Session 04 − **Inflammation**

04.001

Nitroxides regulate protein phosphorylation linked to NOX2 complex activity in neutrophils: prototype of a new anti-inflammatory. Ribeiro ACG[1], Chavasco LS[1], Santos GB[1], Cardoso MHM[2], Brigagão MRPL[1] [1]UNIFAL − Ciências Exatas, [2]UNIFAL − Farmácia

Introduction: Reactive oxygen and nitrogen species (ROS/RNS) released by neutrophils through the Nox2 complex are directly associated with the deleterious actions arise during inflammation processes. Nitroxides are synthetic antioxidants that have been used to protect animal tissues from oxidative/nitrosative damage. In this work, 4-hydroxy 2,2,6,6 tetrametilpiperidiniloxil (Tempol) and 2,2,6,6-tetramethyl-1-piperidinyloxy (Tempo) were used to investigate the nitroxide ability to regulate neutrophil protein kinase and phosphatase activities. Also, this modulatory post-translational event was explored directly associated to Nox2 activity. **Methods:** Inflammatory neutrophils were elicited from mice peritoneal cavity, incubated with nitroxides and then stimulated with phorbol (PMA) or chemotatic peptide (fMLP). Superoxide anion production release was determined spectophotometrically through cytocrome *c* reduction (550 nm). Neutrophil protein phosphorylation linked to Nox2 activation was evaluated by dot blotting. The kinase activity was assessed by luminescent assays (Kinase-Glo® Luminescent Assays) and the phosphatase activity was analyzed by spectrometric assays (Bioclin® Alkaline Phosphatase Test). **Results and Discussion:** The results suggest that nitroxides inhibit the production of $O_2^{\bullet-}$ by Nox2 decreasing the phosphorylation of neutrophil proteins elicited by PMA or fMLP, justified by the inactivation of protein kinase and activation of protein phosphatase. Therefore, the nitroxides are possible prototypes of a new class of anti-inflammatory compounds. **Bibliography:** BABIOR, B.M, *Curr Opin Immunol*, v.16, p.42, 2004. **License authorization number of animal ethic committee:** 205/2009. **Supported by:** CNPq-INCT of Processos Redox in Biomedicina-Redoxoma., FAPEMIG. **Thanks:** UNIFAL-MG, CNPq-INCT of Processos Redox in Biomedicina-Redoxoma, FAPEMIG

04.002

Periodontitis induces functional alterations in rat aorta. Campi P[1], Ceravolo GS[1], Martins Porto R[1], Maia-Dantas A[1], Yamamoto, M[1], Teixeira SA[1], Carvalho MHC[1], Herrera BS[2], Costa SKP[1], Spolidório LC[3], Muscará MN[1] [1]ICB-USP Farmacologia, [2]FO-UNESP − Patologia, [3]UNESP − Patologia

Introduction: Periodontitis (P) is a chronic disease characterized by impairment of the periodontal attachment apparatus and bone loss secondary to the intense inflammatory reaction of the host to bacteria. Based on emerging evidences showing that P can affect cardio-circulatory parameters, in this study we evaluated the effects of P on rat blood pressure (BP) and on the "*in vitro*" reactivity of aorta. **Methods**: The experimental protocol was approved by the CEEA-ICB (protocol 154, fls. 24, livro 2). Male Wistar rats (180-200 g) received daily oral doses of either etoricoxib (ET; 10 mg/kg) or L-NAME (LN; dissolved in the drinking water at 200 mg/L). On the 3rd.-day treatment, the rats had unilateral (first lower molar) ligature-induced periodontitis; sham (Sh) animals had the ligature placed and immediately removed. After 7 days, the rats were killed and thoracic aorta rings (4 mm length) were prepared and mounted for measurement of isometric tension in isolated tissue baths containing Krebs solution bubbled with O_2/CO_2, (95/5%) at 37ºC. Dose-tension curves to cumulative noradrenaline (NA) and acetylcholine (ACh; after NA pre-contraction) were obtained. Tail-cuff blood pressure (BP) was measured in all the animals before starting the treatments and 6 days after the ligature procedure. **Results**: Ligature-induced P had no effect on BP. Both LN and ET treatments resulted in significant BP elevation, but no differences were observed between Sh and P rat responses. The "*in vitro*" maximum contractile response (E_{max}) to NA was higher in aorta rings from P animals in comparison with Sh (1.67 ± 0.03 vs. 0.67 ± 0.01g, $p<0.001$); however, this pattern was reversed when the endothelial layer was rubbed off the rings (Sh: 2.50 ± 0.02 vs. P: 3.07 ± 0.04g, $p<0.001$). No differences in NA pA_2 were observed between Sh and P groups, either in the presence or absence of endothelium. ET treatment resulted in higher NA E_{max} in aorta from Sh animals (0.67 ± 0.01g vs. 2.40 ± 0.01g, $p<0.001$), while LN had no significant effects. Both ET and LN treatments significantly lowered NA E_{max} in aorta from P rats (0.950 ± 0.009g and 1.095 ± 0.003g vs. 1.66 ± 0.03g, $p<0,001$, respectively). ACh E_{max} was not different between P and Sh rats and, in both groups, LN treatment lowered ACh E_{max} (P: 65.1 ± 8.2 vs. 101.9 ± 3.1g, $p<0.001$; Sh: 56.4 ± 10.6 vs. 97.5 ± 2.2g, $p<0,01$) and augmented ACh pA_2 (P: 7.3 ± 0.1 vs. 6.4 ± 0.1, $p<0.01$; Sh: 7.5 ± 0.1 vs. 6.6 ± 0.3, $p<0.05$). ET treatment had no effect on aorta rings from Sh rats, but significantly reduced ACh E_{max} in aorta rings from P animals (83.9 ± 4.5vs.101.9 ± 3.1%,$p<0,05$) without changes in ACh pA_2 values. **Discussion**: Based on the above shown results, we can suggest that despite unilateral periodontitis is not enough to induce changes in BP in rats (even under NOS or COX-2 inhibition), endothelial dysfunction secondary to the oral disease is evident from the hyperresponsiveness pattern of aorta rings to NA and the complete reversal of this response by removal of the endothelial layer. In addition, the endothelium-dependent vasorelaxant activity of ACh is significantly dependent on COX-2, which supports a major role for prostacyclin in the maintenance of vascular tone homeostasis during the course of periodontitis. **Financial support**: CNPq, FAPESP, CAPES.

04.003

Effect of ovariectomy on LPS-induced acute lung inflammation in female mice. Gimenes-Júnior JA[1], Ligeiro de Oliveira AP[2], Vitoretti LB[1], Domingos HV[1], Oliveira-Filho RM[1], Vargaftig BB[1], Tavares de Lima W[1] [1]ICB-USP − Pharmacology, [2]ICB-USP − Immunology

Introduction: Acute respiratory distress syndrome (ARDS) is a multifactorial disease characterized by acute lung injury, severe hypoxemia, polymorphonuclear cells infiltration and lung edema. Lipopolysaccharides (LPS) from Gram-negative bacteria wall induce acute lung inflammation. Studies from our laboratory showed that female sex hormones (FSH) may exert protective or deleterious effect on Th2 lung inflammation, depending on the levels of FSH at the time of the sensitization to antigen. In this study, we investigated the role exerced by FSH on Th1 lung inflammation induced by LPS. **Methods:** Seven days after ovariectomy (OVx), female mice (c57Bl/6, n = 5–8/group) were subjected to intranasal instillation of LPS (100 µg/ml, 1 µl/g) or saline (0.9%, 1 µl/g). Twenty-four h thereafter, the animals were euthanized and the cells present in bronchoalveolar lavage (BAL), blood and bone marrow were quantified. Total cell countings were made in Neubauer chambers; differential countings were made upon optical microscopy. Otherwise intact animals were subjected to the same procedures and served as controls (Sham-OVx group). The experiments used in this study were approved by the local Ethics Committee on Animal Experimentation (Certification no. 113, 2009). **Results and conclusion:** The removal of the ovaries *per se* did not alter the total number of cells recovered in BAL compared with the control group (OVx: 13 ± 2 *vs* Sham-OVx: 23 ± 5 x 10^4 cells). Moreover, LPS increased the total number of cells in both OVx (194 ± 14) and in Sham-OVx groups (127 ± 14 x 10^4 cells) regarding their respective controls and the basal groups (14 ± 2 x 10^4 cells). Also, the increase in total cells observed in the OVx+LPS group was significant when compared with Sham-OVx+LPS. We observed an increase in neutrophils and lymphocytes in OVx+LPS (respectively, 146 ± 13 and 24 ± 1 x 10^4 cells) and in Sham-OVx+LPS (respectively 86 ± 12 and 13 ± 3 x 10^4 cells) as compared to the untreated groups (OVx: respectively 0.3 ± 0.1 and 3 ± 0.8 / Sham-OVx: respectively 1 ± 0.8 and 3 ± 0.9 x 10^4 cells). Furthermore, the group OVx+LPS showed a significant increase in the number of these cells as compared to the Sham-OVx group. No changes were observed in the number of macrophages in the lung. OVx induced a fall in bone marrow cells (OVx: 91 ± 30; Sham-OVx: 199 ± 28 x 10^5 cells). After instillation of LPS this reduction was exacerbated in the OVx group (50 ± 9) compared to Sham-OVx+LPS (212 ± 34 x 10^5 cells). No changes in the circulating leukocyte countings were detected among the groups. These data suggest that female sex hormones attenuate the LPS-induced lung inflammation. **Financial support**: FAPESP 09/52782-7

04.004

Initial characterization of toll-like receptor (TLR)4 signaling pathway on pollutant-induced increased neonate mice susceptibility to asthma. Santos KT[1], Florenzano J[1], Peron JPS[2], Muscará MN[1], Rizzo LV[2], Costa SKP[1] [1]ICB-USP − Farmacologia, [2]ICB-USP − Imunologia

Introduction: Low air quality, by traffic pollution, has been linked to increased prevalence of allergic diseases in susceptible individuals such as children (Bråbäck et al., Environ Health, 16(8):17;2009). Supporting this evidence, we have recently shown a mouse model of increased asthma susceptibility by using a chemical agent (e.g. 1,2-naphthoquinone; 1,2-NQ) commonly found in diesel exhaust particles (DEP; Santos et al., 41° SBFTE: Eventus. p.17;2009). **Objective**: This study was designed to characterize the mechanism pathway of 1,2-NQ-evoked increased asthma susceptibility in neonate mice by targeting the toll-like receptor (TLR)4, since these receptors have been involved in DEP-induced airway inflammation DEP (Williams et al., J Allergy Clin Immunol.,119(2):488-97;2007). **Methods**: Both C57BL/6 wild type (WT) and C57BL10/ScCr natural mutant of TLR4 and IL-12 (MT) neonates mice were used under the approved animal use protocol (ICB/USP: n. 170, book 2, page 80). Mice were nebulized with 1,2-NQ (100 nM; 10 mL) or vehicle (10 mL; PBS: 99,008%, Tween 80: 0,001%, DMSO: 0.001%) at day 6, 8 and 10 for 15 min. Eight weeks later, mice were sensitized/challenged with ovalbumin (OVA). The bronchial hyperresponsiveness (BHR) to MCh was measured via enhanced pause (Penh) test and inflammatory biomarkers assays were evaluated 24 h after last exposure to OVA. Data are presented as mean ± SEM. of n= 6-8 mice. Stats were performed by ANOVA followed by Bonferroni's test. $P<0.05$ was taken as significant. **Results**: neonate WT mice exposed to 1,2-NQ and later to OVA exhibited a marked increasing number of total cells at an adult stage (TC; 93 ± 11.0* x 10^4 cells/BAL), characterized by lymphocytes (LP; 22 ± 3.0** x 10^4 cells/BAL) and eosinophils (EO; 22 ± 4.0* x 10^4 cells/BAL) in the bronchoalveolar lavage (BAL) as compared to WT mice exposed to vehicle/OVA (TC: 65 ± 4.0, LP: 13 ± 2 and EO: 11 ± 1.0 x 10^4 cells/BAL). In parallel, the same trend was observed in the blood and in eosinophils maturation in the bone marrow. The Elisa assay in the BAL of WT mice exposed to 1,2-NQ/OVA showed a significant increase of INF-g (142 ± 16.0* pg/mL) and IgE (94 ± 8.0** pg/mL) when compared to WT group treated with vehicle/OVA (INF-g: 88 ± 9.0 pg/mL and IgE: 48 ± 2 pg/mL). The Penh responses were similar in both control and 1,2-NQ-treated groups. Inversely, in MT mice the same treatment with 1,2-NQ/OVA evoked a significant decreasing in the cell number in the BAL (TC: 18 ± 3.0**, LP: 2 ± 0.4*, EO: 5 ± 2.0*** x 10^4 cells/BAL) in comparison to MT mice exposed to OVA only (TC: 67 ± 10.0, LP: 6 ± 1.0 and EO: 30 ± 8.0 x 10^4 cells/BAL). The same decrease was also seen in the peripheral blood and bone marrow of these mice. Levels of INF-g (1.5 ± 0.1 pg/mL), but not IgE, in the BAL (119 ± 16.0* pg/mL) of MT mice treated with 1,2-NQ/OVA were reduced in comparison with MT mice treated with OVA (INF-g: 1.6 ± 0.1 pg/mL; IgE: 76 ± 09 pg/mL). **Conclusions:** This pilot study provides strong evidence that TLR4 pathway could be a contributing factor to mediate 1,2-NQ-induced increased neonate mice susceptibility to allergic response at an adult stage. This might explain partially the common pulmonary illnesses in children exposed to traffic pollution. **Acknowledgments:** FAPESP, CAPES, CNPQ. We thank Barreto MA for technical support.

04.005

Platelet activating factor participate in the control of adiposity and inflammatory process in epididymal adipose tissues of mice fed with palatable diet. Menezes Z[1], Oliveira MC[1], Shang, FLT[1] Lima RL[2], Teixeira MM[2], Ferreira AVM[3], Souza DG[1] [1]UFMG − Microbiologia, [2]ICB-UFMG, [3]UFMG − Enfermagem e Nutrição

Introduction: Obesity is increased in almost all societies in the world. Several studies have demonstrated that adipose tissue of obese individuals secretes several pro-inflammatory mediators generating a chronic inflammatory low grade. Platelet activating factor (PAF) is a potent phospholipid inflammatory mediator secreted by different cell types showing various effects, including chemotactic action. This study evaluated the role of the PAF receptor on the inflammatory response in adipose tissue of mice fed palatable diet. **Methods**: C57B/6 mice with genetic deletion of the PAF receptor ($PAFR^{-/-}$) and wild type (WT) were divided in four groups according to the diet given: WT control diet (WT-C), WT palatable diet (WT-P), $PAFR^{-/-}$ with diet control ($PAFR^{-/-}$-C) and $PAFR^{-/-}$ with palatable $PAFR^{-/-}$-P diet for 8 weeks. The animals were sacrificed and samples of epididymal (EAT), mesenteric (MAT) and retroperitoneal adipose tissue (RAT) were weighed. The plasma triacylglycerol (TAG) and total cholesterol was performed using enzymatic kits. The concentration of cytokines in the EAT was determined by ELISA. Through flow cytometry the percentage of inflammatory cells was determined using GF-/F4 80 + and CD4 + CD5 + markers, present in the stroma vascular EAT. Isolated adipocytes were used for real-time PCR and primary culture with hyperglycemic media (25 nM). **Results:** Was observed that the mRNA for the PAF receptor was present in adipocytes. A palatable diet was able to increase the weight of epididymal adipose tissue, mesenteric and retroperitoneal compared to controls mice (2 ± 0.18 vs. 0.74 ± 0.08, 0.53 ± 0.08 vs. 0.32 ± 0.04, 0.45 ± 0.07 vs 0.2 ± 0.04 g/100g BW, respectively). In the absence of PAF receptor palatable diet promoted a substantial accumulation of adipose tissue in the three sites examined, compared to its respectively controls (3 ± 0.21 vs 1 ± 0.09, 0.85 ± 0.05 vs 0.32 ± 0.04, 1 ± 0.1 vs 0.23 ± 0.03 g/100g BW). The concentration of triacylglycerol was increased in animals $PAFR^{-/-}$-P compared to control animals (101 ± 8 vs 45 ± 4). The diet promoted an increase in total cholesterol compared to control group (90 ± 3 vs 73 ± 2 mg/dL), however this increase was higher in group $PAFR^{-/-}$-P compared to control (126 ± 2 vs 84 ± 3). The cytokines TNF-α, IL-10, CCL5, IL-6, CCL3 and IL-β were increased by diet in the wild type fed palatable diet (1405 ± 87 vs 1056 ± 153, 1304 ± 65 vs 991 ± 95, 1279 ± 64 vs. 951 ± 77, 701 ± 79 vs 504 ± 50, 424 ± 20 vs 321 ± 33, 559 ± 29 vs 341 ± 35). In absence of PAF receptor palatable diet caused a decrease of the cytokines evaluated in comparison to the respectively control group (599 ± 83 vs 1485 ± 131 and 683 ± 87 vs 1152 ± 102, 721 ± 107 vs. 1259 ± 104, 274 ± 39 vs 604 ± 51, 259 ± 26 vs 420 ± 33, 219 ± 29 vs 513 ± 25). Palatable diet increased the percentage of cells expressing GF-/F4 80^{+} markers (74 ± 6 vs 55 ± 8), however, genetic deletion of PAF receptor did not alter this profile. Animals $PAFR^{-/-}$-P showed an increase in population of CD4 + CD25 + cells compared to control group (37 ± 4 vs 17 ± 1). **Discussion:** The results indicate that PAF receptor is important for the control of adiposity and plasma lipids as well as the increased of inflammatory mediators in the epididymal adipose tissue in mice fed with palatable diet. Our data suggests that inflammation in epididymal adipose tissue modulates the expansion of the tissue, partly by increasing the lipolytic rate. **Financial support**: FAPEMIG, CAPES, CNPq and FAPEMIG. CETEA: 264/08

04.006

Anti-inflammatory properties of fullerol in irinotecan-induced intestinal mucositis in mice. Arifa RDN[1], Madeira MFM[1], de Paula TP[1], Ávila TV[2], Souza DG[1], Menezes Z[3], Lima RL[4] [1]UFMG − Microbiologia, [2]UFPR − Farmacologia, [3]UFMG − Fisiologia e Farmacologia, [4]ICB-UFMG

Introduction: Irinotecan is a drug used to treat several solid tumors through the inhibition of DNA toposoimerase I. However, this drug has several side effects as mucositis and severe diarrhea which can lead to a disruption of the treatment. The use of nanocomposite could be an important pharmacology tool, because of its wide range of biomedical applications, and an important strategy for minimize those side effects. An example is Fullerol, a nanocomposite which is highly water soluble formed by hexagon and pentagon of carbon and 18-24 OH radicals. Fullerol have been shown to be excellent antioxidant and also demonstrated to absorb many oxygen radicals as well as antimicrobial effect by intercalation into biological membranes. Whereas free radicals are involved in inflammatory cascade is interesting to analyze the effect of this nanocomposite in intestinal inflammatory diseases. **Objective:** Thus the aim of this present study was investigate the effects of Fullerol in Irinotecan-induced mucositis in small intestine, in mice. **Methods:** Male C57BL/6 mice were divided in three groups of six animals. Intestinal mucositis was induced by intraperitoneal (i.p.) administration of Irinotecan. The first group (untreated) received i.p. Irinotecan (75mg/kg) daily for four days. The second group (treated) received Irinotecan (75mg/kg) and one dose of Fullerol i.p. (3mg/kg) twelve hours and one hour before of the first application of Irinotecan. The others doses were administered each 12 hours during six days. The control group received two daily administration i.p. of PBS (vehicle) during six days. The mice were sacrificed on seventh day after the first dose of irinotecan, tissues were removed and homogenized for analysis of leukocytes infiltrate, cytokines production, GSH dosage and histology. Animal procedures were performed in accordance with the Animal Care and Use Committee of UFMG. **Results and Discussion:** Compared to control, the untreated group has increase of neutrophils influx in small intestine (Control 6,21 ± 1,44, untreated 36,01 ± 8,47 , treated 6,42 ± 2,43 $p<0,05$), increase of lymphocytes in peritoneal cavity (Control 3,27 ± 0,72, untreated 17,86 ± 6,48 , treated 2,03 ± 0,43 $p<0,05$), increased IL-1β production in duodene (Control 775,8 ± 147,2, untreated 1688 ± 343,9 , treated 1235 ± 103,9 $p<0,05$), decrease of the intestine length (Control 39,83 ± 0,60 , untreated 35,33 ± 1,17 , treated 37,83 ± 0,69 $p<0,05$). Interestingly, all these parameters were decrease by Fullerol treatment. Further, compared to control group, untreated mice presented a great weight loss (Control 102,8 ± 0,51, untreated 90,21 ± 2,9 , treated 93,57 ± 2,59 $p<0,05$) and increase of eosinophils in ileum (Control 0,052 ± 0,012, untreated 0,196 ± 0,034 , treated 0,212 ± 0,0191 $p<0,05$). Nevertheless, these parameters were similar in treated and untreated animals. These results suggest an anti-inflammatory effect of Fullerol in Irinotecan-induced mucositis in small intestine in mice and its potential therapeutic use for treatment of inflammatory intestinal diseases. **Supported by** CNPq and FAPEMIG. License number of the Ethics Committee: 135/2010

