such as IKKepsilon and TBK-1 to the transcription factor IRF-3 that is the major inducer of the IFN enhanceosome driving IFNbeta expression. IFNbeta than induces a cascade of gene expression events of so-called interferon stimulated genes (ISGs) that turn cells into an antiviral-state. It is not surprising that many if not all RNA viruses have evolved strategies to circumvent or suppress this powerful antiviral response. The non-structural protein (NS1) of influenza viruses is one of the most prominent interferon antagonists known and it is the major viral modulator to shape cell responses in favor of efficient virus replication. However, recently other influenza viral proteins were also identified to exhibit interferon antagonistic activity, including polymerase genes PB2, PB1 and PA or the PB1 gene derived non-structural protein PB1-F2. Novel aspects, on function and regulation of these interferon antagonistic proteins will be discussed.

Symposium #4 - Regulation of inflammation

Leptin and Immunomodulation: back to the drawing board

Alexandre A. Steiner, Elizabeth A. Flatow, Evilin N. Komegae, Monique T. Fonseca, Camila F. Brito, Florin M. Musteata, José Antunes-Rodrigues

University of São Paulo, São Paulo, SP, Brazil;
Albany College of Pharmacy and Health Sciences, Albany, NY, USA.

BACKGROUND AND PURPOSE: To elucidate the role of leptin in acute systemic inflammation, we investigated how its infusion at low, physiologically relevant doses affects the response to LPS in a time- and site-dependent manner. **EXPERIMENTAL APPROACH:** Physiological and molecular aspects of the response to LPS (500 μg/kg, i.v.) were assessed in rats infused with leptin s.c. (0-20 μg/kg/h) or i.c.v. (0-1 μg/kg/h). The relationship between leptin dose and plasma level was traced. Besides, cultured resident macrophages were studied at leptin concentrations (1-100 ng/ml) that are low compared to previous studies. **KEY RESULTS:** Using LPS hypothermia and hypotension as response biomarkers, we identified the phase extending from 90 to 240 min as the most susceptible to modulation by leptin. In this phase, leptin suppressed TNF-α without affecting IL-10, prostaglandins or corticosterone. The suppression of TNF-α was attained with s.c. leptin, but not with i.c.v. leptin. At its minimally effective dose, s.c. leptin elevated plasma leptin to a physiologically relevant level (5.9 ng/ml). Our results also revealed that, when primed by food deprivation, LPS-stimulated peritoneal macrophages can be inhibited by leptin at a concentration that is lower than the concentrations reported to promote macrophage activation. **CONCLUSIONS AND IMPLICATIONS:** When infused at a physiological dose, leptin exerts an anti-inflammatory rather than a pro-inflammatory effect. This effect involves an action outside the brain and selective suppression of TNF-α. The potential of leptin to inhibit macrophages deserves further investigation. **Support:** FAPESP, AHA, CNPq & CAPES.

Immunomodulatory Effects of Siglecs on Neutrophil Functions

Fernando Spiller

Department of Pharmacology, Federal University of Santa Catarina, UFSC, Florianopolis, SC, Brazil.

Neutrophil activation, recruitment, and killing of bacteria are the key events involved in controlling infections. These events are guided by activatory receptors expressed on neutrophils that respond to bacteria and their products and/or endogenous molecules. Emerging data suggest that overstimulation of these activatory receptors impairs neutrophil migration to sites of infection, leading to neutrophil infiltration in distant organs and subsequent multiple organ dysfunction and septic shock. Given the lack of target therapies for sepsis, there is a great need for understanding how these activatory receptors are regulated. Inhibitory receptors play important roles in fine-

tuning activatory receptors and are of therapeutic interest because dampening activatory receptors is predicted to improve neutrophil migration to sites of infection and prevent neutrophil-induced tissue injury. Inhibitory receptors on neutrophils include members of the Siglec (sialic-acid-binding immunoglobulin-like lectins) family that have strong immunomodulatory properties through their ability to recruit phosphatases to dampen cellular signaling. Indeed, a growing number of studies have shown that Siglec-5 and -9 on human neutrophils modulate neutrophil responses. Therefore, our lab is interested in understanding which activatory receptors on human neutrophils are regulated by Siglecs. This presentation will show the effect of Siglec activation on neutrophil response and the regulation of the Siglecs on LPS-induced human neutrophil activation. **Funding:** FAPESP, SCRIPPS.

The role of pentraxin 3 (PTX3) on the acute gout attack

Geraldo da Rocha Castelar Pinheiro

Gout is the most common form of inflammatory arthritis in male adults and its prevalence is increasing in the last two decades. Despite being an ancient disease, first recognized by the Egyptians more than 4.500 years ago, having a well-established etiopathogenesis, hyperuricemia due to overproduction, hypoexcretion or both, and effective treatment drugs, gout is still a major cause of suffering and increased mortality. Recent advances in our understanding of the mechanisms of MSU crystal-induced inflammation, in particular the role of the NLRP3 inflammasome (IL-1β), diagnosis, specially new imaging technics (high resolution ultrasound and dual-energy computed tomography) and treatment of gout (IL-1β inhibitors) and hyperuricemia (febuxostato, lesinurad and pegloticase) make it an exciting era to see gout in the clinic. Gout is caused by the deposition of monosodium urate (MSU) monohydrate crystals in joints. The early events involved in acute gout attack are the contact and the phagocytosis of MSU by resident cells that culminate in the maturation and release of IL-1β through NLRP3 inflammasome assembly. However, the precise mechanisms in these initial steps are still poorly understood. Pentraxin 3 (PTX3), a pivotal component of the innate immune system, may have a pivotal role in acute gout. Experimentally, PTX3 levels in plasma and synovial fluid during human gout flares we found it elevated and with a positive correlation with IL-1β levels (synovial fluid). PTX3 seems to be an important molecule during gout attack and may be involved in facilitating the phagocytosis of MSU crystal that amplifying joint inflammation.

Potential of Pegylated Toll-like receptor 7 ligands for controlling inflammation and Functional changes in Mouse Models of asthma and silicosis

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Prior investigations show that signaling activation through pattern recognition receptors can directly impact a number of inflammatory lung diseases. While toll-like receptor (TLR) 7 agonists have raised interest for their ability to inhibit experimental asthma, the putative benefit is limited by adverse effects. We evaluated the therapeutic potential of two PEGylated purine-like compounds, TMX 302 and TMX 306, characterized by TLR7 partial agonistic activity. In vitro mechanisms and translation to murine models of obstructive and restrictive lung diseases were explored. In vitro studies with human PBMCs showed that both TMX-302 and TMX-306 marginally affects cytokine production as compared to the TLR7 full agonist, TMX-202. The PEGylated compounds did not induce monocyte-derived DC maturation or B cell proliferation, differently from what observed after stimulation with TMX-202. Impact of PEGylated ligands on lung function and inflammatory changes was studied in animal models of acute lung injury, asthma and silicosis following LPS, allergen (ovalbumin) and silica inhalation, respectively. Subcutaneous injection of TMX-302 prevented LPS- and allergen-induced airway hyper-reactivity (AHR), leukocyte infiltration and production of pro-inflammatory cytokines in the lung. However, intranasal instillation of TMX-302 led to neutrophil infiltration and failed to prevent allergen-induced AHR, despite inhibiting leukocyte counts in the BAL. Aerolized TMX-306 given prophylactically, but not therapeutically, inhibited asthma features. Treatment with intranasal instillation of TMX-306 reduced pulmonary fibro-granulomatous response, number of silica particles in lung and improved respiratory function in silicotic mice. These findings highlight the potential of TMX-306, emphasizing its value in drug development for lung diseases, and particularly silicosis.

Symposium #5 - Metabolism and inflammation

IL-1b-TLR2 axis induce heart arrhythmias through CaMKII oxidation in type 1 diabetes

Emiliano Medei

Diabetes mellitus (DM) encompasses a multitude of secondary disorders, including heart disease. One of the most frequent and potentially life threatening disorder of

DM-induced heart disease is ventricular tachycardia (VT). However, the potential role of inflammation underlying VT generation has remained elusive. Herein we demonstrate that TLR2 and NLRP3 inflammasome activation in cardiac macrophages mediate the production of IL-1β in DM mice. We also show that IL-1β prolongs the action potential duration, induces a decrease in potassium current (Ito) and an increase in calcium sparks in cardiomyocytes, hence critical cellular changes underlying arrhythmia propensity. IL-1β-induced spontaneous contractile events were associated with CaMKII oxidation and phosphorylation. Thus, our study assigns a critical role to the DM-induced inflammation process in the heart to elicit VT. We further demonstrate that DM-induced ventricular arrhythmias can be successfully treated by inhibiting the IL-1β axis with an IL-1 receptor antagonist, or alternatively, by inhibiting the NLRP3 inflammasome with a NLRP3 inhibitor, MCC-950. Our results establish IL-1β as an inflammatory connection between metabolic dysfunction and ventricular arrhythmias in DM.

Metabolic programs controlling immune cell function

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In the last few years, the field of immunometabolism has gained strong attention, expanding our knowledge on how intracellular metabolism regulates immune cell differentiation and function. In dendritic cells (DCs), the maturation process that leads to the upregulation of costimulatory molecules and the production of cytokines required for the initiation of adaptive immune responses has been linked to metabolic reprogramming. Also in macrophages (MØ), immunological function is accompanied by metabolic changes. How these changes in metabolism directly impact on the key processes required for anti-microbial immunity however, is not well understood. Here we investigated the influence of intracellular bacterial infection on the metabolic program of DCs and MØ with a particular focus on fatty acid metabolism. Using novel mouse models that target specific metabolic checkpoints and a combination of state-of-the-art methods, we dissected the role of de novo fatty acid synthesis and mobilization during mycobacterial infection and its relevance for immunity against this pathogen. Our results highlight remarkable differences between the metabolic requirements of DCs and MØ during infection and suggest that the crosstalk between these cells might also contribute to immune cell function.

The adipokine chemerin links metabolic dyslipidemia and bone loss

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The adipokine chemerin was identified as an inflammatory and metabolic syndrome marker. Elevated levels of chemerin have been found in obese, type-2 diabetes and osteoporotic patients. Considering that the association between metabolic syndrome and bone health remains unclear, the present study aimed to study the role of chemerin in the pathophysiology of bone loss induced by dyslipidemia, mainly focusing in osteoclastogenesis. In vitro analyses showed that mature osteoclasts express chemerin receptor CMKLR1. Although chemerin did not modify osteoclasts formation and osteoclastogenic-associated genes (NFATc1 and TRAP), it increased the expression of osteoclasts activity markers such as actin-ring formation and bone resorption activity. Incubation of osteoclasts with CMKLR1 antagonist (CCX832) effectively inhibited the increased bone resorption activity induced by chemerin. Chemerin boosting mature osteoclasts activity involves ERK5 phosphorylation. Morphometric analysis showed that HFD-treated C57/BL6 and db/db mice (two models of dyslipidemia) exhibited increased alveolar bone loss compared to respective control mice. The bone loss was associated with an up-regulation of serum chemerin in both mice model of dyslipidemia, which also exhibited increased level of chemerin, CMKLR1 and cathepsin K mRNA expression in the gingival tissue. The treatment of db/db mice with CCX832 effectively inhibited the alveolar bone loss. Antagonism of chemerin receptor also inhibited the expression of cathepsin K in the gingival tissue. Our results show that chemerin not only increases osteoclasts activity in vitro, but also that increased level of this key adipokine, in dyslipidemic mice, plays a critical role in bone homeostasis.

POSTER

Chronic inflammatory diseases

Premolis semirufa's caterpillar poison induces the release of inflammatory mediators by human chondrocytes

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The Brazilian moth Premolis semirufa, usually called as

pararama in its larval stage, belongs to the Erebidae family and inhabits rubber plantations found in the Amazon forest. The contact with the bristles, in most cases, causes an intense itching sensation, followed by symptoms of the acute inflammation. On the other hand, a chronic inflammatory reaction frequently occurs in individuals after multiple accidents, which is characterized by articular synovial membrane thickening with joint deformities. **Objective:** Evaluate the possible toxic effect of the Premolis semirufa's bristles extract on human chondrocytes. **Material and methods:** Chondrocytes monolayer cultures (5×10^4 cells/mL) were treated with increased concentrations of Premolis semirufa's bristles extract for 24, 48 and 72 hours. After each treatment, the supernatant was evaluated for the production of cytokines and chemokines by flow cytometry, and of matrix metalloproteinases (MMPs) and complement system components by ELISA. **Results:** The viability of chondrocytes was reduced in dose- and time-dependent manners. Analyses of the supernatants showed that the extract was able to induce the release of IL-6, IL-8 and MCP-1, as well as of MMP-2, MMP-9 and MMP-13 also in a dose- and time-dependent ways. The analysis of the production of some complement system components showed increase in the production of C1q and C4. Discussion and conclusion: The results presented here shows that Premolis semirufa's bristles extract can activate human chondrocytes, promoting the production of cytokines, chemokines, complement components and MMPs, which may contribute to the osteoarticular manifestations observed in pararama envenomed patients. **Financial Support:** Fapesp, GSK, CNPq, CAPES.

Altered expression of glucocorticoid receptor and high basal levels of IL-10, in lung tissue, could be involved in the defective allergic inflammatory response in low-birth-weight rats induced by intrauterine malnutrition

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We have investigated the impact of cytokine and corticosterone regulation on the attenuation of allergic lung inflammatory response in low-birth-weight rats, induced by intrauterine malnutrition. Low-birth-weight offspring were obtained from dams that were fed 50% of the nourished diet of counterparts. At 12-week-of age, the response to sensitization and challenge by ovalbumin was evaluated. Cell counts were performed in bronchoalveolar lavage and peribronchial tissue; we also measured IL-4, IFN-γ and IL-10 levels (Multiplex), PGE_2 (EIA) and glucocorticoid receptor (Western Blot), in lung tissue. Corticosterone hormone levels were evaluated, in serum. After challenge, low-birth-weight rats presented reduced cell infiltration into the airways and lung tissue, accompanied by reduced PGE_2 but increased IFN-γ expression, compared to normal-birth-weight rats. Although low-birth-weight rats showed higher basal levels of IL-4, IL-10 and corticosterone than normal-birth-weight rats, after challenge, no difference was observed between groups. After challenge, different from low-birth-weight rats, normal-birth-weight rats presented reduction in glucocorticoid receptor expression. In a previous study, we showed that high corticosterone levels contribute to increased IFN-γ level and impair the IL-4 expression, affecting the development of the pulmonary allergic inflammation in low-birth-weight rats. Here, we observed that high IL-10 basal levels, an anti-inflammatory cytokine, and a defective regulation in glucocorticoid receptor expression could contribute to the reduced cell infiltration, in low-birth-weight rats. These data, associated to reduction in PGE_2 levels might be closely linked to attenuated allergic lung inflammation presented by low-birth-weight rats.

Paciente com Doença de Crohn e o enfrentamento do luto: atendimento interdisciplinar

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Objetivo: Relatar a análise da experiência de um paciente com Doença de Crohn em relação às fases do luto durante o tratamento cirúrgico. **Materiais e Métodos:** Estudo de caso, elaborado por meio de consultas ao prontuário médico e atendimentos psicológicos e de enfermagem na enfermaria cirúrgica de Coloproctologia de um hospital universitário. **Resultados:** Paciente de 30 anos, com diagnóstico de Doença de Crohn há seis anos e perda de 30kg, submetido à confecção de uma ileostomia, apresentou peritonite e necessitou de duas reabordagens cirúrgicas, contudo, evoluiu para óbito. Foram realizados seis atendimentos interdisciplinares da psicologia e da enfermagem conjuntamente, nos quais o paciente apresentou instabilidade emocional, e fases de luto: negação, raiva, barganha, depressão e aceitação. Durante o seguimento, sequencialmente o paciente manifestou raiva por não conseguir aceitar os procedimentos; passou a negociar com Deus, caso recebesse alta, cuidaria melhor da sua saúde; depressivo, totalmente voltado para si, com negação da sua condição; e tornou-se consciente de sua realidade, expressando aceitação e sentindo-se preparado para a sua finitude. Em sua última noite, este não dormiu, dizendo às enfermeiras que "a hora estava perto". **Discussão:** Este atendimento possibilitou suporte psicológico para o paciente durante a internação, no enfrentamento do luto, com acolhimento, validação de sentimentos e intermediação na busca de sentido do adoecimento, evidenciando a

necessidade da assistência interdisciplinar. **Conclusão:** O suporte psicológico favoreceu o processo de finitude do paciente e a prestação da assistência perioperatória.

Investigation of the profile of cytokines and classic/non classic markers for monocytes in chronic obstructive pulmonary disease

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Introduction: Chronic Obstructive Pulmonary Disease (COPD) is characterized by progressive airflow limitation and intense lung inflammation. Macrophages that can be class into M1 (proinflammatory) and M2 (immunoregulatory) phenotypes which numbers were altered in COPD. These cells derived from blood monocytes $CD14^{++}/CD16^{-}$ (classical), $CD14^{++}/CD16^{+}$ (intermediate) and $CD14^{+}/CD16^{+}$ (non-classical). The monocyte profile seems to favor the macrophage phenotype. The objective of this study was to characterize the profile of monocytes and cytokines in patients with COPD. **Materials and Methods:** Monocytes were obtained from the blood of patients followed at the COPD and anti-tabagism outpatient Service from the Department of Pulmonology. 2.5 10^5 cells were seeded in 24-well plates. Monocytes were stimulated by 24h with 0.1 µg/mL Lipopolysaccharide (LPS) and the release of IL-8, IL-6 and TNF-α were analyzed by ELISA. In parallel monocyte subpopulations were evaluated according to the expression of CD14 and CD16 proteins by flow cytometry. **Results:** A higher number of intermediate monocytes were observed in the COPD group when compared to the control group. There was a lower release of IL-8 in the COPD group than in the control group. The IL-6 cytokine produced by these cells were increased in parallel with the severity of COPD while IL-8 levels decreased. **Conclusion:** COPD patients have a greater number of intermediate monocytes in the blood that can favor a M2 profile of macrophages in the lung.

The role of different immune cell populations on visceral leishmaniasis patients

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Visceral leishmaniasis is an inflammatory chronic disease caused by the protozoan Leishmania infantum in Brazil. Although most patients present asymptomatic forms of the disease, some develop symptoms that eventually result in death. Our study aims to identify differentially expressed genes between groups of VL patients and correlate their expression with the disease outcome. RNA sequencing was performed employing samples from symptomatic and asymptomatic patients, as well as symptomatic patients after 180 days of treatment and healthy controls. The comparison of male asymptomatic and active disease patients resulted in greatest number of differentially expressed genes. The functional analysis of expression profiles demonstrated that symptomatic patients present expression of genes related to neutrophils and citotoxic T cells, possibly contributing to tissue lesions on those patients, while asymptomatic ones revealed an expression profile related to eosinophils responses. Murine validation of those observations using eosinophils deficient mice infected with L. infantum demonstrated that knockout animals present greater responses of neutrophils and T $CD8^{+}$ cells in spleen, confirming the patients observations. Our data indicated that eosinophils might play a regulatory role on inflammation during L. infantum infection. Further studies to comprehend this mechanism are being performed.

Establishment of an experimental model of emphysema: effect of the phosphodiesterase (pde) 4 inhibitor cilomilast

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Objectives: Chronic obstructive pulmonary disease (COPD) is a degenerative and irreversible dysfunction that has no effective treatment until now. PDE4 enzyme has been shown to be an important target in chronic inflammatory processes. Thus, this project aimed to establish an experimental model of emphysema in mice in order to further identify new PDE4 inhibitor compounds. **Material and methods:** Balb/c and C57Bl6 mice were intranasally instilled with elastase (PPE) (0.2 and 0.6 IU) and the following parameters were evaluated: i) pulmonary function (resistance and elastance) and airway hyper-reactivity to the bronchoconstrictor agent methacholine (invasive plethysmography); ii) morphology and morphometry (area of hyperinflation, elastic and collagen fibers); iii) quantification of tissue myeloperoxidase (MPO). **Results and discussion:** We noted that the lung area of hyperinflation was shown to be of similar intensity when comparing both strains of mice used and to be dependent on the dose of PPE used. In parallel, we observed a significant increase in airways resistance and a decrease in the lung elastance. Treatment with the standard PDE4 inhibitor cilomilast reduced the

area of hyperinflation, tissue levels of MPO and fibrosis, as well as improved lung function of C57Bl6 mice stimulated with PPE (0.2 IU). **Conclusions:** Our findings show that intranasal instillation of PPE in mice leads to a short-term and reproducible model of emphysema, which seems to be a useful tool when searching for anti-emphysematous compounds such as new PDE4 inhibitors. **Financial support:** FIOCRUZ, CNPq, FAPERJ e CAPES (Brazil).

Novel solid lipid microparticles for curcumin: characterization and antiinflammatory activity

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Objective: To synthesize curcumin-loaded lipid microparticles (SLMCur) and to evaluate its anti-inflammatory efficacy compared to free curcumin (Cur) in an animal model of Complete Freund Adjuvant-induced arthritis (AIA). **Methods:** SLMCur were obtained using the hot melt homogenization method. Microparticles were lyophilized and analyzed by X-ray and infrared (FTIR) for encapsulation compravation. For the AIA induction, Holtzman rats were randomly divided into five groups: I – Normal; II – AIA; III – AIA + CUR 50 mg/kg; IV – AIA + SLMCur 25 mg/kg; AIA + SLMCur 50 mg/kg. Animals treatment was performed in a single daily dose for a period of 21 days. Arthritis was induced by administration of ACF in the left hind paw. The left hind paws (injected with ACF) and right (non-injected with ACF) were analyzed by digital plethysmography for evaluation of edema development. **Results:** SLMCur was obtained with high encapsulation efficiency. Thermal analysis (X-ray and FTIR) demonstrated absence of characteristic curcumin bands. About the biological response, it was observed that the rats of group III presented a significant decrease in the paw edema development, on the injected and non-injected paw, only on days 17 and 21. However, the rats of group IV and V presented significant inhibition on the development of edema on the injected paw from day 9 and, on the non-injected paw, from day 13. **Discussion and conclusion:** Together, our results demonstrated: 1) SLMCur was obtained satisfactorily demonstrating its encapsulation through thermal analysis and, 2) SLMCur demonstrated a better antiinflammatory efficacy when compared to Cur, inhibiting the development of paw edema in the AIA-induced arthritis.

Influence of aging on experimental arthritis

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Objective: investigate the influence of aging on Adjuvant Induced Arthritis (AIA). **Material and methods:** young (2 months) and middle-aged (12 months) Holtzman rats received 100 µL intradermal injection of Freund's Complete Adjuvant Suspension (heat-inactivated M. tuberculosis, suspended in 0.5% mineral oil) into the left hind paw of the rats. The volume of the injected and contralateral paws was determined by digital plethysmography on days 1, 3, 6, 9, 13, 15, 17, 21, 24 and 28. The ponderal evolution was also determined. Data were statistically analyzed using ANOVA followed by Dunnett test. Differences were considered significant at $p < 0.05$. Experimental protocol was approved by Ethics Committee on Animal Use of the State University of Maringá (CEUA / UEM nº 5410190516). **Results and Discussion:** the inflammatory response in the injected paw of middle-aged arthritic animals (AIAmi) was significantly earlier and more intense when compared to young arthritic animals (AIAj). However, on the 13th day when the immune system was involved in the AIA, inversion was observed in the evolution of the inflammatory response between the experimental groups, with a significant reduction of the edema in the AIAmi group when compared to the AIAj. Regarding the weight evolution, there was a decrease in the weight gain of the AIAmi animals, which coincided with the early onset of the inflammatory response in these animals. In contrast, the weight loss observed in AIAj animals was late, concomitant with the involvement of the immune system. **Conclusion:** our results showed that aging favored early inflammation as well as compromised immune response in AIA; the likely mechanisms involved in these responses are under investigation. **Sources of research support:** CAPES.

Short-term model of acute exacerbation in chronic obstructive pulmonary disease by combining cigarette smoke inhalation and H1N1 infection in mice

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Infections can exacerbate symptoms of chronic obstructive pulmonary disease (COPD) and no appropriate therapy is available, increasing morbidity and mortality. We studied here whether influenza virus would exacerbate cigarette smoke (CS)-induced lung inflammation in C57Bl/6 mice. Forty-two female mice were equally distributed into 6 groups including those exposed to ambient air (AA), those

exposed to CS for 12 days (CSE), AA infected intranasally with 100 (AA100) or 1000 pfu (AA1000) of H1N1 virus at day 7, and CS infected with 100 (CS100) or 1000 pfu (CS1000) of virus. Weight loss, lung mechanics, as well as inflammatory and oxidative/anti-oxidative changes in lung tissue were assessed 24 h after the last exposure to CS. We found that only mice infected with 1000 pfu, exposed or not to CS, lost weight significantly. Levels of neutrophil accumulation in BAL fluid, as also lung MPO, TNF-α, IL-6, KC, MIP-1-α and MCP-1 appeared significantly exacerbated in CS1000 group when compared to the infected but non-exposed ones (AA100 and AA1000), although in CSE group of mice, none of those parameters was altered. Viral infection alone decreased catalase activity and augmented lipid peroxidation when compared to the control AA group while CS alone did not. CS exposure exacerbated lipid peroxidation in the CS100 group only. Our results show that the combination of CS and H1N1 infection led to synergistic exacerbation of pivotal lung inflammatory changes in C57Bl/6 mice. This model may be helpful to investigate mechanisms and putative therapies relevant in COPD exacerbations. **Financial support:** CNPq and FAPERJ.

Vancomycin treatment increases regulatory t cells and prevents the type 1 diabetes in murine model

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Objective: We aimed to evaluate the effect of vancomycin treatment in the type 1 diabetes (TD1) pathogenesis. **Material and Methods:** C57BL/6 mice were treated by oral route with 0.5g/L of vancomycin daily in water during 7 days before and 7 days after the administration with streptozotocin (STZ/40mg/Kg). Blood glucose levels and body weight were monitored weekly. The pancreatic lymph nodes (PLN) were removed to assess the regulatory T cell (Treg) and dendritic cells (DC) frequency and number by flow cytometry. The pro and anti-inflammatory cytokine levels were determined in pancreatic tissue homogenates by ELISA assay. **Results:** STZ-injected mice treated with vancomycin developed lower hyperglycemia and none of mice becoming diabetic. In agreement, these mice have reduced inflammatory infiltrate (insulitis) and augmented insulin content into pancreatic islets by second week. In addition, the vancomycin treatment caused an increase in Treg cell number and tolerogenic DCs in pancreatic lymph nodes (PLN). Despite theIL-17 or IL-10 levels were not altered in the pancreatic tissue or ileum, the IFN-γ levels decreased of significant manner in mice treated with vancomycin. **Conclusion:** These results suggest that the vancomycin treatment might represent a therapeutic target for T1D since boosted the Treg number and prevented the autoimmune T1D onset. **Financial support:** CNPq and FAEPA.

Involvement of adenosine A2A receptor in the lung fibrosis caused by silica particles in mice

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Objectives/background: Adenosine is a nucleoside that has been reported to be implicated in fibrosis, being considered as a potential therapeutic target for fibrotic diseases. In this study, we investigated the involvement of adenosine in pulmonary fibrosis in silicotic mice. Lung fibroblast reactivity was also evaluated in vitro. **Methods and results:** Mice were instilled with silica and the analyzes performed 7 and 28 days later. The parameters included lung function (resistance and elastance) and tissue morphology/morphometry. Lung fibroblast reactivity (proliferation and MCP-1) was evaluated in vitro. Expression of CD39 and CD73 enzymes, responsible for adenosine generation, was increased in the lungs at 7 days of silicosis. Adenosine receptor expression was altered in the lung of the silicotic animals, reflecting increase of A_1 and A_{2B} receptors, reduction of A_{2A} receptor and no alteration of A_3. Lung fibroblasts stimulated with IL-13 and adenosine, alone or in combination, led to an increase in proliferation and MCP-1 production, phenomena sensitive to A2A receptor antagonists ZM 241385 and SCH-58261 Fibroblasts from A_{2A} receptor knockout mice were less responsive to IL-13 and adenosine stimuli. CD39 and CD73 inhibitors also suppressed IL-13 stimulated fibroblast proliferation. Receptor A_{2A} silicotic knockout mice showed reduction of lung function decrease and fibrotic granulomatous responses. **Conclusion:** Our results show that adenosine seems to be implicated in the fibrotic response associated with silicosis in mice, by a mechanism, at least partially dependent on its ability to synergize with IL-13 and its action on A2A receptors. **Financial support:** CNPq, FAPERJ, CAPES.

Evaluation of the protective effect of rolipram in a model of nephropathy induced by doxorubicin

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The chronic inflammation has been associated to an ineffective resolution of inflammation response. Thus, the development of therapy based in induction or activation of inflammation resolution program must be an innovative

approaches to treatment of unresolved inflammation. In this study, we have been investigating the effects of treatment with rolipram, a selective PDE4 inhibitor with putative pro-resolutive actions, on pathogenesis of chronic nephropathy induced by doxorubicin. The nephropathy was induced by single dose of doxorubicin (10mg / kg) in the tail vein of Balb/c mice. All experimental animals injected with doxorubicin developed nephropathy that was maximal at day 14 after injection. To verify the effects of rolipram, doxorrubicin-injected mice were daily treated with rolipram (6.0mg/kg) from day 7, when histological changes and renal injury has already been established, to day 14 after exposure to doxorrubicin. Control mice received vehicle dose (PBS+DMSO) at the same period that was administered the treatment with rolipram. Treatment with rolipram induced recovery in serum total protein and albumin and reduced weight loss and prostration of mice. In addition, rolipram decreased glycoproteins accumulation in glomerulus and renal tubules and ameliorate histophatological renal damage. Futhermore, the treatment with rolipram reduced the number of macrophage and apoptotic cells in renal tissue. Taken togheter, this preliminary study provides evidence that rolipram may have pro-resolutive effects in established chronic inflammation during nephropathy induced by doxorubicin.

Avaliação da intensidade do exercício físico sobre o seu efeito antinociceptivo em modelo experimental de neuropatia periférica utilizando a máxima fase estável do lactato como marcador da capacidade aeróbica

Jorge William Martins

O objetivo deste estudo foi investigar o efeito antinociceptivo do exercício físico em diferentes intensidades, 50 e 75% da máxima fase estável do lactato, quantificar o nível plasmático de corticosterona sobre o desenvolvimento da alodínia em animais com lesão de nervo periférico. O modelo experimental de dor neuropática consistiu em ligação parcial e unilateral do nervo periférico, onde simula o estresse físico frequentemente observado em humanos com inflamação crônica. A atividade física foi quantificada através da máxima fase estável do lactato. O protocolo de treinamento foi constituído por 5 sessões de treino, natação, de 20 minutos com intervalo de 48 horas nas intensidades 50 e 75% da máxima fase estável do lactato. A quantificação hormonal foi através da técnica de radioimunoensaio a sensibilidade mecânica foi através do teste de Von Frey. 10 horas pós treino o nível hormonal foi maior em animais que nadaram numa maior intensidade. 10 horas pós treino a melhora da sensibilidade mecânica foi maior em animais que nadaram em uma menor intensidade. 7 dias pós treino os níveis hormonais dos animais que nadaram em maior intensidade ficam menores do que o grupo controle. 7 dias pós treino a melhora da sensibilidade mecânica foi melhor em animais que nadaram em maior intensidade. Os resultados corroboram com a ideia da supercompensação onde a adaptação é relacionada a intensidade do estimulo e de que o repouso corrobora com o processo de adaptação do organismo, no caso, melhorando a analgesia de animais com dor crônica de origem neuropática.

AhR activation in Th17 cells induces microRNAs implicated in arthritis

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Introduction/objective: Rheumatoid arthritis (RA) is a complex multifactorial autoimmune disorder and Th17 cells have a central role in the induction and progression of the disease. Environmental factors as cigarette smoke may exacerbate the symptoms of RA. AhR is a transcription factor activated by ligands that mediate toxicity of environmental contaminants, and its activation participates in the differentiation and activation of Th17 cells. However, the mechanisms of AhR-mediated immune regulation remain poorly understood. In this context microRNAs are important modulators of protein-encoding genes expression at post-transcriptional level. Their contributions in the differentiation and activation of immune cells, and also in the pathogenesis of diseases, have been extensively studied. Our objective is to determine the miRNAs induced after the AhR activation, their roles in Th17 and the implications in arthritis. **Methods and results:** We differentiate TH17 cells in vitro and TCD4^{+}IL-17^{+} cells were collected for microarrays hibridization after 24hs with AhR agonist FICZ. We identify microRNAs induced by AhR activation, highly conserved between mice and human. AhR activation by cigarette components increases their expressions, which in turn are decreased by inhibition with antagonist. These microRNAs were detected in experimental arthritis mice models and their expression also increases in RA patients compared to health individuals. **Discussion/conclusion:** Our results identified miRNAs induced by AhR activation in Th17 cells implicated in arthritis. These data will contribute to a better understanding of the genetics and molecular basis of the AhR activation, related to the development and activation of Th17 cells in RA. Grant #2012/02438-0, #2013/08216-2 São Paulo Research Foundation (FAPESP).

Structure- and pharmacophore-based virtual screening to design novel potential PAD4 inhibitors with interest in inflammatory diseases

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Rheumatoid Arthritis (RA) is a chronic progressive autoimmune disorder that ultimately leads to a destruction of the cartilage surrounding the joint. It is the second most common type of arthritis, with symptoms first appearing in patients between 40 and 60 years of age. Protein Arginine Deiminase (PAD4), which catalyzes the conversion of peptidyl-arginine to peptidyl-citrulline, is widely believed to play a causative role in RA disease onset and progression because RA-associated mutations in the PAD4 gene have been identified in a variety of populations and RA patients produce autoantibodies that recognize citrulline containing proteins. Interestingly, the anti-citrulline autoantibodies are considered to be the most specific diagnostic marker of this disease and there is a direct correlation between the levels of citrullinated proteins and disease severity, especially in the formative stages of RA. In total, the serological and genetic data suggest that PAD4 activity is dysregulated in RA. In addition , new selective PAD4 inhibitors binding a calcium-deficient form of PAD4 have validated the critical enzymatic role of human and mouse PAD4 in both histone citrullination and neutrophil extracellular trap formation. The therapeutic potential of PAD4 inhibitors can now be explored. Here, we have used structure- and pharmacophore-based virtual screening approaches to design novel potential PAD4 inhibitors as drug candidates for RA treatment. We have used structural information reported in the BindingDB database for 60 PAD4 inhibitors and two PAD structures deposited in the Protein Data Bank in complex with inhibitors (GSK147 - PDB code 4X8C, at 3.1 Å resolution; GSK199 - PDB code 4X8G, at 3.29 Å resolution). Crystallographic pose of GSK147 was used as a template for molecular docking-based virtual screening, due to its highest resolution crystallographic, by using the GOLD 5.2.2 software. Also, derivation of a pharmacophoric hypothesis common to the other compounds was additionally carried out by using the Discovery Studio 4.0 software. After applying toxicity and pharmacokinetic filters, 19 potential PAD4 inhibitors with suitable ADME/Tox properties were selected and purchased for experimental assays.

Effects of adjuvant-induced-arthritis on the daily sperm production and sperm transit

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Objectives: Rheumatoid arthritis is an inflammatory disorder that involves articular's synovial membrane, but also implies several extra-articular manifestations. Studies suggested that it can affect the male gonads. Since male population is less affected by the disease, few researches addressing this group have been developed. Then, the present study aimed to evaluate the effects of adjuvant-induced-arthritis on the daily sperm production and epididimal sperm transit. **Material and methods:** Male Wistar rats were divided into control and adjuvant-induced-arthritis groups. The induction was performed after anesthesia with Mycobacterium tuberculosis (50mg/mL), intradermal via, in the paw plant of the animals. After 41 days of induction, reproductive organs were dissected and weighed. Testis and epididymis were frozen (-20°C) to perform the analyses. Statistical analysis was perfomed by T-student test. Values of $p<0.05$ were considered statistically significant. **Results:** There was significant decrease in body weight and wet weights of seminal glands and prostate from adjuvant-induced-arthritis rats when compared to control group. Significant difference was also observed in daily sperm production from adjuvant-induced-arthritis group. No significant difference was detected on sperm transit among the groups. **Discussion:** The adjuvant-induced-arthritis causes important damage to the testicular function. These findings corroborate with previous studies that have shown the degenerative effects of arthrithis on male genital system. **Conclusion:** The adjuvant-induced-arthritis affected the daily sperm production in rats as well as wet weights of male acessory glands. Then, the next steps is to evaluate the degree of structural changes in seminiferous epithelium that lead to disruption in the spermatogenesis process.

Effects of AIA (adjuvant induced arthritis) on plasmatic oxidative stress profile

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Objectives: To investigate if eventual changes of the redox balance promoted by AIA are influenced by testosterone. **Methods:** Male Wistar rats were submitted to bilateral orchiectomy (ORQ), followed by AIA. Plasmatic oxidative stress was evaluated through the measurement of total antioxidant capacity (FRAP), nitrite/nitrate and lipid peroxidation (FOX). ANOVA – two-way, Tukey's post-test ($P<0.05$). **Results:** FRAP levels did not alter due to AIA and/or ORQ. AIA increased nitrite/nitrate levels in ORQ animals (from 15,40±4,4 to 44,43±6,7; $P<0,002$). AIA reduced FOX levels in SHAM-ORQ animals (from 17,33±1,7 to

10,43±1,2; P<0,02). **Discussion:** Since no differences were observed in FRAP levels, it is possibly to state that oxidative stress in AIA and/or ORQ animals is not sufficiently high to consume its plasma antioxidant defenses. In turn, our study demonstrated higher levels of nitric oxide end-products due to AIA in ORQ animals. Apparently, excessive oxidative stress in the inflamed joints is reflected as an increased concentration of nitric oxide in peripheral blood. However, in our study, this increase was only significant in the absence of testosterone. Lastly, our study observed a reduction rather than an increase in FOX levels due to AIA in SHAM-ORQ group. It is suggested that patients with rheumatoid process have defective defense mechanisms against ROS. Additionally, discrepancies among FOX levels between studies might be explained by the fact that FOX is not a very specific marker. **Conclusion:** The data suggest that testosterone influences the oxidative stress increase due to AIA. **Financial Support:** CAPES. CEUA – FAMEMA (protocol nº 1026/14).

Immunometabolism in immune function and inflammatory disease

Protective role of adenosine monophosphate activated kinase (AMPK) on the progression and severity of experimental tubulointerstitial nephritis

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Adenosine monophosphate activated kinase (AMPK) is an energy sensor and a master regulator of metabolism. We aimed to investigate its role through activation by metformin (Met) on chronic kidney disease, hypothesizing that it could have a positive impact on disease progression, by leading macrophages (MØ) to a less inflammatory phenotype. We induced tubulointerstitial nephritis (TIN) by feeding of adenine-enriched diet to C57BL/6 mice for 10 days. They were gavaged daily with either saline or Met 200 mg/kg. In a second experiment, we administered intraperitoneal injections of clodronate (Clo), 50mg/kg. An in vitro experiment on a cell lineage of murine renal tubules was also performed, in which TGF-β 10 ng/mL, alone or with Met 20mM, was used to induce epithelial to mesenchymal transition (EMT), assessed by quantification of the myofibroblast marker alpha smooth muscle actin (αSMA). Met caused a less marked deterioration of renal function, while significantly reducing the expression of inflammatory cytokines TNF-α and IL-6, as well as fibrosis markers TGF-β and collagen I. Clo turned to be as effective as Met in protecting against TIN. Interestingly, mice treated with both drugs faired even better. The in vitro experiment revealed that αSMA was highly expressed on cells incubated with TGF-β only, while negligible expression was observed on cells incubated with TGF-β plus Met. Thus, we conclude that, in experimental TIN, activation of AMPK sensibly reduces the disease severity, by modulating MØ to a less pro-fibrotic phenotype, and by turning renal tubular cells resistant to EMT. **Funding:** CNPq and FAPESP.

Project "The role of succinate and its receptor GPR91 in experimental psoriasis"

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Psoriasis (PsO) is an inflammatory disease of the skin that affects 2-5% of world population. The etiology of PsO is complex and is not completely elucidated. It is known that accelerated proliferation and early maturation of keratinocytes are responsible for the clinical manifestations of the disease, such as thickening of the epidermis and the appearance of psoriatic plaques. However, the mechanism of the pathogenesis remains unclear. In this context, succinate is a metabolic intermediate of citric acid cycle, an important step of cellular metabolism. In addition to metabolic functions, succinate shows different functions by interacting with its receptor GPR91, a G-protein coupled receptor. GPR91 is expressed in many tissues and it has been described that the interaction succinate-GPR91 can modulate several physiological functions such as hematopoiesis and retinal angiogenesis. In immune system, GPR91 is important to dendritic cells and macrophages activation, and recently was showed their role in experimental arthritis. Considering the immunomodulatory effects of GPR91, the aim of this study is to evaluate the role of this receptor in experimental psoriasis. To investigate the role of GPR91 in the development of psoriasis, psoriasis-like skin inflammation was induced by topical application of imiquimod (IMQ) on the skin of C57BL/6 mice or GPR91 knockout mice. We showed strongly ameliorated the development of psoriasis-like skin inflammation and reduced the levels of proinflammatory cytokines in the skin of the GPR91-/- mice. Moreover, H&E staining from skin sections showed that in lacking of GPR91 reduced acanthosis, leukocytes infiltration, when compared with the WT control group. Finally, flow cytometry analysis showed reduced frequency of Langerhans and Th17 cells after treatment in GPR91 knockout mice. Taken together, our results demonstrate that GPR91 receptor is important to development of the experimental psoriasis, enhancing Langerhans cells and Th17 cells responses.

Low birth weight rats present reduced cell infiltration in acute lung inflammation by influence of corticosterone

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Wistar rats with 12 weeks-old with low birth weight present reduced adhesion molecules expression and increased circulating levels of corticosterone in basal conditions. We investigated the role of corticosterone on acute lung inflammatory response in this model. Low birth weight rats (LBW), induced by intrauterine malnutrition, and normal birth weight rats (NBW), both with 12 weeks old were adrenalectomized (ADX) and received corticosterone replacement (3mg/kg/day, i.p.) or saline for seven consecutive days. In the 8th day the acute lung injury was induced by LPS i.n. (750µg/100µL). Another group of LBW and NBW rats received metyrapone i.p. (50mg/kg) one hour prior instillation of LPS. The cellular infiltration was analyzed in bronchoalveolar lavage fluid (BALF) and lung tissue. Corticosterone and ACTH hormones were quantified in serum (by Multiplex). LBW rats presented a reduction in leucocytes (neutrophils mainly) infiltration into lung tissue and bronchoalveolar lavage, however, after reducing levels of corticosterone, both by ADX and metyrapone, increased leucocytes number similarly to observed in NBW rats. LBW rats presented high circulating levels of corticosterone, and after inflammatory stimulus no alteration occurred. No difference of ACTH levels were observed between NBW and LBW rats. Adrenalectomy and treatment with metyrapone decreases corticosterone levels in animals. Thereafter LBW rats, after stimulus with LPS, increase the number of total cells in the BALF, having a similar response to NBW rats. Our preliminary results indicate that high levels of corticosterone presented by LBW is influencing in reduced cell infiltration in acute lung inflammation induced by LPS. **Supported by** FAPESP-2012/51104-8, 2010/01404-0, CNPq and CAPES.

Lack of eosinophils on fat pad expansion upon the intake of different dietary components

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Eosinophils are generally related to helminth infections or allergy. It has been recently demonstrated also their association with metabolic alterations and adipose tissue remodeling. However, the events related to adipose tissue expansion in obesity triggered by reduction of eosinophil presence has not yet been described. **Objectives:** Our aim was to evaluate the participation of eosinophils on adipose tissue expansion related to metabolic and inflammatory alterations. **Material and Methods:** Male BALB/c wild-type (WT) mice and GATA-1 knockout (GATA-$1^{-/-}$) mice were fed with chow (C) or high-refined-carbohydrate diet (HC) or high-fat (HF) for 8 weeks. **Results:** Lack of eosinophils induced insulin resistance associated with increased adipose tissue and serum leptin levels in GATA-$1^{-/-}$ mice. Cytokines levels were augmented in adipose tissue, but they were lower when released by adipocytes in culture medium. GATA-$1^{-/-}$ mice showed increased rolling leukocytes in adipose tissue related with the presence of neutrophils and proinflammatory macrophages. Bone marrow transplanted from WT mice to GATA-$1^{-/-}$ mice showed a modest improvement of glucose metabolism, but a partial reversion in increased adipose tissue mass that was associated with the same levels of cytokine production by adipose tissue compared with GATA-$1^{-/-}$ phenotype. After intake of HC and HF diet, only GATA-$1^{-/-}$ mice fed with HF diet demonstrated an expressive adipose tissue expansion, worse glucose metabolism, however, interestingly, associated with lower inflammation. **Conclusions:** We suggest that eosinophils control not only the metabolic homeostasis, but also adipose tissue expansion through modulation of inflammation on adipocytes, since lower inflammation intensifies fat pad expansion.

Effects of increased adipose tissue on alveolar bone loss in mice

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This study aimed to evaluate the effect of increased adipose tissue in alveolar bone conditions in mice. Groups of mice were maintained on standard or HF diet on 12 weeks. After, euthanasia was performed and the following parameters were determined: adiposity index, alveolar bone loss, neutrophils influx, expression of cytokines (ELISA), bone mineral density (BMD) and bacterial load on periodontal tissues. Hereafter, groups of mice on standard or HF diet were treated with chlorhexidine (CHX) and the same parameters were evaluated. We also evaluate cytokines (ELISA) in periodontal tissues and alveolar loss bone in leptin receptor deficient (db/db$^{-/-}$) mice. HF diet was associated with increased adiposity, spontaneous alveolar bone loss, decreased of BMD when compared to standard diet. Additionally, mice fed with HF diet presented decreased IL-10 levels in periodontal tissues and significantly less adiponectin in serum. Moreover, HF diet induced

increased neutrophil influx in periodontal tissues and was also associated with disbiose in oral cavity. Depletion of oral microbiota by CHX impaired alveolar bone loss and changes in BMD associated with HF diet. The db/db$^{-/-}$ mice also presented alveolar bone loss when compared to WT mice. These results indicate that increased adiposity may change the oral biofilm and the production of inflammatory mediators and this way may contribute to alveolar bone health. Uniterms: Periodontal disease. High fat diet. Mice. Oral microbiota. **Supported by:** CAPES, FAPEMIG, CNPq.

The glycolytic enzyme pyruvate kinase M2 (PKM2) contributes to autoimmune neuroinflammation by modulating TH17 cell differentiation

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Multiple Sclerosis is an inflammatory autoimmune disorder characterised by autoreactive Th17 cell response. Recent evidences demonstrate that Th17 cells undergo metabolic reprogramming, which is critical for their differentiation and effector function. The glycolytic enzyme Pyruvate Kinase-M2 (PKM2) converts phosphoenolpyruvate into pyruvate. Apart from its catalytic activity, it has been reported that PKM2 can be phosphorylated and translocated into the nucleus, controlling gene expression. Thus, this study aims to investigate PKM2 involvement in Th17 differentiation and its influence on the pathogenesis of Experimental Autoimmune Encephalomyelitis (EAE). EAE was induced by immunising mice with MOG_{35-55}. Naïve T-CD4^{+}CD25^{-} cells were cultured under Th17-polarizing conditions (IL-6–20ng/mL; TGF-β–2,5ng/mL). By performing qPCR, immunofluorescence or immunoblotting, we found that PKM2 is overexpressed in dLNs and spinal cord of EAE-bearing mice, in which PKM2 phosphorylation degree was associated with disease severity. Treatment with shikonin (PKM2 inhibitor; 4mg.kg^{-1}) or CD4–specific Pkm2 deletion (CD4^{cre}PKM2$^{flox/flox}$) noticeably reduced EAE symptoms along with a decrease of IL-17^{+}CD4^{+} cells frequency and downregulation of inflammation-related genes in the spinal cord. In vitro, besides gene expression of PKM2 being augmented over differentiation, phospho-PKM2 was also highly expressed in Th17 lymphocytes. Moreover, inhibition or specific-deficiency of PKM2 reduced Th17 polarization. Interestingly, the absence of PKM2 in Th17 cells caused a decrease in expression of Rorc and Rora genes, which transcribe master transcription factors of Th17 cells. Hence, these findings imply an important role for PKM2 in autoimmune disorders by regulating Th17 differentiation. **Financial Support:** FAPESP

Oncostatin M induces osteoblast differentiation: a new signaling pathway involving SHC1, STAT3 and ERK

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Pro-inflammatory cytokines accelerate bone cell metabolism, increasing both bone resorption and formation. Oncostatin M (OSM), a cytokine belonging to the IL-6 family, is known to participate in intramembranous bone healing. The detailed intracellular signaling pathway associated with osteoblast differentiation induced by OSM remains elusive. Here, we describe a new signaling pathway for osteoblast differentiation, involving SHC1 as adaptor protein, which recruits STAT3 and ERK to induce osteoblast differentiation. To understand the role of SHC1 in osteoblast differentiation, we used siRNA targeting SHC1 to knock-down this RNA and consequentially reduce the protein levels. Silencing of SHC1 in osteoblasts using siRNA reduced the SHC1 mRNA and protein levels, as confirmed by qPCR and western blot. Osteoblasts exposed to OSM (100ng/mL) increased alkaline phosphatase activity, and deposition of bone mineral nodules as assessed by Alizarin red staining, and these effects were suppressed by SHC1 silencing. Treatment of osteoblasts with OSM led to increased expression of osteoblast phenotypic markers (Sp7, Alpl) and this effect was also suppressed by SHC1 silencing. Osteoblasts exposed to OSM exhibited increased phosphorylation of SHC1, ERK and STAT3, and the phosphorylation of these proteins was decreased by SHC1 silencing. In conclusion, the protein SHC1 is important for effects by OSM on osteoblasts differentiation and matrix mineralization. This mechanism is dependent of the phosphorylation of ERK and STAT3.

Immunoregulatory Features of Leptin in Macrophages Rely on mTOR Signaling Network

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Leptin is an energy regulating hormone produced by the adipose tissue and induces satiety and energy expenditure. In obese individuals, leptin resistance leads to hyperleptinemia. We aimed to explore the effects of leptin on macrophages. We hypothesized that leptin acts through mTOR pathway, coupling cellular activation to environmental nutritional status. We used bone marrow derived macrophages from wild type mice. Macrophages were treated with leptin (25 and 50 ng/mL) and lipopolysaccharide-LPS (100 ng/mL). Leptin enhanced LPS-induced secretion of TNF and IL-6. Leptin also enhanced LPS-induced glycolysis. Analysis

of genes regulated by mTOR pathway in macrophages derived from leptin deficient mice indicate that leptin directly regulates LPS-induced mTOR signaling pathway and mTOR-target genes. We next investigated whether the immunoregulatory effects of leptin could also be involved in resident macrophage regulation. We used the microglial cell line BV-2. BV-2 cells were treated with leptin (50 ng/mL) and LPS (100 ng/mL). The phosphorylation of STAT3 was similar when cells were treated with leptin and LPS or treated with leptin alone. LPS induced the activation of both the mTORC1 and mTORC2 pathways and leptin only activated the mTORC1 pathway. BV-2 cells pre-treated with leptin also failed to activate the mTORC2 signaling pathway. Our data suggests that leptin inhibits LPS-induced mTOR2 pathway and that leptin may augment the inflammatory process, at least partially, through mTORC1 activation.

Hif-1a modulates the metabolic profile of adipose tissue macrophages in obesity

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Obesity-induced inflammation is a risk-factor for several chronic pathologies and diseases. Investigating the crosstalk between metabolism and immune function under obesigenic conditions may provide insights underlying inflammation-induced insulin resistance. A central player that regulates the crosstalk between adipocytes and macrophages is the hypoxia-induced factor 1alfa (HIF-1a). Our aim is to understand how hif-1a regulates the immune and metabolic profile of adipose tissue macrophages (ATM) in obesity. Bone marrow-derived macrophages (BMDM) treated with LPS displayed increased expression of Tnf, Il-12, Glut-1, Pfkfb3 and Ldh and coincided with Hif-1a. The expression of these genes peaked at 6h after LPS stimulation. Glucose uptake, glycolytic rate and lactate production in BMDM also had maximum values 6 hours after LPS stimulation. The oxidation of glucose into CO_2 was marginally increased 6h after LPS treatment and reached higher levels at 18h. The inhibition of HIF-1a in LPS-stimulated macrophages resulted in reduced secretion of TNF and IL-6. M1 macrophages isolated from the adipose tissue (AT) of obese mice had increased glycolytic capacity and increased expression of Tnf, Hif-1a and Hif-1a target genes (Pfkfb3 and Ldh) in comparison to M1 and M2 ATMs isolated from lean mice and M2 ATMs isolated from obese mice. Metabolomic profile of M1 and M2 ATMs isolated from lean and obese mice indicate that obesity alters the overall metabolism of M1 ATM. Our results suggest that Hif-1a regulates the metabolic tonus of adipose tissue macrophages and that Hif-1a-induced glycolysis is required for macrophage activation.

Intolerância alimentar para glúten, trigo e farelo de trigo – níveis de Imunoglobulina G (IgG)

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Intolerância alimentar é caracterizada por reações não tóxicas, causada por alimentos levando a reações mediadas principalmente por imunoglobulinas do tipo G (IgG). Esses alimentos, substâncias e/ou macromoléculas presente em alimentos, inflamam a mucosa do intestino aumentando a permeabilidade da barreira intestinal por onde estas substâncias caem na circulação e são reconhecidos pelo sistema imunológico como elementos agressores. Estes elementos são combatidos pelo sistema imunológico, formando imunocomplexos antígeno-anticorpo. O objetivo deste trabalho foi analisar a concentração de Imunolglobulina G (IgG) em pacientes com sensibilidade alimentar para glúten, trigo e farelo de trigo. Neste trabalho foram avaliados 120 resultados (60 feminino e 60 masculino). Os dados dos níveis de IgG foram obtidos através do doseamento em amostra de soro. A IgG alimentar foi doseada pelo método microarray colorimétrico rápido baseado em Elisa. Genarrayt Microarray ® 200+. Após obtenção dos resultados de normalidade (Kolmogorov-Smirnov). A análise estatística foi utilizada no Software Prism para nível de significância para $p<0,05$. Os resultados revelam que 48 homens apresentaram IgG para trigo com média de 44,4±1,35; 22 para gluten com média de 54,73±3, e 31 para farelo de trigo com média de 35,89 ±.1,96. Para o sexo feminino os dados foram 52 apresentaram IgG para trigo (42,58 ± 1,2) 21 para glúten (58,19±5,00) e 6 para farelo de trigo (39 ± 4,2). Podemos afirmar que a concentração de IgG para gluten foi maior nas mulheres que nos homens enquanto para trigo e farelo de trigo não houve diferença significativa na concentração de IgG.

Impact of low birth weight on 12-week rats: Relation between adiposity and inflammation

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Objetive: We have investigated the repercussions to the adipose tissue development in adult (12-week old) rats submitted to in utero undernourishment (which causes small birth weight and hypercorticosteronemia. **Methods:** Female Wistar adult (12-16 weeks old) rats in estrus were mated and, after confirmed the presence of spermatozoa in

vaginal swab, were divided into two groups: G1 – fed with normal diet (commercial pellets) and water ad libitum; G2 –50% food restriction and free water access. G1 mothers gave birth to normal weight (PNN) and G2 to underweight puppies (BPN). These rats were killed by euthanasia and their perivascular fat pads were weighted and inflammatory parameters were measured. **Results:** Body weight (g) at 12 weeks old: PNN= 327,4±7,337 vs. BPN= 363,1±3,45* (p=0,0003). Perivascular fat pad (g): PNN= 2,556±0,2605 vs. BPN= 1,835±0,1123*(p=0,0293). Adipocyte diameter (μm): PNN= 61,36±1,820 vs. BPN= 68,46±1,56. Adipocyte count/fat pad: PNN= 18,65x106±10,61 vs. BPN= 10,93 x106±0,612* (p=0,0001). Insulin receptor and IRS1 (insulin receptor substrate 1) gene expression were reduced in BPN animals while TNFα (tumor necrosis factor α) and IL6 (interleukin 6) gene expressions were unaltered. **Discussion:** The above results indicate that BPN animals reached a higher body weight with adipocyte hypertrophy and adipose tissue hypoplasia. Previous studies demonstrated a strong positive correlation between adipocyte hypertrophy and tissue inflammation which were not reproduced in our model, since we did not show any alteration in TNFα and IL6 mRNA levels. **Conclusion:** Animals that were previously submitted to in utero undernourishment did not develop alterations in their inflammatory profile. **Supported by** FAPESP- 2014/15210-3, 2012/51104-8, 2010/01404-0 and CNPq.

mTOR complex 2 negatively impacts plasmablast formation and B cell isotype class switching

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mTOR is a kinase composed of two distinct complexes, mTORC1 and mTORC2. It has shown to play a crucial role in regulating immune B cell metabolic and immune changes. However, little is known about how each mTOR complex can interfere with B cell fate decision. Thus, we aimed to investigate the role of mTORC2 in B cell metabolic and immune properties. For this purpose, we used mice in which Rictor (essential for mTORC2 function) was excised from their B cells (Rictor$^{\Delta B}$). To investigate the impact of mTORC2 on metabolic pathways and plasmablast formation, B cells were isolated from Rictor$^{\Delta B}$ or CT, and stimulated with LPS for 72 hours in vitro. Rictor$^{\Delta B}$ B cells required a longer period to reach their peak of glucose uptake, presented impaired aerobic glycolysis, whereas oxidative phosphorylation (OXPHOS) was optimized. In the same conditions, plasmablast differentiation from Rictor$^{\Delta B}$ isolated B cells was elevated, as well as isotype switching from IgM to IgG1 when IL-4 was added to LPS. Taken together, our results show that mTORC2 deficiency affects B cell aerobic glycolysis, however favors OXPHOS, plasmablast differentiation and isotype class switching. **Financial support:** FAPESP; CAPES; CNPq.

Fructose 1,6-bisphosphate, a glycolytic metabolite, tunes the metabolic reprogramming of pro-inflammatory macrophage

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Fructose 1,6-bisphosphate (FBP) is an endogenous intermediate of glycolytic pathway. Exogenous administration of FBP showed to exert protective effects, which are attributed to sustain glycolysis, increase ATP production and adenosine levels, however mechanism is not fully understood. We hypothesized that metabolic reprogramming by FBP could modulate the macrophage inflammatory response. Here, we show that FBP enhances IL-10 production by LPS-activated macrophages, because the treatment with FBP can increases cellular metabolism, and this leads enhance production of lactate, ATP and IL-10, dependently the glycolytic pathway. Mechanistically, FBP boots glycolysis pathway, increasing synthesis and secretion of ATP, which is rapidly catabolized into adenosine, as was also increased levels in presence of FBP, and this was abolished when used glycolytic pathway inhibitors. Indeed, the inhibition of Pannexin-1, an ATP-releasing channel, or the ectonucleosides CD39 and CD73 blocked the enhanced production of IL-10 by FBP. Moreover, we found that inhibition of adenosine receptor A2a (A2aR), blocked the enhanced production of IL-10 by FBP. In line, FBP failed to enhance IL-10 production in LPS-activated macrophages from A2AR or Pannexin-1 KO mice and also in presence of adenosine deaminase. Finally, in colitis or peritonitis model, treatment with FBP was able to reduce score of disease, besides increased levels of IL-10 in colon, serum or peritoneal lavage. Taken together, these data implicate FBP as a key molecule that increasing the cellular metabolism that leads enhance production, release and hydrolysis of ATP into adenosine by CD39/CD73 pathway in macrophages, self-limiting their activation state. **Financial Support:** CNPq, CAPES, FAEPA, FAPESP.

Anti-inflammatory effects of glutamine metabolism on IL-1β production by LPS-activated macrophages

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In recent years the metabolism of immune cells has been extensively investigated and it has shown a tight regulation between metabolic profile and cell phenotype and function. Although it is well known that microenvironment are very important for regulation of transcriptional and metabolic level, the role of glutaminolytic pathway in macrophage polarization still not clear. Here, we investigated the involvement of glutamine metabolism in the LPS-activated macrophages. Data mining of GEO datasets revealed LPS down-regulates gene expression of glutamine metabolism enzymes as Got1 and Glud1 in human M1 macrophages, as well as, Gls1, Got, Glud1 and c-Myc in murine macrophages. We show that pharmacological inhibition of glutaminase (GLS) or glutamine transporter (ASCT2) increases IL-1β production in LPS-activated macrophages, but doesn't alter TNF and IL-6 levels in BMDMs. In contrast, the inhibition of transaminase (GOT and GPT), glutamate dehydrogenase (GLUD) and GFAT wasn't able to increase IL-1β production, suggesting a mechanism in an independent manner of TCA cycle and hexosamine biosynthesis pathway. We observed that the inhibition of GLS increase IL-1β secretion, but not Il1beta mRNA levels neither NF-κB activation. The secretion of IL-1β was NLRP3, ASC and caspase-1-dependent, indicating the IL-1β secretion promoted by GLS inhibition was due to inflammasome activation. Finally, treatment of GLS-inhibited BMDMs with glycolysis inhibitor 2-DG was able to block IL-1β secretion. In conclusion, our study reveals a central role of glutamine metabolism pathway as negative control for IL-1β production in activated macrophage and suggests a potential therapeutic target for treatment of inflammatory diseases. **Financial Support:** FAPESP; CAPES; CNPq.