04.007

The role of 5-lipoxigenase in an experimental periodontal disease by *Aggregatibacter actinomycetemcomitans* in a murine model. Madeira MFM[1], Silva TA[2], Corrêa JD[3], Mitre GC[1], Marprates CVB[1], Souza DG[1] [1]UFMG − Microbiologia, [2]UFMG − Patologia, Clínica e Cirurgia Odontológicas, [3]UFMG − Farmacologia

Introduction: Periodontal disease (PD) is a chronic inflammatory disease of the attachment structures of the teeth. The bacterial biofilm attached to the surface of the tooth in close association with periodontal tissues, is the etiologic factor of this disease. *Aggregatibacter actinomycetemcomitans* (*Aa*) is a Gram-negative periodontophatogen with a range of potential virulence factors. Arachidonic acid metabolites, such as leukotrienes, are inflammatory mediators that are likely to be involved in the pathogenesis of PD. Leukotrienes are short-lived lipid mediators that have potent pro-inflammatory biological activities and their production in inflammatory cells begins with the cleavage of arachidonic acid from nuclear membrane glycerophospholipids. The enzyme 5-lipoxygenase (5-LO) catalyses the conversion of arachidonic acid in Leukotriene B4 (LTB4), a potent lipid mediator with various biological activities mediated by specific cell surface receptors (BLTs). **Objective:** In the present work, our aim was to assess the role of 5LO in the experimental model of periodontal disease induced by *Aa*. **Methods:** C57BL6 wild type (WT) or 5LO deficient mice ($5LO^{-/-}$) received a direct injection of 1×10^9 CFU/mL of *Aa* strain FDC Y4 (diluted in 10 µL of PBS) into palatal gingival tissue. Immediately after, was performed an oral administration of the same inoculum diluted in 100 µL of PBS with 1,5% of carboxymethylcellulose. The protocol was repeated 48 and 96 hours later. Negative controls received only PBS (NI). After 30 and 60 days of infection, animals were sacrificed and tissues removed, processed and analyzed by ELISA, myeloperoxidase content, and histology, in order to evaluate the levels of cytokines and chemokines, neutrophil influx, inflammatory infiltrate and alveolar bone loss. CP 105696, a BLT1 antagonist, was used in order to evaluate, *in vitro*, the osteoclast activity in RAW 246-7 activated by *Aa* Y4 LPS. **Results:** WT mice presented increase of myeloperoxidase ($p<0,01$) after 30 days of infection and of IL-6 production ($p<0,001$) after 60 days of infection. Furthermore, this infection was followed by important alveolar bone loss after 30 ($p<0,01$) and 60 days ($p<0,001$) of infection, and by gingival epithelium hyperplasia. $5LO^{-/-}$ mice presented no significant increase of myeloperoxidase when compared to WT mice. Interestingly, although a significant production of TNF-α on 30^{th} day pi ($p<0,05$), and IL-6 on 60^{th} day pi ($p<0,05$) in $5LO^{-/-}$ mice, no significant alveolar bone loss or TRAP-positive cells was observed. Furthermore, the use of BLT1 antagonist on RAW 264-7 cells decreases the number of TRAP-positive cells after LPS activation. C**onclusion:** The leukotriene A4 appears to develop an important effect on osteoclast activity once $5LO^{-/-}$ mice presented less bone loss even with a significant production of IL-6 and TNF-α, and osteoclast-like RAW 264-7 cells had less differentiation in presence of BLT1 antagonist.

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04.008

Microspheres loading prostanoids as modulators of phagocytosis. Pereira PAT[1], Gelfuso GM[1], Santos DF[1], Nicolete R[2], Bitencourt CS[1], Faccioli LH[1] [1]FCFRP − Análises Clínicas, Toxicológicas e Bromatólogicas, [2]UNICEUMA

Introduction: Prostaglandins (PG) are arachidonic acid metabolites, which play an important role in inflammatory processes, by modulating cytokines release in both adaptive and innate immune responses. These compounds, however, possess poor hydro solubility and chemical instability, what make difficult the *in vivo* administration. It is well established that microspheres prepared with suitable polymers are able to improve stability and sustain the release of several pharmaceutically active substances, as well as to target their deliveries, depending to the pathology to be treated. **Objectives:** The aim of this work was, therefore, to prepare and characterize microspheres containing PGD2 or PGE2, following by the *in vitro* evaluation of their capability to be phagocyted by alveolar macrophages. **Methods:** PLGA [poly-(lactic acid-glycolic acid)] microspheres were prepared by the emulsification-solvent evaporation technique. Size distribution and zeta potential of them were evaluated in aqueous media by Light Scattering, and their morphology was analyzed by Scan Electronic Microscopy (SEM).The phagocytic index was stipulated by the number of phagocytic macrophages multiplied by the number of yeast phagocytosed, divided by the total number of macrophages counted. **Results and Discussion:** These analyses revealed that all the microspheres presented spherical shape, with no pores on their surfaces, mean size equal to 4.0 (± 2.5) µm, 3.7 (± 2.1) µm (for loaded-PGD2 and loaded-PGE2, respectively), and negative zeta potential (-10.1 ± 7.9 mV and -13.7 ± 5.7 mV, respectively). These size and charge features make the obtained microspheres proper to be administered through the intranasal route and able to attempt the lung. Phagocytosis profiles of both microspheres, as well as the unloaded ones, were determined after 4 h of their incubation (1 mg) with 2 x 10^5 cells of Wistar rats' alveolar macrophages. Our findings showed that the unloaded microspheres were phagocyted by the cells as expected. Phagocytic index of the unloaded microspheres (123.8 ± 3.3) was 65% improved by incorporation of PGD2, and decreased 77% by PGE2 incorporation. Equally, percentage of phagocytosis, i.e., the percentage of cells that phagocyted at least one microsphere, followed the same pattern with an increase of 36% for PGD2 and a decrease of 43% for the PGE2-loaded microspheres. **Conclusions:** The delivery systems obtained in this work presented features that make possible their intranasal administration. Even in different extensions, both loaded PGs were phagocyted by alveolar macrophages, suggesting their potential application to treat pulmonary inflammatory infections. **Financial support:** FAPESP and CNPq. **Ethics Committee**: 09.1.375.53.

04.009

Evidence of adenosine receptors in the inosine anti-inflammatory effects in a murine model of ovalbumin-induced asthma. Lapa FR[1], Ligeiro de Oliveira AP[2], Golega BA[2], Tavares de Lima W[2], Cabrini DA[1], Santos ARS[3] [1]UFPR − Farmacologia, [2]ICB-USP – Farmacologia, [3]UFSC − Ciências Fisiológicas

Introduction: Endogenous purines, including inosine, have been shown to exert immunomodulatory and anti-inflammatory effects in a variety of disease models, such as acute lung injury and ischemia reperfusion (Liaudet et al., Ann. Surg., 235:568, 2002; Mabley et al., Shock, 32(3):258, 2009). The aim of this study was to investigate the involvement of adenosine receptors in inosine effects in a murine model of ovalbumin-induced asthma. **Methods:** Female Balb/c mice were sensitized with ovalbumin (OVA, 10 µg) (day 0) and boosted (day 7) subcutaneously with OVA (10 µg). At day 14 and 15, mice were challenged with aerosolized OVA (1% in PBS) and treated 30 min before the OVA challenges by intraperitoneal route with inosine (10 mg/kg) or with the adenosine receptors antagonists. In addition, other group of animals was treated with specific adenosine receptor antagonists A_1 (DPCPX), A_{2A} (ZM241385), A_{2b} (alloxazine) or unspecific adenosine receptor antagonist caffeine 30 min. before the inosine treatment. On the day of experiments (24 h after the last OVA-challenge), the bronchoalveolar lavage (BAL) was obtained to perform the cell counting. Lungs fragments were obtained under sterile conditions and cultured-24 h (explant) for determination of cytokines. This study was approved by Ethics Committee of Federal University of Paraná (320) and USP (58). **Results:** Inosine significantly reduced lung inflammation, as indicated by decreased numbers of total leukocytes, macrophages, lymphocytes and eosinophils recovered in the BAL, when compared with the allergic control group, with ID_{50} of 0.3 (0.05-0.35) mg/kg and inhibitions of 60 ± 2%, 49 ± 3%, 67 ± 6%, 97 ± 6% at dose of 10 mg/kg, i.p., respectively. Pre-treatment with ZM241385 (1.5 mg/kg, i.p.) reverted 70 ± 5.1% and 97.6 ± 8.1% the inosine effects (10 mg/kg, i.p.) on the total leukocyte cell migration and macrophage count in the BAL, respectively. In addition the pre-treatment with caffeine reverted 86.5 ± 7.9%; 96.2 ± 4.1% and 68 ± 14,5% the total leukocyte counts, macrophage and lymphocyte count in the BAL. The treatment with DPCPX and alloxazine, did not alter the inosine effects. Furthermore, the treatment with inosine (10 mg/kg, i.p.) reduced the levels of IL-4 and IL-5 in explants challenged and not challenged with OVA with inhibitions of 40 ± 3%; 76 ± 4% and 68 ± 8%; 46 ± 13%, respectively. The pretreatment with caffeine reverted 96.5 ± 3.4% and 78 ± 8.4% the inosine inhibitory effects on release of IL-4 and IL-5, respectively, only in explants not challenged. In contrast, pretreatment with A_1 and A_{2A} antagonists did not interfere with inosine effects in the explants cultures. **Discussion:** inosine reduced total and differential cells count in the BAL from mice with ovalbumin-induced asthma. The effects observed with pre-treatment with ZM241385 and caffeine, suggests a partial involvement of A_{2A} receptor in inosine effect on cell migration. Moreover, inosine effects might involve a possible inhibition on cytokine release that might be related with A_2 and/or A_3 adenosine receptors. **Financial supports:** REUNI, CAPES, CNPq and FAPESP

04.010

Role of inflammatory chemokines and their decoy receptor D6 in modulation of the inflammatory response associated with murine GVHD. Castor MGM[1], Rezende B[2], Bernardes PTT[3], Reis AC[3], Teixeira MM[2], Locati M[4], Pinho V[2] [1]UFMG − Fisiologia e Farmacologia, [2]ICB-UFMG − Bioquímica e Imunologia / Morfologia, [3]ICB-UFMG − Morfologia, [4]Universitá di Milano

Understanding pathophysiological mechanisms involved in graft versus host disease (GVHD), a common disease following bone marrow transplant, could lead to the development of new co-adjuvant therapies for transplant patients. It has been established that leukocytes recruited by inflammatory chemokines orchestrate the inflammatory response and consequent injury associated with GVHD. We believe that a better understanding of the role of chemokines and chemokine receptors in GVHD may open new opportunities as adjuvant therapy in transplant patients. On the basis, the present study verified the role in GVHD of D6, a chemokine "decoy" receptor which has been shown to bind and degrade several inflammatory CC chemokines. Transplant of splenocytes D6-deficient leads a lesser clinical disease and retard of mortality compared to WT donor cells. This is associated with myeloid suppressor cells that are increased in spleen of D6-deficient mice. Transplant of splenocytes D6-deficient depleted of myeloid suppressor cells lead a reversion of protector effect observed in allogeneic transplanted mice with splenocytes D6-deficient. In the other hand, transplant of allogeneic lymphocytes and myeloid suppressor cells results in the same protection observed thru allogeneic D6-deficient splenocytes. Also D6-deficient mice are partially protected when submitted to GVHD. This protection is correlated to increase circulating levels of CCL2. Others studies realized in our laboratory have revealed an association between increased CCL2 levels in blood and mobilization from bone marrow of myeloid suppressors cells, which displayed an immunoregulatory profile acting at T cells activation, proliferation and differentiation. On these bases, D6 may play a role on the mobilization of myeloid suppressors cells and these cells could be responsible by partial protection observed in D6 deficient mice. Now we are starting study the functional characterization of these cells from D6-deficient mice and from WT mice *in vitro*. **Financial support**: Capes, Cnpq, Fapemig and Innochem

04.011

Exacerbation of dengue disease by the blockage of NADPH-oxidase complex and nitric oxide production. Avila TV[1], Costa VV[1], Fagundes CT[1], Silveira KD[2], Morcatty TQ[1], Valadão DF[1], Santos AG[1], Prosperi T[1], Souza DG[1], Silva TA[1], Teixeira MM[2] Souza DG[1]
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Introduction: Dengue is one of the most important vector-borne viral diseases in the world and constitutes a serious world health problem. It's caused by four dengue related virus serotypes (DENV 1-4) and is transmitted by *Aedes* mosquito. Dengue is characterized by thrombocytopenia, increased levels of cytokines, increased vascular permeability, hemorrhage and shock. Although, knowledge about the mechanisms involved in the pathogenesis of this disease is incipient. There are several reports documenting the generation of free radicals (ROS and RNS) after viral infections. These radicals can modulate the permissiveness of cell to viral replication and an important role is attributed to them during the host response to infection. However the role of NADPH-oxidase complex and nitric oxide effects in dengue virus infection is controversial. **Objectives:** Evaluate the role played by NADPH-oxidase enzymatic complex and NO during dengue virus infection. **Methods:** This project was previously approved by CETEA/UFMG (113/09). Wild-type (WT), NADPH-oxidase inhibitor (Apocynin) treated mice (20mg.kg-1) on days 0 to 7 (APO 0-7) or 5 to 7 (APO 5-7) p.i and knockout mice for iNOS ($iNOS^{-/-}$), all background C57/BL6 were infected with 10PFU/100µL of the adapted DENV-3 by i.p route. Using FACS analysis, ROS production was measured with the help of the DCF fluorescence. IHQ for iNOS on liver of WT mice and nitrite measurement in supernatant of DCs culture (Griess assay) was performed. On day 7 p.i, evaluation of hematological signals: hematocrit (%), platelets counts and hepatic transaminases (AST/ALT) levels in serum (Bioclin/Quibasa Kits), inflammatory parameters (cytokines and chemokines) by ELISA (R&D systems) and neutrophils recruitment by the evaluation of MPO activity were performed. Also, appreciation of viral load by plaque assay was conducted. Tissue damage was verified by histological analyses in liver (H&E staining). **Results and Discussion:** ROS levels were significantly increased, mainly on day 5 and 7 p.i, in WT mice when compared with non-infected controls and. iNOS expression is enhanced in WT mice on days 5 and 7 p.i and nitrite production is elevated in culture 72hs p.i. APO 0-7 treated mice and $iNOS^{-/-}$ showed more severe disease manifestation when compared to control littermates, assessed by an increase in thrombocytopenia, hemoconcentration and elevated systemic levels of IFN-g TNF-a, IL-6 and CXCL-2. In addition, there were elevated serum concentrations of AST/ALT, which is closely correlated with higher tissue damage seen in liver when compared with WT controls. This enhanced susceptibility to infection was correlated with the elevated viral load in APO 0-7 treated and $iNOS^{-/-}$ mice. However, APO 5-7 treated mice showed no difference in all parameters analyzed when compared with WT mice, suggesting that ROS production is important in the early stages after infection. Here, we suggest that ROS producing by NADPH-oxidase complex and NO production induced by iNOS are fundamental pathways during response to DENV-3 infection, mainly in the control of viral replication. In absence of these free radical species, there is reduced ability of viral replication control by host, resulting in a more severe disease manifestation. **Financial support:** INCT em dengue **(**CNPq), CAPES and FAPEMIG.

04.012

Change of skeletal muscle caffeine-induced contracture by sepsis. Pachá BP[1], Borges RS[1], Rico, T B[2], Carmo PL[1], Benjamim CF[2], Zapata-Sudo G[1], Sudo RT[1] [1]UFRJ − Fármacos, [2]UFRJ − Farmacologia Celular e Molecular

Introduction: Sepsis is a systemic inflammatory response syndrome (SIRS) caused by infection that significantly contributes to the high morbidity and mortality. During the SIRS, hyperthermia/hypothermia, tachycardia, tachypnea and dysfunction of multiple organs can be observed in the patients [1].Sepsis has been associated to severe manifestation of malignant hyperthermia (MH) crisis during general inhalational anesthesia. MH is a pharmacogenetic disorder characterized by abnormal increase of Ca^{2+} release from sarcoplasmic reticulum (SR) triggered, in susceptible patients, by volatile halogenated anesthetics and/or succinylcholine [2]. MH is caused by genetic mutation in the channel of Ca^{2+} release from the SR (RYR1). The purpose of this work was to evaluate if the caffeine-induced contracture in skeletal muscle is modified by systemic sepsis. **Methods:** The Ethics Committee for Animal Investigation at the Federal University of Rio de Janeiro approved this protocol (DFBCICB 028). Swiss female mice (18-25 g) were subjected to cecal ligation and puncture (CLP) model, whereby the cecum was partially ligated and puncture four times with a 21G needle. Sham-operated mice were used as control. Twenty four hours later the animals were killed under anesthesia by ether and the extensor digitorium longus (EDL) and soleus (SOL) muscles dissected and prepared for isometric tension recording. Muscles were mounted in vertical chambers (15 ml) in which one end of each muscle was attached to a force transducer (Grass FT03) and the other to a fixed clamp. Chambers were filled with Krebs solution (in mM: NaCl 135; KCl, 5; $MgCl_2$ 1; $CaCl_2$ 2; $NaHCO_3$, 15; $NaHPO_4$ 1; glucose, 11; pH 7.4; in 95% O_2, 5% CO_2) at 37 ± 0.1^oC. The muscles were electrically stimulated (S88H Grass Stimulator) with 40-50 V, 2 ms duration, 0.2 Hz frequency. The signals generated by the transducers were collected by Cyberamp, digitalized (Digidata 1322) and stored for later analysis using the program Axoscope 8.0 software on a computer. After stabilization of muscle contraction (ca. 40 min), the muscles were exposed to increasing concentrations of caffeine (0.5 − 32 mM) added into solution. The results were expressed as the relation between maximal muscular contracture in different caffeine concentrations and muscular twitches (Pt) measured at the beginning of the experiments. **Results and Discussion:** The caffeine-induced contractures in SOL (n= 8) and EDL (n= 8) from sham was compared to SOL (n= 6) and EDL (n= 10) from CLP muscles. In the SOL muscles a significant difference was observed at higher caffeine concentrations. At 8 mM, the caffeine-induced contracture in sham and CLP muscles were 2.96 ± 0.39 and 5.57 ± 0.98 ($P<0.05$), respectively, and 6.67 ± 0.67and 10.09 ± 1.27 at 16.0 mM, respectively. In the EDL muscles a more significant response ($P<0.05$) was observed from CLP (3.72 ± 0.52) than sham (1.65 ± 0.46) at 8 mM caffeine. The higher caffeine contracture in SOL and EDL muscle from CLP than sham suggest an increase of Ca^{2+} release from sarcoplasmic reticulum caused by systemic inflammation. Thus, the sepsis could be a clinical manifestation to increase the MH expression during general anesthesia. **References**: [1] Davies MG et al. *Br J Surg* 84:920,1997; [2] MacLennam DH et al. *Science* 256:789,1992. **Financial support**: FAPERJ, CNPq, INCT/INOFAR, FUJB, PRONEX.