Infection triggering inflammation

Chronic alcohol consumption compromises neutrophil chemotaxis in mice during pulmonary aspergillosis.

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Our objective was to elucidate the effects of chronic alcohol abuse in the susceptibility of fungal pulmonary infection, using a model of murine aspergillosis. Five weeks old C57/BL6 mice, were treated with ethanol (20% v/v) in drinking water for 12 weeks (ethanol group - EG). Control group (CG) received water during these 12 weeks. After ethanol treatment, animals were intranasally infected with $3x10^8$ conidia of A. fumigatus. Mice were euthanized 24/48 hours after infection and bronchoalveolar lavage (BAL), lungs and serum were collected. Results show that EG had higher mortality rates associated with more severe pathological score and a higher fungal load in lungs in EG compared to mice from CG after A. fumigatus infection. Moreover, neutrophils recruitment to the airways was also reduced in EG, despite the normal bone marrow function. This phenotype was associated with lower levels of rolling and adhesion of leukocytes in microvasculature. Higher levels of serum CXCL1 in EG, with induced internalization of CXCR2 receptors in circulating neutrophils could be responsible for the defective chemotaxis in the alveoli of EG animals. Besides this, we also investigated the function of neutrophils in terms of phagocytosis and ROS production. Our results demonstrate that ethanol treated neutrophils have lower capacity fungal phagocytosis and a defective production of ROS. Taken together, our results demonstrate that chronic alcohol consumption alters immune and inflammatory response in aspergillosis and this involves chemotaxis and killing function of neutrophils, which will be responsible for mice susceptibility to A. fumigatus infection. **Financial support:** CAPES, FAPEMIG e CNPq

Annexin A1 controls bacteria proliferation and inflammation during pneumococcal pneumonia

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Objective: Evaluate the effect of the glicocorticoid-regulated protein annexin A1 (AnxA1) in a pneumococcal pneumonia model. **Material and methods:** Balb/C wild type (WT), AnxA1 Knockout (KO), C57BL/6 wild type and FPR2 (AnxA1 receptor) Knock-Out mice were infected with $5x10^4$ CFU of Streptococcus pneumoniae intranasally and euthanized in different time points post-infection (p.i.). Bronchoalveolar lavage fluid (BALF) and lungs were harvest to access inflammatory parameters. Lung function was also evaluated. In addition Balb/C WT mice were infected with 10^5 CFU of S. pneumoniae intranasally, treated with Ac2-26

12 hours p.i. and euthanized 24 hours p.i. for inflammation and bacterial assessment. In parallel, vehicle and Ac2-26-treated mice were accompanied for lethality rates. **Results:** AnxA1 KO mice exhibited more marked inflammatory response and more bacterial counts in the BALF than Balb/C WT mice. Also, AnxA1 KO mice presented bacteremia in earlier time points and worsening of lung function. Similarly FPR2 KO mice presented more inflammation than C57BL/6 WT mice. Additionally, treatment with Ac2-26 reduced bacterial counts and inflammation, leading to a partially protection from lethality. **Discussion:** Altogether, our data corroborates with the literature by showing that the absence of AnxA1 or its receptor contributes to the exacerbated inflammation, in this case induced by S. pneumoniae in the lungs. In this regard, AnxA1 active peptide plays an important role controlling inflammation and dissemination of bacteria. **Conclusion:** Therefore, modulation of the inflammatory response can be beneficial during severe pneumococcal pneumonia and treatment with AnxA1 peptidomimetics can be an interesting strategy to control overwhelming inflammation. **Financial support:** FAPEMIG, CNPq and CAPES.

Study of inflammatory response in a murine model of pneumococcal pneumonia

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Objectives: Evaluate the effect of Sivelestat, a neutrophil elastase inhibitor that has protective properties in AnnexinA1 cleavage, in inflammation caused by pneumococcal pneumonia. **Materials and methods:** Balb/C wild type (WT) and AnxA1 Knock-Out (AnxA1 KO) mice were infected with 10^5 CFU of S.pneumoniae intranasally, treated 12h post-infection (p.i) and euthanized 24 pi.. Bronchoalveolar lavage fluid (BALF) was collected, and leukocytes were counted. The bacteria in BALF were also analyzed. The influx of neutrophils was evaluated by myeloperoxidase assay (MPO) in the lung tissue. **Results:** AnxA1KO mice treated with Sivelestat showed lower levels of leukocytes on BALF than vehicle-treated AnxA1KO mice. On the other hand, WT mice treated presented only a tendency to reduce leukocyte infiltrate. In addition, there wasn't difference between infected groups on neutrophil recruitment to the lungs. Also, the number of bacteria on BALF remained the same in all infected groups. **Discussion:** Reduction of neutrophils in BALF of AnxA1KO mice suggests a role for Sivelestat on decreasing recruitment and elimination of neutrophils which's independent of AnxA1. Although only a tendency was observed in WT mice, the experiment needs to be repeated with a larger number of mice to get more consistent data. **Conclusion:** Neutrophil elastase contributes to tissue damage and protein cleavage and it's also related to leukocyte recruitment. By the data gathering so far a direct correlation couldn't be established between elastase and leukocyte recruitment. However, the inhibition caused by Sivelestat proved to be beneficial. The treatment modulated the inflammatory response but didn't change the capacity to eliminate the bacteria.

Treatment with ethanolic extract of Trichoderma stromaticum leads to protection against experimental cerebral malaria by modulation of brain expression of IFN-γ and ICAM-1

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Objectives: Evaluate the antimalarial properties of crude ethanolic extract of the fungus Trichoderma stromaticum (Ext-Ts) in a mouse model of experimental cerebral malaria (ECM). **Material and methods:** Clinical, parasitological, histological and immunological features of experimental cerebral malaria were assessed in C57BL/6 mice infected with Plasmodium berghei ANKA and treated or not with Ext-Ts. **Results:** Treatment with Ext-Ts was able to significantly increase survival, prevent neurological signs and decrease parasitemia levels on treated mice when compared with control mice. In addition, blood-brain barrier (BBB) breakdown, cytoadherence and mRNA expression of IFN-γ and ICAM-1 in the brain were attenuated in Ext-Ts-treated in relation to untreated mice. **Discussion:** Upregulation of IFN-γ in cerebral tissue is a common hallmark during ECM. IFN-γ signaling leads to increase in ICAM-1 expression, an adhesion molecule that allows cytoadherence of infected red blood cells and leukocytes to blood vessel endothelium causing obstruction and disruption of BBB with consequent extravasation of toxic substances towards the neuronal tissue that leads to neurological alterations, coma and death. T. stromaticum is a fungus producer of antimicrobial molecules. In this study, administration of Ext-Ts conferred significant protection against ECM through reduction of IFN-γ and ICAM-1 expression in the brain, leading to increase of survival and attenuation of pathological features of the disease. **Conclusions:** These results suggest that Ext-Ts is a source of antimalarial and immunomodulatory compounds that could improve the current treatment of cerebral malaria.

A model of Chikungunya infection

that emulate most of clinical and inflammatory manifestations of human disease

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The aim of this work was to implement an experimental model of Chikungunya virus (CHIKV) infection in C57/BL6 mice. Four-week-old animals were infected with $1x10^6$ PFU of La Reunion CHIKV through intraplantar route. Clinical and inflammatory parameters such as paw edema, body weight, inflammatory articular hypernociception, leukocyte recruitment (mieloperoxydase activity - MPO) and production of inflammatory mediators by ELISA were analyzed at different time points (1, 3 and 7 days for all parameters, except for edema and hypernociception that were analyzed until day 28th). Viral loads were retrieved from different organs by plaque assay. Our results revealed that intraplantar inoculation of CHIKV induced inflammatory hypernociception from days 1 to 21 of infection, returning to baseline levels at day 28th. Moreover, infection was not associated with any change in body weight and paw volume. Virus was recovered from popliteal lymphonode (LNP) at day 1 of infection, but not from spleen and serum. Corroborating those results, increased levels of the chemokines CCL5 and MCP-1 and the cytokine IL-6 were also found in paw tissue at day 1 of infection when compared to control mice. Finally, MPO activity in paw tissue was increased from day 1 peaking at day 7 after CHIKV inoculation. Thus, we describe a model of CHIKV infection in immunocompetent mice that emulate most of the clinical and inflammatory parameters found in human infection. This model will be useful for further studies investigating CHIKV pathogenesis and therapeutic testing. **Financial support:** INCT Dengue, CAPES, CNPq, FAPEMIG.

Protective role of CXCR1/2 antagonism during influenza and post-influenza pneumococcal murine infections

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Introduction: Influenza A infections are a leading cause of morbidity and mortality worldwide frequently associated with secondary pneumococcal infections. Inflammation is important to control pathogen proliferation but may also cause tissue injury and death. **Objectives:** Investigating the role of CXCR1/2, receptors relevant for neutrophil recruitment, during influenza, pneumococcal and post-influenza pneumococcal infections. **Material and methods:** Mice were infected with influenza A virus (IAV) or S. pneumoniae and then treated with the CXCR1/2 antagonist (DF2162) daily. To study secondary pneumococcal infection, mice were infected with a sublethal inoculum of IAV, treated therapeutically (three days after IAV infection) with DF2162, and infected with S. pneumoniae, 14 days after IAV infection. Lethality and weight loss, inflammation, virus/bacteria counts and lung injury were assessed. **Results:** High levels of CXCL1 and CXCL2 were detected during IAV infection. DF2162 treatment decreased morbidity associated with decreased neutrophils infiltration in the lungs, lung damage, and viral titers. During S. pneumoniae infection, DF2162 treatment decreased neutrophil recruitment, lung damage and lethality rates, without affecting bacteria burden. Therapeutic treatment with DF2162 during a sublethal IAV infection reduced the morbidity associated with virus infection and also decreased inflammatory magnitude, lung damage and bacteria numbers in the blood of infected mice after secondary pneumococcal infection. **Conclusions:** Modulation of the inflammatory response by blocking CXCR1/2 improves disease outcome, without compromising immune response during respiratory influenza and pneumococcal infections. Inhibition of CXCR1/2 may be a valid therapeutic strategy for treating lung infections caused by these pathogens. **Financial support:** CNPq, CAPES, Fondation Mérieux.

Bradykinin B_2 receptor activation induces severe dengue disease via enhancement of viral replication

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OBJECTIVES: The aim of the study was to evaluate the

role of Bradykinin (BK) receptors (B_1R and B_2R) in the development of severe dengue. **METHODS/RESULTS:** BK receptors (B_1R and B_2R) expression was upregulated in liver and spleen of DENV-3-infected mice. DENV-3 infection of WT mice induced increased viremia, hepatic damage, hemoconcentration, thrombocytopenia and augmented vascular permeability. Moreover, infection resulted in a massive release of pro-inflammatory cytokines such as TNF-α, IFN-γ and CXCL-1. $B2R^{-/-}$ mice exhibited increased survival rates when compared to WT and $B_1R^{-/-}$ mice. In accordance, the lack of B_2R resulted in increased platelets level, prevented the increase of vascular permeability and the decrease of those pro-inflammatory cytokines. In addition the $B_2R^{-/-}$ mice presented lower viral loads in liver and blood when compared to WT infected mice. **DISCUSSION:** B_2R activation is associated with increased dengue pathogenesis. Our data indicates that B_2R activation contributes to increased inflammatory response and excessive viral replication. **CONCLUSION:** These results show that B_2R activation contributes to dengue pathogenesis, given the induction of pro-inflammatory cytokines an exacerbating viral replication. **FINANCIAL SUPPORT:** INCT-Dengue, CNPq, CAPES, and FAPEMIG.

Blockade of the Fosfatidilinositol 3-quinase-γ pathway prevent major manifestations of Dengue virus infection

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The aim of this study was to evaluate the role of PI3Kγ pathway activation in the pathogenesis of DENV-3 infection and to verify the therapeutic potential of the specific inhibitor of PI3Kγ pathway, AS605240, against infection. Two different mouse models of DENV-3 infection were used. A lethal model induced by an adapted strain of DENV-3 and a "dengue fever" like model induced by a clinical isolate, in the presence or absence of pan-flavivirus antibody (4G2), which mimics secondary infection. Infection of wild type (WT) mice with the adapted strain resulted in PI3Kγ pathway activation, through AKT phosphorylation. Severe disease manifestation, as represented by massive thrombocytopenia, hemoconcentration, leucocyte recruitment and elevated viral loads to target organs, followed by lethality were observed after virus inoculation. $PI3K\gamma^{-/-}$ mice were massively protected from all DENV-3-induced parameters mentioned. In a model of mild disease using the clinical isolate, DENV-3 infected WT mice showed transient thrombocytopenia, hemoconcentration and increased vascular permeability at 24 and 48hs after virus inoculation. Previous treatment of WT mice with 4G2 antibody exacerbated those manifestations. In this model, $PI3K\gamma^{-/-}$ and AS60524-treated mice were protected from primary and secondary disease manifestations induced by DENV-3. Indeed, viral loads recovered from supernatant of primary culture of peritoneal macrophages of $PI3K\gamma^{-/-}$ cells were reduced in comparison to WT littermates. Therefore, targeting PI3Kγ pathway seems to be beneficial to prevent the major outcomes of DENV infection. **Financial support:** CNPq, FAPEMIG , CAPES, INCT em dengue and PRONEX.

Lipoxin A4 impairs the clearance of Staphylococcus aureus in joint that worsens tissue damage during septic arthritis

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Objectives: The aim of this study was to investigate the role of 5-lipoxygenase (5-LO) in joint inflammation following S. aureus infection. **Methods:** Experimental septic arthritis was induced by intra-articular injection of S. aureus (10^7 CFU/mL) in WT (SV129) and $5\text{-}LO^{-/-}$. The inflammatory parameters were analyzed at 4 or 7 days post infection (dpi). After euthanasia, the articular lavage was performed for cellular counting and the articular tissue was collected to recovery bacteria, measurement of cytokines, for histopatological analysis. Analyzes of the cellular content in lymph node was performed by flow cytometry. **Results:** $5\text{-}LO^{-/-}$ mice have decreased neutrophil accumulation, cytokine production, hypernociception and bacterial load in joint and reduced articular damage at 7dpi compared to WT. At 4 dpi, $5\text{-}LO^{-/-}$ presented increase of activated $CD11c^+$ cells and T lymphocytes in the joint and lymph node, not observed in WT mice. From the 4 dpi, there is an increase of lipoxin A_4/leukotriene B_4 ratio in the joint, byproducts from 5-LO metabolism. The blockade of the lipoxin A_4 receptor ALX/FPR2 (BOC2 – 10ug/Kg i.p.) in WT mice decreased the bacteria load in joint. Corroborating, the injection of lipoxina A_4 in $5\text{-}LO^{-/-}$ increased bacterial load. Mechanistically, lipoxina A_4-treated human dendritic cells have reduced chemotaxis under CCL21 stimulation. **Discussion:** Lipoxin A_4, a pro-resolutive molecule, could negatively regulate the antigen presentation or cell migration, decreasing the ability of bacterial control. Thus, the inhibition of 5-lipoxygenase could be used as a complementary therapeutic option in articular inflammation caused by S. aureus infection. **Financial support:** CAPES, CNPq and FAPEMIG.

Different KPC-2-producing Klebsiella pneumoniae strains induce distincts

types of cell death in macrophages

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OBJECTIVE: We evaluated host defense strategy against different strains of Klebsiella pneumoniae (Kp), in vitro and in vivo. **MATERIAL AND METHODS:** BMDM from C57BL/6 (WT) or Casp1$^{-/-}$/Casp11$^{-/-}$ mice were incubated with A06 and A70 strains of Kp (MOI 1:5) during 90 min for internalization, and after 4h CFU recovery and H_2O_2 susceptibility assay were evaluated. For efferocytosis assay, A06 and A70-infected cells were co-incubated with BMDM (1:3) for 2h, and then CFU were evaluated. For in vivo, WT mice were infected intranasally (10^7 Kp), and after 48h, CFU and cytokines were evaluated in lung and differential cell counts from BALF. **RESULTS:** A70 strain showed more susceptibility to killing by BMDM than A06 strain. Unexpectedly, A70-infected mice showed higher CFU from lung, followed by less neutrophil recruitment and low levels of KC, TNF-α and IL-1β, when compared with A06-infected mice. Moreover, A06-infected macrophages released higher amount of LDH and IL-1β than A70 strain, suggesting that A06 strain induce cell death by pyroptosis. This cell death induced by A06 strain on macrophages increases H_2O_2 sensitivity compare to A70 strain. Furthermore, intracellular A06 strain from Casp1$^{-/-}$/Casp11$^{-/-}$ macrophages became more resistant to H2O2 activity. Similarly, the efferocytosis of A06-infected macrophages leads higher bacterial clearance than A70 strain, and absence of Casp1$^{-/-}$/Casp11$^{-/-}$ impair the microbicidal activity against A06. **DISCUSSION:** Taken together, A06 strain induces pyroptosis, high levels of IL-1β, and efferocytosis of A06-infected macrophages increases bacterial clearance compared to A70 strain. **CONCLUSION:** Cell death by pyroptosis can improve host defense against Klebsiella pneumoniae.

Defective inflammatory response of neonatal monocytes to Zika virus infection

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Objectives: Investigate the differences between the response of adult and neonatal monocytes to Zika virus (ZIKV) infection. **Material and Methods:** Mononuclear cells were isolated from freshly collected peripheral blood (adults) or cord blood (newborns) samples by Ficoll density gradient. Monocytes were then purified by negative magnetic labeling and led to rest overnight. Cells were incubated with ZIKV for 1h (1 monocyte per 100 viral particles) and then washed twice to remove free viruses. Monocytes were cultured for additional 3 and 24h. At each time point, supernatants were collected for analysis. Cytokines (IL-1β, TNF-α and IL-10) were measured by ELISA and viral loads were assessed by RT-PCR/Taqman Assay. As controls, we compared ZIKV infection to uninfected (UN) and Dengue type 1 (DENV)-infected cells. **Results:** ZIKV was able to induce a significant production of inflammatory cytokines, but not IL-10 secretion, in monocytes. This response, however, had a lower magnitude than DENV infection. Even though adult and neonatal monocytes showed comparable viral loads, the latter group was unable to produce IL-1β and TNF-α in response to the flavivirus infection. **Discussion:** ZIKV potential to elicit a robust inflammation in adult, but not neonatal, monocytes suggest that the infection may have an immune silent course in newborns. In addition to the circulatory nature of monocytes, these cells may work as efficient Trojan horses, helping the virus to reach distant anatomical sites. **Conclusions:** Monocytes are a permissive niche for ZIKV infection but neonatal cells are unable to mount a significant inflammatory response. **Funding:** FAPESP (Grant 2016/09764-1)

FcγRIIb and FcγRIII have opposite functions in controlling bacterial clearance and joint inflammation in Staphylococcus aureus-induced arthritis

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The aims of this study are to understand the role of Fcγ receptors in the control of inflammatory response and bacterial clearance in an experimental model of S. aureus-septic arthritis. To do this, wild type mice (WT) and mice deficient for FcγRIIb receptor (FcγRIIb$^{-/-}$) and FcγRIII receptor (FcγRIII$^{-/-}$) were infected (i.a.; 10^7 CFU) with S. aureus (ATCC6538). Seven days after infection (peak of inflammation in WT mice), FcγRIIb$^{-/-}$mice had an expressive reduction of cells in the synovial cavity and periarticular tissue (MPO activity assay) in relation to WT. Importantly, FcγRIIb$^{-/-}$ mice also had reduced bacterial load in the joint cavity and nociception (electronic anestesiometer) compared to WT mice. However, FcγRIIb$^{-/-}$ mice had higher amount of neutrophils at earlier time point (4 days after infection), indicating a faster local inflammation that is important for the control of joint infection compared to WT mice. On the other hand, FcγRIII$^{-/-}$ mice had comparable amount of cells in joint with WT mice 7 days after infection. However, FcγRIII$^{-/-}$ mice kept high amount of cells, especially neutrophils, 14 and 28 days of infection, indicating a prolonged joint inflammation. In conclusion, the absence of

the inhibitory FcγRIIb facilitates faster control of infection and local inflammation by S. aureus while FcγRIII seems to have essential function in controlling bacterial clearance and, consequently, joint inflammation.

Reduction of CCR5 in the heart leads the susceptibility of NFAT-1 KNOCKOUT mice to T cruzi infected-mice

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Recently, have been reported that NFAT also participate in exhaustion of T_{CD8}^{+} cells and anergy of T_{CD4}^{+} cells. As the T cruzi acute infection is a balance between the presence of pro-inflammatory cytokine and T cell exhaustion, we evaluated the role of NFAT-1 during the acute T. cruzi infection. NFAT-1 knockout mice showed more susceptible to infection to Y strain of T. cruzi with higher levels of burden parasites in the blood, heart and skeletal muscle, by qPCR. In the histology analysis they present significantly reduction of inflammatory sites in heart with high nest of amastigotes indicating a failure of immune system to fight the parasite. Analyses of macrophage derivated of bone marrow indicating that the nitric oxide production is over expressed in nocaute mice, probably in an attempt to contain the infection. The reduction of inflammation in the heart is not due to dificulties in lymphocytes proliferation however we identified a reduction of IFN-γ produced by both T_{CD4}^{+} and T_{CD8}^{+} and IL-17 produced by T_{CD8}^{+} cells in the heart of infected mice. Besides the pro-inflammatory cytokynes reduction, the decrease of inflammatory cells was intriguing. The most important chemokyne during the acute phase of Chagas disease é CCR5 and its ligant CCL5. Both were drastically reduced in the heart of T.cruzi infected NFAT-1 knockout mice. The mecanisms by which the absence of NFAT1 alters the expression of CCR5 in the heart is still unknown. However appears to be a crutial transcription factor for the resistance to T cruzi infection.
Financial support: CAPES, CRID .

Atypical Chemokine Receptor-2 (ACKR2) modulates the CCL5:CCR5 axis and the Interferon-dependent antiviral immune response in murine Influenza A virus infection

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The responses against viral infections involve several mechanisms including the inflammatory response. During influenza A virus (IAV) infection, the inflammation triggered is important to mount antiviral innate and adaptive responses leading to clearance of the virus and return to lung homeostasis. However, overproduction of chemokines and cytokines may lead to an overwhelming response and intense lung injury. Among the chemokines induced by IAV infection, CCL5 is important to recruitment and activation of lymphocytes and also to induction of IFNs, which in turns, activate the expression of interferon induced genes (ISGs) upon IAV infection, an important mechanism to control virus replication. In the present study we evaluated the role of the chemokines scavenger receptor ACKR2 during IAV infection. We showed for the first time that ACKR2 expression is induced during IAV infection in mice. Moreover, ACKR2 KO mice presented increased CCL5 levels and consequently IFNs and ISGs expression. The increased production of CCL5 in the absence of ACKR2 leaded to a higher recruitment of lymphocytes and cells expressing CCR5 into the airways. In other hand, CCR5 KO mice displayed increased susceptibility to IAV infection. All together, these events lead to an early viral clearance associated to decreased mortality of ACKR2 KO mice. Collectively, our data suggests that ACKR2 receptor may control CCL5 levels that consequently modulates the recruitment of CCR5+ leukocytes and production of IFNs leading to an anti-viral immune response. Therefore, the atypical chemokine receptor ACKR2 plays an important pathogenic role during IAV infection.

Standardization of methods for screening of antileishmanial compounds using high-throughput screening

Natalia Carvalho Pellison; Dario Simões Zamboni

Leishmaniasis is a neglected disease caused by Leishmania spp. infection. The current treatments available are expensive, toxic and show problems with increased parasite resistance. In this sense, there is a need in development of new drugs as a treatment solution. The aim of this work was to develop standard parameters to data acquisition and analysis of bone-marrow derived macrophages (BMDMs) infected with Leishmania amazonensis. To evaluate this, we used ImageXpress, a high-content screening equipment to assess the replication of RFP expressing L. amazonensis in C57BL/6 BMDMs. We initially tested three different BMDMs concentrations using four different multiplicity of infection. Different amphotericin concentration was used as a proof-of-concept to validate the screening results. Our preliminary results indicate that our conditions are appropriate to perform the drug screenings. In the next step, we will perform the screening using the automated high-throughput screening to for the discovery new drugs against L. amazonensis. **Supported by** FAPESP, CRID/FAPESP, PEW, CNPq, INCT/CNPq and CAPES.

Interleukin-17 drives pathogenesis in a Legionella longbeachae model of experimental Legionnaires' disease

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Legionella longbeachae is an environmental bacterium that can cause acute pneumonia in humans. This disease can be recapitulated after intranasal infection of C57BL/6 mice which succumb to infection. We have shown the crucial role of the bacterial Dot/Icm type IV secretion system for in vivo bacterial proliferation and host response. However, little is known about the host components that exacerbate the pathogenesis. In this context, we used infection of gene deficient mice and found that Interleukin-17 receptor knockout (Il-17ra$^{-/-}$) mice infected with L. longbeachae are more resistant to infection. Infected Il-17ra$^{-/-}$ mice presented reduced mortality and bacterial counts in the lungs and spleen in comparison to C57BL/6 mice. We observed that IL-17 production peaks after 48h and 72h of infection. Flow cytometry analyses demonstrated that TCRγδ+ T cells are the main source of IL-17 in the lungs of L. longbeachae-infected mice. To verify the role of IL-6 in host resistance and IL-17 production after infection, we infected C57BL/6 and Il6$^{-/-}$ mice and observed that the absence of IL-6 also confers increased host resistance to bacterial replication. However, IL-17 production in the bronchoalveolar fluid as well as the frequencies of IL-17+ TCRγδ+ cells in the lungs were not affected in Il6$^{-/-}$ mice after L. longbeachae infection. Interestingly, IL-6 levels were reduced in the absence of IL-17R signaling. Therefore, we concluded that IL-17 is involved in pathogenesis of L. longbeachae, possibly through induction of IL-6 during infection, a feature that increases pulmonary inflammation and culminates with death of the infected mice. **Supported by** FAPESP, CRID/FAPESP, INCTV/CNPq, PEW and CAPES.

Inflammatory response triggered by secondary pneumococcal infection post Flu: a murine model

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Introduction: Influenza A infections are a leading cause of morbidity and mortality worldwide frequently associated with secondary pneumococcal infections. Inflammation is important to control pathogen proliferation but may also cause tissue injury and death. **Methods:** Mice were infected with a sublethal inoculum of influenza A virus (IAV – 500 PFU) and after 7, 14 e 21 days of infection, mice were secondary infected with a sublethal inoculum of S. pneumoniae (serotype 3 – 10^3 CFU). Single infected animals (IAV or S. pneumoniae) were used as controls. Lethality and weight loss were accompanied during for 10 days after the pneumococcal infection. In another set of experiments, IAV infected mice were secondary infected with S. pneumoniae after 14 and 21 days of virus infection. After 2 days of the second infection, mice were euthanized and inflammation, virus/bacteria counts and lung injury were assessed. **Results:** IAV infection increases the susceptibility to pneumococcal infections up to 21 days after virus infection. After 7 days of IAV infection, the peak of loss of weight and virus in the lungs, all the animals secondary infected with S. pneumoniae succumbed to infection after 1 day of pneumococcal challenge. The increased lethality was also observed during pneumococcal infections after 14 and 21 days of IAV. Importantly, after 14 and 21 days of infection, no virus can be recovered from the lungs of infected mice. In addition, a massive influx of neutrophils into the airways and lungs of socndary infected mice was observed. This was related to an increased proliferation of bacteria in the airways of mice and dissemination to the bloodstream. All together, it led to an increased lung injury and death of those animals. Single infected mice (IAV or pneumococcus) did not presented increased number of neutrophils or dissemination of bacteria. **Conclusion:** In this study we established the kinetics of the inflammatory parameters that follows the secondary pneumococcal pneumonia following IAV infection in a murine model. We could demonstrate that inflammation was demonstrated to be an important determinant of morbidity after infection as seen by the huge neutrophil influx, protein leakage/lung injury and cytokine production in infection. In addition, uncontrolled proliferation and dissemination of bacteria contributed to the increased lethality of secondary infected mice.

Comparison of Legionella pneumophila and Legionella longbeachae infections in macrophages and the role of L. longbeachae type IV secretion system (Dot/Icm) for cytokine production

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Legionella species are Gram-negative bacteria that cause Legionnaires' disease in humans. Legionella pneumophila and Legionella longbeachae, are mainly found in environmental sources like water reservoir and soil, respectively, The contamination of humans occurs after the inhalation of aerosols containing the bacteria. After inhalation, both species can subvert the normal vesicle traffic within alveolar macrophages and form the LCV (Legionella-containing vacuoles). This process is mediated by the injection of hundreds of bacterial effectors through a type IV secretion system called Dot/Icm. In this study, we used bone marrow

derived macrophages (BMDM) to compare the infection with L. pneumophila and L. longbeachae and to study the role of the Dot/Icm secretion system from L. longbeachae in the production of cytokines, restriction of infection and pore formation. We demonstrated that the L. longbeachae mutants for the type IV secretion system produce more inflammatory cytokines than the WT bacteria. In addition, the pore formation in the macrophages infected with L. longbeachae was reduced when compared to cells infected with L. pneumophila mutants for flagellin (flaA$^-$). Consequently, macrophages infected with L. longbeachae fail to restrict intracellular replication as compared to cells infected with flaA$^-$. Collectivelly, our data suggest that L. longbeachae is more virulent than L. pneumophila and the Dot/Icm is critical for this process.