04.013

Evaluation of mechanisms of action of fatty acids from vegetables oils on wound healing. Brogliato AR[1], Figueiredo JB[2], Branco AMC[1], Almendra LR[2], Martins V[1], Monte-Alto-Costa A[3] Benjamim CF[4] [1]UFRJ − Farmacologia, [2]ICB-UFRJ, [3]UERJ − Histologia e Embriologia, [4]UFRJ − Farmacologia Básica e Clínica

Introduction: Wounds of difficult healing affect 2% of population in developed countries. Despite clinical relevance, in Brazil there aren´t statistical data until now. Researchers have been showing that fatty acid from vegetal origin improves the wound healing. Currently, oils for wound treatment have been used in Brazil but do not have scientific endorsement. Therefore, our aim was to describe the effect of LM1, LM2 and LM3 oils as well as Curatec AGE® (commercial product of these oils) in wound healing.
Methods: The wound was surgically induced in mice and treated daily with the separated oils and Curatec AGE®. Mineral oil was used as control. This protocol was approved by ethic committee (process number: DFBCICB 028). **Results:** All oils induced an increase of neutrophil accumulation in the wound at day 1 after injury, at days 4, 7 and 14 (except for Curatec AGE® that showed a peak at the 4th day) no differences were observed compared with control. High levels of IL-6 at day 7 were observed in LM2 group when compared with other groups. LM2 and Curatec AGE® showed an increase of new blood vessels. Fibroblasts are predominant in LM3 group. However no differences in wound closure were observed between all groups. Currently, we have been doing immunohistochemistry for new blood vessels (CD34), cytokines such as IL-1B and TGF beta. In addition, we intend to evaluate the wound cellular profile by flow citometry and the collagen I formation by Picro Sirius. **Discussion:** The oils were pro-inflammatory only in early days after injury. The presence of new blood vessels and fibroblasts indicate granulation tissue formation that was not evident in control group. Data suggest that fatty acids from these oils affect the wound microenvironments leading to an improvement of new tissue formation after injury. **Financial support**: LM Farma Comércio e Indústria Ltda., CNPQ,CAPES and FAPERJ.

04.014

Cooperative DP1- and CRTH2-activated signaling is required to elicit enhanced leukotriene C_4 synthesis induced by prostaglandin D_2 within eosinophils. Mesquita-Santos FP[1], Bakker-Abreu I[2], Luna-Gomes T[2], Bozza PT[3], Diaz BL[2], Bandeira-Melo C[2] [1]UFRJ / FIOCRUZ − Inflamação / Imunofarmacologia, [2]IBCCF-UFRJ − Inflamação, [3]FIOCRUZ − Imunofarmacologia

Introduction: During allergic response several inflammatory mediators are produced. Among these products, prostaglandin D_2 (PGD_2) have been described as a major metabolite of arachidonic acid released by activated mast cells, that is able to induce eosinophil migration. Recently, we described that PGD_2 is able to trigger eosinophil activation, inducing lipid bodies (LB) biogenesis and leukotriene C_4 (LTC_4) synthesis (Mesquita-Santos, F.P., *J. Immunol*. 176(3):1326-30, 2006). PGD_2 is known to exert its effects through 2 receptors constitutively expressed on eosinophils, DP1 and CRTH2. Specifically concerning PGD_2 ability to trigger eosinophil chemotaxis, simultaneous activation of DP1 and CRTH2 displays opposing effects. Here, we investigated how PGD_2 receptors interact to elicit LB biogenesis and LTC_4 synthesis. **Methods:** For *in vivo* studies, we have used a model of PGD_2-induced eosinophilic inflammation (approved by CEUA/FIOCRUZ − L002/09). Briefly, *Swiss* mice (18-20g) sensitized with $Al(OH)_3$ and ovalbumin at day 0 and 7, were stimulated with intrapleural injection of PGD_2 (35 pmol/cavity) at day 14, 30 min after pre-treatment with DP receptors antagonists. Eosinophils influx, LB biogenesis and LTC_4 synthesis were evaluated within 24 h. For *in vitro* studies, human eosinophils were isolated from peripheral blood of health volunteers with a negative selection kit (StemCell Technologies; human studies approved by 052/09 CEP UFRJ/HUCFF). PGD_2-stimulated human eosinophils, antagonists and agonists of DP1 and CRTH2 receptors (BWA868c, BAY-u3405 or CAY10471 20 mM – 30 min before stimulation) were employed to identify the PGD_2 receptor responsible for LTC_4 secretion. The signaling pathways involved were also investigated. **Results:** *In vivo* and *in vitro* assays with receptor antagonists showed that PGD_2-triggered cysLTs secretion depends on activation of both DP1 and CRTH2 receptors. Moreover, DP1 and CRTH2 agonists were able to elicit cysLTs production solely under simultaneous activation of both receptors. By immuno-detecting intracellular LTC_4, we demonstrated that LTC_4 synthesis, but not LTC_4 transport/export, is the event activated by dual PGD_2 receptor stimulation on eosinophils and that lipid bodies (lipid droplets) are the intracellular compartments of DP1/CRTH2-driven LTC_4 synthesis. Although not sufficient to trigger LTC_4 synthesis, DP1 activation *per se,* signaling via PKA activity, was accountable for activating eosinophil lipid body biogenic process, identified as a prerequisite for successful PGD_2-induced LTC_4 synthesis. Concurrent CRTH2 activation added complementary signaling which was shown to be necessary to mount an effective LTC_4 synthesizing activity.

Discussion: Based on pivotal roles of cysLTs to allergic inflammatory pathogenesis and the collaborative interaction between PGD_2 receptors unveiled here, our data suggest that both DP1 and CRTH2 antagonists might be attractive candidates for anti-allergic therapies. **Financial support**: CNPq / FAPERJ

04.015

L-arginine up-regulated and protects the skeletal muscle tissue after resistance training for production of collagen, TGF-β and decreased TNF-α. Morais, SRL[2], Mello, WG[2], Oliveira SHP[1] − [1]UNESP-Araçatuba − Farmacologia, [2]UNESP-Araçatuba − Fisiologia

Objectives: Resistance training (RT) promotes the increase of cytokines and free radicals, leading to oxidative damage, mainly in skeletal muscle. The aim of this study was to investigate the inflammatory mediators and the mechanisms involved in the practice of RT, supplementation with L-arginine in order to analyze a possible protective role of this amino acid on the effects of RT. **Methods:** Male Wistar rats, 180 − 200 g were divided into four groups (post-exercise, 8 hours after exercise, 24 hours after exercise and 48 hours after exercise), each group being divided into supplemented (n = 10) with L-arginine (1g/kg) and not supplemented (n = 10) (carrier vehicle), both by gavage one hour before the start of training. The animals were subjected to RT-oriented practice for four days, through a staircase with 80° tilt, with the overload apparatus corresponding to 80% relative to the body weight. We collected peripheral blood, skeletal muscle (extensor digitorum longus), for analysis of myeloperoxidase, TGF-β and collagen type 1 mRNA expression by RT-PCR and CINC-2 and TNF-α production by ELISA. The samples were collected shortly after the last session of the program oriented RT offered and the 8, 24 and 48 hours after exercise and kept in a freezer at -70°C until the early tests. **Results:** Our results demonstrate that the peak of neutrophil migration into the muscle tissue was observed 24 hours after training oriented (10.260 ± 1.750), interestingly; the L-arginine reversed the process (5.269 ± 0.8814). Regarding the TGF-β expression we observed that there was increased expression in muscle tissue of animals treated with L-arginine (average: 7208) immediately after exercise declining to control levels after 24 hours (average: 4222). Conversely the expression of type 1 collagen in the muscle tissue increased 24 hours (average: 9552) after exercise remained stable until 48 hours (average: 1075). A high production of TNF-α in muscle tissue was observed 24 hours after exercise (2043 ± 28.7), being reduced by treatment with L-arginine (674.2 ± 14.43). For CINC-2 there was a high production of this chemokine in the muscle tissue 8 hours after exercise decreasing after 48 hours and pretreatment with L-arginine tended to change only the production 8 hours post-exercise, but it was not statistically significant. There was a considerable decrease in the concentration of C-reactive protein on two occasions, eight hours post-exercise (33.17 ± 5.448) (9.953 ± 2.131) $p < 0.001$, and 24 hours post-exercise (22.26 ± 1.934) (11.97 ± 0.9029) $p < 0.05$. **Conclusion:** We can conclude that the peak of inflammation resulting from RT was more evident 24 hours after exercise, since we observed increased neutrophil infiltration into the muscle tissue. Supplementation with L-arginine was effective in decreasing inflammation in this period favoring the regeneration process of skeletal muscle fibers, since we observed an increased production of type 1 collagen possibly due to activation of local resident cells by TGF-β. Taken together our data suggest a possible protective role of L-arginine after RT. **Financial** support: CAPES; FAPESP.

04.016

Effects of resveratrol on the pruritogenic and inflammatory events evoked by trypsin in mice. Lazarotto LF[1], Pereira PJS[2], Souto AA[3], Campos MM[4], Morrone FB[3] [1]PUCRS − Farmácia, [2]PUCRS − Medicina e Ciências da Saúde, [3]PUCRS − Biologia Celular e Molecular, [4]PUCRS − Cirurgia-Odontologia

Introduction: Pruritus is an unpleasant cutaneous sensation leading to the desire to scratch (Steinhoff et al., J Invest Dermatol., 126, 1705, 2006). It serves as a physiological self-protective mechanism against harmful agents and is a main symptom of many diseases. In some cases, especially when pruritus becomes severe, conventional therapies fail to alleviate this condition (Ständer et al., Arch Dermatol., 139,1463, 2003). Resveratrol (3, 4´, 5-trihydroxystilbene) is a polyphenolic compound produced in response to stress in plants such as Vitis vinifera, and it is present in red wine, grapes, berries and peanuts (Harikumar et al., Cell Cycle., 7, 1020, 2008). It has been reported that resveratrol exhibits its anti-inflammatory properties by inhibition of NF-kB and COX-2 activity and it also suppresses TNFα and IL-1β production (Baur and Sinclair, Nat Rev Drug Discov., 5, 493, 2006; Elmali et al., *Inflammation*, 30, 1, 2007). Resveratrol has also been shown to inhibit PI3K/Akt pathway (Roy et al., *Pharm Res*., 26, 211, 2009). This study investigated the effects of resveratrol in the pruritogenic and inflammatory responses evoked by trypsin. **Methods:** Male Swiss mice (n=8, 25-30 g) were used. All the experimental protocols were approved by the Local Ethics Committee (07/03611-PUCRS). Animals were pretreated orally with resveratrol (50 and 100 mg/kg), 30 min before trypsin. The control groups received saline by the same route. Scratching behavior was induced by a 50 ml intradermal (i.d.) injection of trypsin (200 mg/site) in the mouse dorsum. The animals were observed for 40 min, and the scratching behavior was measured as the number of scratches with forepaws and hindpaws close to the injected site, and/or behind the ears. Another parameter evaluated was the neutrophil recruitment to the site of injection (dorsum) by the indirect assessment of myeloperoxidase (MPO) activity. In order to analyze the effects of resveratrol in the paw edema produced by trypsin, mice received a 50 µl intraplantar (i.pl.) injection of trypsin (30 µg/paw) in the right hindpaw. The left paw received 50 ml of saline and it was used as the control. Edema was determined with a plethysmometer, at different time points (0.5 to 6 h). **Results:** The administration of resveratrol at the dose of 50 mg/kg promoted a significant reduction of scratching behavior elicited by trypsin (45 ± 8%), while the administration of 100 mg/kg produced a partial, but not significant reduction of this parameter (33 ± 12%). The animals administered with resveratrol (50 mg/kg) showed a 37 ± 11% inhibition of MPO activity. The treatment with resveratrol (50 mg/kg) was also able to significantly reduce the paw edema formation. The percentage of inhibition, calculated based on the area under the curve was 39 ± 5%. **Discussion:** Our data suggest that oral administration of resveratrol was able to prevent the pruritogenic and inflammatory effects elicited by trypsin. It is possible to infer that resveratrol might be useful to control symptoms of inflammatory skin diseases. Other studies are being conducted to verify the effects of a topical preparation of resveratrol in our experimental paradigm. **Financial support:** BPA-PUCRS, CNPq.

04.017

Evaluation of selective phosphatidylinositol-3 Kinaseγ inhibitors in the inflammatory, nociceptive and pruritogenic responses induced by different agents in mice. Pereira PJS[1], Lazarotto LF[2], Leal PC[3], Calixto JB[4], Morrone FB[5], Campos MM[6] [1]PUCRS − Medicina e Ciências da Saúde, [2]PUCRS − Farmácia, [3]QMC-CFM-UFSC, [4]UFSC − Farmacologia, [5]PUCRS − Biologia Celular e Molecular, [6]PUCRS – Cirurgia-Odontologia

Introduction: It has been described that PI3Kγ plays a pivotal role in inflammation, and it is involved in chronic inflammation and autoimmune diseases (Fougerat et al., Circulation, 117, 1310, 2008). This study investigated the effects of selective PI3Kγ inhibitors in the nociceptive, inflammatory and pruriceptive responses induced by different pro-inflammatory agents in mice. **Methods:** Male Swiss mice (8 per group, 25-30 g) were used. All the experimental protocols were approved by the Local Ethics Committee (09/00101-PUCRS). Mice were treated orally with the selective PI3Kg inhibitors AS605240 (1 to 30 mg/kg), AS041164 and AS252424 (both 1 mg/kg), 30 min before. The control groups received saline. Edema was induced by an intraplantar (i.pl.) injection of the protease trypsin (30 µg/paw, 50 µl) in the right hindpaw. The left paw received 50 ml of saline. Edema was determined with a plethysmometer, at different periods of time (0.5 to 6 h). Another parameter analyzed was the scratching behavior evoked by trypsin (200 mg/site, 50 ml) or by the mast cell depletor CP48/80 (10 µg/site, 50 ml) in the mouse dorsum. Scratching was measured for 40 min, as the number of scratches with forepaws and hindpaws close to the injected site and/or behind the ears. For evaluating spontaneous nociception, mice received an i.pl. injection of trypsin (300 mg/paw, 20 ml) or the active red pepper principle capsaicin (1.6 µg/paw, 20 ml) into the right hindpaw, and the amount of time (in s) spent licking and/or biting the injected paw was recorded during 10 min. **Results:** AS605240 administered orally produced a significant and dose-dependent reduction of paw edema induced by trypsin. The percentages of inhibition, calculated based on the area under the curve, were: 24 ± 7%, 46 ± 3%, 40 ± 7% and 42 ± 3%, for the doses of 1, 3, 10 and 30 mg/kg, respectively. In addition, the treatment with AS605240 produced a marked reduction of scratching behavior elicited by trypsin (200 µg/site). The inhibition percentages were 60 ± 8%, 57 ± 12% and 51 ± 8% for the doses of 1, 3 and 10 mg/kg, respectively. However, the administration of AS041164 and AS252424 was not able to significantly affect trypsin-induced scratching response. Moreover, AS605240 (1 mg/kg) was also capable to produce a partial, but significant inhibition of the scratching bouts elicited by CP 48/80 (25 ± 6%). Finally, AS605240 promoted a significant reduction of spontaneous nociception induced by trypsin in the mouse paw, at the dose of 1 mg/kg (61 ± 7%). On the other hand, a higher dose of AS605240 (10 mg/kg) did not significantly alter this response. Likewise, the administration of AS605240 (1 mg/kg, 30 min) did not modify capsaicin-evoked nociception. **Discussion:** The present results suggest that AS605240 was the most effective PI3Kg inhibitor in the inflammatory and nociceptive models tested herein. It is tempting to suggest that this compound might well represent a promising alternative for treating inflammatory-related painful conditions. **Financial support:** CAPES, CNPq, PUCRS.

04.018

Effect of Annexin-1 derived peptide AC2-26 on allergic lung inflammation in mice. Matheus-Souza D[1], Trentin PG[2], Arantes ACS[2], Ferreira TPT[2], Pires ALA[2], Flower RJ[3], Perretti M[3], Martins MA[2], Silva PMR[2] [1]FIOCRUZ − Inflamação, [2]IOC-FIOCRUZ − Fisiologia e Farmacodinâmica, [3]William Harvey Institute − Biochemical Pharmacology

Introduction: Asthma is a chronic inflammatory disease characterized by bronchoconstriction and airways hyperreactivity as well as eosinophil accumulation mucus secretion and remodeling. During the establishment of an inflammatory process, endogenous mediators are released in order limit the progression of the pathological process. Glucocorticoid hormones are considered as critical based on their potent anti-inflammatory activity, which is at least partially dependent on the release of intermediate factors such as the protein annexin-1. It has been reported that the Ac2-26 peptide, derived from the N-terminal region of annexin-1, is able to reproduce the suppressive action of the full protein. Based on these observations, in this study we aimed to investigate the potential effect of the Ac2-26 peptide on the allergic lung inflammatory response in mice. **Methods**: BALB/c mice were sensitized with a subcutaneous injection of ovalbumin (OVA, 50 µg) plus aluminum hydroxide (5 mg). On day 14, animals were boosted intraperitoneally with OVA (25 mg) and on days 19, 20 and 21 post-sensitization, mice were challenged intranasally with OVA (25 µg). Treatments were performed by intranasal administration of Ac2-26 peptide (50-200 µg), 1 h before the challenge. The analyses were made 24 h after the last provocation and included: i) lung function (resistance and elastance) and airways hyperreactivity to methacholine (3-27 mg/ml) by invasive plethysmography (FinePoint,), ii) morphology, peribronchial eosinophil infiltration and mucus production by histological techniques using H&E, Giemsa and PAS, respectively; iii) cytokine generation evaluated by ELISA. All the experimental procedures were approved by the Ethics Committee of Animal Use (CEUA) FIOCRUZ (License 0213-4). **Results:** We showed that treatment with the Ac2-26 peptide, at doses of 50, 100 and 200 µg, markedly inhibited airways hyperreactivity noted in sensitized animals subjected to allergen challenge. The increase in the levels of total leukocytes and eosinophils in the BAL fluid were suppressed by the Ac2-26 peptide. Moreover, the eosinophil accumulation in the peribronchial areas and the mucus production as well the generation of the cytokine IL-13 and the chemokines eotaxin 1 and 2 were clearly sensitive to treatment with the peptide. **Conclusion:** Our findings show that annexin-1 derived peptide Ac2-26 showed the ability to impair several phenomena associated with allergic lung inflammatory responses in mice, clearly indicating that the peptide may constitute a promising therapeutic approach for the treatment of diseases such as asthma. **Financial support:** FIOCRUZ, CNPq, CAPES, FAPERJ (Brazil).

04.019

Effects of an anti-TNF-α therapy on *Aggregatibacter actinomycetemcomitans*-induced alveolar bone loss in mice with experimental arthritis. Queiroz Júnior CM[1], Coelho FM[2], Madeira MFM[3], Candico LCM[2], Sousa LFC[2], Teixeira MM[2], Souza DG[3], Silva TA[4] [1]UFMG − Farmacologia, [2]UFMG − Bioquímica e Imunologia, [3]UFMG − Microbiologia, [4]UFMG − Patologia, Clínica e Cirurgia Odontológicas

Introduction*:* Periodontal disease (PD) and rheumatoid arthritis (RA) are arguably the most prevalent chronic inflammatory bone diseases in humans. Although PD is primarily triggered by an infection and RA is an auto-immune process, both conditions share several pathogenic features, such as bone resorption, inflammatory cell infiltration and release of inflammatory mediators, *i.e.* tumor necrosis factor (TNF)-α, in the affected sites. A growing number of clinical studies point towards a potential association between PD and RA, with RA patients probably being more susceptible to severe forms of PD. Nevertheless, little is known about the mechanistic events that could explain such association. Therefore, this study aimed to evaluate whether RA could alter the progression of PD and to outline the role of TNF-α on this process. **Methods***:* RA was induced in C57BL/6 immunized mice, challenged with methylated Bovine Serum Albumin (100µg/20µL of saline/joint) in the femorotibial joint. These animals (PD+RA) and saline-challenged mice (PD) were orally inoculated with *Aggregatibacter actinomycetemcomitans* (10^9 FCU diluted in 100µL 1.5% carboximetilcelulose) 30 days before the challenge. Negative controls (C) comprised sham-infected and saline-challenged mice. Mice were also treated with an anti-TNF-α antibody (Infliximab, 10mg/kg, 3x/week, i.p.) in the groups PD and PD+RA, during the course of RA. After 45 days of oral infection, all animals were sacrificed and maxillae, femorotibial joints and serum were collected for morphometric, histopathological and/or immunoenzimatic evaluations. This study was approved by the Institutional animal ethics committee (Nº 165/09). **Results and Discussion***:* Experimental PD induced a significant alveolar bone loss, which was increased in ~15% in PD+RA mice (C: 0.53 ± 0.04; PD: 0.83 ± 0.03; PD+RA: 0.95 ± 0.01 mm^2). Anti-TNF-α therapy prevented this effect in both PD and PD+RA groups (T+PD: 0.48 ± 0.03; T+PD+RA: 0.54 ± 0.03 mm^2). PD+RA femur-tibial joints presented histological evidence of an ongoing inflammatory process, *i.e.* hyperplasia of the synovial membrane, bone resorption lacunae and presence of mononuclear inflammatory cells, which was significantly decreased in treated mice. Despite oral bone loss distinction, PD and PD+RA groups showed a similar increase in myeloperoxidase activity, TNF-α, IL-6 and IL-10 concentrations in periodontal tissues in relation to C. Serum KC concentration was higher in both PD-infected groups, but IL-6 levels only were significantly increased (~650%) in PD+RA. Infliximab treatment did not reduce the concentrations of TNF-α in maxillae of PD+RA mice, although it did in the group PD. Nevertheless, the anti-TNF-α drug significantly decreased the concentrations of IL-6 in maxillae and serum of PD+RA mice. In conclusion experimental RA seems to worsen *Aggregatibacter actinomycetemcomitans*-induced alveolar bone loss in mice through a mechanism involving TNF-α and IL-6. **Financial** support: CNPq, FAPEMIG

04.020

Pancreatic injection of phospholipases A_2 causes abdominal hyperalgesia mediated by NK-1 receptors without leading to systemic toxicity in rats. Zanoni CIS[1], Camargo E[2], Teixeira SA[1], Martins Porto R[1], Santos KT[1], Florenzano J[1], Muscará MN[1], Costa SKP[1] − [1]USP − Farmacologia, [2]UFS − Fisiologia

Introduction: We have previously shown that common bile injection of phospholipase A_2 (PLA_2) from *Crotallus durissus terrificus* snake venom (*Cdt*) or bovine pancreas induces acute pancreatitis (AP) in rats (Silva et al., 41° SBFTE Congress, pg. 12; 2009). We have now investigated the participation of neurokinin-1 receptor in the inflammatory/hyperalgesic effects in this animal model of pancreatitis and possible systemic effects. **Methods**: All experimental protocols were approved by the CEEA/USP (n°055 pg 44 book 2). Male Wistar rats (250g; n=7) were anaesthetized with isoflurane (2-3% in O_2) and the injection of PLA_2 *Cdt* and PLA_2 bovine (300µg/kg) or saline was performed in the common duct biliopancreatic. After 4 hours, animals were killed, and peripheral blood and pancreatic tissue were collected. Total and differential cell counts were performed in the peripheral blood in addition to the measurement of serum amylase, creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), urea, γ-glutamil-transferase (γ-GT) and angiotensin-converting enzyme (ACE) activities / concentrations via commercial kits. As required, a group of animals were pretreated with the NK-1 receptor antagonist SR140333 (250 nmol/kg; i.v.) 15 min prior *Cdt* or bovine PLA_2 injection and 4 hours thereafter, serum amylase, myeloperoxidase activity and oedema index in the pancreatic tissue, as well as abdominal hyperalgesia via electronic von Frey were assessed. The statistical analysis was performed by ANOVA followed by Bonferroni's test. $P<0.05$ was taken as significant. **Results:** The serum amylase was significantly increased by either *Cdt* PLA_2 (367 ± 69 U/L) or bovine PLA_2 (439 ± 66 U/L) in comparison with the control group (177 ± 25 U/L). No differences were found in the serum levels of γ-GT, creatinine, AST, urea or ACE for both bovine and *Cdt* PLA_2. However, a significant reduction in the levels of ALT was observed in the group treated with bovine PLA_2 (45.3 ± 3.4 U/L) as compared to control group (87.1 ± 14.6 U/L). The total number of leukocyte was significantly increased in the peripheral blood of rats with pancreatitis evoked by bovine PLA_2 (93 ± 4 $x10^5$ cells/mL) when compared with the control group (72 ± 2 $x10^5$ cells/mL). In contrast, the total leukocyte number in the blood of animals with pancreatitis induced by *Cdt* PLA_2 was significantly reduced (53 ± 6 $x10^5$ cells/mL). Interestingly, the numbers of neutrophils and eosinophils in the blood of rats with pancreatitis evoked by bovine PLA_2 (47 ± 4$x10^5$ cells/mL were larger when compared with the values of *Cdt* PLA_2 group (22 ± 4$x10^5$ cells/mL). The simultaneous treatment of rats with SR140333 prevented the abdominal hyperalgesia observed 4 hours after the injection of *Cdt* PLA_2 (-15.8 ± 5.8g) or bovine PLA_2 (-15.6 ± 6.1g); however, SR140333 neither inhibited the increase in the activity of serum amylase nor abolished the oedema and increased MPO activity in the pancreas. **Conclusion:** The blockade of the NK-1 receptors significantly reduced the abdominal secondary hyperalgesia, but not the inflammatory parameters, thus indicating that these receptors are only involved in mediating the pain in this animal model of pancreatitis evoked by either mammal PLA_2 or *Cdt* PLA_2. Moreover, at the tested dose none of the PLA_2s induced signs of systemic toxicity. **Acknowledgements**: FAPESP, CNPq e CAPES, MA Barreto, I Gouvea for their technical assistance.

04.021

Amphetamine decreases inflammation and TH2-cytokines production in murine model of asthma. Hamasato EK[1], Ribeiro A[1], Ferraz-de-Paula V[1], Pinheiro ML[1], Ligeiro de Oliveira AP[2], Palermo-Neto J[1] [1]FMVZ-USP − Patologia, [2]ICB-USP − Imunologia

Introduction: Amphetamine (AMPH) is a drug of abuse that exert profound effects on behavior, biochemistry, and immunity. Since we observed that AMPH altered the leukocyte distribution in murine model of asthma, we searched the mechanisms possibly involved in the AMPH treatment could result in our findings of cell migration. **Methods:** Animals were housed and used in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, USP, Brazil (protocol No. 1633/2009, FMVZ –USP). Male Balb/c mice were divided in 3 groups: naïve (N), control (C), and experimental (E). In order to induce lung allergic inflammation mice of the groups C and E were sensitized (i.p.) with ovalbumin (OVA, 10µg) on days 0 and 7 and challenged on days 13 and 14 with OVA nebulization (1% in PBS). AMPH (2mg/kg) or saline (0.9%NaCl) treatment was perform 1 hour before OVA nebulization by i.p route. On day 15, 12 h after the last OVA challenge, we collected BAL (bronchoalveolar lavage), bone marrow and peripheral blood to leukocyte counting. Cytokines (IL-4 and IL-13) levels in BAL supernatant and seric IgE levels were determinated by ELISA. **Results:** AMPH treatment decreased ($F(2,16)= 7.525$; $p<0.005$) the total number of inflammatory cells in the BAL (N:8.688 ± 1.143; C:39.64 ± 20.55; E:18.88 ± 8.460) and increased ($F(2,18)=2.529$; $p<0.005$) bone marrow cellularity (N:15.25 ± 4.668; C:11.38 ± 2.218; E:15.96 ± 4.078); however, no effect was observed ($F(2,22)=0.412$; $p>0.05$) in the number of leukocytes in peripheral blood (N:6.525 ± 2.520; C:7.000 ± 2.068; E:5.989 ± 2.279). Moreover, AMPH treatment decreased IL-4 ($F(2,11)=88.89$; $p<0.0001$) (N:7.788 ± 4.547; C:61.58 ± 5.165; E:28.33 ± 7.848) and IL-13 levels in BAL supernatant ($F(2,11)= 17.47$; $p<0.0004$) (N:63.05 ± 16.98; C:137.8 ± 21.43; E:70.57 ± 23.86) and also decreased ($F(2,11)= 46.15$; $P<0.0001$) (N:4.710 ± 3.437; C:17.79 ± 1.601; E:12.77 ± 1.200) IgE serum levels compared to control group (Mean ± SD). **Discussion:** AMPH treatment changes leukocyte distribution in a murine model of asthma. In addition, we showed that AMPH decreased the production of cytokines IL-4, IL-13, and IgE. In asthma Th2 cells orchestrate the inflammatory response by releasing IL-4 and IL-13, which stimulate B cells to synthesize IgE. These data taken together raise the possibility that the decrease the number of inflammatory cells in BAL of AMPH-treated mice could be associated with a decrease in the activation of these cells and thereby decreasing the release of this cytokines. **Financial support:** FAPESP (2009/01826-4, 2009/51886-3) and CNPq.

04.022

Intravital imaging by confocal and multiphoton microscopy: a new tool for understanding dengue pathogenesis. Santos AG[1], Costa VV[2], Menezes GB[3], Fagundes CT[2], Paula AM[4], Valadão DF[1], Morcatty TQ[2], Vilela MC[5], Pinho V[2], Teixeira MM[2] Souza DG[1] [1]UFMG − Microbiologia, [2]UFMG − Bioquímica e Imunologia, [3]UFMG − Patologia Geral, [4]UFMG − Física, [5]ICB-UFMG

Introduction: Dengue disease is caused by four serotypes (Den-1-4) of the most prevalent arthropod-born virus affecting humans nowadays. This disease is characterized by thrombocytopenia, increased levels of cytokines, increased vascular permeability, hemorrhage and shock. As the mechanisms involved in the physiopathology and lethal outcome of this disease had not been clearly defined, a better understanding of disease mechanisms is fundamental for the development of adequate therapeutic approaches. For this, we have developed a mouse model that displays the hallmarks of severe human disease (Souza DG, 2009) and used liver multiphotonintravital microscopy (LIMV) to visualize hepatic microvasculature during dengue infection. **Objectives:** Thus the aim of this study was to investigate the interaction between leucocytes, platelets and endothelium *in vivo* after dengue virus infection and to correlate these findings with the systemic disease. **Methods:** This project was previously approved by CETEA/UFMG on number access 113/09. BALB/c mice were infected with 10 PFU/mouse of the adapted Den-3 by intraperitoneal route. Disease signals were evaluated at days 3, 5 and 7 after infection (Hematocrit (%), platelets counts in blood and in liver microvasculature, AST and ALT quantifications in serum). Tissue damage was verified by liver histological analysis (stained by H&E) and viral load in blood and liver was quantified by plaque assay. The experimental setup for liver intravitalmultiphotonmicroscopy was based on an Olympus confocal microscope adapted to intravital studies. **Results and Discussion:** I.p infection of BALB/c mice causes hemoconcentration, trombocitopenia and increased hepatic transaminases mainly at day 7 after infection. Also, high viral load in blood and liver as demonstrated at same time point and H&E analysis of liver shows increased liver injury after infection. These systemic data can be correlated with our findings in liver microvasculature that reveals morphological alterations at the peak of infection (7^{th} day). Intravital microscopy revealed that platelets and leucocytes accumulated in liver microvasculature and sinusoidal perfusion was remarkably reduced, probably due to an increase in hepatocyte diameter, which occluded liver sinusoids. In this sense, we suggest that thrombocytopenia may be directly correlated with platelet accumulation in organs as the liver. These findings may help to elucidate the mechanisms for thrombocytopenia displayed during dengue infection. In conclusion, we are expanding our understanding about dengue virus pathogenesis by visualizing *in vivo* cell and organ response using intravitalmultiphoton microscopy. **Financial support:** INCT em Dengue (CNPq), CAPES and FAPEMIG.

04.023

Effects of formaldehyde inhalation on allergic lung inflammation: role of female sex hormones. Amemiya RM, Lino dos Santos Franco A, Ligeiro de Oliveira AP, Oliveira-Filho RM, Tavares de Lima W USP − Pharmacology

Introduction: Formaldehyde (FA) is a common indoor and outdoor pollutant that is found in many products including particle board, plywood, floor coverings and office furniture. Other major indoor sources are tobacco smoke, urea-formaldehyde foam insulation and cosmetics, deodorants, solvents, disinfectants and fumigants. FA cause lung inflammation and may impair the lung cell recruitment after an allergic stimulus. Considering the positive relationship between female sex hormones (FSH) and asthma, we evaluated the role of female sex hormones on allergic lung inflammation after FA inhalation.
Methods: Female Wistar rats were ovariectomized (OVx) or not (Sham OVx). After 7 days the rats were subjected to FA exposure (1%, 3 days, 90 min / day) and subsequently sensitized with ovalbumin (OVA)-alum the subcutaneous route. Two weeks later the rats were challenged with aerosolized OVA (1%, 15 min) (FA/OVA group). Another group was sensitized and challenge with OVA without FA exposure (OVA/OVA group). After 24 h the OVA challenge the bronchoalveolar lavage (BAL) and the mieloperoxidase enzyme activity (MPO) were determined. In a parallel set of experiments, group of rats 7 days after OVx were treated with estrogen (280 mg / kg, sc) or progesterone (200 mg/kg, sc) 4h before each FA inhalation and subsequently the rats were sensitized and challenge with OVA. After 24h the OVA challenge the BAL and MPO were evaluated. **Results:** The OVx in allergic rats submitted to FA inhalation did not alter the total cells recovered in BAL and the MPO activity when compared to Sham OVx group. However, the OVx in allergic rats reduced the total cells in BAL and the MPO activity when compared to Sham OVx group. The treatment with estrogen and progesterone increased the neutrophils and eosinophils in BAL of FA/OVA OVx group when compared to non-treated group. On the other hand, these same treatments reduced the MPO activity in FA/OVA OVx group when compared to non-treated group. The treatment with estrogen but not progesterone increased the neutrophils and eosinophils in BAL of OVA/OVA OVx group when compared to non-treated group. **Discussion:** Our data showed that the OVA-induced allergic lung inflammation in rats is blunted by previous exposure to FA and the FSH did not interfere in this response. On the other hand, the FSH modulates the allergic lung inflammation. We suggest that FA impairs the ability of mast cells to adequately degranulate after an antigen challenge, thence blocking the functions involved with the late allergic response We showed also that the estrogen and progesterone exert inflammatory effects in this model. Thus, the data obtained in this study may contribute to the understanding of mechanisms underlying the effects of HSF in the presence pollutants such as FA. **License authorization number of the Ethics Committee:** 22 **Financial support:** FAPESP (2008/58108-3).

04.024

Phospholipase A2 group V in leishmaniasis: role in immunity. Zamith-Miranda D[1], Poublan LE[1], Araújo Souza PS[2], Siqueira EA[1], Viola JPB[2], Diaz BL[3] [1]UFRJ-IBCCF, [2]INCa − Biologia Celular, [3]IBCCF-UFRJ − Imunobiologia

Introduction: Secretory Phospholipases A_2 ($sPLA_2$) are low molecular weight enzymes that share the ability to hydrolyze sn-2 position of phospholipids generating lysophospholipids and free fatty acids. Particularly, Group V sPLA2 (PLA_2-GV), acting alone or in concert with cytosolic PLA2, can release arachidonic acid for eicosanoid synthesis. Such lipid mediators are crucial players in inflammation and infection and modulate Th cell polarization. Susceptibility to *Leishmania major (L. major*) infection seems to be modulated by arachidonic acid cascade products. Our aim was to investigate the role of PLA_2-GV on *L. major* infection. **Methods**: PLA_2-GV null mice (PLA_2-$GV^{-/-}$) and their wild-type littermates (WT, Balb/c background) were infected with *L. major* metacyclic promastogotes in hind footpads. Footpad swelling was monitored weekly with a digital caliper and was calculated by subtracting the thickness of the uninfected contra-lateral footpad from the thickness of the infected footpad, for up to 8 weeks. Draining lymph nodes from 3 weeks-infected mice were homogenized and total cells were stimulated *in vitro*. IFN-γ, IL-4 and IL-17 levels were determined by ELISA. Cytometric analysis was used to access lymphocyte populations present in the infected lymph nodes. The parasite burden in lymph nodes was accessed by limiting dilution assay. **Results**: Infected mice deficient in PLA_2-GV showed marked smaller lesions from the third week on than wild-type controls (35% reduction by week 5). PLA2-$GV^{-/-}$ lymph nodes had a ~50% increase in total cell number, but no difference in relative lymphocyte populations and parasite burden when compared to wild-type controls. *In vitro* activation of PLA2-$GV^{-/-}$ total lymph node cells yielded equal production of IFN-γ and IL-17, but a 75% reduction in IL-4 production. **Discussion**: *L. major* resistance is mediated by a Th1 dominated response by the host, while susceptibility is promoted by Th2 response. Our results point to a role for PLA2-GV in promoting a Th2 response during *L. major* infection what may explain the observed reduction in lesion size. In fact, PLA2-GV is expressed by *in vitro* differentiated Th2 (Ho *et al*, JBC. **276**:18321-2001). Thus PLA2-GV may participate in the production of a paracrine/autocrine lipid mediator with effect on Th2 polarization and ultimately *L. major* susceptibility. **Financial support**: CNPq, FAPERJ. **Ethics Committee**: IBCCF 065.

04.025

Role of 5-Lipoxygenase products in acute respiratory distress syndrome induced by severe sepsis. Monteiro APT[1], Pinheiro CS[1], Benjamim CF[2], Soledade ES[3], Rocco PRM[4], Canetti C[1] [1]IBCCF-UFRJ, [2]UFRJ − Farmacologia Básica e Clínica, [3]UFRJ − Farmacologia, [4]UFRJ − Investigação Pulmonar

Introduction: Sepsis is the main cause of death in intensive care unit worldwide. Sepsis can be defined as a systemic inflammatory response syndrome that occurs in a presence of a infection. A frequent outcome of sepsis is the acute respiratory distress syndrome (ARDS), a pathological state in which the patient develops dyspnea or tachypnea, refractory hypoxemia, and short lung compliance. ARDS is characterized by overwhelming inflammation with increased microvascular permeability that causes diffuse lung edema and mechanical dysfunction leading to respiratory failure. Leukotrienes are lipid mediators of inflammation derived from arachidonic acid metabolism by 5-lipoxygenase (5-LO). We previously demonstrated that 5-LO knockout mice (5-LO$^{-/-}$) presented less mortality and less protein extravasation in peritoneum and kidney after sepsis. The aim of this work is to evaluate the participation of 5-LO products in ARDS induced by sepsis. **Methods**: 5-LO$^{-/-}$ mice and their background lineage (SV129; WT) were submitted to cecal ligation and puncture (CLP; Licença de autorização do Comitê de Ética Animal: DFBCICB028). Briefly, the animals were anesthetized with ketamin and xilazine and a 1-1.5 cm laparatomy was performed. The cecum was exposed, ligated, and punctured 2 times with an 18G needle. As control, mice were submitted to sham surgery, in which the cecum was only exposed, not ligated nor perfurated. In a set of experiments, 16 h after surgery, mice were sacrificed, perfused, and bronchoalveolar lavage performed and lung tissue collected. Histological and morphometric analysis were also performed. In another set of experiments, pulmonar mechanics analysis was performed through occlusion at the end of insufflations with constant influx as previously described (Bates, J.H.T. *J. Appl. Physiol* 65: 408–414, 1988; Bates, J.H.T. *J. Appl. Physiol* 64: 2204–2214 1988). **Results**: Histological analysis of lung tissue from WT mice submitted to CLP showed a massive inflammatory cell infiltration, and loss of tissue architecture. In contrast, 5-LO$^{-/-}$ mice lungs were protected from sepsis induced ARDS. Furthermore, morphometric analysis of lungs from CLP-5-LO$^{-/-}$ mice also revealed less collapsed alveolar than CLP-WT mice. Pulmonar mechanics results also confirmed that 5-LO$^{-/-}$ mice are more resistant, since the increase in elastance evoked by CLP was ameliorated in those animals compared to WT mice. **Discussion**: These results suggest that 5-LO$^{-/-}$ mice are resistant to lung injury induced by sepsis. 5-LO$^{-/-}$ mice presented preservation of the pulmonary components and less infiltration of inflammatory cells, observed in histological and mechanical analysis. **Financial support**: CNPq, FAPERJ, FUJB