Recombinant Influenza viruses carrying murine IFNγ gene as tools to evaluate the role of that cytokine during influenza virus infection

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Objectives: It is known that cytokines, such as IFNγ play important roles during influenza virus infection, either improving or worsening its outcome. However, their role are still far from to be completely elucidated. Therefore, the aim of our study was to establish a new approach to evaluate the role of IFNγ, when locally produced in lungs, to immunopathogenesis of influenza virus infection. To this aim, we constructed recombinant influenza viruses which are able to encode the murine IFNγ sequence. **Material and methods:** Replication-defective influenza viruses encoding IFNγ sequence were generated by reverse genetics. Recovered viruses were purified twice by limit dilution on MDCK cells prior to the preparation of seed and work stocks. Further, they were characterized about their genetic stability by PCR and sequencing. Their phenotype were evaluated by lysis plaques on MDCK cells under agarose overlay and their ability to produce IFNγ in cell culture and in lungs of infected mice were assessed by ELISA. **Results:** The recombinant viruses were found genetically stable and able to produce IFNγ in cell culture as well as in lung of infected mice at different time-points after inoculation. Moreover, we were able to demonstrate the biological activity of the viral-encoded IFNγ by using bone marrow differentiated macrophages (BMDM). **Discussion & Conclusions:** Our preliminary findings strongly suggest that recombinant influenza viruses encoding cytokines would be remarkable tools to allow us better understand the role of those immunomodulatory proteins, when produced locally in lungs, to the immunopathogenesis of influenza virus infection. **Financial support:** FIOCRUZ/PDTIS-Vacinas, and National Institute for Vaccine Development and Technology (CNPq/FAPEMIG Nº 015/2008), Universal FAPEMIG and Universal. CNPq provided fellowships to AMVM.

Aggregatibacter actinomycetemcomitans mutant with leukotoxin A gene deletion as a tool to understand dysbiotic biofilm microbial interactions

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Aggregatibacter actinomycetemcomitans, the etiological agent of aggressive periodontitis, is a candidate for "keystone pathogen" – a low-abundance microbial pathogen in bucal biofilm that can orchestrate inflammatory disease by remodeling a normally microbiota in a dysbiotic biofilm are still unknown. In order to clarify these interactions, this study aimed to generate mutants by gene deletion of virulence factors involved in the pathogenesis of periodontal disease (PD). The leukotoxin A is an important virulence factor which interacts with β2 integrin, accounts for the selective killing of leukocytes. Thus, a gene deletion cassette targeting leukotoxin A gene was constructed by homologous recombination in Saccharomyces cerevisiae. The cassette will be used for Aggregatibacter actinomycetemcomitans leukotoxin A knockout strain construction. Our strategy is a conjugative system using a strain of Escherichia coli capable of mobilizing pVT1460. Selection for mutants will be done by spectinomycin resistance gene. After, we intend to use a murine model of PD using A. actinomycetemcomitans strains in order to evaluate host-pathogen interactions, emphasizing the relationship between leukotoxin A and virulence, in terms of alveolar bone loss, bacterial growth and association with early colonizers, together with in vitro experiment of oral biofilm. **Supported by:** CAPES, FAPEMIG, CNPq.

Leishmania infantum mCherry as a biological tool for the visceral leishmaniasis study in murine model

Bruna Araújo David, Cleyson Oliveira, Carla Duque Lopes, Franciele Pioto, João Santana da Silva

Objectives: Investigate the possibility of Visceral Leishmaniose's model induced by L. infantum mCherry in relation to the WT strain and evaluate the fluorescence maintenance of these parasites through the infection. **Material and Methods:** prostaglandina production by parasites was measured by PCR and stability of fluorescence

was measured in Bio Station after 12 passages in culture. The fluorescence maintenance was observed in Bio Station after transformation promastigotes forms in amastigote in macrophages derived from bone marrow in culture. The infective capacity of parasites, in vivo, was measured and presence of parasite in liver was availed by intravital microscopy. **Results:** Different clones of L. infantum mCherry shows expression of more than one isoform of prostaglandines as the WT strain. The fluorescence stability was kept in 100% of parasites. Transgenic L. infantum mCherry is able to infect macrophages in culture and keep the fluorescence after the transformation of the promastigote to amastigote form. Model of infection in vivo, is observed that virulence of fluorescent parasite is like of non modified parasites. Also, was possible to locate the leishmanias in mice's liver 6 weeks after infection by intravital microscopy. **DISCUSSION:** Besides the transformation suffered for insertion of fluorophore in its original constitution, we show by different ways that parasite don't show major difference in infectious process, being important tool for the disease monitoring once is possible to locate the parasite in any form intracellular and extracellular. **CONCLUSIONS:** The L. infantum mCherry generated in our lab is a excellent biological tool for study of visceral leishmaniases in murine model. **FINANCIAL SUPORT:** FAPESP, CRID, CPID, CNPq, CAPES.

Decreased number and low suppressor function of CD4$^+$Foxp3$^+$ cells as consequence of CCR4 deficiency increase the Th1 cell-mediated inflammation and the susceptibility to M. tuberculosis chronic infection

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The increased number of Foxp3$^+$ or CD25$^+$ regulatory T cells (Treg) and their ability to down regulate the production of IFN-γ has been associated with high pathogen load in M. tuberculosis infection. On the other hand, the magnitude of an IFN-γ mediated immune response may promote an intense lung damage that favors the dissemination of extracellular bacilli. Therefore, the balance of protective immune response and Treg cells is essential to infection control and protection against tissue damage. In attempt to investigate this question, we used CCR4 deficient mice (CCR4$^{-/-}$) because this receptor is associated with the recruitment of Treg cells to the lung. Wild type (WT) and CCR4$^{-/-}$ mice were infected with 1x10^5 bacilli by intra-tracheal route. Fifteen, thirty and seventy days post infection, Colony-Forming Unit (CFU) number in lungs and spleens, lung inflammation, populations of CD4$^+$ and cytokines were evaluated. The frequency of CD4$^+$Foxp3$^+$ cells was reduced 15, 30 and 70 days post infection in the lungs of CCR4$^{-/-}$ compared to WT mice. However, at the chronic, but not in the initial phase of the infection, CCR4$^{-/-}$ mice were susceptible and presented a higher lung inflammation. The increased susceptibility was associated with a highest frequency of IFN-γ-producing CD4$^+$ cells. The proliferation frequency of CD4$^+$CD25$^-$ effector cells was higher when these cells were co-cultured with CD4$^+$CD25$^+$CCR4$^-$ cells compared with CD4$^+$CD25$^+$CCR4$^+$ cells. To ascertain the role of CCR4 in the suppressor function, CFU number and IFN-γ were evaluated in the lungs of infected CCR4$^{-/-}$ mice that did undergo Foxp3-GFP$^+$ cell transfer. These mice showed a decreased bacterial load and reduced IFN-γ levels compared with CCR4$^{-/-}$ mice that did not undergo Treg cell transfer. In conclusion, the regulation of an excessive IFN-γ-mediated immune response by Foxp3$^+$ cells at the chronic phase of tuberculosis is crucial to balance the infection control and lung inflammation. **Financial Support:** FAPESP, CAPES, CNPq.

Dynamics of phagocyte migration to the liver after infection by Leishmania infatum

Cleyson Oliveira, Bruna Araújo David, Carla Duque, Franciele Pioto, João Santana da Silva

Objective: Evaluation of migration dynamics and establishment of non-liver phagocytes in murine model of visceral leishmaniasis induced by Leishmania infatum. **Materials and Methods:** C57Bl/6 WT mice were infected with Leishmania infantum mCherry and CX3CR1$^{wt/gfp}$CCR2$^{wt/rfp}$ reporter mice were infected with Leishmania infantum WT. Images of the mice's livers were obtained by intravital microscopy six weeks post infection. In addition, liver function test was performed to measure the functional impairment of the organ caused by the infection. **Preliminary results:** The livers of infected animals had regions with absence of hepatic parenchyma coinciding with regions of parasite accumulation. These regions are absent of Kupffer cells (F4/80$^+$) that present accumulation of CX3CR1$^+$ and CCR2$^+$ cells, indicating a possible role of cells in the containment and fight against the parasite. Most of the parasites seen in the liver are found in these spaces and in small numbers within liver macrophages. In addition, mice infected by Leishmania infantum exhibit a small impairment of liver function six weeks after infection when compared to uninfected animals. **Discussion:** In the visceral leishmaniasis CCR2 and CX3CR1 phagocytes are important, but it is still unclear how such cells participate in visceral leishmaniasis in the liver. Our data show the indicative of how this dynamics occurs for the formation of the immune response, but more study is still needed to clarify the role of these cells. **Conclusion:** Our data show evidence the

participation of CCR2 and CX3CR1 phagocytes in visceral leishmaniasis, demonstrating the importance of these cells in the infection. **Financial suport:** FAPESP, CRID, CPID, CNPq, CAPES.

Acute-phase proteins during inflammatory reaction by bacterial infection: Fish-model

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Objective: Based on the importance of establishing new experimental models and the advantages of using teleosteal fish to study the pathophysiology of the inflammatory reaction, considering that the main endogenous glucocorticoid in fish is the cortisol, similarly to humans, the present investigation evaluated the modulation of serum levels of acute phase proteins (APPs) after inoculation of Aeromonas hyrophila into the swim bladder. **Material and Methods:** 40 tilapias, Oreochromis niloticus (240.0 ± 10.2 g) were randomly divided into four aquariums with 250 L of water (n = 10), to establish two treatments: inoculated with A. hydrophila and inoculated with saline, to be sampled 6 and 24 hours post-inoculation (HPI) for blood collection and determination of the electrophoretic fractionation of the APPs (SDS-PAGE), in-gel protein digestion and mass spectrometric identification. **Results:** Electrophoretic traces of 30 protein fractions were found by computerized densitometry whose molecular weights ranged from 22 to 200 kDa. By LC-MS/MS analysis, proteins including ceruloplasmin, complement C3, α2 macroglobulin, albumin, transferrin, haptoglobin, apolipoprotein A1, complement C3 isoform X1, complement factor 3 and apolipotrotein Eb were identified. **Discussion:** Similarly to mammals, tilapia inoculated with A. hydrophila showed decrease ($P < 0.05$) in the amount of albumin and transferrin when compared to control animals 6 and 24 HPI, as well as, presented increase ($P < 0.05$) of ceruloplasmin, Alpha2 macroglobulin and C3 complement. **Conclusion:** The response profile of APPs in tilapia during the infectious disease resembles that observed in mammals, demonstrating the potential of this experimental model.

Control of ZIKV virus infection is associated with monocytes inflammatory recruitment to CNS in the immunocompetent C57BL/6 mice

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Zika virus (ZIKV) infection is associated with increased rates of neurological disorders, as well as congenital disorders, including microcephaly and Guillain-Barre syndrome. There is a need to develop an animal model to study the pathogenesis of the disease. In this study, we have established a model of infection ZIKV in immunocompetent C57BL/6 mice and investigated the migration of leukocytes to the central nervous system (CNS). We showed that the viral peak in the CNS occurred at 24 hours and decreased. The reduction of viral load was associated with increased IFN-α and IFN-β expression. The IFN type I receptor deficient mice ($IFNAR^{-/-}$) showed a high viral load at 72 hours p.i. In addition, we isolated cells from CNS of infected C57BL/6 mice and phenotyped the leukocytes by FACS. At 48 hours p.i, we identify two distinct subpopulations of inflammatory monocytes, $CD11b^{+}Ly6C^{hi}$ and $CD11b^{+}Ly6C^{lo}$, know to be responsible for production of pro-inflammatory cytokines and interaction with T lymphocytes, respectively. In the $IFNAR^{-/-}$ mice the increase of both monocytes populations occurred only at 72 hours p.i. Our data suggest that C57BL/6 mice are resistant to ZIKV infection due to the capacity to recruit inflammatory monocytes soon after infection, contributing to control of viral replication in a type I IFN dependent manner. The development of a model of ZIKV infection will provide insight the immunopathology of the virus and possible mechanisms of control. **Financial Support:** FAPESP; CAPES; CNPq.

The impact of obesity on the immune response to Mycobacterium tuberculosis infection

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB) that affects 10.4 million people worldwide. Epidemiological studies show that people who are overweight or obese are associated with a lower risk of active pulmonary tuberculosis. Furthermore, obesity

significantly increases the risk factor for pulmonary diseases such as asthma and chronic obstructive pulmonary disease, which are associated with high levels of inflammation and lung injury. Therefore, our aim was to evaluate the impact the obesity in the Mtb infection. Female C57BL/6 mice were fed either a low-fat diet (LFD) or a high-fat diet (HFD) for period of 90 days. At 60 days of feeding, mice were infected with 1 x 10^5 bacilli of Mtb by intra-tracheal route. At 30 days post infection, the glucose tolerance test (GTT), the body weight and adipose tissue accumulation were determined. The lungs were collected to assay colony forming unit (CFU), flow cytometry assay and PCR array analysis. HFD infected mice showed higher body weight and adipose tissue, glucose intolerance and lung CFU number compared with LFD infected. Also, there was a significant increased in IFNγ- and IL-17-producing $CD4^+$ cells as well as in the pro-inflammatory genes such as IL-17, IFN-α, IL-10, which were up regulated in lung of HFD infected mice compare to LFD infected mice. In conclusion, the up-regulation of pro-inflammatory genes and the increase of IFNγ- and IL-17 producing $CD4^+$ cells in the HFD infected mice, was impaired and triggered the increase of susceptibility to Mtb infection.

N-acetylcysteine prevents the effects mediate by PKC delta on platelets of rats injected with lipopolysaccharide

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Objective: PKC delta isoform activity is elevated in stress oxidative condition. The aim of this study was evaluate the effect of the antioxidant N-acetylcysteine (NAC) and the role of PKC delta on the decreased aggregation and increased AKT and VASP phosphorylation in platelets of lipopolysaccharide (LPS)-injected rats. **Methods:** N-acetycysteine (NAC) (150mg/kg, i.p.) was injected into the rats 30 min before saline or LPS (from E. coli, 1 mg/kg, i.p.) treatment. After 6h, arterial blood was collected. Washed platelets were obtained by differential centrifugation and using citrated buffer (pH 6.0). Aggregation was carried out in absence or presence of PKC delta inhibitor rottlerin (5μM). Phosphorylation of AKT and VASP were determinate by Immunoblotting assay. **Results:** Injection of rats with LPS reduced the aggregation and increased Thr308-AKT and Ser239-VASP phosphorylation. ADP-induced aggregation and AKT and VASP phosphorylation was similar to the saline-injected rats when platelets were incubated with rottlerin. However, the effects of rottlerin on platelet aggregation and AKT and VASP phosphorylation were prevented with the pre-treatment of LPS-injected rats with NAC. **Conclusion:** PKC delta mediates the decreased aggregation and the increased of AKT and VASP phosphorylation in platelets of LPS-injected rats, which is dependent of stress oxidative condition. **Supported by:** CAPES

Evaluation of IL-1/IL-1 receptor axis during obesity induced and its involvement in the inflammation and susceptibility by Mycobacterium tuberculosis infection.

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Tuberculosis (TB) is an airborne infectious disease caused by Mycobacterium tuberculosis. According to World Health Organization (WHO), in 2015, it was estimated 10.4 million new (incident) TB cases worldwide. As TB, obesity is another global health problem in the world and the WHO experts have estimated that obesity affects 500 million people worldwide and it is predicted to increase to one billion people by 2030. The adipose tissue of obese individuals is characterized to present a condition called metainflammation or inflammation of lower magnitude which is source of IL-1β, IL-6 and TNFα. The metainflammation induced by adipose tissue affects the immune response increasing the risk of obese individuals to infection diseases. However, the influence of obesity and their inflammatory mediators on the progression of TB remains unknown. To evaluate IL-1/IL-1 receptor axis involvement during obesity induced and its involvement in the inflammation and susceptibility by Mycobacterium tuberculosis infection, Females C57BL/6 wild type (wt) and $IL\text{-}1R^{-/-}$ knockout (ko) mice were induced to obesity with high-fat diet or low-fat diet for 81 days. At 60 days of feeding, they were infected with M. tuberculosis (1 x 10^5 bacilli) by intratracheal route. Twenty-one days post infection, the glucose tolerance test (GTT), the body weight and adipose tissue accumulation were determined. The lungs were collected to quantify colony forming unit (CFU) and IL-1β production. HFD mice showed higher body weight, adipose tissue and IL-1β production compared with LFD. GTT results showed that LFD (ko) and HFD (ko) were more intolerant than LFD (wt) and HFD (wt) respectively. Moreover, there was an increase of bacterial burden of HFD (wt) and knockout mice compared with LFD (wt). These results suggest that mouse model of diet-induced obesity impairs the pulmonary immune response under the involvement of IL-1 and the absence of IL-1R maybe affect the glucose metabolism which could be involved with susceptibility to Tuberculosis.

Leishmania (L.) amazonensis promastigotes and amastigotes induce different IL-1β production profiles upon infection of murine macrophages

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Introduction: Leishmaniasis is an infectious disease that affects people worldwide under cutaneous, mucocutaneous and visceral forms. Upon infection of host macrophages, promastigotes rapidly transform into amastigotes and live inside a unique parasitophorous vacuole. Among the mechanisms used by the host cell to help control parasite growth is the activation of inflammasomes, such as NLRP3 that, with caspase-1 activation, leads to IL-1b and nitric oxide (NO) production, restricting the replication of the parasite inside macrophages. **Objective:** Our main goal was to determine if different forms of Leishmania (L.) amazonensis were able to activate inflammasomes. **Methods:** Bone marrow-derived macrophages (BMMs) from C57BL/6 mice were primed with LPS (25ng/mL) and infected with L. (L.) amazonensis stationary phase promastigotes or amastigotes. IL-1β production was detected at 6h after infection, and caspase-1 inhibitor YVAD was used at 96h-infection to determine susceptibility to infection. **Results:** We could see that L. (L.) amazonensis promastigotes can activate inflammasomes inducing IL-1β production in LPS-primed BMMs in a MOI that ranges from 0.5 to 5. On the other hand, when we used both axenic and lesion-derived amastigotes, we saw no production of IL-1β in MOIs from 0.5 to 2. Using an MOI of 5, IL-1β production was restored. The importance in caspase-1 activation in the first hour after infection is shown in the 96h time point, when we difference in caspase-1 inhibition just for the infection with stationary phase promastigotes. **Conclusion:** Our data suggest that low MOIs of L. (L.) amazonensis amastigotes are not able to activate inflammasomes, suggesting that there could be differences in the behavior of either form during infection of macrophages.

Blimp-1 controls hepatic-inflammation induced by T. cruzi infection

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Chagas disease is caused by infection with the intracellular protozoan Trypanossoma cruzi. IFN-γ is critical for controlling many intracellular infections, but also contributes to tissue lesions. The molecular mechanism underlying regulation of the IL-12-IFN-γ axis during infection is poorly understood. The transcriptional factor Blimp-1 has emerged as a multifunctional regulator of T lymphocyte differentiation. Here, we investigated the role of the Blimp-1 in the Th1 response regulation induced during T. cruzi infection. We demonstrated that T. cruzi infection induces higher Blimp-1 expression in spleen and liver at 14 days post-infection (dpi). We found that 100% of the T cell conditional Blimp-1-deficient mice (CKO) were succumbed by infection within 15 dpi, whereas WT mice survived more than 50 dpi. The Blimp-1 deletion resulted an excessive hepatic inflammation with high levels of IFN-γ, CXCL10 and TNF compared to WT mice. The IFN-γ was highly produced by $CD4^+$ T cells from spleen and liver than by those of WT mice. However, the Foxp3 expression was reduced in the CKO mice compared to WT mice. In this context, parasite burden was similar between both groups. Furthermore, the CKO mice showed an increase in myeloid cells in the liver accompanied with a high IL-12 and low IL-10 expression compared to WT mice. In contrast, in vitro, T. cruzi-infected Blimp-1 deficient BM-derived dendritic cells (BM-DCs) showed a high IL-10 production and a reduction of IL-12 compared to WT BM-DCs. Therefore, our data demonstrated that IL-12-induced Blimp-1 controls Th1-mediated hepatic inflammation during acute T. cruzi infection. **Financial Support:** FAPESP, CAPES and CNPq.

Innate immunity as a target for inflammatory diseases

In vitro anti-inflammatory activity of novel imidazole small molecules

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Objectives: Analyze the anti-inflammatory activity of eight imidazoles in non-cytotoxic concentrations by their ability to inhibit nitric oxide (NO_x), and pro-inflammatory cytokines (TNF-α and IL-6) in a J774 macrophages cell culture. **Methods:** The cytotoxic effect of the compounds was evaluated by Alamar Blue (resazurin) assay. The cytotoxic concentration (CC_{10}) was used to investigate the inhibition of the compounds on NO_x, TNF-α and IL-6 levels after cell stimulation with lipopolysaccharide (LPS). Briefly, cells were incubated for 1 h with the imidazole at CC_{10}, and next LPS (1 µg/mL) was added to the culture and incubated for 24 h. After that, cell culture supernatants were collected for further investigations. **Results:** From the eight tested small molecules, one was excluded from the anti-inflammatory analysis for its high cytotoxic effect (TR334a). Five compounds showed significant inhibitory effect on NO_x, TNF-α and IL-6 secretion ($p < 0.05$). **Discussion:** Amongst the under development anti-inflammatory small molecules, imidazoles express inhibition on several inflammatory parameters. Since its discovery in the early 1840's these compounds have been used to different

medicinal approaches like anticancer, antifungal and anti-inflammatory. Regarding anti-inflammatory activity, imidazoles have been shown inhibitory effect on different inflammatory parameters, which include cyclooxygenase 2 (COX-2), nuclear factor κ B (NF-κB) and p38 mitogen activated protein kinase (p38 MAPK), which might explain the anti-inflammatory effect described in this study. **Conclusion:** Five imidazoles tested showed an in vitro anti-inflammatory activity, and this effect was directly linked to the inhibition of nitric oxide metabolites production and pro-inflammatory cytokines secretion.

Activation of inflammasome by toxins isolated from bothrops venoms in macrophages and effect on C2C12 myotubes

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Introduction: Toxins from snake venoms develop distinct biological activities that are crucial for the pathology observed in envenomations. Jararhagin (JAR) and bothropstoxin-I (BthTX-I) isolated from B.jararaca and B.jararacussu, respectively are involved in the inflammation and tissue injury observed in the accidents by these snakes. Components from immune system are responsible for both the elimination of the toxins as well as resolution of tissue damage. The inflammatory response is initiated by the recognition of pathogen-associated molecular patterns (PAMPs) or endogenous danger signals (DAMPs) by several Pattern Recognition Receptors (PRRs), including inflammasomes, in immune cells, as the macrophages. Inflammasomes are a family of cytosolic multiprotein complexes that after sensing PAMPs or DAMPs catalyze proteolytic cleavage of pro-interleukin-1β (pro-IL-1β) resulting on IL-1β release, which can result in cell death. **Objectives:** To evaluate whether JAR or BthTX-I are able to activate inflammasome in macrophages and their action on myotubes formed from C2C12 mouse muscle cells. **Methods and Results:** We analyzed the effect of JAR and BthTX-I on bone marrow derived macrophages (BMDM) or macrophages from peritoneum of wild type (WT), Caspase 1/11$^{-/-}$, NLRP3$^{-/-}$ and ASC$^{-/-}$ mice. The ability of JAR and BthTX-I to induce myotoxicity was assessed in vitro in myotubes from skeletal muscle cell C2C12. The results showed that only BthTX-I was able to induce release of CK and ATP in cultures of myotubes from C2C12 indicating myotoxic activity. In addition, BthTX-I induced secretion of IL-6 and MCP-I, whereas JAR promoted only the MCP-1 secretion in these cell culture. In macrophages cultures, only BthTX-I induced secretion of IL-1β (WT mice) by BMDM from WT mice, after 9h of incubation, however this cytokine secretion was not detected in BMDM cultures from caspase 1/11$^{-/-}$, NLRP3$^{-/-}$ and ASC$^{-/-}$ mice. Similar results were obtained in peritoneal macrophages incubated with BthTX-I for 6 h. We also verified that the cell death induced by BthTX-I was dependent of caspase 1/11 in peritoneum macrophages cultures. Secretion of IL-6 was detected in cell culture of BMDM or peritoneum macrophages incubated with BthTX-I. This cytokine induced by JAR was not verified because it was showed that this toxin is able to degrade IL-6. **Conclusions:** Both toxins are able to activate myotubes from C2C12 inducing secretion of cytokines/chemokines, but only BthTX-I exerted myotoxic activity. IL-1β secretion induced by BthTX-I was dependent on NLRP3, ASC, Caspase, as well as the viability of these cells. **Financial support:** FAPESP (2014/09880-6) e CNPq.

Zymosan promotes IL-1β processing through NLRP3/ASC/Capsase-1 inflammasome activation

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Zymosan (ZY) is an insoluble preparation of cell wall from Saccharomycescerevisiae.Itiswidelyusedtostudytheinnate immune system and to induce inflammatory response in pre-clinical studies. However, the inflammasome components involved in the maturation of IL-1β triggered by ZY is still unclear. Here, we investigate the molecular mechanisms by which ZY promote IL-1β maturation in macrophages focusing in the role of inflammasome. **Methods.** Peritoneal naive macrophages (MΦ) were harvested from C57BL/6 wide type (WT), NLRC4$^{-/-}$, NLRP3$^{-/-}$, ASC$^{-/-}$, Caspase-1$^{-/-}$ and P2X7$^{-/-}$ mice and incubated with ZY (10-100 µg/mL) from 6 to 12 hours. MΦs from WT mice were pre-incubated with KCl, glyburide (potassium channel inhibitor), cytochalasin D (phagocytosis inhibitor), carbenoxolone (pannexin-1 inhibitor), acetylcysteine (ROS scavenger). Culture cells supernatants were used to measure the levels of IL-1β and TNF by ELISA. Mitochondrial and total ROS were also meansured. This study was approved by Local Ethical Commission in Animal Research: Protocol n° 055/2016. **Results.** ZY promotes the release of active IL-1β from WT MΦ in a concentration-dependent manner. In contrast, IL-1β release was severe impaired when MΦ obtained from NLRP3$^{-/-}$, ASC$^{-/-}$, caspase-1$^{-/-}$ and P2X7$^{-/-}$ mice. On the other hand, TNF levels released by MΦ obtained from all knockout mice and WT did not diverge. ZY-induced IL-1β release in WT MΦ, but not TNF, was inhibited by KCl, glyburide and acetylcysteine, but not by carbenoxolone or cytochalasin D. **Conclusions.** ZY induces the maturation/release of

IL-1β though a mechanism dependent of NLRP3/ASC/caspase-1 inflammasome, which seems to be independent on phagocytosis. **Supported by:** CAPES, CNPq, FAPESP.

Heatshock protein 60 induces hypertrophy and increases complement system expression in cardiomyocites

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Introduction: The immune system leads to interface between several other systems and tissues including cardiovascular system. Cardiac response may be initiated by Toll-like receptors (TLRs) [pathogen-related molecular (PAMPs) or damage-related (DAMPs)], through the complement system (SC) or by both combined responses. In the inflammatory process, C3a component of SC is released in large amounts and binds to the C3aR receptor, which we have already known that is influenced by the activation of TLRs, inducing the transcription of inflammatory factors through the translocation of nuclear transcription factor kappa B (NF-kB) to the nucleus. **Objective:** The present study aims to evaluate the participation of Heat shock protein 60, a DAMP, and its impact on SC activation through TLR4 pathway in vitro. **Methods:** we used primary culture of cardiomyocytes from. The cells were treated with the TLR4 agonists (HSP60 and LPS). Real-time PCR was used to analyze gene expression. Analysis of variance (ANOVA) followed by Tukey test for comparison of 3 or more experimental groups. The comparison between two groups was made through T-test Student. For all comparisons, $p<0.05$ was considered significant. **Results:** There were performed treatments of 1uM, 5uM and 10uM of HSP60 and of 100uM of LPS on the primary cardiomyocytes. After 24h, there was a significant increase in alpha-actin expression (cardiac hypertrophy molecular marker) in the 1uM and 10uM HSP groups, as well as in the 100uM LPS ($p <0.05$) compared to the untreated cells. HSP60 1uM and 10uM groups promoted a significant increase on factor 3 (C3) and factor B (CfB) in relation to the control cells ($p <0.05$). **Conclusion:** These data suggest that both treatments with TLR4 agonists were able to modulate cardiomyocyte tropism in vitro. In addition, data suggests that the complement system is modulates after DAMP and PAMP molecules treatment. **Financial Support:** FAPESP 2016/11685-0, 2015/19107-5.

Evaluation of miR-34c-5p role during Trypanosoma cruzi infection in murine macrophages

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The interaction between host and parasite involves several changes in the gene expression of host cells to respond to the pathogen. The pathway of interfering RNAs, mainly represented by microRNAs, is linked to post-transcriptional regulation of several genes involved in the interaction parasite-host. The role of microRNAs during T. cruzi infection in macrophages has not been evaluated. To better understand this regulation we performed RNAseq of small RNAs and messenger RNAs from murine macrophages after infection with T. cruzi (CL strain). miR-29b-3p, miR-143-3p and miR-34c-5p were differentially expressed in relation to the control and, their mRNA targets were predicted. We are evaluating the role of these miRNAs in murine macrophages during infection by T. cruzi (CL strain). We performed a gymnotic transfer of a miR-34c-5p inhibitor into BMDMs. Different concentrations of miR-34c-5p inhibitor were tested, 2mM and 48 hours of treatment were chosen. BMDMs were infected with T. cruzi with a MOI of 5. Treated cells presented higher frequency of parasite number by infected cell and higher percentage of infected cells. Cellular RNA was extracted 8 hours post-infection and gene expression evaluated by qPCR. Genes related to cell cycle and innate immune response were affected by the inhibition of miR-34c-5p. We are analyzing the differential expression of genes involved in these pathways during infection with T. cruzi (control) and during treatment with miR-34c-5p inhibitor. We anticipate that this study will help to better understand the different mechanisms that host cells use to respond to T. cruzi infection. **Supported by** FAPESP, CRID/FAPESP, INCTV/CNPq, PEW and CAPES.

IL-1α is essential to parasite control and confers resistance during Trypanosoma cruzi infection

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Trypanosoma cruzi, parasite of the Chagas disease, triggers a response IL-1R-dependent, which it is essential for resistance to this infection. Notably, both IL-1α and IL-1β signal through IL-1 receptor. In the study, we investigated the mechanisms responsible by IL-1α production and it role during experimental T. cruzi infection. Interesting, just the live T. cruzi, in the presence of IFN-γ or not, induces the production of the IL-1α in bone marrow macrophages (BMMs) at 48 hours post-infection (p.i) in a caspase-11, TLR2, IRAK4 and MyD88- dependent manner. In vivo, IL-1R$^{-/-}$ and IL-1α$^{-/-}$ mice succumbed to infection at 25 dpi with a high parasitemia compared with WT mice. At 10 dpi the IL-1R$^{-/-}$ and IL-1α$^{-/-}$ mice showed high parasitism in the

heart accompanied of lower score inflammatory, NOS2 and IFN-γ production compared with WT. Differently, at 17 dpi the IL-1R$^{-/-}$ and IL-1α$^{-/-}$ mice showed an increased heart myocarditis with higher IFN-γ and NOS2 production in the heart than WT. Interestingly, the persistence of the parasite remains throughout the period evaluated in the absence of the IL-1R signaling pathway, suggesting the importance of IL-1α to parasite control. Corroborating with these data, IL-1R$^{-/-}$, IL-1α$^{-/-}$, IL-1β$^{-/-}$ and IL-1αβ$^{-/-}$ BMMs (DKO) not controlled the parasite compared with WT BMMs. However, WT and IL-1α$^{-/-}$ BMMs IFN-γ-primed killing efficiently the trypomastigotes and produced higher amount of nitric oxide (NO), IL-1β, NOS2 and activation NF-κB than IL-1R$^{-/-}$, IL-1β$^{-/-}$ and DKO BMMs. Our data suggesting that IL-1α is essential to control the T. cruzi infection in vitro and in vivo. **Financial Support:** FAPESP.

Inflammasome activation by Loxosceles laeta venom in human keratinocytes

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Few studies have been conducted to investigate the recognition of animal venoms by the innate immune system. Some inflammatory features of the loxoscelism suggest that the recognition of this venom by the innate immune system in keratinocytes occurs by activation of inflammasomes, triggering the cascade of inflammatory events that culminates with the development of a necrotic lesion. Thus, this study aims to investigate the possible activation of inflammasomes by the venom of Loxosceles laeta and its central toxin, the sphingomyelinase D (SMase D), in human keratinocytes. Using IL-1β secretion, as a parameter of inflammasome activation, it was possible to observe that Loxosceles laeta venom, but not the SMase D toxin, was able to induce the secretion of this cytokine within 72 hours of treatment. The processing of IL-1β induced by the venom was completely dependent on caspase-1 activity and partially dependent on caspase-4 and 5 activity. The venom was able to induce an increase in concentrations of pro and mature forms of caspases-1, 4 and 5, as detected by western blot. Furthermore, the activity of caspase-1 seems to be an important factor for the loss of viability of keratinocytes due to the action of venom, since cell death, detected by MTT method, was significantly reduced in the presence of the specific caspase-1 inhibitor, but not caspase-4 and 5. These data indicate that another component of the venom, in addition to SMase D, plays an important role in the recognition of venom by the innate immune system, initiating the inflammatory process. **Support:** FAPESP

Differencial Role of the Inflammasomes in Diffuse and Localized Cutaneous Leishmaniasis

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Leishmania amazonensis infection can induce different types of disease in humans, such as the Localized Cutaneous Leishmaniasis (LCL), characterized by round papules in the skin and the Diffuse Cutaneous Leishmanisis (DCL), a more severe form of the disease, characterized by multiple nodules in the skin, which contain high numbers of parasites. We have previously reported the protective function of the NLRP3 inflammasome during L. amazonensis infection in mouse, thus we aimed to evaluate if the inflammasome is involved in the outcome of the LCL or DCL disease. To evaluate the role of the inflammasome during infection, we used with clinical isolates of L. amazonensis from LCL or DCL patients. We infected bone marrow-derived macrophages from C57BL/6 (WT) and Nlrp3$^{-/-}$ mice with parasites isolated from 5 LCL and 7 DCL patients. We observed that the isolates did not differ in the ability to trigger NLRP3-induced IL-1β production and Caspase-1 activation. Next, we performed in vivo infections using 10^6 parasites in the ear of WT and inflammasome deficient mice and found that parasites isolated from LCL patients induced higher lesions in the absence of the inflammasome in comparison to WT mice. In contrast, infections with DCL isolates resulted in similar lesion development in WT and inflammasome-deficient mice. These data suggest that the resistance to the inflammasome may underlie the development of controlled (LCL) or disseminated (DCL) Leishmaniasis. Our data is consistent with the hypothesis that parasite-intrinsic factors related to resistance to the inflammasome might be determinant for the outcome of diseases caused by L. amazonensis. **Supported by** FAPESP, CRID/FAPESP, INCTV/CNPq, PEW and CAPES.

Interferon type 1 and 2 play diferent role in a mice model of saint louis encephalitis virus infection

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OBJECTIVE: study host antiviral response against Saint Louis Encephalitis virus (SLEV) in the Central nervous system (CNS) **METHODS:** wild type mice (WT), IFN-1 receptor (ABR$^{-/-}$) and IFN-2 (IFNγ$^{-/-}$) knockout mice were intracranially infected with $2x10^2$ PFU of SLEV. Viral load accessed by plaque assay. Inflammatory parameters were evaluated by total/differential cell count, indirect colorimetric assays, ELISA, qPCR. **RESULTS:** type I and II IFNs play contrasting roles in St. Louis encephalitis pathogenesis. Deficiency in type I IFN signaling associates to early and increased mortality, uncontrolled SLEV

replication, increased levels of pro-inflammatory cytokines and impaired interferon-stimulated gene (ISG) expression, notably of the helicase RIG-I when compared to WT mice. In contrast, IFNγ$^{-/-}$ mice were moderately resistant to SLEV infection. IFNγ deficiency led to a reduction in SLEV titers in the brain, which was associated to an increased basal expression of RIG-I, when compared to WT mice. **DISCUSSION:** St. Louis encephalitis virus is the causative agent of severe neurological disease. IFNs are not always protective to the host. The identification of RIG-I as an important immune effector against SLEV agrees with the extensive literature demonstrating the protective roles of RNA helicases against flaviviral infections. Therefore, therapies promoting RIG-I activity could be beneficial in the context of St. Louis Encephalitis. **CONCLUSION:** type I IFN is essential to viral control whether type II IFN is not associated to protection in the brain. Both phenotypes might be linked to the expression of RIG-I, indicating that this innate immune effector is crucial for the antiviral response to SLEV.

Fc_γIII and Fc_γIIB receptors play opposite roles in intestinal reperfusion injury

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Objective: Our aim was to evaluate the role of FcγRIII and FcγRIIb receptors in intestinal inflammation induced by intestinal reperfusion injury. **Metodology:** Intestinal ischemia was performed for 30 minutes in C57BL/6 WT, FcγRIII$^{-/-}$ and FcγRIIb$^{-/-}$ mice, followed by three hours of reperfusion. Then, mice were euthanized and their intestines were collected for analysis. Results: Intestinal repefusion injury led to IgG deposition in intestines, suggesting that FcgRs could play important roles in this inflammatory pathology. Hence, Fc_γRIII$^{-/-}$ mice were protected from intestinal reperfusion injury, showing higher survival rates and lower tissue injury in addition to reduced influx of neutrophils and release of pro-inflammatory mediators. On the other hand, we observed that Fc_γRIIb$^{-/-}$ mice presented earlier and higher lethality rates than WT mice during intestinal reperfusion injury. This higher lethality was associated with a greater tissue injury and bacterial translocation to other organs. Increased susceptibility to reperfusion injury was associated to higher IgG deposition in tissue and alterations in the amount and repertoire of circulating IgG in Fc_γRIIb$^{-/-}$ mice, suggesting that Fc_γRIIb controls the generation of injury-promoting IgG. Hence, we observed that Fc_γRIIb$^{-/-}$ mice presented an increase in total IgG and in intestinal antigens-reactive IgG at the eighth week of age, but not at the seventh week of age. In accordance, Fc_γRIIb$^{-/-}$ mice were more susceptible to reperfusion-induced lethality at the eighth week of age, but not at the seventh week of age. **Conclusion:** Fc_γRIII and Fc_γRIIb plays a important role in intestinal reperfusion injury.

NLRP1 is a protection factor for the development of multiple sclerosis

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Objective: Experimental models showed that inflammasome plays a role in the exacerbation of the mustiple sclerosis (MS). Little is known about the involvement of inflammasome in the pathogenesis and/or predisposition of MS in humans. Distinct single nucleotide polymorphisms (SNPs) in inflammasome genes were associated with both autoimmune and neurodegenerative diseases. Aim of this project is to evaluate the contribution of inflammassome genetics in the development of MS. **Material and methods:** 204 unrelated individuals with different clincal presentation of MS were enrolled at the Ambulatory of UNFESP, 142 healthy donors (HD) were used for case/control analysis. Selected SNPs in inflammasome genes NLRP1, NLRP3, IL18 and IL1B were genotyped using allele specific assays and qPCR. Case/control and MS cohort studies were executed by multivariate analysis. **Results and discussion:** The NLRP1 gain-of-function missense variant L155H (rs12150220) resulted more frequent in controls than in MS group, suggesting a protective effect of this SNP against the development of MS. The studied SNPs apparently do not affect the severity and/or the clinical presentation of the disease, nor the response to treatment as previously related. L155H variation lead to an increased inflammasome activation, an augmented IL-1ß/IL-18 release and/or cell pyroptosis. NLRP1 is expressed not only in inflammatory cells but also in neurons. This variant was previously associated to autoimmune disease and Alzheimer disease. **Conclusion:** Our preliminary data shown that the gain-of-function polymorhism rs12150220 in NLRP1 confers protection against MS, but further investigations are needed to elucidate the effect of this variant on MS development.

Monocyte-derived macrophages from newborns and adults respond similarly to IFN-I adjuvants stimulation

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Objectives: Evaluate gene expression of cytosolic sensors, components of inflammasome and antiviral restricting factors in monocyte-derived macrophages from newborns and adults, and the immunomodulatory effect of IFN-I adjuvants in these cells. **Material and Methods:** Macrophages were differentiated from umbilical cord blood and adult peripheral blood monocytes and stimulated with TLR7/8 agonist (CL097), STING ligand (cGAMP) and TLR3 agonist (Poly-I:C). After 3h of stimulation, mRNA levels were evaluated by real-time PCR. Cytokine production were assessed by CBA after 24h of stimulation. **Results:** Our data show that newborn and adult macrophages express the same levels of cytosolic sensors, components of inflammasome and antiviral restricting factors. They also suggest that CL097, cGAMP and Poly-I:C were able to induce the expression of some cytosolic sensors and antiviral restricting factors, but only CL097 induced the expression of inflammasome proteins. We have also observed that CL097 induced the production of IL-1β, IL-6, TNF-α and IL-10 similarly in adult and newborn macrophages. However, adult cells produced greater amounts of IL-12p70 than newborn cells after stimulation with CL097. The other two adjuvants had no effect on cytokine production. **Discussion:** Increased susceptibility of newborns to infections may not be related to lower expression levels of innate immunity components. Besides, newborn macrophages can be as much responsive as adults for gene expression and cytokine production under stimulation, with the exception of IL-12p70. **Conclusion:** Newborn macrophages appear to be immunocompetent and capable of responding similarly to adult macrophages when stimulated with IFN-I adjuvants. **Financial support:** LIM-56/HC-FMUSP/FAPESP.

AIM2 inflammasome activation in intestinal macrophages contributes to TYPE 1 diabetes resistance

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Introduction: Recently, our group demonstrated that gut microbiota translocates to pancreatic lymph nodes (PLNs), activate the NOD2 receptor in myeloid cells and contributes to type 1 diabetes (T1D) onset. Aim: Based on this evidence, we aimed to evaluate if another innate immunity receptors, like AIM2 inflammasome, contributes to gut microbiota translocation to PLNs and consequently T1D development. Methods: For the induction of T1D, C57BL/6 and AIM2KO mice were treated with 5 low doses of streptozotocin (STZ) (40 mg/Kg) and had glucose monitored on days 7 and 15 after treatment with STZ. Subsequently, serum, ileum, PLNs, and pancreatic samples were used for cytometry, ELISA, PCR and histopathological analysis. Results and Discussion: Our results showed that AIM2 is expressed in intestinal macrophages in the gut mucosal. In addition, AIM2-deficient mice are more susceptible to T1D development since had elevated blood glucose levels associated with gut microbiota translocation to the PLNs. Interestingly, diabetic AIM2KO mice have increased claudin-2 expression and decreased in occludin expression in the gut mucosal, which leads to more intestinal permeability in these mice. It is known that Th17 cells are important in modulating these tigh-juction expression. In accordance, we observed that AIM2-deficient mice display impaired Th17 cell response in ileum and PLNs, during the STZ-induced T1D development. Conclusion: The activation of AIM2 inflammasome appears to control of gut microbiota translocation to PLN and consequently prevents the T1D onset.

The role of the AIM2 inflamassome in the innate immune response to L. amazonensis

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Several species of Leishmania can induce the Leishmaniasis. In Brazil, species such as L. braziliensis and L. amazonensis are prevalent to trigger cutaneous leishmaniasis. It was reported that L. amazonensis promotes the endoplasmic reticulum stress on the host cells, which damaging the host mitochondrial and releases mtDNA in the cytosol. Because mtDNA is a bona-fide AIM2 ligand, we hypothesized that L. amazonensis activate the AIM2 inflammasome. Thus, we aimed to evaluate the role of the AIM2 inflamassome on the innate immunity during the L. amazonensis infection. To address this issue we infected bone marrow derived macrophages (BMDMs) from C57BL/6, Aim2$^{-/-}$, Asc$^{-/-}$ and Nos2$^{-/-}$ mice with stationary-phase L. amazonensis (MOI 10) and evaluated caspase-1 activation, secretion of IL-1β and IL-1α, NOS2 expression and the intracellular replication of the parasites. We found that Aim2$^{-/-}$ BMDMs show lower levels of IL-1α, but not IL-1β and caspase-1 activation. Aim2$^{-/-}$ BMDMs also show a reduced expression of NOS2 and a higher frequency of infected cells, suggesting that the AIM2 is important for the in vitro control of L. amazonensis. Additionally, we observed that AIM2 deficient mice infected, on the ear, with 10^6 stationary-phase L. amazonensis, displayed higher lesion thickness than C57BL/6. These data also suggest an in vivo role for AIM2, in this model of infection, through mechanisms that will be further investigated. **Supported by** FAPESP, CRID/FAPESP, INCTV/CNPq, PEW and CAPES.

Alarmin S100A9: a key driver in the development of psoriasis

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INTRODUCTION: Psoriasis (Ps) is an immune-mediated chronic inflammatory skin disease, characterized by accentuated proliferation and abnormal differentiation of keratinocytes and infiltration of inflammatory cells. S100A9 is an alarmin that is produced by keratinocytes and myeloid cells in inflammatory conditions. However, the role of this molecule in the development and maintenance of the inflammatory response in Ps remains not well understood. Herein, we investigated the role of S100A9 in the development of psoriasis. **METHODS AND RESULTS:** Immunofluorescence staining showed that S100A9 was overexpressed in the lesional skin from Ps patients compared with paired samples of nonlesional psoriatic skin, which was positively correlated with the expression of keratin-17, a keratinocytes activation marker. To investigate the role of S100A9 in the development of Ps, psoriasis-like skin inflammation was induced by topical application of imiquimod (IMQ) on the back skin of S100A9-deficient mice ($S100A9^{-/-}$) or paquinimod (10mg/kg, v.o) pretreated mice, a S100A9 inhibitor preventing its binding to their receptors (RAGE and TLR4). IMQ exposure induced s100a9 mRNA and S100A9 protein expression in a rapid and time-dependent manner in the epidermis of mice and remained elevated until the end of the experiment (6^{th} day). Notably, inflammation assessed by epidermal thickness measurement and H&E-stained histological sections was significantly reduced in $S100A9^{-/-}$ or paquinimod treated-mice compared with wild-type (WT) control mice. Moreover, the expression of IL-23 in the skin was significantly reduced, which can explain the significantly reduction of IL-17 frequency by gamma-delta T cells in the lymph nodes in $S100A9^{-/-}$ or paquinimod-treated mice. **CONCLUSION:** We show that the alarmin S100a9 plays an important role on development of psoriasis. Thus, targeting S100A9 could be a future strategy for treatment of psoriasis and this protein can be used as a marker of disease activity.

Methyl gallate modulates macrophage activation through TLR-2 pathway

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Methyl gallate (MG) is a prevalent phenolic acid in the plant kingdom, and its presence in herbal medicines might be related to its remarkable biological effects, such as its antioxidant, antitumor and antimicrobial activities. Recently, we demonstrated that MG (0.7-70 mg/kg) inhibited zymosan-induced experimental arthritis in a dose-dependent manner. The oral administration of MG (7 mg/kg) attenuated arthritis induced by zymosan, affecting edema formation, leukocyte migration and the production of inflammatory mediators (IL-1β, IL-6, TNF-α, CXCL-1, LTB_4 and PGE_2). Zymosan is recognized through TLR-2 and dectin-1 receptor, resulting in the activation of reactive oxygen and nitrogen species, such as the nitric oxide (NO) and NF-κB thereby triggering the production of inflammatory cytokines and chemokines. Here, we investigated the MG effect in vitro to improve knowledge about its mechanism of action in macrophage stimulated with toll-like receptor 2 (TLR-2) agonists. To assess the effect of MG on macrophage activation, we evaluated the production of nitric oxide, TNF-α and nuclear factor-κB (NF-κB) translocation on RAW 264.7 cell line stimulated with zymosan and IFN-γ or specific TLR-2 agonists (Pam_3CSK_4 and Pam_2CSK_4). MG impaired zymosan-stimulated macrophages by inhibiting NO and TNF-α production and translocation of NF-κB into the nucleus. Besides, RAW 264.7 stimulated with specific TLR-2 agonists produced NO, which was inhibited by MG. Our results showed that MG has direct effect on macrophage stimulated with TLR-2 agonists, suggesting that this substance modulates TLR-2 pathway.

Participation of IL-1β, IL-18 and TNF-α in Ehrlich tumor-induced pain in mice

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This study aims to evaluate the role of inflammatory mediators in Ehrlich tumor induced pain model (CEUA #14543.2013.03). Mechanical and thermal thresholds were evaluated using a digital analgesimeter and hot plate test respectively, and the hind paw size was measured using a caliper in wild type, $IL\text{-}1r^{-/-}$, $IL\text{-}18^{-/-}$ and $Tnfr1^{-/-}$ mice. Mice were inoculated with $1x10^6$ cells/25µl of Ehrlich tumor cell suspension the hind paw. Paw growth, mechanical and thermal thresholds were evaluated prior to inoculation and every 2 days (12 days). Mice were sacrificed and paw tissue, tumor mass and spinal cord were collected and stored at -80oC to further ELISA, western blot, PCR and immunofluorescence analyses. We observed that IL-1β, IL-18 and TNF-α deficient mice were protected from mechanical but not thermal hyperalgesia caused by Ehrlich tumor. However, cytokines deficiency did not affect tumor growth. We also evaluated

Etanercept, daily treatment effect (10mg/Kg; i.p.) and our results showed that TNF-α pharmacological inhibition also reduces mechanical but not thermal hyperalgesia; and tumor growth. Additionally, we demonstrated that IL-1β and TNF-α levels are increased in paw tissue and spinal cord, 8 and 12 days after inoculation, respectively. IL-1β, IL-18 and TNF-α expression increase was also observed in the 12th post inoculation, in the spinal cord tissue. Etanercept treatment also inhibited glial cells activation in spinal cord 12 day after tumor inoculation. Thus, our study shows the participation of IL-1β, IL-18 and TNF-α cytokines in the Ehrlich tumor induced pain model providing a possible therapeutic target for cancer induced pain treatments.

Absence of Type I interferon signaling plays a protective role in intestinal ischemia and reperfusion injury

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Introduction: The ischemia of an organ may cause severe damage and cell death if prolonged. Thus, the desired course of action after ischemia is the reestablishment of blood flow, dubbed reperfusion, to the affected region. However, reperfusion may lead to inflammation enhancing damage to the affected tissue. Type I Interferons (IFN-I) play important roles in multiple infections, as well as in autoimmunity and inflammation. The role of IFN-I in diseases affecting the small intestine is not clear, therefore requiring further investigation. Our work intends to evaluate IFN-Is role in the inflammatory response resulting from ischemia and reperfusion injury (IRI) in the small intestine. **Results and Methods:** Type I Interferon receptor knockout mice ($ABR^{-/-}$) and wild type (WT) controls were submitted to severe superior mesenteric artery ischemia and subsequent reperfusion. Intestine and lungs were harvested for evaluation of inflammation. We observed that $ABR^{-/-}$ mice are protected from intestinal IRI, presenting prolonged survival when compared to WT mice. $ABR^{-/-}$ mice increased survival correlates with reduction in neutrophil recruitment to the small intestine. Expression of cytokines IL-1β, IL-10 and TNF-α, which are hallmarks of IR damage, were altered in $ABR^{-/-}$ mice when compared to WT. $ABR^{-/-}$ mice expressed higher levels of IL-1β and IL-10, but decreased levels of TNF-α. **Conclusion:** Our results indicate that the absence of IFN-I signaling is protective in small intestine IRI. Our findings are in accordance to literature, where protection is associated with reduced neutrophil recruitment and differential cytokine expression. Further studies will determine the mechanism underlying $ABR^{-/-}$ mice resistance, specifically the contribution of the evaluated cytokines to the protection phenotype. **Financial aid:** CNPq, CAPES, Fapemig.

Evaluation of the role of galectin-1 and -4 on experimental infection by L. (L.) amazonensis.

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Leishmaniasis is a neglected disease caused by protozoa of the genus Leishmania. This infectious disease is a worldly serious health problem with ineffective treatment and increasing incidence. Studies have shown that the resolution of this infection is dependent of homeostatic immune response from the host. However, there are still many gaps in understanding its pathogenesis. In the literature, the role of galectins, proteins that recognize glycans with beta-galactoside motifs, have been described in several infectious diseases. Nevertheless, there are few reports on the involvement of galectins in Leishmaniasis. Therefore, the aim of this work is to evaluate the role of galectins 1 and 4 (exogenous and endogenous) in experimental infection by Leishmania sp. We demonstrated that galectin-1 deficiency in mice with the BALB/c background ($Lgals1^{-/-}$-BALB/c), whether not in $Lgals1^{-/-}$-C57BL/6, promotes restriction of L. (L.) amazonensis infection. This outcome was related to increased IFN-γ production and decreased IL-4, IL-10, IL-12p70, and TNF-α and decreased lesion size and parasite load at the infection site. Galectin-1 did not bind to this parasite, suggesting that the susceptibility of wild type BALB/c was not due to a direct interaction between galectin-1 and Leishmania. Full length Galectin-4, and not their truncated forms, recognizes L. (L.) amazonensis in dose and carbohydrate-dependent manners and the highly complex glycoconjugate lipophosphoglycan (LPG) from these parasites is a molecular target for full length Galectin-4. Further studies on the involvement of Gal-4 in murine leishmaniasis will be developed. These results demonstrate that galectins can participate in the immune response against L. (L.) amazonensis and open new avenues for therapeutic or diagnostic strategies applicable to this neglected disease.

NLRP3/Casp1/IL-1β axis is involved in the prolongation of QTc induced by renal ischemia/reperfusion

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Patients with chronic renal ischemia have a high risk to develop cardiovascular diseases. Therefore, understanding the

pathogenic links between renal ischemia and cardiovascular disease is of utmost importance. Previous work of our group showed a systemic peak of IL-1β on the day 8 after renal ischemia-reperfusion (IR) in mice. This finding was associated with prolonged QTc on the EKG, an arrhythmogenic marker, sustained up to day 15. We have also previously demonstrated the important role played by resident heart macrophages in IL-1β release upon NLRP3 inflammasome activation and in cardiac electrical remodeling. Thus, in this work we tested whether the activation of NLRP3 Inflammasome axis and IL-1β release by macrophages is involved in cardiac electrical disturbances promoted by renal IR. Our data showed that the genetic ablation of NLRP3 (NLRP3$^{-/-}$) or caspase-1 (Casp-1$^{-/-}$) prevented either the QTc prolongation or the IR-induced systemic peak of IL-1β on day 8. In fact, renal IR failed to prolong the QTc interval in IL-1r$^{-/-}$ mice. The depletion of macrophages on day 7 post-IR was able to prevent the IR-induced prolonged QTc observed at day 15 post-IR. Finally, the daily treatment with IL-1r-antagonist from day 8 to day 15 reverted the IR-induced longer QTc. The data presented here demonstrates that NLRP3/Casp1/IL-1β axis is involved on the induction of prolonged cardiac QTc by renal ischemia/reperfusion. Additionally, the block of the IL-1r was found to be a potential therapeutic approach to revert the IR-induces cardiac changes.

Complement activation contributes to local and systemic inflammation induced by Naja annulifera venom

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Knowing that the complement activation contributes to several events from inflammatory response, the goal of this study was to evaluate if the N. annulifera's venom is able to trigger complement activation and, if so, the impact of this activation in inflammatory response in vivo. We showed that the venom has Cobra Venom Factor and proteins containing Mannose and N-Acetylglucosamine residues, being all these components able to trigger complement activation. By different assays, it was observed that the venom interferes with the alternative, lectin and classical pathways activity. These interferences were related to the activation of the system, since it was detected the presence of the anaphylatoxins C3a, C4a and C5a and soluble terminal complement complex in the normal human serum samples treated with venom. In addition, the venom contains proteases, which cleave C2, C3, C4, C5 and C6. The C3, C4 and C5 cleavage was functional since it promote the generation of anaphylatoxins. N. annulifera's venom was not able to cleave complement membrane bound regulators; however, it can promote hydrolysis of FH, a soluble C-regulator. Finally, we demonstrated that inflammatory events promoted by venom in mice are complement mediated, since the modulation of C5a-C5aR1 and C5a-C5aR2 axis decreased oedema, leukocytosis, neutrophilia and IL-6 and MCP-1 plasma levels. N. annulifera's venom promotes complement system activation, which could contribute to the pathology observed in envenomation. Complement modulation, thus, can be a therapeutic target in accidents. **Supported by:** FAPESP, CNPq and CAPES.

Infliximab/methotrexate combined therapy in rheumatoid arthritis patients: effect on peripheral blood neutrophils' and the complement system's activation status

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Objective: To analyze the peripheral blood neutrophils' and the complement system's activation status in rheumatoid arthritis patients with active and inactive disease undergoing infliximab/methotrexate combined therapy. **Material and methods:** This study enrolled 60 female rheumatoid arthritis patients who were followed at the rheumatology outpatient clinic at Ribeirão Preto Medical School Hospital of the University of São Paulo (Ribeirão Preto, SP, Brazil), and treated with infliximab (3-5 mg/kg every 90 days) associated with methotrexate (10-15 mg/week). The oxidative metabolism and FcγRIIa/CD32, FcγRIIIb/CD16, and CR1/CD35 expression were analyzed by luminol-enhanced chemiluminescence and flow cytometry, respectively. Hemolytic activity of the classical and alternative pathways of the complement system was determined by spectrophotometry. Serum levels of the complement system's activation fragments C5a and Bb were determined by ELISA. **Results and discussion:** Compared with healthy individuals, rheumatoid arthritis patients with active and inactive disease exhibited increased neutrophil oxidative metabolism activity and expression of FcγRIIa/CD32, FcγRIIIb/CD16, and CR1/CD35; slower hemolytic activity of the alternative pathway of the complement system; similar hemolytic activity of the classical pathway of the complement system and serum levels of Bb. These parameters were similar in patients with active and inactive disease. Compared with sera from patients with inactive disease, sera from patients with active disease exhibited increased levels of C-reactive protein and C5a, and stronger ability to induce neutrophil chemotaxis. **Conclusion:** The infliximab/methotrexate combined therapy modulates activation of the complement system but not the neutrophil oxidative metabolism and expression of Fcγ and

complement receptors in rheumatoid arthritis patients.

NLRP1 plays a protective role in T1D by inhibiting pathogenic TH17 cells

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Type 1 diabetes (T1D) is an autoimmune disease that leads to the specific destruction of the pancreatic β cells. Recently, polymorphisms in the innate immune receptor NLRP1 were found in patients with T1D, thus demonstrating a possible role for this receptor in the pathogenesis of the disease. However, the precise mechanisms by which NLRP1 is involved in T1D remain elusive. Thus, the aim of this study was to evaluate the contributions of NLRP1 in the pathogenesis of T1D. For this, naïve C57BL/6 wild-type (WT) or NLRP1$^{-/-}$ mice were inoculated intraperitoneally with 40mg/kg/day of streptozotocin (STZ) for 5 days to induce STZ-induced T1D diabetes. Surprisingly, we observed that NLRP1$^{-/-}$ mice were more susceptible to STZ-induced T1D than WT mice. This increased susceptibility was correlated with an increase in IL-6, but not IL-12-producing DCs in the pancreatic lymph nodes (PLNs), together with an increase in IL-17 – producing T CD4^{+} and T CD8^{+} lymphocytes in the PLNs. Of note, no differences were found in Th1 and Tc17 cells. We also observed that both naïve and STZ-injected NLRP1$^{-/-}$ mice presented a robust translocation of gut bacteria to the PLNs, thus indicating a possible role for this receptor in maintaining the gut epithelial barrier, thereby contributing to disease susceptibility. Accordingly, antibiotics treatment before the STZ injections in NLRP1$^{-/-}$ mice was able to fully protect these mice from STZ-induced T1D. Together, our results demonstrate that the NLRP1 receptor plays a protective role in T1D, possible by its role in maintaining the gut epithelial barrier, thus preventing the translocation of bacteria from the gut to the PLNs during T1D development.

NETs (neutrophil extracellular traps) are linked to severity of pediatric sepsis and are potential targets for therapeutic intervention

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Introduction: Neutrophil Extracellular Traps (NETs) are an innate defense mechanism of neutrophils that are also implicated in the pathogenesis of organ dysfunction. However, the role of NETs in pediatric sepsis is unknown. **Objective:** To investigate the role of NETs in the pathogenesis of the pediatric sepsis. Methods: C57BL/6 mice (infant and adults) were subjected to polymicrobial or LPS-induced sepsis. Neutrophil infiltration, bacteremia, organ injury, and concentrations of cytokine and NETs in the plasma were measured. Production of reactive oxygen and nitrogen species and release of NETs by neutrophils were also evaluated. To investigate the functional role of NETs, mice undergoing sepsis were treated with antibiotic plus rhDNase (Pulmozyme®) and the survival, organ injury and levels of inflammatory markers and NETs were determined. Blood samples from pediatric and adult sepsis patients were collected and the concentrations of NETs measured. **Results:** The higher level of NETs developed in the infants may account for the increased pathology and mortality in pediatric sepsis. Mechanistically, the increased NETs were associated with elevated expression of Padi4 and histone H3 citrullination in the neutrophils. Furthermore, treatment of infant sepsis mice with antibiotics plus rhDNase markedly attenuated sepsis. Importantly, pediatric sepsis patients contain higher levels of plasma NETs than that in the adult sepsis patients and the severity of pediatric sepsis is positively correlated with the level of NETs. **Conclusions:** Therefore, this study highlights an underappreciated mechanism of pediatric sepsis susceptibility suggesting that NETs represent a new potential target to enhance the clinical outcomes. **Financial Support:** CNPq, FAPESP, CAPES.