04.026

Rosiglitazone potentiates alveolar bone loss due to ligature-induced periodontitis in rats. Martins Porto R[1], Teixeira SA[1], Maia-Dantas A[1], Herrera BS[2], Campi P[2], Costa SKP[1], Nucci G[3], Spolidório LC[2], Muscará MN[1] [1]ICB-USP − Farmacologia, [2]FO-UNESP − Patologia, [3]UNICAMP

Introduction: Rosiglitazone (RTZ) is a peroxisome proliferator activator receptor (PPAR)-gamma agonist widely used for treatment of type-2 *diabetes mellitus.* The aim of this study was to investigate the effects of RTZ on periodontitis-induced alveolar bone loss (ABL) and gene expression of some markers of inflammation and bone metabolism. **Methods**: The experimental protocol was approved by the CEEA-ICB (protocol 077, fls. 33, livro 2). Male Wistar rats (180-220g) received RTZ (10 mg/kg/day) during 3 weeks, either as the pure maleate salt (i.p.) or the commercial formulation (Avandia from GlaxoSmithKline, p.o.); control animals received 1 ml/kg of the respective vehicles (DMSO or 0.5% CMC). Two weeks after the beginning of treatments, the rats had unilateral (first lower molar) ligature-induced periodontitis (P); sham (Sh) animals had the ligature placed and immediately removed. The following groups were thus defined: untreated Sh and P, CMC-treated P (PC), DMSO-treated P (PD), pure RTZ-treated P (PR) and Avandia-treated P (PA). After 7 days, the rats were killed, and the jaws removed for radiographic measurement of ABL (as the distance between the cemento-enamel juntion and the alveolar bone crest). RNA was isolated from alveolar bone samples and analyzed by qPCR for markers of both osteoblast (RUNX2 and Osterix) and osteoclat (TRAF6, TRAF2, RANKL) activation and differentiation, as well as isoforms of nitric oxide synthase (e, n and iNOS) and PPARs (α, β e γ). RTZ pharmacokinetics from each formulation was also studied from plasma RTZ concentrations measured at selected time-points (HPLC-MS/MS). **Results**: PA and PR groups had more ABL than the respective controls PC (0.57 ± 0.02 vs 0.45 ± 0.01 mm; $p<0.05$, n=10) and PD (0.56 ± 0.01 vs 0.44 ± 0.01 mm; $p<0.002$, n=10). Compared with Sh animals, periodontitis resulted in increased RUNX2 (1.9 ± 0.3 vs. 1; $P<0.05$, n=6) and decreased PPARγ (0.19 ± 0.09 vs. 1; $P<0,05$, n=8) and PPARα (0.15 ± 0.08 vs. 1; $P<0.05$, n=8) expression. PR group showed less Osterix expression (0.35 ± 0.11 vs. 1; $P<0.01$, n=8) than PD,, as well as higher RANKL (18,1 ± 6.0 vs. 1; $P<0.05$, n=6) and eNOS (3.9 ± 0.7 vs. 1; $P<0.01$, n=7). A trend towards higher TRAF6 expression was also observed in PR rats (123,7 ± 66,3 vs. 1, n=8), albeit was not significant. Compared with the CMC-treated P rats (PC), PA animals showed decreased TRAF2 (0.11 ± 0.02 vs. 1; $P<0.001$, n=8) and increased iNOS expression (29.5 ± 9.3 vs. 1; $P<0.05$, n=7). Other comparisons were of no statistical significance**.** The administration of a single 10 mg/kg RTZ dose by either i.p or p.o. route resulted in similar pharmacokinetic profiles, as well as the respective plasma RTZ concentrations measured 24 h after the 21st. dose (4.7 ± 1.5 and 2.5 ± 0.8 mg/l). **Discussion**: RTZ, either from the pure maleate salt (i.p.) or the commercial Avandia (p.o.), resulted in aggravated periodontitis-induced ABL in rats. Despite resulting in similar plasma RTZ concentrations, signaling mechanisms seem to depend on the administered formulation (i.e., while i.p RTZ affected RANKL, TRAF6 and Osterix, p.o. RTZ increased iNOS and TRAF6 expression), which could be due to vehicle related effects (DMSO vs. CMC). This hypothesis is under current investigation). **Financial support**: FAPESP, CNPq, CAPES.

04.027

Ligature-induced periodontal disease affects salivation and saliva composition in rats. Maia-Dantas A[1], Campi P[1], Martins Porto R[1], Teixeira SA[1], Herrera BS[2], Costa SKP[1], Spolidório LC[2], Muscará MN[1] [1]USP − Farmacologia, [2]UNESP − Patologia

Introduction: Diminished salivation can produce deleterious effects on oral health. Several problems, such as difficult deglutition, impaired taste sensation, higher susceptibility to tooth loss and periodontal diseases, have been associated with this condition. Periodontal diseases (PDs) are infections in which injurious effects are unleashed by the interaction between the presence of bacteria and exacerbated host responses. PDs are among the most common oral cavity diseases and can be classified according to the affected area (for example, gingivitis only involve gums, while during periodontitis, tooth support tissues are affected, including bone loss). The presence of periodontitis has been associated with systemic consequences, like cardiovascular alterations of the host and low-weight pre-term neonates delivered by mothers with periodontitis. Considering that saliva is an important entrance barrier to oral microorganisms, and periodontitis is also associated with systemic alterations, the aim of the present work was to study the effects of ligature-induced PD in rats on salivary composition and saliva secretion rate during the course of the disease. **Methods**: The experimental protocol was approved by the CEEA-ICB (protocol 020, fls. 29, livro 2). Under deep anesthesia (80 mg/kg ketamine plus 20 mg/kg xylazine, i.p.), male Wistar rats (180-200g) had unilateral (first lower molar) ligature-induced periodontitis; sham (Sh) animals had the ligature placed and immediately removed. After 3, 7 and 14 days of the disease induction, in half of the animals salivation was stimulated by the administration of the muscarinic agonist pilocarpine (1 mg/kg, i.p.; Allergan□); salivation rate was measured 15 minutes after pilocarpine injection, and saliva was collected for analysis of amylase activity, calcium and protein concentrations. Submandibular salivary glands (SMG) were surgically removed from the remaining animals and amylase release was assessed "*in vitro*" in response to carbachol (10 nM). **Results**: Three days after ligature, salivary flow rate increased 44% relative to the Sham group ($p<0.05$) but remained unaltered at days 7 and 14. Salivary amylase (in mU/ml) was not different between the groups at any period, however, its "*in vivo*" secretion rate (in mU/min) was increased in animals with PD at day 3 (104%) and decreased at day 7 (by 26%; $p<0.05$), probably as a result of salivary flow rate, since no differences in specific activity (in mU/mg of protein) due to PD were observed at any time. Salivary calcium and protein contents showed similar patterns. In all the groups, left and right SMGs showed similar amylase content (in mU/g tissue). "*In vitro*", SMGs collected on day 3 spontaneously released 73% more amylase than controls ($p<0.05$), and carbachol-induced amylase release was 213% and 112% higher for SMGs collected at days 3 and 14, respectively ($p<0.001$). **Discussion**: Saliva alterations at different stages of PD are partially mediated by SMGs. However, the participation of parotid and/or sublingual glands deserves further investigation. **Financial support**: FAPESP, CNPq, CAPES.

04.028

Reparixin, via CXCR1/CXCR2, does not reduce fever induced by PGE_2 mediators. Yamashiro LH[1], Soares DM[1], Melo MCC[2], Teixeira MM[3], Souza GEP[2] [1]USP − Farmacologia, [2]FCFRP-USP − Física e Química, [3]UFMG

Introduction: We showed before that CINC (cytokine induced neutrophil chemokine)-1, centrally administered promotes an integrated febrile response along with an increase in the prostaglandin PGE_2 content in the cerebrospinal fluid (CSF) of rats [(1)]. Moreover, treatment of animals with reparixin (Repa), a non-competitive allosteric inhibitor of CXCR1/2 receptors reduced fever induced by LPS in rats[(2)]. Therefore, the aim of this study was to investigate the effectiveness of this antagonist on fever induced by PGE_2-dependent (IL-1β, TNF-α, IL-6) and -independent (CRF) endogenous pyrogen. **Methods**: Reparixin 150, 300 and 600 ng per animal was injected intracerebroventricularly right before the injection of CINC-1, IL-1β, TNF-α, IL-6 and CRF in male Wistar rats (200g). Control animals received saline (Sal). Rectal temperature was measured by telethermometry every 30 min for up to 6 h. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations in conscious animals set by the Ethical Committee of USP, Campus of Ribeirão Preto, SP, protocol number 050/2008. **Results**: The i.c.v. injection of reparixin abolished the fever induced by CINC-1 in all the doses and during all the time observed (Repa 150 ng + CINC-1: -0.08 ± 0.08; Repa 300 ng + CINC-1: 0.2 ± 0.08; Repa 600 ng + CINC-1: 0.3 ± 0.06; Sal + CINC-1: 0.8 ± 0.09). Therefore, the dose of 150 ng per animal was selected for further experiments. Reparixin, at the same dose and route of administration did not alter the febrile response induced by IL-1β, TNF-α, IL-6 and CRF. **Discussion**: These results show that fever induced by CINC-1 depends on the activation of CXCR1/CXCR2 receptors. Since i.c.v. injection of CINC-1 increases PGE_2 in the CSF[(1)] and reparixin does not affect the fever induced by PGE_2-dependent (IL-1β, TNF-α, IL-6) or independent (CRF) endogenous pyrogens it is plausible that this chemokine directly stimulates the PGE_2 to produce fever in rats. **Support**: FAPESP, CNPq. (1) Soares et al., *Brain Research*, v.1233, p.79, 2008. (2) Yamashiro et al., unpublished Results**:**

04.029

Essential oil of *Mansoa standleyi* exerts anti-inflammatory effect by inhibition of macrophage activity. Santos IVF[1], Magalhães RC[1], Nascimento MVL[1], Zoghbi MGB[2], Maués LAL[1], Bastos GNT[1], Do Nascimento JLM[1] [1]UFPA − Neuroquímica Molecular e Celular, [2]Museu Emilio Goeldi

Introduction: *Mansoa standleyi* (Bignoniácea) is known in Brazil as "Cipó d'alho" due to garlic odor. This herb has been used in native medicine to treatment of cough, sickness, rheumatism, constipation. The aim of this study is validate the anti-inflammatory effect of this herb. **Methods**: The anti-inflammatory activity was analyzed in the carregenan-induced air pouch model and them rats were randomly assigned in three groups: Control, Essential oil of Mansoa standleyi (OEMS) 0, 1% and 0, 01%, the samples was collected and the numbers of exudate cells were quantified. The peritoneal macrophage cultures also were used to analysis the level of nitrite using Griess reaction, and to cell viability the MTT assay. All procedures involving animal care and experimentation were performed in accordance with the guidelines of the Ethical Committee for Research with Experimental Animals of the Universidade Federal do Pára (BIO001-09). **Results and Discussion**: The results demonstrated a decrease the cellular migration of exudates fluid (Lymphocytes: 33.38 ± 9.38; 15 ± 2.02; 27.74 ± 10.18; Macrophage: 16.63 ± 3.47; 11 ± 1.6%; 11 ± 3.3%; respectively to Control, 0.1 and 0.01% of OEMS), the OEMS could not decrease the percentage of neutrophils and bands neutrophils. The level of nitrite (μM, mean ± SD) by peritoneal macrophage was significantly decreased when OEMS were administrated (16.73 ± 1.25; 3.98 ± 3.6; 5.26 ± 3.38; respectively to Control, 0.1% and 0.01% of OEMS). OEMS is not cytotoxic as demonstrate by the MTT assay (percentage of viable cells, mean ± SD) (92.36 ± 7.45; 84.6 ± 1.2; 80.53 ± 1.38; 79.76 ± 1.45; Control, 0.1, 0.01 and 0.001% of OEMS). Our results indicate that OEMS exerts anti-inflammatory effect interfering with nitrite pathway and macrophage activity. **Supported by:** FAPESPA, CNPQ. CEPAE-UFPA: BIO001-09

04.030

Efficacy of H_2S in the management of pruritus and oedema evoked by different mediators in the mouse skin. Rodrigues L[1], Florenzano J[1], Ekundi-Valentim E[1], Teixeira SA[1], Muscará MN[1], Costa SKP[1] [1]USP − Pharmacology

Introduction: Pain and itch sensations are induced by depolarization of C-fibers and possibly other fiber types. In a recent study, we have shown that exogenous delivery of H_2S to the knee joint produces a significant anti-nociceptive and anti-inflammatory effect in a rat model of synovitis (Ekundi-Valentim et al., *Br J Pharmacol*., 159: 1463-1474; 2010). We have now investigated the role of H_2S pathway in the skin oedema and itching (measured as bouts of scratches) observed in response to intradermally-injected histamine, compound 48/80 (C-48/80) or bradykinin (BK) in BALB/c mice. **Methods**: Experiments were carried out in male mice (BALB/c; 25-30g) under the approved animal use protocol (CEAA/USP; n. 33, book 2, page 85). Following anesthesia with isoflurane in oxygen, the ^{125}I-bovine serum albumin (BSA; 0.03 MBq/0.1 ml) was i.v. injected and a single (or up to six) intradermal injection (0.05 ml) of test agents or vehicle was made by a blind technique by the investigator into the shaved dorsal skin. The bouts of mouse scratches were recorded by a video camera (Samsung SC-D382 Mini DV) following 40 min injection. The skin oedema was assessed by the extravascular accumulation of ^{125}I-BSA, accordingly (Costa et al., *Vascul Pharmacol*., 45(4): 209-214; 2006). Data are mean ± SEM of n=4-8. Statistical analysis was performed by ANOVA followed by Dunnett's test. $P<0.05$ was taken as significant. **Results**: As expected, the i.d. injection of histamine (3-30 nmol/site) and BK (3-30 nmol/site) evoked a significant and dose-dependent plasma extravasation in the mouse skin. Likewise, the i.d. injection of C-48/80 (3 µg/site) caused a significant plasma extravasation. Neither the co-injection of Lawesson's reagent (LR, 10-300 nmol/site, n=4-7) nor Na_2S (1-1000 nmol/site, n=4-8) with histamine (30 nmol) reduce the plasma extravasation evoked by this agent. However, the oedema evoked by C-48/80 (122 ± 20 µl/g; n=8) was significantly reduced by co-injection of LR, at doses of 30 nmol/site (47 ± 14 µl/g; $P<0.05$), 100 nmol/site (39 ± 13 µl/g; $P<0.01$) and 300 nmol/site (57 ± 21 µl/g; $P<0.05$), or by Na_2S at doses of 30 nmol/site (20 ± 6 µl/g; $P<0.01$) and 100 nmol/site (22 ± 9 µl/g; $P<0.01$). The BK (10 nmol/site)-induced plasma extravasation (57 ± 3 µl/g; n=6) was unaffected by LR at all tested doses but it was significantly inhibited by Na_2S at 10 nmol/site (24 ± 3 µl/g; $P<0.01$) and 30 nmol/site (22 ± 5 µl/g; $P<0.001$). Histamine (1 µmol/site)-induced pruritus (133 ± 60 bouts of scratch; n=4) in the mice dorsal skin was significantly reduced by Na_2S (26 ± 8 bouts of scratch; n=4), at a smaller dose (1 nmol/site) than that used in the microvascular permeability assay. Neither the i.d. injection of Na_2S nor LR caused itch or plasma extravasation *per se*. **Discussion**: These results show an important role for H_2S in the plasma extravasation evoked by C-48/80 and BK in the mouse dorsal skin. Moreover, this pilot study provides strong evidence that H_2S pathway could be a contributing factor to mediate histamine-induced pruritus. Thus, a distinct separation between mast cells or sensory fibers-dependent effects is suggested. **Acknowledgments:** CNPq, CAPES and Fapesp for **Financial support**. Gouvea IM and Barreto MA for their technical assistances.

04.031

Anti-inflammatory activity of new isatin derivatives. Zardo RS[1], Figueiredo GSM[2], Silva BV[3], Matheus ME[1], Pinto AC[4], Fernandes PD[1] [1]UFRJ − Farmacologia Básica e Clínica, [2]ICB-UFRJ − Farmacologia, [3]IQ-UFRJ − Química Orgânica, [4]UFRJ − Química

Introduction: Isatin (1H-indole-2,3-dione) was first discovered by Erdmann and Laurent (1840) as a product arising from the oxidation of indigo. Various substituted have also been identified in plants, fungi and marine molluscs. In mammals, isatin is found as an endogenous molecule distributed in tissues and body fluid. It synthetic flexibility permits the synthesis of a great variety of derivatives. These substances demonstrate a diverse array of pharmacological activities including anticonvulsant, antibacterial, antifungal, anti-inflammatory and anticancer properties. The objective of this work was to evaluate the anti-inflammatory properties of new synthetic isatins derivatives. **Methods**: The use of animals in this work was approved by the ethical committee of animal experimentation from Centro de Ciências da Saúde (UFRJ), and received the number ICBDFBC-015. Male Swiss mice (20-25g, n=5-7) were used in the licking response induced by formalin (2.5%, intraplantar) and in the subcutaneous air pouch (SAP) model. Animals received oral administration of isatin derivatives (ISA003, ISA127, ISA147) at doses of 0.1, 1, or 10 mg/kg, 1h before formalin injection (in formalin model) or 1h before and 23h after carrageenan (1%) injection (in SAP model). Statistical analyses was performed by ANOVA followed Bonferroni's post test (*p<0.05). **Results**: Pre-treatment of mice with ISA003 (control=267.1 ± 48.2 sec; 0.1mg/kg=68.1 ± 9.3* sec; 1.0mg/kg=181.6 ± 13.7* sec; 10.0mg/kg=155.5 ± 5.3* sec) or ISA127 (control=267.1 ± 48.2 sec; 0.1mg/kg=129.4 ± 16.7* sec; 1.0mg/kg=135.2 ± 9.8* sec; 10.0mg/kg=101.7 ± 8.2* sec), significantly inhibited the second phase of licking response induced by formalin. ISA147 demonstrated inhibitory effect only when used at 10 mg/kg dose (control=267.1 ± 48.2 sec; 10.0mg/kg=170.5 ± 45.8*). The pre-treatment of mice with the same doses of isatin derivatives also significantly inhibited the cell migration into the SAP. ISA003 reduced in 54.8%, 64.9%, and 71.9% (control=45.6 ± 10.2 x 10^6 leukocytes/mL *versus* 20.6 ± 7.5*, 16.4 ± 7.4*,12.8 ± 6.0*, to 0.1, 1, and 10mg/kg, respectively); ISA127 reduced in 84.2%, 72.8%, and 48.2% (control=45.6 ± 10.2 x 10^6 leukocytes/mL *versus* 7.2 ± 3.0*, 12.4 ± 3.1*,23.6 ± 4.5*, to 0.1, 1, and 10 mg/kg, respectively), and ISA147 reduced in 68.2%, 60.3%, and 17.5% (control=45.6 ± 10.2 x 10^6 leukocytes/mL *versus* 14.5 ± 3.4*, 18.1 ± 5.4*,37.6 ± 3.3*, to 0.1, 1, and 10 mg/kg, respectively). All three substances also inhibited IL-6 [control=1.6 ± 0.1pg/mL; ISA003=1.34±0.1pg/mL*(0.1mg/kg), 0.4±0.14*pg/mL(1.0mg/kg), and 1.2±0.1*pg/mL(10.0mg/kg); ISA127=0.3±0.2*pg/mL(1.0mg/kg) and 1.05±0.2*pg/mL (10.0mg/kg); ISA147=0.4±0.1*pg/mL(1.0mg/kg), and 0.7±0.1*pg/mL(10.0mg/kg)] and TNF-a [control=1.1±0.3pg/mL; ISA003=0.6±0.05*pg/mL(0.1mg/kg); 0.4±0.1*pg/mL (1.0mg/kg), and 1.2±0.1*pg/mL(10.0mg/kg); ISA127=0.7±0.1*pg/mL(0.1mg/kg), 0.3±0.02* pg/mL(1.0mg/kg), and 0.4±0.01*pg/mL(10.0mg/kg); ISA147=0.6±0.1*pg/mL (0.1mg/kg), 0.7±0.1*pg/mL(1.0mg/kg), and 0.4±0.1*pg/mL(10.0mg/kg)] production in exsudates of SAP. **Discussion:** These results indicate that the three new isatin derivatives significantly reduced the carrageenan-induced inflammation, even when administered orally at doses of 0.1mg/kg, indicating that these substances could become new candidates for prototype drugs. **Financial support:** CAPES, CNPq, and FAPERJ.