Paclitaxel Reeducates Tumor-Associated M2-like Macrophages to M1 phenotype in a TLR-4/NF-κB dependent-manner and reduces tumor growth

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The efficacy of most of anticancer drugs mainly relies on the cytotoxic and antiproliferative mechanisms. Recent studies suggest that these agents may directly influence antitumor immune responses. Paclitaxel (PCX), a drug commonly used to treat breast cancer, has been shown to induce a LPS-like effect. However, whether PCX could modulate antitumor immune response is still a matter of debate. Then, we

investigated if PCX could influence macrophage polarization and whether that mechanism would modulate tumor progression. Murine bone marrow macrophages (BMDM) from wild-type (WT) or toll-like deficient mice ($TLR4^{-/-}$) were in vitro incubated with PCX (10, 30, 100 μM), LPS (M1-like condition), IL-4 (M2-like condition) or IL-4 plus LPS or PCX. After 48h of incubation M1 and M2 markers (TNF, IL-12, iNOS and CCL22, IGF-1, CD206, respectively) were measured. In order to investigate the LPS-like effect of PCX in in vivo conditions, WT, TLR-$4^{-/-}$ or $Tlr4^{fl/fl}$/LysM-cre mice were inoculated with B16 tumor cells, a murine melanoma cell line, and were treated with saline of PCX. PCX induced naive macrophages polarization towards a M1 phenotype. In addition, PCX reprogrammed M2 macrophages to a M1 phenotype, However, PCX failed to induce M1 polarization in $TLR4^{-/-}$ cells. Furthermore, PCX attenuated tumor development in $TLR4^{-/-}$ and $Tlr4^{fl/fl}$/LysM-cre mice through the increase of M1 phenotype. Therefore, PCX shifts tumor phenotype in a TLR-4 dependent-manner. We conclude that PCX has a cell-cycleindependent mechanism acting on a macrophage-directed therapy induced tumor reduction may be at least in part dependent on TLR4 signaling.

Molecular cloning and expression of the murine receptor interacting Serine/ Threonine Kinase 2 (RIPK2)

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Introduction/Aim: Intracellular nucleotide binding and oligomerization domain (NOD) receptors recognize antigens, including bacterial peptidoglycans and initiate immune responses by triggering the production of pro-inflammatory cytokines through activating NF-kB and MAP kinases. Receptor interacting serine/threonine kinase 2 (RIPK2) is critical for NOD-mediated NF-kB activation and is involved in the development of chronic inflammatory diseases such as rheumatoid arthritis. Thus, we have hypothesized that RIPK2 inhibitors represent an important new class of targeted therapeutics to treat inflammatory diseases. **Methods/Results:** In this context, we used ligand shape–based virtual modeling techniques to identify novel RIPK2 inhibitors previously identified as chemotypes. Using this approach, twenty compounds were select and purchase from commercial chemical libraries to perform subsequent enzymatic inhibition assays. To develop the assay with the selected compounds, the murine RIPK2 gene was amplified using cDNA from bone marrow derived macrophages as a template; then, cloned and expressed in the pGEX plasmid under tac promoter fused with GST tag. IPTG was used as an inducer for RIPK2-GST expression. Next, affinity purification of recombinant RIPK2-GST was performed using a GST-column. The protein purity was confirmed by SDS-PAGE and the obtained molecular mass was 62 kDa. Conclusions: In this present work, we demonstrated that the full length of RIPK2 was successfully expressed and purified. Now, we can perform further studies to test the capacity of the compounds to inhibit RIPK2 activation. **Financial Support:** FAPESP/CAPES/CNPq.

Neuro-immune interaction in inflammatory diseases

Stress-mediating inflammatory changes in a neurodevelopmental animal model of schizophrenia

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Introduction: Post-weaning social isolation (pwSI) is an early stress paradigm used to study the neurobiology of schizophrenia (SZ); however, investigation of inflammatory markers through this model remains scarce. **Aims:** to measure cytokines (IL-6, TNF, IL-10) at the peripheral blood and brain tissues [hippocampus (HIPPO), pre-frontal cortex (PFC)], and the respective cytokines mRNA at the same brain areas of rats under pwSI. **Methods:** Male wistar rats (n=10/group) were kept isolated or grouped since weaning during 10 weeks. After, animals were submitted to the Open Field (20 min). Cytokines were measured (Milliplex MAP; pg/mL), and qRT-PCR (fold change) performed using TaqMan mastermix. We conducted repeated measures ANOVA for behavioural analysis, and T test to compare cytokines between the groups, for each brain area. **Results:** Isolated animals presented hyperlocomotion at periphery and centre of the arena when compared to grouped animals (group & local & time interaction; p=0.005), indicating that early-life stress affected adult behaviour. In addition, isolated rats showed lower IL-10 plasmatic levels (27.19±6.54 vs. 96.08±31.77 pg/mL; p=0.039), whereas no difference was found for IL-6 or TNF plasmatic levels (p>0,05). In the PFC, isolated rats had reduced IL-6 (8503.22±682.89 vs. 11976.22±1334.21 pg/mL; p=0.034), as well as its mRNA (0.66±0.11 vs. 1.11±0.16; p=0.039). In the HIPPO, lower levels of IL-10 (716.0±124.12 vs.

1089.26±89.86 pg/mL; p=0.031), and higher levels of TNF mRNA were found (1.97±0.40 vs. 0.93±0.10; p=0.039). **Conclusion:** Our results showed stress-mediating systemic and central inflammatory changes in animals submitted to the pwSI, which resembles what is hypothesised in the neurobiology of schizophrenia.

Low plasmatic concentrations of NR1 and NR2 subunits of N-Methyl-D-Aspartate receptor as a possible biomarker for first-episode of psychosis

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Background: Dysfunctions in the glutamatergic system are implicated in the neurobiology of psychosis. **Aims:** to investigate the plasmatic concentrations of NR1 and NR2 subunits of the N-Methyl-D-Aspartate receptor (NMDAR) and of IgG antibodies anti-NMDAR in first-episode psychosis (FEP), compared with siblings and community-based controls. **Methods:** NR1 and NR2 concentrations and IgG antibodies were quantified by ELISA. Data were analyzed by chi-square, multivariate analysis and ROC curve. **Results:** The sample comprised 166 patients (mean age=30.3±12.2 years; 64% men), 76 siblings (mean age=31.5±11.0 years; 30.3% men) and 166 controls (mean age=31.4±12.0 years; 63.9% men). Patients were diagnosed as schizophrenia spectrum (SZ=84), bipolar disorder (BD=51) and psychotic depression (PD=31). The frequency of IgG antibodies was significantly higher (p=0.046) in BD (11.8%) than in the remaining groups (SZ=2.4%; PD=0; siblings=1.3%; controls=3.6%), even though the detection of antibodies anti-NMDAR is not specific. NR1 and NR2 concentrations were significantly lower in the FEP group, compared with siblings and controls (p<0.001). NR1 concentrations lower than 17.65 pg/ml (AUC=0.621) showed a sensitivity of 42.8%, a specificity of 84.3%, a positive predictive value (PPV) of 73.2% and a negative predictive value (NPV) of 59.6. Individuals with NR2 concentrations lower than 2.92 ng/ml (AUC=0.801) presented a 10.61-fold increased risk of FEP, with a sensibility of 71.9%, a specificity of 80.6%, a PPV of 79.0% and an NPV of 73.9%. **Conclusions:** This is the first study reporting the measurement and the reduction of NR1 and NR2 plasma concentrations in FEP. The NR2 subunit may be a candidate plasmatic biomarker for psychosis.

Related mechanisms of C5a/C5aR during neuropathic pain

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Introduction: Emerging data indicate that C5a and its receptor, C5aR, participate in acute and chronic pain, although the related mechanisms are largely unknown. The present study aimed to further address the peripheral and spinal mechanisms by which C5a/C5aR signalling mediates neuropathic pain development. **Methods:** Neuropathic pain was induced by PSNL (peripheral sciatic nerve ligation). Nociceptive behaviour was evaluated by von Frey filaments (mechanical), Hargreaves (heat) and acetone test (cold). Spinal WDR neurons response was evaluated by in vivo extracellular single cell electrophysiology. Molecular analyses were done by qRT-PCR, ELISA/Miliplex, FACS and Immunofluorescence. **Results:** Mechanical threshold was reduced in a dose-dependent manner from 1 up to 24 hours after i.t. injection of C5a recombinant. Directly spinal administration C5a recombinant promoted facilitation of WDR neurons response after paw mechanical stimulation with both noxious and innocuous filaments. The absence of C5aR in male and female mice submitted to PSNL resulted in less development and maintenance of mechanical, cold and heat nociceptive responses. Systemic and i.t. treatment with DF2593A, a C5aR antagonist, reduced mechanical and cold allodynia. Spinal administration of PMX-53, a C5aR antagonist, reduced the excitability and the mechanical evoked response of spinal WDR neurons to noxious and mainly innocuous filaments. Sciatic nerve, DRG and spinal cord express C5aR in baseline conditions, decreasing in this order, and there is an increase in C5aR mRNA expression and in C5a protein released in sciatic nerve in the first hours after lesion, between 3 and 7 days in the DRG and after 10 and 14 days in spinal cord. This result is corroborated by the very similar time profile of CD45+ cells in these tissues. In both, sciatic nerve and DRG, the most present cells are neutrophils and macrophages and those are also the main cells of C5aR expression. In the absence of C5aR, the release of several cytokines are reduced. **Conclusion:** Taken together, these results indicate that C5a/C5aR are clearly involved in both, genesis and maintenance of neuropathic pain, participating in response to polimodal stimulus. These effects seem to occur in peripheral and spinal sites, by among others, modulation in cytokines and chemokines release by neutrophils and macrophages in an ascending communication and sensitization of pain pathways.

N-Methyl-D-Aspartate (NMDA) receptor blockade prevents neuronal death induced by Zika Virus infection

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OBJECTIVES: Exploit the hypothesis that ZIKV-induced neurodegeneration can be rescued by blocking N-Methyl-D-aspartate (NMDR) overstimulation with memantine. **MATERIAL AND METHODS:** Neuronal cultures were prepared from the cortex and striatal regions of E15 (embryonic day 15) C57BL6j wild type mice. Commercially available drugs: Memantine (Eurofarma), MK801 (Calbiochem), Agmatine sulphate (CHEM-IMPEX INT'L INC) e Ifenprodil (Tocris) were used in vitro to blockade NMDA receptors on primary cultured neurons. In vivo experiments were conducted in FNα/βR$^{-/-}$ mice inoculated with 4x10^5 PFU of ZIKV via intravenous route. Memantine (Eurofarma) treatment (from day 3-6 post infection) was performed orally (B.I.D) in vivo. **RESULTS and DISCUSSION:** Our results show that ZIKV actively replicates in primary neurons and that virus replication is directly associated with massive neuronal cell death. Interestingly, treatment with memantine or other NMDAR blockers, including MK801, agmatine sulphate or ifenprodil, prevent neuronal death without interfering with the ability of ZIKV to replicate in these cells. Moreover, in vivo experiments demonstrate that therapeutical memantine treatment prevents the increase of intraocular pressure (IOP) induced by infection and massively reduces neurodegeneration and microgliosis in the brain of infected mice. **CONCLUSION:** Our results indicate that the blockade of NMDAR by memantine provides potent neuroprotective effects against ZIKV-induced neuronal damage, suggesting it could be a viable treatment for patients at risk for ZIKV infection-induced neurodegeneration. **Financial support:** INCT-Dengue, CNPq, CAPES, FINEP, FAPEMIG.

Zika virus infection of immunocompetent pregnant mice induces neurological and ophthalmological abnormalities of the offspring

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OBJECTIVE: Our aim was to evaluate the effects of early ZIKV infection on the neurodevelopmental and ophthalmological abnormalities of the offspring born from infected dams. Additionaly, we also evaluated the potential role of antibody-dependent enhancement in the exacerbation of those abnormalities. **MATERIAL AND METHODS:** C57BL/6 pregnant dams were inoculated with 1x10^6 PFU of ZIKV (HS-2015-BA-01 strain) by intraperitoneal (i.p.) route on gestational day 5.5 in the presence or absence of anti-envelope pan-flavivirus antibody (4G2). Negative and positive dam controls were injected with PBS or polyinosinic-polycytidylic acid potassium salt poly I:C) by i.p. route, respectively. Intraocular pressure (IOP), behavioral tests (basal locomotion and time in the center of arena, open field, sucrose preference test, y-maze and others) and magnetic resonance imaging (MRI) started at four, eight and twelve-week age of offspring, respectively. **RESULTS AND DISCUSSION:** A marked increase in IOP levels, indicative of ophthalmological abnormality, was detected in the offspring of 4G2-ZIKV and Poly I:C inoculated dams in comparison to ZIKV and PBS littermates. Preliminary behavior analysis revealed slight alterations on basal locomotion, indicative of anxiety-like behavior, and a reduced glucose preference, suggesting anhedonia. Interestingly, MRI revealed reduction in whole brain volume of all infected groups in comparison to PBS control. **CONCLUSION:** Thus, our results reveal that early maternal ZIKV infection is associated to increased IOP, slight behavior alterations and reduced whole brain volume of the offspring in adulthood. Those results provide insights on clinical and neurodevelopmental consequences of early maternal ZIKV infection. **Financial support:** INCT Dengue, CAPES, CNPq, FAPEMIG, FINEP.

Identification of miRNA regulatory networks in leprosy and integrated analysis with inflammatory mediators

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This study aimed to analyze miRNA regulatory networks in skin lesions from leprosy patients and associate them with host-bacteria interaction in this disease. Moreover, in an integrative approach, we analyzed the interplay between miRNA and inflammatory mediators based on their expression profiles aiming to identify inflammatory pathways modulated by direct control of miRNAs in leprosy.

In the first step, 75 differentially expressed miRNAs were selected and their potential targets were predicted using Ingenuity Pathway Analysis Software allowing us to identify 927 experimentally validated targets. The miRNA regulatory networks and enriched canonical pathways involving them were also predicted and the top enriched ones were related to cell cycle regulation. Besides that, the "neuregulin signaling" canonical pathway was enriched. Nodal molecules in the enriched pathways included up-regulated genes such as ERB2 and ERB3 receptors as well ERBB2 interacting protein and ERBB receptor feedback inhibitor. Mycobacterium leprae has a unique ability to invade Schwann cells and it is able to bind Erb2 which further induce Erk1/2 signalling leading to a myelin breakdown and damage in axons providing advantages for M. leprae survival. Our results show that miRNAs may play a central role in modulation of this mechanisms being involved with the ability of infect Schwann cells as well as the consequent nerve damage. In the second step, miRNAs and inflammatory mediators with opposite expression levels were integratively analysed revealing thirteen down-regulated miRNAs regulating four up-regulated inflammatory targets: IL-18, CXCL9, CCL5 and CCL13 which points to a role of miRNAs in regulating immulogical aspects in leprosy.

Antiviral drug (RS-574) prevents Zika virus in in vitro and in vivo models of infection

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Objectives: Study the effect of the antiviral drug (RS-574) in in vitro and in vivo models against Zika virus (ZIKV) infection. **Methods:** Primary neurons were infected with ZIKV and treated once a day with RS-574 in different concentrations. Supernatant for titrations and the cells for viability were assessed after 72 hours of infection. In in vivo experiments, IFNα/β$^{-/-}$ mice were infected with ZIKV and treated once a day with 10mg/Kg with different treatment intervals. The animals were euthanized on the 6th day post infection and the brain was collected for viral titers and inflammation parameters. **Results:** In in vitro experiments, RS-574 completely prevented viral replication in different concentrations. In animal experiments, when the treatment started two days before the infection, it prevented viral replication and disease itself, showing no increase in the measured pro-inflammatory cytokines and tissue damage. In contrast, when the treatment started on the third day post infections, it did not present the same results. The treatment was not efficient in preventing viral replication and inflammatory patterns remains elevated. As a middle ground, we started the treatment on the first day post infections, and it prevented partial lethality and viral replication showing a good perspective of treatment. **Discussion:** Many articles with perspective antiviral drugs have been published on the last year, but only a few of them showed in vivo results, therefore, showing the importance of our study. **Conclusion:** We presented an antiviral drug that prevented ZIKV replication both in vitro and in vivo showing good perspectives for treatment.

Novel key role of suppressor of cytokine signalling 2 in bipolar disorder

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Bipolar disorder physiopathology occurs due to a multifactorial complex interaction in the neuroimmune-endocrine system. Manic and depressive episodes of bipolar disorder were associated with elevation of proinflammatory cytokines. Lipoxins modulate the expression of suppressor of cytokine signalling 2 protein, which in turn regulates cytokine and neurotrophic factors signalling. We aimed to study the role of suppressor of cytokine signalling 2 and eicosanoids in manic and depressive-like behaviour in mice. Mania induced by GBR12909 (dopamine reuptake inhibitor) in mice pre-treated with zileuton (5-lipoxygenase inhibitor) and depression-like behaviours were evaluated in open field and forced swim tests, respectively. The expression of suppressor of cytokine signalling 2 and production of cytokines and neurotrophic factors were evaluated in different regions of the brain using western blot, cytometric bead array and enzyme linked immune sorbent assay, respectively. The results showed that suppressor of cytokine signalling 2 knock out mice had a basal depressive behaviour. Once mania was induced, suppressor of cytokine signalling 2 expression was reduced in prefrontal cortex. Deficiency in this protein resulted in delay of manic behaviour onset and inability to restore basal behaviour over the time, as well as increase of proinflammatory cytokines and reduction of neurotrophic factors on brain. Pre-treatment with zileuton prevented the manic episode, and altered the levels of cytokines and neurotrophic factors in a suppressor of cytokine signalling 2 -dependent manner. In conclusion, this protein is a key regulator of experimental manic and depressive-like behaviours and prophylactic effect of zileuton by regulating cytokines and neurotrophic factors on brain.

Contribution of CCR2+ and CX3CR1+ cells to neuropathic pain

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The aim of the present study is determine whether the induction of neuropathy, by itself, can lead to CCR2$^+$ and CX3CR1$^+$ cells infiltration, into spinal cord and dorsal root ganglia (DRG), as well as, to investigate the mechanisms of this recruitment, the source of these cells and how they can contribute singly to neuropathic pain. So, we evaluated by RT-qPCR, immunofluorescence and flow cytometry, the infiltration of myeloid cells in the spinal cord and DRG after peripheral nerve injury model (spared nerve injury- SNI) in wild type (WT) and CX3CR1$^{+/GFP}$/CCR2$^{+/RFP}$ animals. In the spinal cord samples, after SNI induction, we observed that there was an increase in the immunostaining for CX3CR1, although we do not observed any change of CCR2+ expression by RT- qPCR. Oppositely, in the DRG, we observed the presence of CD11b$^+$CX3CR1$^+$ cells (by flow cytometry) at seven days after SNI induction, as well as, an enhance in expression of CCR2+ and CX3CR1+ and chemokines, such as MCP-1, in relation to naïve mice (by PCR and immunofluorescence). Additionally, we induced SNI in WT and CCR2 knockout mice and observed there was no difference in the expression of CX3CR1, whereas the production of IL-1 and TNF-α cytokines was increased in WT animals in relation to CCR2 knockout mice. Here, we found that there are no myeloid cells entering in the spinal cord and the microglial activation is independent of monocytes infiltrate. In addition to the role that leukocytes play at the lesion site, CCR2 + and CX3CR1 + cells play a role in the GRD after SNI induction and appear to contribute in a differential way in the induction and maintenance of neuropathy.

Regulatory T cells accumulate in the injured nerve and reduce neuropathic pain by suppression of T_H1 cells

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Neuropathic pain is a debilitating condition caused by damage to the somatosensory nervous system, such as peripheral nerve injury. The immune system, and in particular the adaptive T cell response, plays a key role in mediating such pain. Regulatory T (Treg) cells are a small subpopulation of inhibitory T cells that prevent autoimmunity, limit immunopathology and maintain immune homeostasis. It was recently shown that Treg cells reduce neuropathic pain following peripheral nerve injury; however, the mechanisms by which these cells act remain unclear. Here, we showed that adoptive transfer of Foxp3$^+$ cells or pharmacological expansion of this cell subpopulation reduces hyperalgesia post-PSNL (partial nerve sciatic ligation); meanwhile, Foxp3$^+$ cells depletion significantly increases neuropathic pain, followed by a massive leukocyte infiltration, in particular T CD4$^+$ lymphocytes, at the site of the injury. Although the transcriptional profile of anti-inflammatory cytokines was not changed in nerves of mice with no Foxp3$^+$ cells, we observed increased levels of IFNγ and T-bet transcripts, but not Rorgt, IL-17 and GATA-3, which suggests that Treg could modulate T_H1 response during nerve injury and consequently reduce hyperalgesia. In contrast to previous reports, we did not find Treg infiltration in dorsal root ganglia (DRG) and spinal cord post-PSNL, however Treg absence was responsible to increase the levels of IBA-1 and ATF3 at DRG, showing that peripheral inflammation observed Foxp3-deficient mice could induce activation of DRG resident cells. Altogether, our data show that regulatory T cells acts at the site of the injury reducing Th1 immune response and indirectly controlling DRG resident cells activation indirectly, which contributes to reduction of neuropathic pain development and maintenance.

Regulation of inflammation

Leptin And Immunomodulation: Back To The Drawing Board

Alexandre A. Steiner

BACKGROUND AND PURPOSE: To elucidate the role of leptin in acute systemic inflammation, we investigated how its infusion at low, physiologically relevant doses affects the response to LPS in a time- and site-dependent manner. **EXPERIMENTAL APPROACH:** Physiological and molecular aspects of the response to LPS (500 μg/kg, i.v.) were assessed in rats infused with leptin s.c. (0-20 μg/kg/h) or i.c.v. (0-1 μg/kg/h). The relationship between leptin dose and plasma level was traced. Besides, cultured resident macrophages were studied at leptin concentrations (1-100 ng/ml) that are low compared to previous studies. **KEY RESULTS:** Using LPS hypothermia and hypotension as response biomarkers, we identified the phase extending from 90 to 240 min as the most susceptible to modulation by leptin. In this phase, leptin suppressed TNF-α without affecting IL-10, prostaglandins or corticosterone. The suppression of TNF-α was attained with s.c. leptin, but not with i.c.v. leptin. At its minimally effective dose, s.c. leptin elevated plasma leptin to a physiologically relevant level (5.9 ng/ml). Our results also revealed that, when primed by food deprivation, LPS-stimulated peritoneal macrophages can be inhibited by leptin at a concentration that is lower than the concentrations reported to promote macrophage activation. **CONCLUSIONS AND IMPLICATIONS:** When infused at a physiological dose, leptin exerts an anti-inflammatory rather than a pro-inflammatory effect. This effect involves an action outside the brain and selective suppression of TNF-α. The potential of leptin to inhibit macrophages deserves further investigation.

Role of the Fructose 1,6-bisfosfato on osteoclastogenesis and bone resorption in vitro

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Bone remodeling is a coordinated metabolic process, where the osteoblasts and osteoclasts participate actively. Therefore, any alteration in this balance may cause a change in the bone mineral density, a condition observed in certain inflammatory diseases such as osteoporosis, rheumatoid arthritis and periodontitis. Recently, there has been a growing interest in assessing the role of the glycolysis on the proliferation, survival, and differentiation of the different cell types. In particular, it has been demonstrated the protective effect of the Fructose 1,6-bisphosphate (FBP), a high-energy glycolytic intermediate. Considering that there is no evidence in the literature that associate FBP with the function of osteoclasts, this work aims to evaluate its role in osteoclastogenesis and bone loss. To this end, murine bone marrow derived pre-osteoclasts were differentiated into osteoclasts in the presence of M-CSF, RANKL and two concentrations of FBP (100 and 300 μM). The results showed that the FBP inhibits the differentiation of osteoclasts in a dose dependent manner, without affecting the cell viability. It was also observed that the treatment with the FBP decreases the expression of marker genes such as Nfatc1, Trap and Cathepsin K ($p < 0.01$) and the NFATc1 and Cathepsin K proteins expression. As well, the treatment with FBP resulted in markedly fewer osteoclast activity after 96 h of culture. Together, these data denote the important regulatory role of the FBP on osteoclastogenesis, proving to be a potential agent for the treatment of inflammatory diseases.

Activation of Aryl Hydrocarbon-receptor controls expansion of Indole-producing bacteria and protects mice from DSS-induced ulcerative colitis

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Objectives: To determine if the production of indole compounds by the indigenous microbiota and consequent activation of AhR plays a protective role during experimental ulcerative colitis. **Materials and methods:** The production of indole compounds in feces was measured using the Ehrlich reagent. Wild animals and $AhR^{-/-}$ were exposed to 2% SDS for 9 days and monitored for body weight, clinical score, CFU of enterobacteria, and total IgG and IgM dosage in faeces. Enterobacterial colonies present in the feces of the $AhR^{-/-}$ animals with colitis were isolated for presumptive identification using the modified Rugai test. **Results:** Increased indole production was observed on the initial days of colitis. $AhR^{-/-}$ animals showed a significant increase in the clinical score from the third day of induction of colitis, with marked weight loss when compared to wild animals and 100% of lethality rate. Despite the greater susceptibility, there were no differences in the enterobacterial content expansion between the two genotypes and greater total IgG and IgM titers were found in the faeces in the $AhR^{-/-}$ animals. However, there was expansion of Vibrio parahaemolyticus and Escherichia coli in feces of $AhR^{-/-}$ animals, enterobacterial species producing indole compounds. In wild animals, there was expansion of Enterobacter spp., a non-indole-producing bacterium. **Discussion/Conclusion:** $AhR^{-/-}$ animals are more susceptible to ulcerative colitis, which suggests the protective effect of indole against the disease. The expansion of indole-producing bacteria in $AhR^{-/-}$ animals suggests that AhR activation is important to control the expansion of these bacteria during the ulcerative colitis induced dysbiosis.

Study of angiotensin 1-7 effect on bleomycin-induced lung fibrosis

Flávia Rago Glória Gonçalves; Rosália Catarina da Silva; Geovanni Dantas Cassali; Mauro Martins Teixeira

Pulmonary fibrosis is a clinical condition that may cause dyspnea, hypoxemia which might culminate in death. It is characterized by a chronic activation of the inflammatory response and continuous production of inflammatory and fibrotic mediators which can activate fibroblast differentiation. Fibroblast activation and differentiation cause the production of extracellular matrix and collagen deposition that will lead to complacency loss and organ dysfunction. Pulmonary fibrosis is an adverse effect of some chronic inflammatory pulmonary diseases such as chronic asthma and the continuous inhalation of aluminum, silicon and titanium. In addition, pulmonary fibrogeneses can be caused by drug administration. Lung fibrosis can be observed in patients under bleomycin chemotherapy treatment. This drug has become the most used method of induced lung fibrosis in mice. Angiotensin 1-7 is a heptapeptide of renin-angiotensin system with anti-fibrotic and anti-inflammatory activity. It has been studied in some models of lung fibrosis, in rats, through subcutaneously administration and by lentivirus-mediated angiotensing-converting enzyme 2 (AEC2). Due to this, we aim to study the inflammatory and fibrotic parameters on Bleomycin-induced lung fibrosis of mice under the treatment with angiotensin 1-7 via gavage. Male C57Bl/6j mice were

treated with angiotensin 1-7, one hour before the induction of lung fibrosis by the instillation of a previously determined bleomycin dose. The animals were euthanized 2, 7 and 14 days after bleomycin instillation. We observed that the pre-treatment with angiotensin -17 prevented mice weight loss and increased survival. The treatment also significantly decreased the levels of mononuclear cells and neutrophils in mice airways and in lung tissue as well as levels of IL-1β in the tissue. Therefore, our results indicate that the pre-treatment with angiotensin 1-7, ireduces inflammatory process in the airways and lung tissue of mice treated, increasing survival, in this bleomycin-induced lung fibrosis model.

Effect of angiotensin- (1-7) in inflammatory resolution in an antigen-induced arthritis model

Livia Corrêa Barroso

Resolution of inflammation is a crucial event that prevents tissue damage and the consequent loss of organ function. Recent studies have advanced our understanding on the role of the renin-angiotensin system in the pathogenesis of several diseases. We now recognize angiotensin-(1-7) [Ang-(1-7)] as having pivotal role in antagonizing cell proliferation, tissue fibrosis and inflammation. Using experimental models of inflammation, we provide strong data for a novel action of Ang-(1-7): resolution of inflammation in arthritis. When administered orally or directly to the site of inflammation, at the peak of the inflammatory process, Ang-(1-7) decreased neutrophil accumulation in the synovial cavity. Ang-(1-7) promoted the resolution of inflammation by inducing apoptosis of neutrophils. These effects were Mas receptor-mediated and depended on the activation of caspase and inhibition of NF-κB. Ang-(1-7) also increased the engulfment of apoptotic leukocytes, ie. efferocytosis. Altogether, our results show that Ang-(1-7) activates events that are crucial for the resolution of the inflammatory process and the return to homeostasis indicating Ang-(1-7) as a novel endogenous pro-resolving mediator.