04.032

Lipopolysaccharide stimulates NFkB and glucocorticoid receptor translocation to the nucleus of A7r5 rat smooth muscle cells. Scheschowitsch K, DalBó S, Assreuy J UFSC − Pharmacology

Introduction: Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria that induces systemic inflammation by increasing a variety of pro-inflammatory cytokines, such as TNF-α and IL-1. It is known that Toll-like receptor 4 recognizes LPS and that the activation of this receptor leads the activation of the nuclear factor transcription *kappa*-B (NFkB). However, the dynamics of glucocorticoid receptors (GR) following LPS is not known. Therefore, this study aims to determine the effects of LPS on GR. **Methods:** Line rat smooth muscle cells (A7r5) were placed on a 12-well culture plate containing glass coverslips pre-treated with gelatin 0,5%. After 24 hours the cells were treated with LPS 1 µg/mL for 2 and 4 hours and then total GR or NFkB in the nucleus were identified by immunofluorescence. The images were made with confocal microscopy (63x immersion) and quantified by the software ImageJ®. The results are expressed as the mean of fluorescence (in arbitrary units, AU) in the nucleus ± SEM, n= 10. Statistical comparisons were made by one-way ANOVA followed by Dunnet's test. **Results:** Two hours after LPS exposure the amount of NFkB and GR in the nucleus of A7r5 cells increased from 0.9 ± 0.26 AU to 36.9 ± 6.4 AU, returning to basal levels 1.5 ± 0.4 AU 4 hours after LPS. In contrast, GR basal levels were 73.3 ± 8.2 AU and rose to 167.8 ± 15.9 AU 2 hours after LPS and increased further (217.1 ± 6.9) 4 hours after LPS. **Discussion:** LPS increased the NFkB translocation to the nucleus, reaching a maximum 2 hours after stimulus. Interestingly, a classical pro-inflammatory stimulus such as LPS also induced the translocation to the nucleus of GR and this was sustained at least until 4 hours after LPS. Our findings suggest that GR migration to the nucleus induced by LPS may be not sufficient to inhibit the cell activation and prevent inflammation, but the modulation of GR translocation can be an interesting target for anti-inflammatory strategies. **Financial support:** CAPES, CNPQ and FAPESC.

04.033

Nicotinic receptors modulate IL-12 production by dendritic cell. Pinheiro ML, Ribeiro A, Ferraz-de-Paula V, Quinteiro-Filho WM, Palermo-Neto J FMVZ-USP − Patologia

Introduction: The inflammatory reflex is a neurophysiological mechanism that regulates the immune system. Recent studies indicate that the vagus nerve can modulate the immune response through a “nicotinic anti-inflammatory pathway”. The aim of this work was to investigate the effects of both cholinergic agonists and antagonists on splenic dendritic cell (DC) cytokines production in ovalbumin (OVA)-sensitized mice. **Material and methods:** The first experiment was conducted with cholinergic agonists: Forty C57BL/6 male mice were used and divided in 4 groups: saline (S1), anabasine 4 mg/kg (nicotinic agonist) (A1), bethanechol 20 mg/kg (muscarinic agonist) (B1) and the combination of these two drugs (AB1). The second experiment was conducted with cholinergic antagonists: Forty C57BL/6 male mice were used and divided in 4 groups: saline (S2), atropine 1 mg/kg (nicotinic antagonist) (A2), mecamilamine 1 mg/kg (muscarinic antagonist) (B2) and the combination of these two drugs (AB2). Each mouse received one single dose (i.p.) and 30 minutes latter mice were sensitized with OVA (50µg/mouse, s.c.). After 7 days, mice were euthanized and spleens were harvested. Splenocytes were cultured with OVA and 12 hours later the culture supernatant was collected to measure cytokines by ELISA. **Results:** Regarding the experiments with agonists, statistical analysis showed that Anabasine was able to decrease IL-12 levels in the supernatant compared to saline group. Thus, the group that received bethanechol showed higher IL-12 levels compared to group Anabasine, but these levels were similar to saline group (S1:74.65 ± 14.1; A1:51.53 ± 7.5; B1:69.49 ± 14.5; AB1:60.02 ± 9.3) ($p<0.05$). There were no significant differences in IFN-gamma dosage and IL-4 was not detected. Thus, in the antagonists’ experiment, IFN-gamma and IL-4 were also not detected in all groups. In relation to the IL-12 dosage, its quantification was possible, but no significant difference between the groups was observed. **Conclusions:** Due to IL-12 plays a central role linking innate and adaptative immune response, it is likely that the cholinergic system may be involved in this process regulation. Moreover, we believe that cholinergic modulation is related to nicotinic receptors action, because only nicotine stimulation produced any effect on dendritic cells cytokine production. **Financial support:** Fapesp.

04.034

The role of suppressor of cytokine signaling 2 (SOCS-2) in an experimental pulmonary disease by pathogenic fungus *Paracoccidioides brasiliensis*. Santos PC[1], Santos DA[1], Machado FS[2], Souza DG[1] Cisalpino PS[1] [1]UFMG − Microbiologia, [2]UFMG − Bioquímica e Imunologia

Introduction: *Paracoccidioides brasiliensis* is the etiological agent of paracoccidioidomycosis, a systemic mycosis endemic in Latin America. The infection can be acquired by inhalation of airborne conidia that reach the lung alveoli, where they transform into yeast cells, resulting in distinct clinical forms of the disease. This infection is associated with intense inflammatory response, however, the control of inflammation is crucial to prevent damage to the host during infection. Then, our aim was assessed the role of enzyme 5-LO and signaling molecule SOCS-2, associated respectively with production and signaling of lipoxin. **Methods:** C57BL/6 (WT), 5-LO (5-LO$^{-/-}$) or SOCS-2 (SOCS-2$^{-/-}$) deficient mice were used in experiments and procedures involving animals and their care were conducted in conformity with CETEA/UFMG (protocol n^{o} 134/2009). The *P. brasiliensis* 18 isolate, which is highly virulent, was used throughout this study. After anesthesia, the animals were infected with 10^6 yeast cells, contained in 30 µL of PBS, by surgical i.t. inoculation, which allowed dispensing of the fungal cells directly into the lungs. Negative controls received only PBS (NI). Pulmonary tissues were analyzed by ELISA, myeloperoxidase and N-acetilglicosaminidase content. The viable microorganisms were determined by counting the number of CFU recovered in bronchoalveolar lavage fluid (BALF) and infected lungs. The BALFs obtained from individual mice were analyzed for leukocyte recruitment into lungs. **Results:** 5-LO deficient animals succumbed to infection at the early stages of acute disease, with excessive pro-inflammatory cytokine production, elevated microbial proliferation and uncontrolled leukocyte infiltration into the lungs. Next, we found increased monocyte and neutrophil counts in the BALF and lung tissue from infected SOCS-2 deficient mice compared with their wild-type counterparts, after 72h of infection. The majority of the pro-inflammatory leukocytes in the BALF of SOCS-2 infected mice were polymorphonuclear (PMN) cells. However, 30 days post-challenge neutrophil and macrophages were present in equal proportions in BALF and lung from both infected animals. SOCS-2 deficient mice infected with fungus also had higher number of CFU in BALF and lung after 72 h of infection compared with WT mice. Thirty days after infection, SOCS-2 deficient mice had significantly higher levels of IFN-g, TNF-a, IL-6, CXCL1 and IL-10 in the BALF and lung, compared with WT mice. These results were consistent with the results found in *P. brasiliensis*-infected 5-LO deficient mice. **Conclusion:** Our results suggest that lipoxin way may be targeted for the modulation of immunity in this disease.

Key words: *Paracoccidioides brasiliensis*, pulmonary disease, SOCS-2. CAPES and CNPq

04.035

Lipoxin A_4 attenuates zymosan-induced arthritis modulating endothelin-1 effects. Conte FP[1], Menezes-de-Lima Jr O[1], Verri Jr WA[2], Cunha FQ[3], Penido C[1], Henriques MGMO[1]
[1]FIOCRUZ − Farmacologia Aplicada, [2]UEL − Ciências Patológicas, [3]FMRP-USP

Introduction: Lipoxin A_4 (LXA_4) is a lipid mediator implicated in the resolution phase of inflammation. Increased LXA_4 levels in synovial fluid and expression of lipoxin A_4 receptor (ALX) in synovial tissues from rheumatoid arthritis patients have been reported. We have previously demonstrated that endothelin (ET)s play a pivotal pro-inflammatory role on acute articular inflammatory response (Conte et al, J Leukoc Biol 2008;84:652-660). This study aims to evaluate the anti-inflammatory role of LXA_4 in neutrophil migration, edema formation and production of inflammatory mediators during the acute phase of zymosan-induced arthritis in mice, focusing on the modulation of ET-1 expression and effects. **Methods:** The anti-inflammatory effects of LXA_4, BML-111 (ALX agonist) and acetylsalicylic acid (ASA) pre- and post-treatments were investigated in a C57BL/6 model of zymosan induced arthritis. Zymosan-induced articular inflammation was assessed by knee joint swelling, neutrophil influx to synovial cavities as well as prepro-endothelin(ET)-1 mRNA, leukotriene (LT)B_4, TNF-α and KC/CXCL1 levels. The direct effect of LXA_4 on ET-1-induced neutrophil activation and chemotaxis were evaluated by shape change and Boyden assays, respectively. **Results:** LXA_4, BML-111 and ASA treatments prior and after zymosan challenge inhibited edema and neutrophil influx into inflamed joints. Zymosan-induced preproET-1 mRNA, KC/CXCL1, LTB_4 and TNF-α levels were also decreased after LXA_4 pre-treatment. *In vitro* ET-1-induced neutrophil chemotaxis and shape change were inhibited by LXA_4 pre-treatment. LXA_4 treatment also inhibited ET-1-induced edema formation and neutrophil influx into mouse knee joints. All procedures were approved by the Committee for Animal Care and Use (CEUA-FIOCRUZ) under L0052-2002 license. **Discussion:** LXA_4 presents anti-inflammatory effects on articular inflammation through a mechanism that involves the inhibition of ET-1 expression and effects. **Financial support**: FIOCRUZ; CNPq

04.036

Anti-hypernociceptive and anti-oedematogenic properties of bis selenide in inflammatory models in mice. Jesse CR[1], Wilhelm EA[2], Bortolatto CF[2], Nogueira CW[2] [1]UNIPAMPA − Nutrição, [2]UFSM − Química

Introduction: Drugs that decrease the inflammatory condition could be successful applied in certain chronic pain states. Taking this into account, several works have described antinociceptive and anti-inflammatory activities of bis selenide in rodents (Savegnago et al., 2006; Jesse et al., 2007; 2008; 2009). In this study, we investigated the properties of bis selenide in the mechanical hypernociception and paw oedema in inflammatory models in mice induced by Complete Freund's Adjuvant (CFA), carrageenan (CG) and prostaglandin E_2 (PGE_2). **Methods:** The behavioral experiments were conducted using male adults Swiss mice (25–35 g). Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources (n°. 23081.018371/2006-94). To produce a persistent inflammatory response, mice received a 20 µl intraplantar injection of CFA (Mycobacterium tuberculosis) into the right hindpaw (Quintão et al., 2005). To assess the effect of the acute (for up to 12 h) treatment on mechanical hypernociception and paw oedema, animals received a single oral (p.o.) dose of the bis selenide (1, 5 or 10 mg/kg) or vehicle 24 h after CFA injection. The mechanical hypernociception was assessed by Von Frey Hair (VFH) filaments and paw oedema was evaluated by micrometer. To investigate the effect of the long-term (for up to 18 days) treatment, bis selenide (5 or 10 mg/kg, p.o.) or vehicle was administered in mice. In other model, mice received an i.pl. injection of 50 µl of CG (300 µg/paw) under the right hindpaw (Quintão et al., 2005). Mice received bis selenide (1, 5 and 10 mg/kg, p.o.) or vehicle 30 min before CG injection. Mice were treated with bis selenide (1, 5 or 10 mg/kg, p.o.) or vehicle 30 min before the injection of PGE_2 (0.1 nmol/paw, Kassuya et al., 2007). The statistical significance of differences between groups was performed by ANOVA followed by Newman-Keuls test. Probability values less than 0.05 (P<0.05) were considered as statistically significant. **Results:** Treatment with bis selenide (5 and 10 mg/kg) demonstrated a significant reduction in the mechanical hypernociception in inflammatory models induced by CFA, CG and PGE_2 injection. Treatment decreased the paw oedema formation induced by CFA at 10 mg/kg and CG and PGE_2 at 5 and 10 mg/kg of bis selenide. The long-term treatment with bis selenide decreased markedly the mechanical hypernociception (5 and 10 mg/kg) and the paw oedema (10 mg/kg) induced by CFA in paw of mice. **Discussion**: Results show a consistent anti-hypernociceptive and anti-oedematogenic effects for bis selenide in the CFA model, when administered in acute and repeated schedules of treatment. Treatment of mice with bis selenide produced anti-hypernociceptive effect for up to 24 h in the CG and PGE_2 acute model of nocicepton. **Acknowledgements:** FAPERGS, CAPES and CNPq. Jesse, C.R., 2007. *Life Sci*. 81, 1694-1702. Jesse, C.R., 2008. Brain Res. 1231, 25-33. Jesse, C.R., 2009. *J. Pharm. Pharmacol*. 61, 623-630. Kassuya, 2007. *Br. J. Pharmacol*. 150, 727-737. Quintão, N.L., 2005*. Anesth. Analg*. 101, 1763-1769. Savegnago, L., 2006. *Pharmacol. Biochem. Behav.* 83, 221-229.

04.037

Effects of hydroquinone inhalation on functions of tracheal tissue. Shimada ALB[1], Ribeiro ALT[1], Hebeda CB[1], Bolonheis SM[1], Lino dos Santos Franco A[2], Tavares de Lima W[2], Farsky S[2] [1]USP- Análises Clínicas e Toxicológicas, [2]USP − Farmacologia

Introduction: Hydroquinone (HQ) is an important phenolic compound from natural or anthropogenic origin. Naturally found in foods and beverage, it is also a component of cigarette and medicines. Moreover, it is obtained from benzene metabolism and it is heavily used in petroleum refining, petrochemical and chemical industries, contributing to the environmental contamination. Since respiratory is an important via of HQ absorption, its effects on cells of this system have been evaluated. Therefore, this work aimed to investigate the effects of HQ inhalation on the secretion of tracheal cells and the *ex vivo* trachea responsiveness to methacholine (MCh). **Methods:** Male Swiss mice were exposed to aerosolized HQ 1.5mg/60mL/1h (25ppm) during 5 days, once a day. Control animals received vehicle (saline with 5% ethanol). One hour after the last exposure, the trachea was removed and processed as following: (1) incubated in culture medium and stimulated or not with LPS (1µg/mL) during 24h. The supernatant of tracheal culture was collected for measurement of cytokines (IL-10; TNF-α; IL-6; MCP-1) by ELISA and NO by Griess Reaction. Concentrations of these mediators were expressed in pg/mg of dry tissue; (2) intact tracheal rings or tracheal rings which lumen was gently rubbed with polyethylene tubing were mounted for the measurement of isometric force quantification (Powerlab®, Lab chart 7.01). Tissues were maintained continuously aerated at 37ºC (95% O_2 and 5% CO_2). After an equilibration period, cumulative dose-response curves to MCh were constructed. In order to identify the effectiveness of epithelial removal, tracheal segments were processed for hematoxylin and eosin staining and histology was evaluated by light optical microscopy. The experiments were conducted according to Ethics Committee in Animal Experiments n.53/2008 – Protocol n.196. **Results:** *In vivo* HQ exposure *per se* induced secretion of TNF-α (82.56%), which was reversed after LPS stimulation, and reduced the expression of MCP-1 (79.77%) evoked by LPS. Secretion of IL-10, IL-6 and NO were not modified by HQ exposure. In addition, *in vivo* HQ exposure increased tracheal reactivity to MCh (193.48%) and epithelial denudation suppressed the effect. Rubbing of tracheal rings lumen was effective in removing the epithelium, as shown by histological images. **Discussion:** Data obtained show that HQ inhalation modifies the abilities of trachea cells to respond to inflammatory stimulus, altering the secretion of chemical mediators and muscle responsiveness. This latter action is dependent on tracheal epithelium integrity. **Financial support**: FAPESP (Process nº08/55382-7 and 09/03964-5) and Capes.

04.038

An exploratory study of H_2S-releasing enzymes in rat synovial tissue. Ekundi-Valentim E, Rodrigues L, Teixeira SA, Munhoz CD, Muscará MN, Costa SKP ICB-USP − Farmacologia

Introduction: We have previously shown that exogenous H_2S delivered to the knee joint can produce a significant anti-inflammatory and anti-nociceptive effect, thus suggesting that H_2S donors can be used as complementary therapies for treatment of joint inflammation (Ekundi-Valentim et al., *Br J Pharmacol. 159:1463-7,* 2010). In this study, we aimed to identify the existence and activity levels of both H_2S-releasing enzymes: cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS) in the rat synovial membrane. **Methods:** experiments were performed in naive Wistar rats according to our Institutional Ethics Committee (protocol nº 64, book 2/2007, and page 46). The synovial membranes were removed from the rat knee (n=4 per group) and homogenized (10.000 g, 5 min; 4^{o} C). The supernatant was collected (400µl) and assays were performed by incubating the samples withL–cysteine (2 mM) and pyridoxal 5 phosphates (2mM) in the presence (or absence) of CSE (DL–propargylglycine; DL –PGly, 2mM) and CBS inhibitors (O-[carboxymethyl] hydroxylamine hemihydrochloride; CHH, 3mM) or both CSE and CBS inhibitors (- 20 min, 37ºC). The kinetic of H_2S generation was determined in different intervals at 670nm. The data were fitted into equation [Y=Bmax*X/(Kd+X)] and results were expressed in terms of absorbance. **Results:** The quantitative assay shows that H_2S is synthesized in the rat synovial membrane in a time-dependent manner. The H_2S generation in the synovial membrane was poorly reduced by PGLy (16%), but greatly inhibited by CHH (70%). The simultaneous administration of CHH and PGLy almost abolished (92%) H_2S generation in the rat synovial membrane. **Conclusion:** These preliminary results clearly show, for the first time, evidence for endogenous H_2S-releasing enzymes in the rat knee, thus indicating that endogenous H_2S pathway might contribute to the pathophsyiology of arthritis. Further studies are required to determine the levels of protein expression and immunolocalization of these enzymes in the knee synovial tissue. **Acknowledgments:** EEV is a recipient of a grant from University Agostinho Neto, Angola. We thank FAPESP, CAPES and CNPq (Brazil) for **Financial support** and Maria Alice Barreto, Irene Gouveia for technical assistance.

04.039

Effects of endogenous glucocorticoid and TSPO ligands on L-selectin expression in rat lymphocytes. Lima CB[1], Palermo-Neto J[2], Farsky S[1] [1]FCF-USP − Experimental Toxicology, [2]FMVZ-USP – Neuroimmunomodulation

Introduction: Benzodiazepines (BZD) are one of the most commonly used groups of anxiolytic drugs, whose effects are mediated through binding on neuronal post-synaptic plasma membrane $GAGA_A$ receptors. However, a second class of benzodiazepine binding sites, translocator protein (TSPO), has been found in many peripheral tissues and cell populations, as immune and endothelial cells. Studies have been shown that TSPO may be related, directly or indirectly, to the modulation of BZD on immune system and suggest the involvement of endogenous glucocorticoids (EG). Therefore, these mechanisms involved in the actions of BZD via TSPO are not fully comprehended. In this context, the aim of this work was to evaluate the effects of TSPO-binding drugs on expression of L-selectin expression in lymphocytes and the participation of EG on the effect. **Methods:** Adult male Wistar rats were divided into 2 groups: 1) vehicle treated (control, C) and 2) treated with the EG cytosolic receptor antagonist RU 38486 (10mg/kg; 18 and 1 h before blood collection). Leukocytes suspensions were obtained after erythrocytes lysis and *in vitro* incubated with RPMI, ethanol (0.001%), or with TSPO ligands PK 11195 (100nM) or Ro 5-4864 (100nM) for 1h. After this period, cells were stimulated or not with fMLP (10^{-8}M, 1 h) and the expression of L-selectin was quantified by flow cytometry. Animals were used in accordance with the guidelines of the Committee on Care and Use of Animal Resources of the School of Veterinary Medicine (nº 1690/09) and School of Pharmaceutical Sciences (nº262/09), USP. **Results:** *In vivo* RU treatment or *in vitro* TSPO ligands treatment did not change expression of L-selectin in lymphocytes. However, *in vivo* RU and *in vitro* Ro5-4864 treatments decreased L-selectin expression (C+Ro=153.6 ± 54.04; RU+RPMI=156.8 ± 61.62; RU+Ethanol= 143.5 ± 34.66; RU+Ro= 71.33 ± 28.79*; p<0.01 vs other groups). The action was specific to Ro 5-4864, as this effect was not observed in cells treated with PK11195. fMLP *in vitro* stimulation of cells obtained from C animals induced L-selectin shedding (RPMI basal= 184.4 ± 20.0; RPMI+fMLP= 151.7 ± 24.01*; Ethanol basal= 150.0 ± 23.43; Ethanol+fMLP= 102.3 ± 35.35*, p<0.05 vs respective basal), and previous *in vitro* treatment with TSPO ligands blocked this effect evoked by fMLP (PK11195 basal= 134.2 ± 42.36; PK11195 + fMLP= 104.7 ± 43.09; Ro5-4864 basal= 153.6 ± 54.04; Ro5-4864+fMLP= 123.3 ± 52.67). In contrast, RU and TSPO treatments did not prevent the shedding evoked by fMLP. (RPMI basal= 156.8 ± 61.62; RPMI+fMLP= 94.62 ± 41.47*; Ethanol basal= 143.5 ± 34.66; Ethanol+fMLP= 105.8 ± 25.36*; PK11195 basal= 129.6 ± 36.25; PK11195+fMLP= 94.07 ± 29.14*; Ro5-4864 basal= 71.33 ± 28.79; Ro5-4864+fMLP= 40.15 ± 17.90*; p<0.05). **Conclusion:** Results herein presented show that EG alone, via cytosolic receptor, do not modulate L-selectin expression in lymphocytes and the effects of TSPO on L-selectin expression involve EG, via cytosolic receptor. Therefore, a connection of L-selectin, EG cytosolic receptor and TSPO has been proposed, and it may be dependent on cell activation state. The *in vivo* biological significance of this connection will be further investigated. **Financial support:** Fapesp nº 2009/52245-1

04.040

Cellular influx and vascular permeability are modulated differentially by formaldehyde exposure in a rat model of allergic lung inflammation. Lino dos Santos Franco A, Amemiya RM, Domingos HV, Ligeiro de Oliveira AP, Breithaupt-Faloppa AC, Oliveira-Filho RM, Tavares de Lima W USP − Pharmacology

Introduction: Exposure to air pollutants such as formaldehyde (FA) leads to inflammation, oxidative stress and immune-modulation in the airways and is associated with airway inflammatory disorders such as asthma. FA is emitted by building materials, furniture carpets, wallpaper, plywood, floor coverings and sterilizing agents. Other major indoor sources are tobacco smoke, urea-formaldehyde foam insulation and cosmetics, deodorants, solvents, disinfectants and fumigants. The purpose of our study was to investigate the effects of exposure to FA on the allergic lung inflammation. The hypothesized link between reactive oxygen species and the effects of FA was also studied. **Methods:** Male Wistar rats were exposed to FA inhalation (1%, 90 min daily) for 3 days and subsequently sensitized with ovalbumin (OVA)-alum via the intraperitoneal route. One week later the rats received another injection with OVA-alum (booster). Two weeks later the rats were challenged with aerosolized OVA. After 24 h the challenge with OVA the pulmonary cellularity, vascular permeability and generation of LTB_4 were determined. In a parallel set of experiments, group of rats were treated 30 min before each FA inhalation with vitamin C (150 mg/kg) and vitamin E (350 mg/kg) by gavage and with apocynin (5 mg/kg) by intraperitoneal route. **Results**: The OVA challenge of rats upon FA exposure induced an elevated release of LTB_4 in lung cells, increased lung vascular permeability, whereas the cell recruitment into lung was reduced. The treatments with vitamins C, E and apocynin reduced the levels of LTB_4 in BAL-cultured cells, while increased the pulmonary cellularity. **Discussion:** Our findings suggest that FA down regulate the cellular migration into the lungs after an allergic challenge and increase the ability of resident lung cells to generate inflammatory mediators, explaining the increased lung vascular permeability. Our data are indicative that the actions of FA involve mechanisms related to oxidative stress, as far as the deleterious effects of this air pollutant on airways are concerned. **License authorization number of the Ethics Committee:** 66 **Acknowledgments:** FAPESP (2008//50766-1).

04.041

Cannabidiol, a nonpsychotropic plant-derived cannabinoid, decreases inflammation and alters leukocyte distribution in a murine model of acute lung injury. Ribeiro A[1], Ferraz-de-Paula V[1], Pinheiro ML[1], Zager A[1], Hallak JEC[2], Zuardi AW[2], Crippa JA[2], Palermo-Neto J[1]
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Introduction: Endocannabinoid system has become a topic of great interest in pharmacology due to its remarkable distribution in mammal organisms and capacity to play a modulatory role on diverse physiological functions, including immune system modulation. Acute lung injury is a murine model of Acute Respiratory Distress Syndrome (ARDS), a condition developed by many patients with sepsis, which affects thousands of people every year. There are reports showing that Cannabidiol (CBD) has anti-inflammatory properties. Therefore, our goal was to analyze the effects of CBD in a murine model of acute lung injury. **Methods:** Eighteen C57BL/6 male mice (Bioethic Commission protocol # 1597/2009) were used and divided randomly in 3 groups (n=5-7/group). Experimental group received CBD 20 mg/kg i.p. 60min prior nasal instillation of LPS (100 ug/mL). Control groups received vehicle i.p. 60min prior nasal instillation of LPS or sterile PBS. Each mouse received 1ul of LPS/g of weight. Twenty-four hours followed LPS or PBS nasal instillation, mice were submitted to euthanasia and bronchoalveolar lavage fluid (BAL), bone marrow and blood were harvested to analyze leukocyte distribution. Moreover, BAL supernatant was collected in order to measure TNF-alfa concentration. Data was analyzed using One-way ANOVA followed by Tukey-Kramer multiple comparison test. **Results:** We observed that vehicle+LPS group presented a remarkable increase in BAL cell counting and CBD 20 mg/kg prevented such effect ($F(2,14) = 24.56$; $p < .0001$). We did not observed statistical significance either in blood or bone marrow cellularity. However, we did observe that vehicle+LPS group presented a tendency to increase blood cellularity and CBD 20 mg/kg tended to prevent such effect ($F(2,15) = 3.125$; $p = .0734$). Additionally, vehicle+LPS group presented a remarkable increase in the TNF-alfa concentration in the BAL and CBD 20 mg/kg prevented such effect ($F(2,15) = 15.58$; $p < .001$). **Discussion:** We showed for the first time that CBD was able to decrease inflammatory parameters, such as BAL cellularity and TNF-alfa concentration, in a model of acute lung injury. Literature data points out for some possible mechanisms involved in the anti-inflammatory action of CBD, such as inhibition of NF-kB. Moreover, there are reports showing that CBD anti-inflammatory properties are sometimes triggered by CB1 and CB2 receptor, Vanilloid receptor and even Adenosine receptors. These possible mechanisms are afterwards intended to be analyzed. **Financial support**: FAPESP (2009/51886-3) and CNPq.

04.042

FPR2/ALX agonist modulates neutrophil migration in mouse air pouch. Sordi R[1], Della Justina AM[1], Menezes de Lima Jr O[2], Fernandes D[3], Assreuy J[1] [1]UFSC − Farmacologia, [2]FIOCRUZ − Farmacologia, [3]UEPG − Ciências Farmacêuticas

Introduction: Lipoxins (LX) are endogenous anti-inflammatory and pro-resolving eicosanoids generated during various inflammatory conditions. LX inhibits neutrophil chemotaxis, adhesion, and transmigration, while stimulating monocyte chemotaxis and phagocytosis of apoptotic neutrophils. The aim of this study is to investigate whether BML-111, a FPR2/ALX (lipoxin receptor) agonist, modulates the immune response in the mouse air pouch model of inflammation induced by carrageenan. **Methods**: Murine air pouch model of inflammation was induced by carrageenan. Total and differential cell counts were performed at 2, 4 and 8 hours after carrageenan. BML-111 (0.1, 1 and 10 ng/pouch) was injected immediately before carrageenan. Furthermore, we evaluated the action of the FPR2/ALX antagonist Boc1 (N-t-Boc-Met-Leu-Phe; 200 ng/pouch) on BML-111 effects. All procedures were approved by our Institutional Ethics Committee (PP00307/CEUA-UFSC). Results: Carrageenan promoted neutrophil influx into pouches in all evaluated periods. Administration of BML-111 (1 and 10 ng/pouch) immediately before carrageenan injection reduced ~50% neutrophil influx into inflamed pouches. The effect of BML-111 treatment lasted for up 8 hours. The FPR2/ALX antagonist Boc1 injected 30 minutes before BML-111 prevented the reduced migration (2 hours: 1.37 ± 0.14; 0.71 ± 0.05; 2.40 ± 1.06 for carrageenan group, BML + carrageenan group, Boc1 + BML + carrageenan group, respectively). **Discussion:** BML-111, a FPR2/ALX agonist, attenuated the inflammatory response by decreasing the neutrophil migration into the pouches. This effect was reverted by FPR2/ALX antagonist Boc1. **Financial support**: CAPES, CNPq and FAPESC.

04.043

The role of CXCR2 in mediating neutrophil accumulation in liver microvasculature depends on the nature of the stimulus. Barroso LC[1], Paula AM[2], Teixeira MM[1], Menezes GB[1]
[1]UFMG − Bioquímica e Imunologia, [2]UFMG − Física

Introduction: Hepatic neutrophil recruitment is a key feature in liver damage during infection and inflammation, and liver failure is one of the major causes of death worldwide. Neutrophils can mediate liver damage during systemic inflammation and contribute to hepatic failure. Chemokines are described to guide neutrophil migration to sites of infection and inflammation; however, there is an increasing body of evidence that the role of chemokines in leukocyte infiltration may vary with the severity of the inflammatory process. In this sense, we investigated the potential differential involvement of CXCR2 chemokines during systemic inflammatory response (endotoxemia) and an injury localized to liver tissue. **Methods**: In order to investigate mechanisms of hepatic neutrophil recruitment, we developed a novel surgical approach that generates an small liver thermal injury restricted to the liver surface, compared to intraperitoneal (IP) administration of *E. coli* LPS. CXCR2 antagonists (repertaxin and DF2156A) were used to investigate the participation of chemokines in liver neutrophil accumulation. Adhesion, crawling and morphology of eGFP-expressing neutrophils within hepatic sinusoids were studied by using two photon confocal intravital microscopy. Blood was collected to liver transaminases (TGO and TGP) dosage, and liver samples from injured and non injured sites (control) were collected to MPO, cytokines (TNF, IL-1beta, KC, IL-10) and histopathology studies. This project was previously approved by CETEA/UFMG (113/09). **Results and Discussion**: Local liver injury caused neutrophil adhesion in sinusoids within 30 minutes of injury with continued cell recruitment up to 4 hours. Highest numbers of cells were present immediately adjacent to the lesion. KC production, but not other cytokines, was significantly increased in injured site in comparison to control site. Serum TGO and TGP levels were increased up to 4 hours after local injury, concomitantly to neutrophil accumulation. Oral pre-treatment with repertaxin (30mg/kg) or DF2156A (15mg/kg) inhibited neutrophil accumulation in injured site, as assessed by MPO activity and confocal intravital microscopy. However, systemic LPS injection (0.5mg/kg; 4h) induced significant accumulation of neutrophils in sinusoids that was independent of CXCR2 chemokines. We herein describe a novel model of liver local neutrophil accumulation, which allows an interesting comparison with neutrophil accumulation due to systemic inflammatory responses. In addition, we suggest that CXCR2 chemokines are released during local liver inflammation and guide neutrophils precisely to the site of injury, contrasting to the mechanisms during systemic inflammatory responses. **Support**: INCT-Dengue, CNPq and FAPEMIG.

04.044

Participation of PI3K/AKT pathway in the pathogenesis of dengue virus infection. Valadão DF[1], Costa VV[1], Santos AG[1], Morcatty TQ[2], Fagundes CT[2], Cisalpino D[2], Silveira KD[3], Ávila TV[4], Sousa LP[5], Tavares LD[4], Teixeira MM[2], Souza DG[1] [1]UFMG − Microbiologia, [2]UFMG − Bioquímica e Imunologia, [3]UFMG − Fisiologia e Biofísica, [4]UFMG − Fisiologia e Farmacologia, [5]UFMG − Patologia Clínica

Introduction: Dengue is the most prevalent mosquito-borne tropical disease in humans. It caused by four dengue related virus serotypes (DENV 1-4) and is transmitted by *Aedes* mosquito. Among a number of viral infections, a wide variety of antiviral and virus-supportive signaling pathways are induced. Phosphoinositide 3-kinase (PI3K) is a recent addition of to the growing list of the signaling mediators that are activated after some virus infections. Represent a family of enzymes that have important roles on signal transduction, regulation of cell activation, growth, differentiation, survival, migration, proliferation and adhesion. Recent works have also demonstrated the participation of this pathway in virus replication *in vitro,* but until now the participation of this pathway in dengue virus infection is unclear. **Objective:** Thus, the aim of this study is to evaluate the participation of the PI3K/AKT pathway in an experimental model of dengue virus infection. **Methods:** This project was previously approved by CETEA/UFMG on number access 113/09. Wild type mice (C57BL/6) and knockout mice for PI3K-γ (PI3K-γ $^{-/-}$) were infected with the adapted Den-3 virus by the intraperitoneal route. After infection, lethality of animals was accompanied each 12 hours until day 14 p.i. Western Blotting analysis were made to examine the kinetics of AKT and NFκß phosphorylation. We made the evaluation of disease signals (hematocrit, platelets and hepatic transaminases levels in serum).Tissue damage was verified by histological analyses in liver and viral load in target organs was quantified by plaque assay. **Results and Discussion:** Phosphorylation of AKT and NFκß occurs on day 5 and 7 p.i, respectively, showing the downstream activation of this pathway after Den-3 infection. PI3K-γ$^{-/-}$ mice exhibit a protective phenotype after infection (86% survival) in comparison with WT mice that showed 80% of lethality. The index of platelets in Den-3 infected mice was 16% higher if compared with WT mice and there was a reduction in 31% in hematocrit levels, showing better clinical state of these animals. Hepatic transaminases levels in PI3K-γ$^{-/-}$ mice were lower in comparison with WT littermates, AST were 6X lower and ALT were 20X lower. This data can be directly correlated with the less tissue damage observed in histological analysis in PI3K-γ$^{-/-}$ mice. And viral load in spleen and blood were reduced in PI3K-γ$^{-/-}$ mice in comparison to WT mice. Our data suggests an important participation of PI3Kγ/AKT pathway in Denv infection. PI3K-γ$^{-/-}$ mice are protected from severe disease probably because of both, better clinical signs and lesser viral load showed in these animals. **Financial support:** INCT em dengue -CNPq, CAPES and FAPEMIG

04.045

Blockade of angiotensin converting enzyme and AT_1 receptor in T cells during malaria infection: mechanisms of t-cell regulation mediated by angiotensin II. Silva-Filho JL[1], Morrot A[2], Costa MFS[3], Souza MC[3], Henriques MGMO[3], Savino W[4], Caruso-Neves C[1], Pinheiro AAS[1] [1]IBCCF-UFRJ − Ciências da Saúde, [2]FIOCRUZ − Imunologia, [3]FIOCRUZ − Tecnologia em Fármacos, [4]FIOCRUZ − Pesquisa Sobre o Timo

Introduction: Malaria is one of the most serious infectious diseases in humans. The murine model *Plasmodium berghei* ANKA has provided valuable contributions to the understanding of disease pathogenesis. Here, we use the malaria infection experimental model to verify the *in vivo* role of angiotensin II (Ang II) in activated T lymphocytes as well as its contribution to malaria pathogenesis. **Methods**: C57BL/6 mice (L0004/08) infected with *P. berghei* ANKA (8–12 wks) were divided into three groups (6 per group) treated with vehicle, losartan or captopril and sacrificed at day 6 post infection for isolation of splenic T lymphocytes. In addition, T cells from infected mice received the same treatment in culture to verify the direct effect of Ang II. **Results and Discussion**: *In vivo* or *ex vivo*, losartan or captopril reduced the migration capacity of T cells by 90% and 78%, respectively. Ang II in the lower chamber did not restore T-cell migration from losartan-treated infected mice. Losartan or captopril reduced the frequency of activated endothelial cells with adhered T cells by 43% and 51%, respectively. Both treatments reduced conformational changes in the actin cytoskeleton by contact with extracellular matrix proteins, as analyzed by confocal microscopy. FACS analysis of cells showed that Ang II induced up-regulation of chemokine receptors such as CCR2 (MIF: 4.35 ± 0.2 naïve; 9.92 ± 1.5 infected; 5.50 ± 0.3 losartan- and 5.20 ± 0.3 captropil-treated mice) and CCR5 (MIF: 4.03 ± 0.06 naive; 6.06 ± 0.5 infected; 4.84 ± 0.3 losartan- and 4.63 ± 0.1 captopril-treated mice). Treatment with these drugs reduced the percentage of activated T cells by 50%; and the decrease of naïve T cells during infection was partially blocked with treatments (47.6 ± 0.8% naïve; 25.7 ± 2.4% infected; 34 ± 0.7% losartan and 34 ± 1.0% captopril). Effector memory T cells enhanced during infection but were reduced in treated mice (21.59 ± 1.3% naïve; 33.2 ± 1.4% infected; 21.6 ± 0.8% losartan- and 22.3 ± 0.7% captopril-treated mice) and central memory T cells showed the same profile (11.56 ± 0.7% naïve; 14.7 ± 1.2% infected; 6.95 ± 0.2% losartan- and 7.47 ± 1.1% captopril-treated mice). $Foxp3^+$ T cells were reduced to control levels in treated mice (6.5 ± 1.4% naïve; 9.5 ± 0.5% infected; 4.9 ± 0.8% losartan- and 7.1 ± 0.5% captopril-treated mice). These data were supported by a similar decrease in the frequency of IFN-γ-, IL-17- and IL-10-producing $CD4^+$ T cells. Immunoblotting analysis revealed a 4.3- and 3.3-fold increase in the expression levels of AT_1 and $AT_{(1-7)}$ receptors, respectively, in activated cells and *in vivo* losartan treatment was able to suppress it. AT_2 receptor levels were not modified. Moreover, serum TNF-α and IFN-γ was reduced by losartan only. These results indicate, for the first time, a role of Ang II, through the AT_1 receptor, in regulating T-cell functions and recruitment to inflamed tissues. The blockade of Ang II signaling can provides a strategy for adjunctive therapy to improve treatment outcomes of malaria disease. **Financial support**: FAPERJ, CAPES, and CNPq

04.046

Immature thymocytes are released into the periphery of *Trypanosoma cruzi* acutely infected mice by a S1P-dependent mechanism. Lepletier A[1], Borja GP[2], Einicker-Lamas M[3], Silva Barbosa SD[1], Perez AR[4], Terra-Granado E[1], Carvalho CE[1], Melendes A[5], Savino W[6], Morrot A[1] [1]FIOCRUZ − Imunologia, [2]UFRJ − Imunologia e Microbiologia, [3]IBCCF-UFRJ, [4]Universidade Nacional de Rosario, [5]Glasgow University − Biomedical Research, [6]FIOCRUZ − Pesquisa Sobre o Timo