Endosymbiontic dsRNA virus worsens Leishmania infection by limiting NLRP3 inflammasome activation

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Introduction: Leishmaniasis is a disease that affects millions of people worldwide, ranging from self-healing cutaneous lesions to visceral pathology. Depending on the immune response and intrinsic parasite factors, individuals infected with L. braziliensis, L. guyanensis or L. panamensis may develop mucocutaneous lesions, a more severe form of the disease. A few decades ago, it was described that these species of Leishmania can harbor an endosymbiontic double stranded RNA virus, the Leishmania RNA virus (LRV). However, its relevance in Leishmaniasis has remained largely unexplored. Recently, it was demonstrated that the LRV triggers TLR3 activation and exacerbates the infection. The aim of this work is to evaluate whether (LRV) modulates the innate immune response against Leishmania infection. **Methods and results:** Nlrp3$^{-/-}$ deficient mice were previously shown to be more susceptible to Leishmania infection than WT mice. However, we found that NLRP3 is dispensable for infection with L. guyanensis M4147, a strain that harbors high levels of LRV (L.g.+). In order to study the impact of LRV in disease progression, we ramdomly generated a M4147-derived clone that does not contain LRV (L.g.-). Upon infection with these two different clones, we found that LRV favor parasite replication in WT mice and macrophages (BMDMs). Surprisingly, Nlrp3$^{-/-}$ mice infected with L.g.- displayed increased lesion size and parasite titers, suggesting that LRV favors L. guyanensis replication by interfering with NLRP3 inflammasome activation, both in vivo and in vitro. In BMDMs, LRV induces high levels of inflammatory cytokines such as TNF-α, IL-12 and type I IFN, and a reduced caspase-1 cleavage and IL-1β release. These effects were not observed in Tlr3$^{-/-}$ and Trif$^{-/-}$ BMDMs. Additionally, when we preformed infections in presence of the TLR3 agonist Poly:IC or with IFN-β, the inflammasome activation was decreased. Mechanistically, we determined that autophagy was triggered in the presence of the virus and reduced inflammasome activation. Autophagy was strongly induced by L.g.+ but not L.g.- and addition of poly:IC or IFN-β restored autophagy induction in L.g.- parasites. Accordingly, the inflammasome blockage by L.g.+ was null in Atg5$^{-/-}$ macrophages. Finally, we determined that LRV induces NLRP3 and ASC degradation via TLR3 and autophagy. **Conclusion:** Our data demonstrate an evasion mechanism triggered by LRV that result in increased parasite replication and survival. Mechanistically, Leishmania virus induces a TLR3-TRIF-IFNβ-autophagy signaling pathway that targets NLRP3 and ASC for degradation, impairing inflammasome activation by L. guyanensis. Although TLR signaling is known to prime cells for inflammasome activation, our data demonstrate that, during L. guyanensis infection, TLR3 triggered by LRV result in decreased inflammasome activity and increased parasite replication and disease exacerbation. Therefore, LRV worsens Leishmania infection in vivo and in vitro, and represent an important virulence factor that triggers evasion from innate immunity. **Financial support:** FAPESP, CRID/FAPESP, INCTV/CNPq, PEW and CAPES.

Microbiota-derived metabolites protect mice against colitis Induced by clostridium difficile infection.

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The intestinal colonization by pathogens is restricted by components of the commensal microbiota. Oral antibiotic therapy causes an imbalance in gut that predisposes the organism to infection by Clostridium difficile, a toxigenic bacterium that is associated with many pathological conditions. The aim of this study was to investigate whether short chain fatty acids (SCFAs), metabolites derived from the gut microbiota, affect the development and intensity of C.difficile infection. For that, we used male 8-weeks old C57BL/6 mice treated with SCFAs (acetate or butyrate) in the drinking water. The microbiota depletion was done with a mix of antibiotics orally administered for four days and a single intraperitoneal dose of clindamycin. The infection was induced by gavage with 10^8 CFUs of C. difficile VPI 10463 strain on day latter. We noticed that, in addition to reducing the loss of body weight and the clinical impairment, oral administration of acetate or butyrate significantly increased the survival rate of infected animals (from 20% to 60% and 70%, respectively). Acetate and butyrate had no direct effect on C.difficile growth or colonization in vivo. They also did not affect the production of toxins (TcdA and TcdB) by the bacteria. However, we noted that SCFAs, especially butyrate, modulated the expression of several genes and induced an anti-inflammatory profile in the colon. In addition, SCFAs improved the intestinal epithelial permeability in infected mice: a reduction in the translocation of luminal bacteria and FITC dextran was observed after SCFAs-treatment. These latter effects were associated with increased expression of molecules involved in the maintenance of the intestinal permeability. We conclude that oral treatment with SCFAs has a significant protective effect on the murine model of acute colitis by C.difficile. This effect may be related to SCFAs immunomodulatory effects and/or direct actions on epithelial cells.

The microbial metabolite-sensing Gpr43 receptor and the microbial metabolite acetate are important to host defense and immune modulation against lung bacterial infections

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Klebsiella pneumoniae and Streptococcus pneumoniae are the mostly common cause of pneumonia and death wordwide. The gut microbiota wires local intestinal mucosal immunity and is increasingly recognized as an important modulator of the systemic immune response although the mechanisms are still poorly understood. Here, we hypothesized that one of these mechanism evolved the intestinal production of the metabolite acetate reaching higher systemic levels and binds to their receptor Gpr43 modulating lung inflammation. We found that in the absence of Gpr43, by using Gpr43-deficient mice, these mice display similar susceptibilities to both bacterial pneumoniae, as previously demonstrated in germ-free mice, indicating that the absence of microbiota or metabolite/Gpr43 activation confers no protection and increased lung injury. However, infected mice acetate-treated presented faster resolution of inflammation with enhancement of the cytokine IL-10, decreased of pro-inflammatory cytokines TNF-a and IL-1b, also neutrophils and macrophages, decreasing lung damage and reduction of pathogen growth contributed to rescue 70% of mice from death. Also, alveolar macrophages showed high production of reactive oxygen species in the presence of acetate. This phenotype correlates with reduction of bacteria in the lung from infected mice acetate-treated. However, alveolar macrophages from Gpr43-deficient mice showed a diminished capacity to phagocytose and reduction of ROS related with higher amounts of bacteria in the lung. Thus, we propose that the metabolites produced by gut commensal microbiota protects the host against pneumonia-induced death by fine tuning the immune cells in the lung and contributing to faster return to lung homeostasis through activation of Gpr43 receptor.

Cannabidiol reduces mortality, recruitment of inflammatory cells and intestinal injury in murine Graft-Versus-Host Disease

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Objectives: The aim of the present study was to evaluate the effect of cannabidiol (CBD) treatment in the inflammatory response of mice submitted to experimental GVHD. **Material and methods:** GVHD was induced in balb/c mice by the transplant of $1x10^7$ bone marrow cells and $1x10^7$ splenocytes from C57BL/6 mice. GVHD mice

were treated with three doses of CBD (10, 30 or 60 mg/Kg) 24/24h, starting in the same day of disease induction. After transplant, mortality was assessed every day. Inflammatory response was evaluated in the 6th day after GVHD induction in the intestine and liver, the major target organs of GVHD. Histopathological analysis, quantification of cytokines and chemokine and recruitment of inflammatory cells, by intravital-confocal microscopy were performed to evaluate the inflammation of these organs. To verify the direct effect of CBD in the rolling and adhesion of leukocytes, CBD treatment was performed only 30 min before intravital analysis. **Results:** CBD 30 mg/Kg was the more effective dose in reduce mortality associated to GVHD. It was able to reduce intestinal injury, assessed by histopathological score, and levels of TNF-α, IFN-γ, CCL2 and CCL3. In the intestinal microvasculature, CBD did not alter the number of rolling cells but reduced adherent cells. FACS experiment are been made to evaluated the role of CBD in the accumulation and activation of leukocytes in the GVHD target organs. **Conclusion:** Even now, CBD treatment has reduced mortality of GVHD mice and decreased intestinal inflammation verified by the reduction of inflammatory mediators, leukocytes adhesion and intestinal injury. **Financial support:** FAPEMIG, CNPQ, CAPES, PRPQ/UFMG

Histamine down-regulates TLR4 hyperresponsiveness of human neonatal monocytes

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Objective: Evaluate the immunomodulatory effect of histamine in human neonatal monocytes induced by TLR4-ligand. **Materials and methods:** Human umbilical cord mononuclear cells (MNCs;n=9) and healthy adult subjects (MNCs;n=13) were incubated with TLR4 agonist (LPS) and histamine. Supernatants were evaluated for CCL2-secretion by flow cytometry. Real-time PCR of histamine receptors (HRs) and histidin decarboxylase (HDC) were also performed on MNCs. The evaluation of the gene profile of the purified monocytes was done through an array PCR for 84 relevant genes in the TLR signaling pathway. **Results and discussion:** In NBs MNCs, we found a 5-fold increase in CCL2-secretion upon TLR4 stimulation compared to adults, which was inhibited by histamine in both groups. The effects of histamine were partially reversed by the H1R, H2R and H4R inhibition, suggesting that all three receptors are required for histamine modulation, both in NBs and adults. Analysis of the constitutive HRs and HDC expression showed that H2R mRNA levels were lower in NBs MNCs. Upon LPS stimulation, NBs monocytes showed an up-regulation of genes related to the JAK/STAT/NF-kB and IFN pathway. Strikingly, most of the over expressed genes induced by LPS in NBs were down-regulated by histamine, reaching a similar profile in both adults and NBs. The balance between inflammatory and regulatory factors induced by TLR stimulation is crucial for restraining inflammation during the early stages of life. **Conclusion:** Our work identifies histamine as a potential player in immune homeostasis during the period that can counterbalance the NBs hyperresponsiveness to TLR stimulation. **Financial Support:** LIM56/HCFMUSP/FAPESP.

Genetic screening for identification Coxiella burnetii Dot/Icm effectors that target inflammation using Legionella pneumophila as surrogate host

Robson Kriiger Loterio, Hayley Newton, Dario Simões Zamboni

The intracellular bacterium Coxiella burnetii is an immunologically "silent" pathogen that uses a Dot/Icm secretion system to secrete effectors proteins into host cytosol to promote bacterial survival and pathogenesis. The immune system is often efficient to control infectious diseases, but several microorganisms acquired mechanisms capable to evade the immunological recognition and survival in their host. We aim to identify and characterize new C. burnetii effectors that modulate the intracellular pathways in macrophages. We are conducting a genetic screening of C. burnetii Dot/Icm effectors using Legionella pneumophila JR32 flaA$^-$ as surrogate host. Approximately 70 Coxiella effectors cloned into the plasmid pJB-CAT:FLAG with a Coxiella/Legionella promotor will be used in the genetic screening. These effectors were initially amplified in Escherichia coli DH5α and further used to transform L. pneumophila JR32 that lacks flagellin (flaA$^-$). Transformation were performed by electroporation and transformed bacteria grown in AYE plus chloramphenicol at 36°C until the emergence of isolated colonies. The colonies were expanded for 2 days and evaluated to confirm the effector expression by western blot. We are also building a collection of Legionella expressing Coxiella effectors. Until now, 52 effectors were transformed with success in E. coli, and from these, 25 were transformed in L. pneumophila. We anticipate that this study will provide scientific basis to better understand the different mechanisms that pathogenic intracellular bacteria use to evade the host defense and the development of therapies against immune diseases. **Supported by** FAPESP, PEW and CNPq.

Divergent nitric oxide-immunomodulatory effects on newborn and adult CD4+ T cells

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Newborns are particularly susceptible to infections because their difficulties to develop pro-inflammatory responses. Nitric oxide (NO) is a microbicide molecule, produced mainly by phagocytes and important in innate immune responses. However, in the last few years, NO-immunomodulatory properties (modulating adaptive responses) have gained attention. **Objective:** To evaluate whether known NO concentrations has potential to modulate newborn TCD4+ responses, inducing a pro-inflammatory profile, similar to observed in adults. **Methods:** Mononuclear cells were isolated from peripheral (adults) and cord blood (newborns) and cultivated with phytohemagglutinin (PHA) and an NO donor, NOC-18. After 120 hours, lymphocyte proliferation (by CFSE), activation (CD69) and cell death (AnexinV+) were evaluated by flow cytometry, and cytokine release was assessed in culture supernatants (48 and 120 hours) by Cytometry Bead Array. **Results:** TCD4+ lymphocyte proliferation rate were similar between both groups even when NOC-18 was added with PHA. However, newborn TCD4+ lymphocytes presented greater activation in comparison with adults when cells were stimulated with PHA and NOC-18. Cell death neither changed with stimuli (in both groups) nevertheless NOC-18 addition caused changes in cytokine release, especially in adults. While NOC-18 addition did not have any effect in cytokine release by newborn cells, there were an increase in pro-inflammatory (IFN-γ only after 48 hours and TNF-α, IL-2) and anti-inflammatory (IL-10) cytokines release in adults. **Discussion and Conclusion:** NO-immunomodulatory properties were different between adults and newborns, probably because NO affects different mechanisms in each one. NO is able to modulates newborn responses, but not sufficient to undergo a Th1 response.

Evaluation of non-steroidal anti-inflammatory drugs to inhibit the enzymatic activity of phospholipase A2 from Crotalus durissus terrificus venom

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Verify potential antagonism of non-steroidal anti-inflammatory drugs diclofenac and nimesulide against phospolipase A2 from snake venon. For such, phospholipase A2 was isolated from Crotalus durissus terrificus venon by molecular exclusion chromatographic method. Diclofenac and nimesulide were diluted and incubated with phospholipase A2 for analysis in reverse fase column, to verify the molecular interactions between the enzyme and drugs. The same samples were analysed in circular dichroism for structural analysis, and enzymatic inhibition by 4N3OBA enzymatic assay. Chromatographic and circular dichroism results showed that both nimesulide and diclofenac interacted with enzyme, by altering its α-helix secondary structures. Through enzymatic assay, results showed a drug inhibition of almost 50% of the enzyme activity, corroborating with previously obtained results. Therefore, protein structural alterations indicate that there was a labile interaction in its hydrophobic region, showing that both diclofenac and nimesulide partially inhibit catalytic activity of phospholipase A2, proving that these drugs also operate in inflammatory process beginning. In Brazil, diclofenac and nimesulide are freely used by the population, they have low cost and do not require medical prescription, but their neutralizing capacity for two cyclooxygenase isoforms and phospholipase A2 enzymatic activity might be responsible for several side effects caused by the indiscriminate use of these drugs.

The role of Annexin A1 in the modulation of Caspase 1 and IL-1β in a model of gout

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Objective: This study aimed to characterize the involvement of Annexin A1 (AnxA1) in the caspase-1 and IL-1β activation during monosodium uric acid (MSU) crystals-induced joint inflammation. **Methods and Results:** Experiments were performed in wild-type (WT) and AnxA1-deficient (AnxA1$^{-/-}$) male Balb/c mice. It is known that AnxA1$^{-/-}$ animals exhibit greater joint inflammation compared to WT mice after the injection of MSU crystals. However, the levels of IL-1β (a key cytokine of gouty inflammation) in synovial tissue are comparable between both groups of mice. Interestingly, in the early stage of MSU crystals-induced arthritis (3 hours), we found reduced level of IL-1β mRNA in the synovial tissue in AnxA1$^{-/-}$ compared to WT mice. That result was corroborated in vitro, where there is lower release of IL-1β and cleaved caspase1 in the supernatant of AnxA1$^{-/-}$ Bone Marrow Derived Macrophage (BMDM), when they were stimulated with MSU crystals or ATP, as compared to BMDMs from WT mice. **Conclusion:** These results suggest a temporal role for AnxA1 in caspase-1 and IL-1β activation

in joint inflammation induced by MSU crystals. **Financial support:** CNPq, FAPEMIG, CAPES.

CD300a immunoreceptor is an important regulator of arthritic inflammation in mice

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Objectives: This work aim to evaluate the CD300a participation on arthritic inflammation in mice. **Methods:** Wild type (WT) and CD300a$^{-/-}$ Balb/c mice were immunized i.d. at the base of the tail with 500 µg of methylated BSA (mBSA) in 100 µL of an emulsion of saline and an equal volume of complete Freund's adjuvant (CFA) at day 0. Two weeks later, the mice were challenge with an injection of the 10 µg of mBSA in the knee joint. Negative control received saline in the joint. 24 hours later, the mice were culled for inflammatory analysis. The articular lavage was performed for cellular counting and the articular tissue was collected to measurement of cytokines (ELISA) and to myeloperoxidase (MPO) activity. Further, the expression of CD300a was evaluated on neutrophils from WT AIA mice by flow cytometry. Chemotactic capacity of the neutrophils front different stimuli was also assessed using Boyden chamber. **Results:** CD300a$^{-/-}$ mice presented increased number of neutrophils in the joint and in periarticular tissues, besides presenting higher IL-6 and CXCL1 chemokine in the tissue when compared to the WT littermate. Further, interestingly, a great amount of neutrophils accumulated into the joint express high levels of CD300a on their surface. Neutrophils from CD300a$^{-/-}$ mice showed higher chemotactic ability when compared to neutrophils from WT mice. **Discussion:** CD300a is upregulated in neutrophils present in inflamed joint, participate on neutrophil recruitment and also control cytokine production. **Conclusion:** These results suggest that CD300a is an important receptor that control inflammation in this model. **Financial support:** FAPEMIG, CAPES, CNPq.

The relevance of PI3Kγ for TLR9-dependent cytokine production and inflammation

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Phosphatidylinositol-3-kinase gamma (PI3Kγ) is a G protein-couple receptor lipid kinase that is responsible for a myriad of cell functions like cell migration, cell activation, protein synthesis, cell survival and others. In this study, we evaluated the role of PI3Kγ in two different inflammatory models that DNA sensing is important for the progression of the disease: drug induced liver injury (DILI) and silicosis. Oral administration of acetaminophen (APAP), induces liver necrosis, neutrophil accumulation in the liver and high levels of liver transaminases (ALT) in the blood of wild-type (WT) mice. PI3Kγ$^{-/-}$ mice showed reduced liver necrosis, less neutrophil accumulation and lower levels of ALT. Moreover, PI3Kγ$^{-/-}$ mice, showed less remote lung damage and better respiratory parameters when compared to the WT mice. Mice treatment with AS605240, a PI3Kγ inhibitor, resulted in liver protection in the same extent as gene knock-out. Also, late mice treatment with AS605240 could protect animals from DILI, effect not observed for the gold standard N-acetylcysteine. In another model, silicosis, PI3Kγ$^{-/-}$ mice showed lower number of cells in the lungs, less fibrosis and better respiratory parameters when compared to the WT mice. In vitro analysis of human PBMCs showed that CpG stimulation could induce p-AKT and cytokine production and that PI3Kγ inhibition could dampen cytokine production without harming cell viability. Here we show a non-canonical activation of PI3Kγ via tyrosine kinase receptor and that this enzyme is crucial for full TLR9 function and disease progress. Thus, drugs that target this enzyme could be good candidates for disease treatment. **Financial Support:** CNPq; GlaxoSmithKline; FAPEMIG.

Regulatory T cells and its association with angiogenesis in oral squamous cell carcinomas

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The aim of this study was to evaluate the frequency of regulatory T cells and its correlation with angiogenesis in oral squamous cell carcinomas. Samples from a total of 61 patients with oral squamous cell carcinomas, located on the lip and tongue and floor of the mouth were analyzed for the histopathological malignancy index and immunoexpression of regulatory T cells (forkhead box P3), intratumoral microvessel density and vascular endothelial growth factor A. Similar values were observed for the frequency of regulatory T cells, intratumoral microvessel density values and vascular endothelial growth factor A expression in oral squamous cell carcinomas of the lip and tongue and floor of mouth. A positive correlation between

forkhead box P3 expression with intratumoral microvessel density values was also detected, however, statistically non-significant (P-value=0.682). Furthermore, a significant positive correlation was observed between the frequency of tumor-infiltrating regulatory T cells with vascular endothelial growth factor A expression (P-value=0.029). Suggesting that tumors with increased regulatory T cell infiltration tend to express higher levels of vascular endothelial growth factor A. No correlation was observed between intratumoral microvessel density values and with vascular endothelial growth factor A expression. We can hypothesize that in these oral squamous cell carcinomas samples, other angiogenic factors are being important to result in the observed angiogenic levels. These results suggest that although there is an association between the frequency of regulatory T cells and angiogenesis in oral squamous cell carcinomas, forkhead box P3 expression was not associated with tumor development.

MSU stimulate immune cells to produce nitric oxide which activates TRPV1 to induces nociception and inflammation

OBJECTIVES: To investigate the participation of transient receptor potential vanilloid 1 (TRPV1) and nitric oxide (NO) in the nociception and inflammation in a mice model of acute gout attack. **MATERIAL AND METHODS:** We induced acute gout attack by injecting 10 µg MSU into the right ankle of mice wild type, knock out for TRPV1 ($TRPV1^{-/-}$) or inducible nitric oxide synthase ($iNOS^{-/-}$) (C57/BL6; 4 - 6 weeks old, 15 – 20 g). Nociception was assessed by using von Frey filaments to measure the mechanical sensitivity after MSU injection. To confirm the relevance of NO in this model, we measured the total amount of nitrates in periarticular tissue after MSU injection. As macrophage plays a key role on the inflammation, we evaluate macrophage accumulation in periarticular tissue by measuring NAGase enzyme activity and its functionality by evaluating (in vitro) calcium influx mediated by capsaicin and NO (total nitrite) production after stimulation with MSU. **RESULTS:** After intra-articular MSU injection, we observed a robust nociception from 1 up to 12 hours in WT mice, but not in $TRPV1^{-/-}$ or $iNOS^{-/-}$. Similarly, the ablation of TRPV1 receptor or iNOS prevented the macrophage infiltration and nitrate production. In vitro, MSU stimulated NO production (increase in nitrate production) and potentiate the calcium influx elicited by capsaicin. **DISCUSSION AND CONCLUSION:** MSU, the etiological agent of acute gout attack can activate macrophages to produce nitric oxide, measure as total nitrate, which in turn may act as an activator of TRPV1, also relevant in this condition.

Glycated collagen induces pro-inflammatory factors activation on rat dorsal root ganglion (DRG) primary culture

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Advanced glycation end-products (AGEs) are formed in collagen glycation reaction and interact with receptors to induce pro-inflammatory activation. This process occurs in Osteoarthritis (OA), which is characterized by pain, swelling and stiffness of the joint. The exact mechanisms of pain in OA remain poorly understood and treatments are not effective in reducing pain. This shows the importance of characterizing novel in vitro models for the study of OA pain. **Objetive:** to assess a model that mimics the interaction between OA glycated extracellular matrix and sensory neurons, in an attempt to reproduce one of the conditions involved in the genesis of pain observed in OA. **Material and Methods:** rat DRG cells were incubated with normal collagen or glycated collagen (100 or 500 µg/ml). Cell viability, RAGE expression, MAP Kinase and nitric oxide were analyzed. **Results and Discussion:** Normal and glycated collagen did not change viability on DRG cells. RAGE expression was detected on normal and glycated collagen but not modulated by glycation. Glycated collagen induces p38 and ERK phosphorylation and increase nitrite levels ($p<0,05$). **Conclusion:** Suggest the possible effect of glycation on neuronal activation for inflammatory parameters and this model can be useful to study new targets for drug development in OA treatment. **Supported by** FAPESP Grant number: 2016/12128-0 and Grant number: 2015/50040-4, São Paulo Research Foundation and GlaxoSmithKline.

Other

In silico modeling of major histocompatibility complex class I HLA-G protein and lipid bilayer complex

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Introduction: HLA-G is a nonclassical major histocompatibility complex class I (MHC-I) molecule considered to be tolerogenic, playing an important role in the suppression of the immune response. **Objective:** Since all segments of the molecule may contribute to the suppressive ability and interaction with immune receptors,

it is necessary to generate the complete 3D structure of HLA-G. **Methods:** The HLA-G protein tertiary structure was modeled by homology using Modeller 9.14 software and the residues not contained in the crystallography were modeled by a combination of ab initio techniques using the Rosetta software and the the I-TASSER server, in order to generate the 3D structure for the 39 amino acids located in the transmembrane and intracellular region. The complete HLA-G protein model generated was then inserted in a homogenous lipid bilayer using the CHARMM-GUI server. **Results:** The HLA-G protein model generated allowed the assessment of the complete 3D structure of the protein and its organization on the surface of the lipid bilayer. Discussion: The in silico modeling and prediction of the HLA-G allowed the observation of the complete protein structure, which will be used as an input for molecular dynamics simulation. **Conclusions:** This is the first complete model of the HLA-G protein and it will be important to determine the protein stability and behavior on a lipid bilayer, in an effort to mimic the cellular surface where it is located. **Financial Support:** CAPES.

Caracterização das proteases presentes nas sementes da Morinda citrifolia

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Introdução: Conhecida como Noni, a Morinda citrifolia é usada na medicina popular com diversas aplicações terapêuticas. O presente trabalho tem como objetivo a caracterização das cisteíno proteases presentes nas sementes de Noni para o desenvolvimento de novos fármacos. **Métodos:** As sementes foram trituradas, homogeneizadas com água destilada, centrifugadas, e o sobrenadante filtrado, dosado por Bradford e armazenado à 80ºC. A atividade proteolítica qualitativa foi realizada na presença de fibrinogênio, sendo o perfil eletroforético analisado em gel de poliacrilamida à 14% incubando a enzima com as variações de concentração, tempo, pH, temperatura, íons e inibidores. **Resultados:** A atividade proteolítica na presença de fibrinogênio com a enzima obtida da semente, ocorre a partir da concentração de 20ug; tempo de início a partir dos primeiros 5 minutos; o pH ótimo de 7,0; as temperaturas variam da temperatura ambiente até 50ºC; ocorrendo atividade com os íons bivalentes e o efeito de inibição apareceu na presença da Leupeptina. **Discussão:** O presente trabalho caracterizou as propriedades gerais e o substrato específico da protease presente no extrato aquoso das sementes de Noni, tendo relevância em estudos posteriores de prevenção e tratamento de diversas enfermidades. **Conclusão:** Foi realizada a caracterização do perfil proteico do extrato da semente da Morinda citrifolia. **Financiamento:** CAPES, CNPq e FAPEMIG.

Papel do receptor CCR2 na perda óssea alveolar induzida por artrite séptica em camundongos

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This project aims to evaluate the effects of the septic arthritis (SA) induced in mice in alveolar bone conditions in $Ccr2^{-/-}$ mice. Periodontal disease (PD) is an inflammatory condition of infectious etiology that affects the supporting structures of the teeth. SA is a purulent condition of joint spaces when they are invaded by microorganisms, especially bacteria such as Staphylococcus aureus that results in an inflammatory process around the affected joint, culminating in tissue damage. CCL2 chemokine is known to be involved in the recruitment of monocytes under inflammatory conditions. SA was induced by intra-articular inoculation of S. aureus ATCC 6538 (1×10^7 CFU / mL in PBS) in the knee of C57B6/J (WT) or $Ccr2^{-/-}$ mice. The control group received only PBS. Seven days after inoculation, mice were euthanized and articular and maxillary tissues were removed and processed for histopathological score, alveolar bone loss, neutrophil infiltrate (MPO), and cytokine production (ELISA). AS induced by S. aureus resulted in significant inflammatory cell infiltrate in the knee of both groups, but it was significantly decreased in the absence of CCR2. A significant alveolar bone loss was also observed in infected WT mice when compared to control mice. In addition, WT mice showed higher production of RANKL relative to OPG than $Ccr2^{-/-}$ mice. **Conclusion:** These results indicate that SA is associated with alveolar bone loss in mice and CCR2 may play an important role in controlling this relationship. **Supported by:** CAPES, FAPEMIG, CNPq.

In silico analysis of interaction between oncoprotein E6 and European variants of HPV type 16 with molecular targets E6AP and p53

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Introduction: The European variants (EV) of the E6 oncoprotein of HPV type 16 are the most commonly found and related to cervical cancer. Several studies have investigated SNPs that occur in E6 and their possible relation with differences in the oncogenic potential of the virus, emerging the E6 oncoprotein as a potential therapeutic target. **Objective:** Identify possible differences in the interaction between E6 and EV oncoproteins in

the formation of the E6/E6AP/P53 complex. **Methods:** Molecular docking made using HADDOCK 2.2 software and analysis of the interaction between proteins made using the PyMOL software. **Results:** The docking that generated the E6 (EV)-E6AP complex showed that the proteins have conserved regions of interaction. P53 docking with E6 (EV)-E6AP generated the E6 (EV)-E6AP-P53 complex, where P53 bound in the same region on all proteins, but showed variations in the number and types of binding between bound residues. An important region was also observed where the three proteins interact, showing that the function of E6 and EV can be dependent on the formation of the complex with E6AP-P53. **Conclusions:** The protein interaction regions are conserved, but each of the EV and E6 interacts with E6AP and P53 in a particular way, retaining some similarities. Differences in the formation of complexes may be indicative of variation in the degree of affinity between EV and E6 with their molecular targets, supporting the hypothesis of a possible difference in the degree of oncogenicity between them. This information reveals important data on regions that can be used as therapeutic targets. **Financial Support:** CAPES.

Evaluation of anti-inflammatory activity of DGB1, a synthetic derivative of digoxin

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The present work aims to evaluate the anti-inflammatory activity of a novel synthetic compound derived from digoxin (DGB1). Swiss mice were treated orally with DGB1 at different concentration (0.03, 0.1 e 0.3mg/Kg) or 10 mg/kg Indomethacin, 30 min before subplantar injection of carrageenan in the left hind paw. Paw volume was measured by using a plethysmometer before inflammatory stimulus and 1, 2, 4 and 6 h after injection of the carrageenan. To analyze leucocyte infiltration mice (sham, control, 0.3mg/kg DGB1 and 10 mg/kg Indomethacin groups) were sacrificed 2, 4 and 6 h after the subplantar injection of carrageenan. Footpads samples were fixed in 10% formaldehyde for 24h, processed for paraffin embedding, cut into 5 µm sections and stained with hematoxylin-eosin for light microscope examination. The DGB1 significantly inhibited the paw edema by 54.17% and 75% at doses of 0.1 and 0.3mg/Kg, respectively, 2h after carrageenan injection. As well as, significant inhibition was observed 4h after the inflammatory stimulus at doses 0.1mg/Kg (51.15%) and 0.3mg/Kg (74.14%) and 6h ate dose 0.3mg/Kg (70,24%). The DGB1 at 0.3mg/Kg was unable to inhibits the recruitment of neutrophils into the inflammatory site. Data that corroborate to our results are available in the literature, showing cardiac glycosides can be promising molecules for the development of anti-inflammatory drugs, what will not only provide a drug repositioning, but also the development of an innovative product. Nevertheless, more studies should be carried out.

Bacterial flagellin is able to activate inflammasome in hiv+ dendritic cells: new strategy for adjuvant use in HIV+ individuals

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Objective: Inflammasome activation plays a key role in dendritic cells (DC) activation and in the consequent induction of adaptive immunity. Common vaccine adjuvants activate NLRP3 inflammasome. HIV+ individuals are unable to mount an efficient immune response. Our group have demonstrated that NLRP3 inflammasome do not correctly activate in HIV+ DC. Aim of this project is evaluate alternative ways to activate inflammasome in HIV+ DC, such as bacterial flagellin, nowadays implied as vaccine adjuvant in several pre-clinical trials, which stimulates inflammasome formation through NLRC4/NAIP proteins. **Materials and Methods:** Inflammasome activation is evaluated in HIV+ DC stimulated with flagellin by the meaning of IL-1ß and IL-18 release, caspase-1 activation, specific inflammasome genes expression, speck-like formation. Inflammasome signaling cascade is investigated through the use of specific agonist and/or inhibitors. **Results and Discussion:** HIV+ DC release lower amount of IL-1β compared to healthy donors (HD) DC in response to common NLRP3 agonists (LPS/ATP). NLRP3 inflammasome genes expression also resulted reduced in HIV+ DC with respect to HD DC. Flagellin resulted able to restore the release of IL-1ß at the level of HD-DC, suggesting that while NLRP3 activation is corrupted in HIV+ individuals, the NLRC4 pathway seems to be unaffected by the (chronic) HIV-1 infection. As expected, caspase-1 inhibitor parthenolide abolished IL-1ß release in both HIV+ DC and HD DC. **Conclusion:** Our preliminary results seem to corroborate our hypothesis that it could be possible to activate HIV+ DC through the use of adjuvants known to act on inflammasome other than NLRP3.

The genetics of host influence the activation of NLRP3-inflammasome in human monocyte-derived macrophages in response to Mycobacterium spp

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Objective: Innate immunity plays a central role in susceptibility to M.tuberculosis (Mt), in the elimination of bacillus, and in the development of latent versus active tuberculosis (TB). Inflammasomes are responsible for caspase-1 activation and release of the pro-inflammatory cytokines IL-1ß and IL-18. Experimental models have showed that Mt activates NLRP3-inflammasome and IL-1ß production, however little is known about the role of inflammasome activation in human TB. We aim to investigate the interaction M.tuberculosis/host in humans, in terms of (1) contribution of inflammasome genetics in the development of active pulmonary and extra-pulmonary TB; (2) activation of inflammasome in human peripheral blood monocytes-derived macrophages (MDM) stimulated with pathogenic and not pathogenic Mt strains. **Material and Methods:** Selected inflammasome polymorphisms were genotyped in a case/control cohort of Amazon TB patients. Inflammasome activation was analyzed in MDM stimulated with Mt spp by the measure of IL-1β release, inflammasome genes expression modulation. Experiments were done in the presence of common NLRP3 inflammasome activators (ATP, LPS), or inhibitors (parthenolide, high K+). **Results and Discussion:** Polymorphisms in specific inflammasome genes contributes to control (NLRP3, CTSB) or development (P2X7) of active pulmonary TB, and these variants correlated with IL-1ß release in genotype-guided experiments of Mt-activated MDM. Mt induced release of IL-1β (BCG>H37Rv>Beijing 1741), and NLRP3 and IL1B expression, suggesting that Mt is able to prime (through NF-kB) and activate (through caspase-1) the complex. This activation is significantly inhibited by parthenolide, confirming the involvement of inflammasome in response to Mt in human MDM.

Photoacoustic spectroscopy for evaluation of inflammatory response

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Objective: To apply photoacoustic spectroscopy (PAS) as experimental tool for the evaluation of inflammatory response using the croton oil (CO) and phenol models of skin inflammation. **Material and Methods:** Ear edema was induced on the left ear of Swiis mice (n = 5/group) by applying CO (200 µg) or phenol 10% (v/v). The right ear received the vehicle (acetone 70%) that was used to dilute the irritant agents. Six h after applying CO and 1 h after applying phenol, ear tissue was collected to perform the photoacoustic measurements using a custom-built experimental setup. Experimental protocol was approved by Ethics Committee on Animal Use in Research (ECAE/UEM 045/2012). **Results and Discussion:** The readings from the ear tissue showed the presence of two bands in the left ear spectrum centered around 225 and 270 nm after the CO-induced inflammatory response and 233 and 278 nm after the phenol-induced inflammatory response. These bands were absent in the right ear spectrum. Thus, these bands can be attributed to the presence of components that participate in inflammatory events, as the leukocytes recruited in the inflamed area as demonstrated in a previous study1, showing that PAS detected the inflammatory response in ear tissue. **Conclusion:** The presente results showed that PAS is an effective technique that may be used safely for determining the optical absorption characteristics of inflamed ear tissue, demonstrating the applicability of this non-destructive method for studies on skin inflammation. **Reference:** Y. G. Terentýeva et al. (2016) Methods Appl. Fluoresc. 4, 044010.

The Caspase-11-mediated non-canonical activation of the inflammasome plays an important role on the restriction of leishmania infection.

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Introduction: Leishmania parasites are the causative agent of leishmaniasis in humans, a disease that affects over 12 million people worldwide. The innate immune response against Leishmania spp., including TLRs and NLRP3 inflammasome activation, play an important role on the restriction of Leishmania spp. The function of another inflammatory caspase on Leishmania infection was not described. Thus, the aim of this work is to evaluate the contribution of the non-canonical inflammasome, mediated by caspase-11, in the recognition and restriction of Leishmania infection. **Methods and results:** Bone marrow-derived macrophages (BMDMs) from WT, Nlrp3$^{-/-}$ and caspase-11$^{-/-}$ mice were used on in vitro studies with Leishmania spp. After Leishmania infection, Casp11$^{-/-}$ BMDMs showed impaired Caspase-1 activation and IL-1β secretion when compared to WT mice, and were less capable of restricting L. major, L. amazonensis and L. braziliensis parasites, as showed by FACS and Giemsa staining. Pull-down assays revealed that different species of Leishmania activates Caspase-11. Moreover, mice were infected in vivo in the ear and the lesion was followed by fifteen weeks. After that, parasite titer in their ear and lymph nodes were determined. Casp11$^{-/-}$ mice displayed larger lesion and higher parasite burden compared to WT

mice. **Conclusion:** Our results suggest that Leishmania spp. trigger Caspase-11 activation, an important effector caspase involved in the non-canonical inflammasome activation, which has only been implicated in the control of gram-negative bacterial. Therefore, this work reveals that Caspase-11 is also an important mechanism for the restriction of parasites, with great implications on the course of Leishmaniasis. **FINANCIAL SUPPORT:** FAPESP; CRID/FAPESP; CNPq; INCTV/CNPq, PEW and CAPES.

Polymorphisms in the Transforming Growth Factor Beta 1 Gene Influence Human Papillomavirus Infection

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Objective: To assess the association between genetic variability in transforming growth factor beta 1 (TGFB1) gene and human papillomavirus (HPV) infection susceptibility. **Material and methods:** Cervical swabs and blood samples were obtained from 358 outpatient women, along with socio-demographic and sexual behavioral data. The study population was stratified by absence (n=179) or presence (n=179) of HPV DNA, as tested by PCR. Four single nucleotide polymorphisms (SNPs), c.−1638G>A, c.−1347C>T, c.29C>T, and c.74G>C, in the 5' region of TGFB1 were genotyped using PCR-restriction fragment length polymorphism. **Results:** Each polymorphism had the minor allele frequency higher than 5.0% and the linkage disequilibrium (LD) were higher between SNPs c.−1347C>T and c.29C>T (r2=0.93). Theoretically, twelve haplotypes were provided by PHASE software (version 2.1), however only four (GTCG, GCTG, GTCA, and CCCG) were more frequent than 5.0% (43.9%, 32.0%, 10.2%, and 5.1%, respectively). In a binary logistic regression analysis adjusted to age, tobacco status, and number of sexual partners during lifetime, GTCG haplotype was associated with a lower susceptibility of HPV infection, with odds ratio and 95% confidence interval of 0.57 and 0.34-0.93 (P=0.02). **Discussion:** The GTCG combination has been associated with low TGFB1 plasma level. Therefore, the protection against HPV infection afforded by the TGFB1 GTCG haplotype could be resulted of low TGFB1 level in cervical epithelium and, as consequence, a lower immunosuppressive microenvironment would favor the infection resolution. **Conclusion:** Although further studies are necessary, the presented data demonstrate that GTCG haplotype is significantly associated with protection against HPV infection. **Grants:** Capes, PPSUS, CNPq, Fundação Araucária.

Níveis de Imunoglobulina A salivar pré e pós treinamento contra-resistido

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A imunoglobulina A salivar (IgAs) é caraterizada como proteção primária servindo como indicador de disbiose. A redução em seus níveis pode ser correlacionado com alterações na flora oral, reprodutiva, respiratória e intestinal no entanto outro fator que interfere nos níveis de IgAs é a atividade física. O objetivo foi analisar níveis de imunoglobulina A salivar (IgAs) em relação ao estímulo pré e pós treinamento resistivo. Para realização do estudo participaram indivíduos frequentadores de uma academia na cidade de Juiz de Fora, MG. No treinamento contra-resistido realizaram a sequência: cadeira extensora com 3 séries de 10 repetições com 30kg; leg 45 com 3 séries de 10 repetições com 100kg e mesa flexora com 3 séries de 10 repetições com 30kg.Os dados dos níveis de IgAs foram obtidos através do doseamento em amostra de saliva, a coleta de saliva ocorreu em dois momentos em repouso pré treino e pós o processo de treinamento contra-resistido. O doseamento para IgAs foi realizado pelo método Imunoensaio Enzimatico (Elisa). Após obtenção dos resultados utilizou-se o test t-student. Para análise estatística foi utilizada no Software Prism para nível de significância para p<0,05. Os indivíduos participantes tinham idade entre 25 a 45 anos. O níveis de IgAs no pré treino (211,7 ±36,1 mcg/ml) e pós (360±45,6 mcg/ml) caracterizando aumento de 70% quando compara-se as etapas do treino. Muitos estudos afirmam que os níveis de IgAs diminuem durante a atividade física sendo imunossupressor, os resultados revelam valores de IgAs com aumento significativo, sendo necessários mais estudos nesta modalidade esportiva.

Evaluation of inflammatory cytokines in experimental rat model of abdominal aortic aneurysms with hipercholesterolemic diet

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Abdominal aortic aneurysm (AAA) is a chronic degenerative

disease reaching high morbidity and mortality levels. Weakening of aortic wall and dilatation up 50% are the mainly characteristics of AAA, associated with atherosclerosis and increase of proinflammatory cytokines. Was developed in our laboratory a traumatic model of abdominal aortic aneurysm (AAA) through the combination of two potential causes of metalloproteinases secretion and activation – acute inflammation and blood flow turbulence. The objective of this study was investigate the role of cytokines in this new traumatic model of abdominal aortic aneurysms associated with hypercholesterolemic diet. Methods: Forty-eight male wistar rats were divided into four groups: Control Standard Diet (CSd), Control hypercholesterolemic diet (CHd), Aneurysm Standard Diet (ASd), Aneurysm hypercholesterolemic diet (AHd), and were euthanized in 7 and 30 days post-surgery. Hypercholesterolemia was induced in rats feeding a standard diet supplemented with 4% cholesterol, 1% cholic acid, and 0.5% 2-thiouracil. This hypercholesterolemic diet started to be offered 2 days before surgery. The animals submitted to surgery induction of abdominal aortic aneurysms consumed the hypercholesterolemic diet for more 7 and 30 days. At 7 and 30 days post surgery the animals were euthanized, and the aortas were collected for morphological analyses, immunohistochemistry, Elisa and Western Blot for cytokines analysis [interleukin 6 (IL-6), Tumor necrosis factor alpha (TNF-alpha), transforming growth factor beta (TGF-beta) and Monocyte Chemoattractant Protein-1 (MCP-1)]. Results: The AAAs developed in this experimental model show an extraordinary aortic dilatation ratio with similar morphology to human abdominal aneurysms in just 7 days in approximately 65% of the animals. Macroscopic differences were no observed between Aneurysm Standard Diet (ASd) and in Aneurysm hypercholesterolemic diet (AHd). Beyond the elastic fiber destruction, deposition of collagen and intense remodeling process characterized by a severe inflammatory response, these alterations were directly related to the dramatic expressions increase of the IL-6, TNF-alpha, TGF-beta and MCP-1 in Aneurysm Standard Diet (ASd) and in Aneurysm hypercholesterolemic diet (AHd) compared to controls groups (CSd and CHd); $P < 0.05$. In the Control hypercholesterolemic diet (CHd) was observed a slight increase compared to control group (CSd) $P<0.05$. Conclusion: These results suggest that the IL-6, TNF-alpha, TGF-beta and MCP-1 may play a crucial and an important role in the development and progression in both aneurysms and atherosclerosis diseases. Moreover, this rat model has great potential for improving the understanding of the etiopathogenesis of AAA, as well as for investigating new therapeutic treatments.

Generation of PKM2 knockout HaCaT cell line by CRISPR/Cas-9 mediated genome editing

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Introduction/Aim: Keratinocytes are the cells that play a central role in skin inflammatory responses, participating in the development and maintenance of certain diseases such as psoriasis. It has been show that under inflammatory stimuli cells undergo a process of metabolic reprogramming, expressing genes involved in glycolysis such as PKM2. Recent studies have shown that PKM2 can be activated by inflammatory stimuli and is important for cytokine secretion and cellular proliferation. Results of our laboratory show that PKM2 is highly expressed in the skin of patients with psoriasis and animals with this disease. However, no studies in the literature show the participation of PKM2 in keratinocyte activation. Thus, the aim of this work is to generate a cell line of keratinocytes (HaCaT) knockout for PKM2 enzyme, as a tool for elucidate of the participation of PKM2 in keratinocytes activation, development and maintenance of psoriasis. **Methods/Results:** In this context, we used a method to selectively knockout PKM2 expression from mammalian cells using CRISPR/CAS9 technology. The CRISPR plasmid was generated by using a single-guide RNA targeting exon 10 of the PKM gene, with is responsible for the PKM2 expression, and then transfected several times in the HaCat cell line. Immunoblotting analysis showed that this processes impaired the PKM2 protein expression in some cells, but, consistent with an on-target effect, the PKM1 expression was not altered. **Conclusions:** Our data suggests that this powerful gene-editing technology can be used to dissect and analyze PKM2 signaling networks. **Financial Support:** FAPESP, CAPES, CNPq.

Cyclophosphamide modulated acute inflammatory reaction in fish experimental model

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Objective: The present study investigated the effects of cyclophosphamide (CYP) on the modulation of acute phase

proteins (APPs) and cellular accumulation in exudates present in the swim bladder of tilapias (aerocystitis fish-model). **Material and methods:** 80 tilapias, Oreochromis niloticus (± 150 g) were randomly divided into eight aquariums with 250 L of water (n = 10), to establish four treatments: T1 - Control NAIVE; T2 - Negative Control + Injected saline solution NaCl; T3- Positive Control + Injected A. hydrophila; T4- Cyclophosphamide (200 mg/kg) + Injected A. hydrophila. Samples of exudate and blood were collected 6 and 24 hours post-inoculation (HPI) for cell counts and determination of the APPs electrophoretic fractionation by SDS-PAGE, in-gel protein digestion and mass spectrometric identification. **Results and Discussion:** In the aerocystitis evolution, tilapia treated with CYP presented decreased in the circulating values of ceruloplasmin, complement C3, alpha2 macroglobulin and haptoglobin. The exudate evaluation revealed decrease ($p < 0.05$) the total number of cells accumulated in fish treated with CYP when compared the response observed in positive control fishes (T3), influenced mainly by the decrease of granulocytes. Similarly in mammals, several studies reported the suppressive effects associated the treatment with this alkylating agent. **Conclusion:** The treatment with 200 mg of cyclophosphamide/kg of body weight in O. niloticus modulated acute phase protein responses and cell accumulation at the inflamed site, demonstrating the potential of this experimental model for pathophysiology studies during acute inflammatory reaction.

Low-molecular-weight metabolites from murine melanoma tumor microenvironment induced macrophage lipid droplets formation.

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Melanoma, a malignant neoplasm has a poor prognosis in advanced stages, in addition to presenting high propensity to metastasize and be refractory to chemotherapy treatment. Tumor-associated macrophages (TAM) has been given attention by being somewhat related to promote tumor development. Lipid droplets (LD) also have importance by working like multifunctional organelles in the cellular pathway, activation, synthesis and secretion of inflammatory mediators. Herein, we demonstrated the effect of low-molecular-weight metabolites (LMWM) from murine melanoma B16F10 culture medium, on TAM-LD formation. Also, we observed the TAM inflammatory mediators production after LMWM stimulation. For this, we obtained molecular fractions from B16F10 medium, such as < 30 kDa and < 3 kDa. The culture of Bone Marrow-derived macrophage (BMDM) and total B16F10 medium triggered the LD formation, also induced high production of Nitric Oxide (NO), TNF-α, and modulated the BMDM phenotype. However, when the BMDM was stimulated with fraction < 3 kDa (LMWM) we still observed LD formation, but not others effects on TAM modulation. Those evidence suggests that TAM are involved in complex chemical cross talk with tumour cells. This interplay has the potential to modulate lipid metabolism, which may in turn change the ability of the tumour to thrive. **Financial Support:** FAPESP (2016/22899-3) and CNPq.

Prostaglandin E2 via EP2/EP4 receptors enhance plasticity of TH17 to TH1 cells

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Objectives: This study aimed to evaluate the effect of EP2 and/or EP4 receptors in the plasticity of Th17 to Th1 cells. **Material and Methods:** Naïve CD4+ T cells were isolated from spleen of C57BL/6 mice, activated with anti-CD3/CD28 and cultured for 4 days in Th17 polarizing conditions (IL-6, TGF-β, IL-1β, IL-23 and anti-IL-4/IL-2/IFN-γ). Then, Th17 cells were cultured in Th1 polarizing conditions (IL-2, IL-12 and anti-CD3), and then EP2 and/or EP4 agonist were added to the cell culture at the 4th, 10th and 16th day. Th17 and Th1 cell differentiation was evaluated by Flow Cytometry. **Results:** After 10 days, 51% of Th17 cells treated with EP2 agonist were able to produce IFN-γ, while EP4 agonist treatment induced 60,2% of IFN-γ-producing Th17 cells. On the other hand, on day 16, EP2 receptor seemed to be critical for the induction of IFN-γ-producing Th1 cells, since EP2 agonist led to three times more IFN-γ-producing CD4+ T cells than EP4 agonist. **Discussion:** These results suggest that PGE_2 via EP2 and EP4 receptors has a critical role in the Th17/Th1 plasticity. However, after cells start to change their phenotype towards Th1, EP2 seems to have an important role than EP4 at the final stage of plasticity. Yet, other experiments are needed to understand the kinetics of EP2 and EP4 receptors, in the context of Th17/Th1 cells plasticity. **Conclusion:** Taken together, these findings suggest, for the first time, that EP2 and EP4 can play an important role in different phases of Th17 to Th1 plasticity.

Adenosine production through CD39 contributes to regulatory T cell expansion and sepsis-induced immunosuppression via A2aR

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Aim: We investigate the role of adenosine in the induction of regulatory T (Treg) cells and development of sepsis-induced immunosuppression. **Methods and Results:** We found that adenosine concentration increases in sepsis-surviving mice. Adenosine has immunoregulatory properties through the activation of adenosine receptors, mainly A2aR. Indeed, we also found that blocked of A2aR improves resistance of sepsis-surviving mice against secondary infection induced by Aspergillus fumigatus, suggesting that this pathway may be implicated in the sepsis-induced immunosuppression. One the main pathway the adenosine production is catalyzes the sequential hydrolysis of ATP to adenosine by CD39. Interestingly, deficiency of CD39 also improves resistance of sepsis-surviving mice against secondary infection. Moreover, we also found that the surface expression and activity of CD39 is up regulated in macrophages of sepsis-surviving mice. Since Treg cells play an important role in the development of sepsis-induced immunosuppression, we investigated whether adenosine can induced the expansion of Treg cell population in sepsis-surviving mice. $CD39^{-/-}$ mice avoided the increase of Treg cell number in the spleen 15 days after CLP. Consistently, the blocked of A2aR also prevented the expansion of Treg cells in the spleen of sepsis-surviving mice. We then addressed whether M2 macrophages might induce to Treg differentiation via adenosine. Interestingly, deficiency of CD39 on M2 induced lower differentiation of Treg than WT M2 when co-cultured with WT T cells. **Conclusion:** Our data therefore provide evidence that macrophage-derived adenosine through CD39 promotes Treg cell expansion and mediates the development of sepsis-induced immunosuppression via A2aR. **Financial support:** FAPESP and CNRS.

High sodium levels have no impact on dendritic cell maturation and maintenance of peripheral tolerance

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Objective: We evaluated whether high NaCl concentration during efferocytosis of apoptotic cells (AC) can lead to dendritic cells (DC) maturation and cytokine production, leading to autoreactive CD4+ T lymphocytes. Material and **Methods:** As a source of AC, thymocytes were cultured in RPMI supplemented with 40mM of NaCl and submitted to UV irradiation (1 mJ). Bone-marrow derived dendritic cells from C57BL/6J mice were co-cultured with AC (ratio 1DC:3AC) during 18h in RPMI with or without 40mM NaCl. DCs were cultured in RPMI (40mM NaCl or isotonic) with or without LPS (1μg/mL). After 18h, DC phenotype was assessed by flow cytometry and cytokine production measured by ELISA. **Results:** Apoptosis induced by UV irradiation revealed 92% of thymocytes Annexin-V^+/$7AAD^-$. Also, phosphatidylserine exposure was twice higher on AC cultured with NaCl 40mM compared to isotonic media. Moreover, the presence of higher NaCl concentration improved DC efferocytosis capability. Unexpected, higher concentration of NaCl during efferocytosis had no effect on DC activation, since MHC-II, CD86 and CCR7 molecules expression remained unaltered compared to isotonic media. Regarding cytokine production, efferocytosis in hypertonic condition did not induced production of IL-1β, IL-6 and IL-12 by DCs. **Discussion:** The increased "eat-me" signals on the AC may have led to higher efferocytosis capability, potentiating the anti-inflammatory effects of efferocytosis over the pro-inflammatory role of NaCl. **Conclusion:** NaCl had no effect on DC maturation. Further studies are needed to clarify whether hypertonic condition could also improve release of "find-me" signals by AC and a possible upregulation of efferocytosis receptors on the DC. **Financial support:** FAPESP 2016/03967-2; 2011/17611-7; CNPq- 471945/2012-9; 302097/2010-4

Aryl hydrocarbon receptor (AhR) is crucial for the enzyme Indoleamine 2,3-dioxygenase (IDO) expression in sepsis-surviving mice

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Introduction: Sepsis leads to an immunosuppression state characterized by an increased number of regulatory CD4+/Foxp3+ T cells (Tregs). The molecular mechanism(s) underlying this process remain unclear. Indoleamine 2,3-dioxygenase (IDO), an enzyme involved in tryptophan metabolism, has been implicated in Treg expansion and T cell tolerance. Here, we investigate the role of aryl hydrocarbon receptor in IDO expression in sepsis-surviving mice and the role of IDO in sepsis-induced immunosuppression. **Material and Methods:** Severe sepsis was induced by cecal ligation and puncture (CLP) model in Wild Type (WT) or aryl hydrocarbon receptor (AhR) knockout mice in a B6 backgroud. Mice were treated with antibiotic to improve survival. A B16F10 melanoma mouse model was employed to check immunosuppression development. AhR and IDO expression were analyzed by WB, immunofluorescence or real time-PCR. **Results:** There is an increase in IDO protein expression and IDO enzymatic activity in spleen of sepsis-surviving mice. Pharmacological inhibition of IDO suppressed the enhanced tumor growth observed in sepsis-

surviving mice and decreased the expansion of Tregs. Using immunofluorescence staining we identified that a $CD11C^+$ population of cells are expressing IDO in spleen of sepsis surviving mice. AhR is associated with increased IDO expression found in $CD11C^+$ population. In addition, $CD11C^+$ cells isolated from spleen of sepsis-surviving mice, presented a higher capacity to induce a regulatory phenotype in naïve $CD4^+$ $CD25^-$ T cells than $CD11C^+$ isolated from naïve mice. **Conclusion:** AhR dependent IDO expression in $CD11C^+$ population is important to Treg cells expansion and immunosuppression development in sepsis-surviving mice. **Financial support:** FAPESP.

Prostaglandin E2 produced during efferocytosis of apoptotic-infected cells compromises Th17 cell differentiation by downregulation of STAT3 phosphorylation

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Objective: To evaluate whether PGE2 produced during efferocytosis of apoptotic-infected cells (IAC) interferes in STAT3 phosphorylation and gene expression involved in Th17 differentiation. **Material and methods:** Bone marrow-derived dendritic cells (DC) from C57BL/6J mice were treated in the presence or absence of indomethacin, and co-cultured with E. coli-infected apoptotic cells, for 18h. For T cell differentiation, naive CD4+ T cells were stimulated with anti-CD3/CD28 and incubated with supernatants from co-cultures of DC (CM) or DC treated with indomethacin (CM+Indo). Moreover, naive CD4+ T cells were pretreated with EP agonists/antagonists, as well as, protein kinase A (PKA) or EPAC activators. After 15 and 30 min., STAT3 phosphorylation was evaluated by PhosFlow Cytometry. PGE_2 and cytokines levels were determined from culture supernatant by ELISA. **Results:** Efferocytosis of IAC by DC promotes high levels of PGE2 production, and this prostanoid compromises Th17 differentiation as well as STAT3 activation. Both inhibitory effects were reversed when CD4+ T cells were cultured CM+Indo or EP4 antagonist. Moreover, PKA and EPAC agonists were also able to inhibit Th17 differentiation and STAT3 phosphorylation when CD4+ T cells cultured with CM+Indo. **Discussion:** These results expanded our understanding about the mechanism of action that PGE_2 inhibits Th17 differentiation during efferocytosis of apoptotic E. coli-infected cells. PGE_2 through EP4/PKA-EPAC inhibits STAT3 phosphorylation and impairs Th17 cell differentiation, in the context of IAC-phagocytosis. **Conclusion:** PGE^2 produced during phagocytosis of IAC dramatically inhibits Th17 cells differentiation through EP4/adenylate cyclase/PKA pathway, which leads to the inhibition of STAT3 phosphorylation. Further studies will be needed to completely elucidate this mechanism, evaluating the involvement of SOCS3 in STAT3 inhibition. **Financial Support:** FAPESP: 11/17611-7; 16/10964-5, 12/23580-0. CNPq: 130862/2016-9; CAPES.

Expression of signaling molecules by T lymphocytes in human tuberculosis

Maria Cláudia Magalhães Cavallini

In order to analyze IL-10 signaling pathways and their interactions with other cell profiles in human tuberculosis, we performed analyzes of IL-10 related genes expression (IL10, IL10R1, MAF, PRDM1), to disease-related response profiles (IFNG, IL4, IL17, IFNGR1, FOXP3, GATA3, TBX21, RORC, NOTCH1, NOTCH3) and PDCD1 by qRT-PCR of PBMC cultures of short duration (48 hours) and long (lineage- 15 days), stimulated by specific antigen BCG and anti-CD3, respectively, stemmed from peripheral venous blood samples of patients with active disease, treated and healthy controls (PPD+). Through our analyzes, we observed increases in the expression of IFNG, Th1 standard cytokine, in cured patients, evidencing the importance of this profile in clinical cure. Although no significant changes in IL-10 expression, we reported increases in IL-10´s transcription factors expression, MAF and PRDM1 in cured patients. Other genes such as IL17, IFNGR1 and NOTCH3 decreased their expression in cultures of these patients. Concluding, IL-10 signaling pathways may be able to interfere in different cellular response patterns, such as Th1, Th2 and Th17, and are able to modulate the expression of cytokines, transcription factors and receptors of these different profiles.

Study of the impact of the protective and nonprotective antibodies on the loading and immunogenicity of Histoplasma capsulatum extracellular vesicles

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Extracellular vesicles (EVs) are structures involved with membranes secreted by pathogenic fungi. Studies have shown that these vesicles contain macromolecules, including virulence factors that can modulate the host immune response. In the present work EVs released by Histoplasma capsulatum were evaluated by proteomic analysis in order to evaluate the impact of opsonization with two different monoclonal antibodies (mAbs), a protective 6B7 and non-protective 7B6, on EV loading and its impact on mammalian cells. These both mAbs binds to heat shock protein 60 (Hsp60) located on cell wall surface. EVs content

was regulated in a manner dependent and –independent of the concentration of mAbs. Enzymatic assays demonstrated that laccase activity in EVs was reduced after treating with 6B7, however, urease activity was not altered by any mAb treatment. Interestingly, the uptake of H. capsulatum by macrophages did not change after pre-incubation for 5 or 24 hours with EVs, but was inhibited when macrophages were exposed to EVs 1 hour prior the in vitro challenge. The fungal intracellular proliferation was inhibited in macrophages incubated with EVs from 6B7-treated H. capsulatum. Our findings suggests that EV sorting and secretion are dynamic mechanisms for a fine-tuned response by fungal cells.

Development of heterologous NaV1.8 sodium channel expression system for pain drug discovery

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Introduction/Aim: Neuropathic pain is a debilitating chronic syndrome that often arises from injuries to peripheral nerves. It is believed that to be the result of an aberrant expression and function of sodium channels at the site of injury. The sodium channel NaV1.8 is restricted to the periphery, predominantly in the nociceptive neurons of the dorsal root ganglia (DRG), has been extensively explored in the find for new chemical compounds useful in the treatment of chronic pain conditions. Pre-clinical results using compounds able to selectively block the NaV1.8 corroborate the efficacy of this therapeutic approach in the treatment of pain. Recently, our research group developed a series of compounds with possible activity in NaV1.8 channels. Therefore, this present work has as main objective the cloning of the murine NaV1.8 channel and the generation of a cell-based assay to test these new compounds. **Methods/Results:** Murine NaV1.8 (SCN10A gene) was amplified using cDNA from DRG sensory neurons and then cloned fused to EGFP in pCDNA3.1 plasmid. ND7/23, a neuroblastoma hybrid cell line, was transfected with cloned plasmid. Stable cell line was generated by positive and negative antibiotic selection. Individual resistant targeted ND7/23 clones started to grow and expressed EGFP. EGFP-positive clones were then picked up and screened for NaV1.8 expression confirmed by PCR and Western Blot. We confirm that six selected clones expressed NaV1.8-EGFP. **Conclusions:** Herein, we demonstrated that the NaV1.8-EGFP was successfully expressed in neuronal cell lines (ND7/23). We are currently performing others in vitro experiments, such as electrophysiology and FRET, using ND7/23-NaV1.8-EGFP cells to test the potential NaV1.8-inhibitory activity of the selected compounds. **Financial Support:** FAPESP/CAPES/CNPq.

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