Introduction: Sphingosine 1-phosphate (S1P), a breakdown product of sphingolipid metabolism, is present in all mammalian cells and serves as a second messenger in signal transduction pathways. S1P is released into the extracellular milieu by a variety of cell types, making it one of the most abundant biologically active lysophospholipids in circulation. Autocrine and paracrine interactions between S1P and a family of five different G protein-coupled receptors (S1P1-5) have been implicated in a wide range of physiological activities, including immunity. It has been shown that the S1P1 receptor surface expression on lymphocytes is critical for their egress from thymus and lymph nodes. The biosynthetic pathway of the signaling S1P agonist has been well characterized. Reversible systemic and local synthesis of S1P is mediated by sphingosine kinases (SPHK) and by S1P phosphatases (SPP1 and 2), while irreversible degradation of S1P is carried out by a single enzyme, S1P lyase (SPL). Furthermore, S1P1-dependent chemotactic responsiveness is strongly upregulated in T-cell development before exit from the thymus, whereas S1P1 is downregulated during peripheral lymphocyte activation, and this fate is associated with retention in lymphoid organs. Here we aim to investigate the role of S1P pathway on *Trypanosoma cruzi* induced thymus atrophy. **Methods:** We performed a series of experiments using 4-6 weeks-old Balb/c male mice, infected with hundred of trypomastigote forms of Tuluahuen *T. cruzi* parasites. All the protocols were approved by Foundation Oswaldo Cruz Animal Use Ethical Committee (LW 8/10). In the first approach we quantified the global levels of intrathymic S1P levels by TLC and further characterized the gene expression of the S1P metabolic enzymes from both normal and infected thymus by Real-Time PCR. Moreover we assessed the intrathymic S1P1 receptor expression by immunohistochemistry. The early thymocyte release pattern during *T. cruzi* infection was analyzed by direct *FITC* intrathymic injection. We assessed the chemotactic responses of thymocytes obtained from acutely infected mice to S1P, by using a transwell migration assay. Finally, infected animal were systemically treated with FTY720, a potent agonist of S1P receptor that induces a prolonged down-regulation of the receptor subtypes $S1P_1$ on thymocytes. In all the assays, the total thymic cellularity and thymocyte subsets were determined by citofluorimetric analysis. **Results:** Our findings indicate that *T. cruzi* acutely infected thymus show a decreased level of the signaling S1P receptor agonist associated to a S1P catabolic gene expression pattern. Furthermore, we observed an abnormal increase in the expression of the S1P1 receptor from both cortical and medullary thymic compartments from day 10 to 14 post-infection, when premature release of all thymocytes subsets to periphery takes place. Upon FTY720 treatment, infected mice were able to recover the thymic cellularity, with a significant increase of the intrathymic $CD4^+$ and double negative cell populations. Furthermore, our data indicate that these two thymocyte subsets from chagasic thymus presented a high chemotactic response to S1P. **Discussion:** Achieved data indicate that the S1P signaling pathway exerts a critical role on the premature release of undifferentiated thymocytes during the *T. cruzi* induced thymus atrophy. As the early release of the thymocytes expressing forbidden TCR molecules to periphery may contribute to the immunopathological events seen in Chagas disease, our findings suggest the use of the S1P1 signaling pathway as a therapeutic target in Chagas disease. **Financial support:** CNPq and FAPERJ

04.047

04.047

Effect of repeated treatment with enalapril on the hepatotoxicity induced by acetaminophen in mice. Betto MRB[1], Lazarotto LF[2], Leite CE[3], Watanabe TTN[4], Driemeier D[5], Campos MM[6] [1]PUCRS − Biologia Celular e Molecular, [2]PUCRS – Farmácia, [3]PUCRS − Toxicologia, [4]UFRGS − Patologia e Clínica Veterinária, [5]UFRGS − Veterinária, [6]PUCRS − Cirurgia-Odontologia

Introduction: Acetaminophen (AA) is a potent antipyretic and analgesic agent, with weak anti-inflammatory effects**.** Despite its efficacy, the use of elevated doses of AA is associated with severe hepatotoxicity. It has been suggested that treatment with angiotensin-converting enzyme (ACE) inhibitors, such as captopril, might present protective effects in the acute intoxication evoked by AA (Yeung, Drug Metabol Drug Interact., 6, 295, 1988). The present study investigated the effects of repeated treatment with the highly potent ACE inhibitor enalapril on the hepatotoxicity induced by AA in mice. **Methods:** Male and Female C57BL/6 mice (6-8 per group, 20-25 g) were used. All the experimental protocols were approved by the Local Ethics Committee (09/00119 − PUCRS). Animals were pretreated orally with enalapril (30 mg/kg), once a day, during 4 days before AA administration. On the fifth day, hepatotoxicity was induced by a single dose of AA (400 mg/kg, i.p.). The animals received two additional enalapril doses 1 h before, and 6 h after AA administration. Mice were submitted to euthanasia 24 h after AA injection. The following parameters were assessed: macroscopic and histological examination of livers, serum levels of aspartate transaminase (AST) and alanine transaminase (ALT), and determination of catalase activity (CAT) and glutathione concentration (GSH) in liver slices. **Results:** The oral administration of enalapril produced a significant reduction of hepatotoxicity induced by AA, according to the macroscopic evaluation of livers (71 $\pm$ 10%). The histological evaluation revealed that samples obtained from AA control animals presented necrosis in 1 to 2 layers of hepatocytes, accompanied by layers of centrilobular degeneration, while those pretreated with enalapril showed minimal necrosis, or no change. The treatment with enalapril also displayed a marked inhibition of liver CAT and GSH (72 $\pm$ 11% and 90 $\pm$ 11%, respectively). Additionally, the animals pre-treated with enalapril showed a marked reduction of AST and ALT serum levels (86 $\pm$ 14% and 88 $\pm$ 9%, respectively). **Discussion:** The results of the present study indicate that chronic administration of enalapril was able to prevent the hepatotoxicity caused by AA. We might suggest that patients under treatment with this inhibitor are less susceptible to the effects of AA. **Financial support:** CAPES, CNPq, PUCRS.

04.048

A pharmacological approach to food allergy in mice: novel therapeutic targets. Pereira-Silva PEM, Amaral SS, Noviello MLM, Menezes GB, Cara DC ICB-UFMG − Morfologia

Introduction: The prevalence of allergic diseases has markedly increased in westernized countries since the 1960's. Food allergy, asthma, and atopic eczema are well known members of this group of diseases, being characterized by inflammatory processes involving CD4+ T helper cell responses of the Th2 phenotype, increased IgE concentrations, mast-cell degranulation and eosinophil-mediated inflammation. Food allergy is defined as an adverse immunological response (hypersensitivity) to food proteins, which is particularly frequent among children and may affect their final height and weight. Since food restriction and chronic glucocorticoid treatment cause several side effects, the development of novel therapeutic strategies is necessary. In order to achieve this, we made different pharmacological approaches using an experimental murine model of food allergy, in which ovalbumin-sensitized mice are given egg white solution as the only liquid source [1]. **Methods:** Four weeks old, female BALB/c mice, obtained from CEBIO/UFMG, were sensitized with 10µg Ovalbumin (OVA) adsorbed in 1mg aluminum hydroxide, subcutaneously. A second injection of 10µg OVA was given fourteen days later. Control groups received only saline and adjuvant. Seven days after the second injection, the mice received a 20% EWS (egg white solution) as the only liquid source, for two weeks (antigen challenge). Euthanasia and sample harvesting were performed at the end of this period. The groups were treated with: Acetylsalicylic Acid (ASA; 0.5 mg/kg), Dexamethasone (DEX; 0.4mg/kg), Ondansetron (OND; 1.0mg/kg) or Promethazine (PRO 3mg/kg). All treatments began three days before antigen challenge, and were given daily by gavage (ASA) or subcutaneously (DEX, OND, PRO) until the end of the experiment.
Data was analyzed by One-Way or Two-Way ANOVA, in which the level of significance was set at $p < 0.05$. All animal protocols are in accordance with the Ethics Committee in Animal Experimentation of our Institution (CETEA/UFMG – 214/2007). **Results and Discussion:** Allergic mice presented a significant weight loss (~20%) at the beginning of the antigen challenge. Administration of ASA, OND, PRO and DEX was able to inhibit the weight loss at the first week of challenge, although only DEX and OND treatments maintained this benefic effect throughout the whole challenge period. Regarding serum anti-OVA IgE levels, ASA, OND and DEX caused a reduction of IgE levels in comparison with the untreated allergic group, but not PRO treatment. DEX was expected to reduce serum IgE, but not ASA and OND. A possible explanation is the induction of aspirin-trigged mediators by ASA, such as 15-epi-Lipoxin A4, which possess anti-inflammatory and pro-resolving properties on mucosal surfaces, while antagonism of 5-HT_3 by OND might have reduced allergic inflammation and diarrhea. Both effects on IgE levels can be correlated with less mucosal injury/inflammation and less antigen penetration, what would represent a weaker immunological stimulus to IgE production. Thus, pharmacological antagonism of serotonin 5-HT_3 receptors and administration of aspirin-triggered mediators may consist in alternative therapeutic strategies for food allergy. **References:** [1] Saldanha J.C.S. *et al, Braz J Med Biol Res*. 2004 Jun;37(6):809-16. 2004. **Support:** CNPq and Fapemig

04.049

Quercetin inhibits neutrophil recruitment *in vivo* and *in vitro*: inhibition of actin polymerization. Zarpelon AC[1], Souto FO[2], Staurengo-Ferrari L[1], Fattori V[1], Casagrande R[1], Fonseca MJ[3], Cunha TM[2], Ferreira SH[2], Cunha FQ[2], Verri Jr WA[1] [1]UEL − Ciências Patológicas, [2]FMRP-USP – Farmacologia, [3]FCFRP-USP − Ciências Farmacêuticas

Introduction: Quercetin is a flavonoid with antioxidant properties. It is not completely understood, but it is likely that there is a link between the antioxidant and anti-inflammatory effects of quercetin. An important component of the inflammatory response is the neutrophil recruitment to the inflammatory focus, because neutrophils phagocyte and eliminate infectious agents. Recent *in vitro* data suggested that the quercetin would not affect neutrophil function. Therefore, we evaluated *in vivo* and *in vitro* whether quercetin affects neutrophil function focusing on recruitment. **Methods:** Mice received subcutaneous treatment with quercetin (30-300 mg/kg) or vehicle (20% Tween 80 in saline) 30 min before intraperitoneal injection of CXCL1 (3 ng), CXCL5 (30 ng), LTB_4 (25 ng) or fMLP (3 µg). Neutrophil migration was determined 6h after. Human neutrophils were treated with quercetin (10-100 nM) or vehicle (RPMI with 2% DMSO) for 30 minutes, and then assayed for chemotaxis in Boyden chamber, receptor cell surface expression by flow cytometry or actin polymerization/F-actin assembly by immunefluorescence using CXCL8 (10 ng/ml), LTB_4 (10^{-7}M) or fMLP(10^{-7}M) as stimuli. This study was approved by Ethics Committee on Animal Studies of the FCF of Ribeirao Preto (University of Sao Paulo; protocol no. 04.1.951.53.1) and Human Ethics Committee of the Faculty of Medicine of Ribeirao Preto (University of Sao Paulo; protocol no. 12664/2006). **Results:** The *in vivo* treatment with quercetin inhibited in a dose-dependent manner the recruitment of neutrophils to the peritoneal cavity of mice induced by known chemotatic factors such as CXCL1, CXCL5, LTB_4 and fMLP. Furthermore, quercetin also inhibited in a concentration-dependent manner the chemoattraction of human neutrophils induced by CXCL8, LTB_4 and fMLP in the Boyden chamber. *In vitro* treatment with quercetin did not affect human neutrophil surface expression of CXCR1, CXCR2, BLT1 or FLPR1, but rather reduced actin polymerization. **Discussion:** These results suggest that quercetin inhibits the neutrophil recruitment *in vivo* and *in vitro*, and highlights its possible usefulness to diminish excessive neutrophil migration during inflammation. **Financial support:** FAPESP, CNPq and CAPES and Fundação Araucária.

04.050

Effects of mangiferin on allergic inflammation induced by ovalbumin in A/J mice. Coelho LP, Jurgilas PB, Serra MF, Pires ALA, Cruz CCD, Cordeiro RSB, Silva PMR, Martins MA IOC-FIOCRUZ − Fisiologia e Farmacodinâmica

Aim: Mangiferin has been screened in our laboratory in a systematic effort to discover new antiasthma substances. We had already verified that relaxation effect of mangiferin *in vitro* is mediated by nitric oxide-cGMP pathway. Ridnour and collaborators, (2007) had demonstrated that the nitric oxide-cGMP pathway increases MMP-9 activity. Furthermore, McMillan and collaborators (2004) had showed that MMP-9 deficiency results in enhanced allergen-induced airway inflammation. According with these data, we investigated the bronchorelaxant and anti-allergic action of mangiferin and these relationships with NO production and MMP-9 activity. **Methods:** Sensitized and boosted A/J mice (License no. L034/09) were subjected to i.n. instillation of OVA, 25 µg on days 19 and 20 post-sensitization. Mangiferin (12.5 – 50 mg/kg, v.o.) was administered 1 h before provocation. Airway hyperresponsiveness was determinated by Whole-body plethysmography, 24 h after the last OVA challenge, by aerosolized PBS or methacholine (3 and 6 mg/mL). Eosinophil accumulation was verified in lung digest and its analysis was performed on May-Grunwald-Giemsa stained cytospun. Eotaxin-1 and IL-4 were analyzed in lung tissue by ELISA. We have also investigated the nitric oxide-cGMP pathway *in vivo*, using barometric whole-body plethysmography. Naive A/J mice were treated with 20 mg/kg L-NAME (i.p.), 30 min before the 50 mg/kg mangiferin administration (p.o) or controls (equal amounts) and vehicle (1% Tween 80, diluted in 0.9% NaCl – p.o.) 1 h before the nebulizated PBS or methacholine (3 and 6 mg/mL). The MMP-9 activity was analyzed, in tracheal rings, performed by gelatin zymography. Each tracheal ring group was treated to 30 min with saline, L-NAME (100 µM), mangiferin (0.1 – 10 µM) or L-NAME plus mangiferin (10 µM). After the end of incubation, the cells were lysated and the supernatant was collected to MMP-9 analysis. **Results:** Oral administration of mangiferin (50 mg/kg) inhibited airway hyperresponsiveness triggered by aerosolized methacholine (3 and 6 mg/ml), reduced eosinophils number and inhibits IL-4 and eotaxin-1 production in the lung samples of allergic mice. In naive mice, mangiferin also inhibited airway obstruction triggered by aerosolized methacholine, in a mechanism sensitive to L-NAME. Our result of zymography indicated that mangiferin (10 µM) increased MMP-9 activity and its effect was inhibited by L-NAME (100 µM). **Discussion:** Our findings indicate that oral treatment with mangiferin inhibits airway hyperreactivity and pulmonary eosinophilic inflammation, triggered by antigenic stimulation in mice through a mechanism associated with the inhibition of IL-4 and eotaxin-1. In naive mice the mangiferin inhibited methacoline-induced airway obstruction through the NO-cGMP pathway. Furthermore, the enhanced production of NO by the treatment of tracheal rings with mangiferin led to an increase of MMP-9. According to McMillan and collaborators (2004) this increment in MMP-9 activity at the initial phase of asthma process, may be important to resolution of the disease. Taken together, these results emphasize the possibility that this compound may be beneficial for the treatment of airflow limitation triggered by allergen challenge in humans. **Financial support:** FAPERJ, PAPES-FIOCRUZ

04.051

Inhibition heme oxygenase increases neutrophil migration to the bronchoalveolar spaces and attenuates pulmonary mechanics changes during severe sepsis induced by pneumonia. Czaikoski PG[1], Nascimento DCB[2], Spiller F[1], Rocco PRM[3], Cunha FQ[1]
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Background: Decrease of the neutrophil migration to the focus of infection is associated with poor outcome in severe sepsis. Heme oxygenase (HO) is an enzyme that catalyzes the degradation of heme into carbon monoxide, biliverdin and free iron, and its activity is known to inhibit the rolling, adhesion and migration of neutrophil to the inflammatory site. In the present study we evaluated the effect of the heme oxygenase inhibition on neutrophil recruitment to the bronchoalveolar spaces and pulmonary mechanics during severe sepsis induced by pneumonia. **Methods:** Sepsis was induced in mice by intratracheal administration of different number of *Klebsiella pneumoniae* to induce severe (SS) and mild (MS) sepsis (experiments were approved by ethical committee -CETEA – FMRP-USP, protocol n°. 027/2009). One group of SS mice was pretreated with HO inhibitor (ZnDPBG). **Results:** SS mice presented a reduced number of neutrophil in bronchoalveolar lavage, high number of bacteria in blood, neutrophil sequestration into pulmonar parenchyma and deterioration of lung mechanics when compared to MS mice. However, the HO inhibition in SS mice partially restored the neutrophil migration to the bronchoalveolar spaces, decreased the pulmonary neutrophil sequestration, reduced pulmonary and blood bacterial counts, and minimized pulmonary mechanics changes, as a consequence, these mice are more resistant to sepsis. **Conclusion:** These results show that HO activity mediated the reduction of neutrophil migration to the bronchoalveolar spaces and consequently impaired bacterial control during SS induced by pneumonia.
Grant support: FAPESP, CAPES, FAEPA.

04.052

Effect of thoracic lymphatic duct ligation on the release of lung inflammatory mediators in the model of gut trauma in rats. Breithaupt-Faloppa AC, Vitoretti LB, de Assis Ramos MM, Cavriani G, Sudo-Hayashi LS, Oliveira-Filho RM, Vargaftig BB, Tavares de Lima W ICB-USP − Farmacologia

Introduction: Lung inflammation is characterized by cellular recruitment and increased vascular permeability. As inflammatory mediators are involved in both events, we investigated the involvement of lymphatic system on the lung inflammation caused by intestinal ischemia/reperfusion (I/R). In parallel, we assessed the involvement of lymphatic system on the mechanisms related to leukocyte-endothelial interaction notably on the expression of adhesion molecules and extracellular matrix proteins. **Methods:** Upon anesthesia, male rats were subjected to occlusion of the superior mesenteric artery (SMA) during 45 min, followed by 2 hr of intestinal reperfusion. In parallel, a group of rats had the thoracic lymphatic duct obstructed immediately before the ischemia until the end of reperfusion period. Lung myeloperoxidase activity (MPO) and microvascular leakage were determinated. The levels of IL-1β, IL-10, IL-6 and VEGF were quantified in lung explant and the expression of adhesion molecules (PECAM-1 and E-Selectin) were evaluated in lung sections using immunohistochemistry. **Results:** Intestinal I/R induced neutrophils migration and increased lung vascular permeability and the levels of inflammatory cytokines. The PECAM-1 expression on lung vessels was significantly reduced by intestinal I/R, however the E-Selectin expression was increased. Thoracic lymphatic duct ligation before intestinal I/R reduced neutrophils recruitment, lung plasma leakage and the lung generation of IL-1β, IL-10 and VEGF and also reverted the altered expression of adhesion molecules in lung vessels. **Discussion:** Our data indicates that lung neutrophil recruitment, cell adhesion and lung hemodynamic are mediated by lymph-transported mediators drained from the intestine. We suggest that the lymphatic system modulates lung inflammatory response after gut trauma and promotes the generation of an additional wave of inflammatory mediators in lungs. CEEA Nº 080/2005. **Financial support:** FAPESP and CNPq

04.053

Eosinophils as novel cell source of prostaglandin D_2: autocrine activity and allergy-driven synthesis. Luna-Gomes T[1], Magalhães KG[2], Mesquita-Santos FP[2], Bakker-Abreu I[1], Samico RF[1], Bozza PT[2], Diaz BL[1], Bandeira-Melo C[1] [1]IBCCF-UFRJ, [2]IOC-FIOCRUZ

Introduction: Prostaglandin (PG) D_2 is a key mediator of allergic inflammatory diseases, like asthma. This eicosanoid is a cyclooxigenase product synthesized mainly by mast cells which constitutively express high levels of the terminal enzyme involved in PGD_2 synthesis, the hematopoietic PGD synthase (h-PGDS). Here, we investigated whether eosinophils that like mast cells are effector cells of allergic inflammatory reactions, are also capable to synthesize and, therefore, supply PGD_2 when properly stimulated. **Methods:** Potential PGD_2 synthesis was considered within (i) human eosinophils isolated from peripheral blood of health volunteers with a negative selection kit (as approved by 052/09 CEP UFRJ/HUCFF); mouse eosinophils differentiated *in vitro* from mouse bone marrow cells (Dyer JI, 181:4004-4009, 2008); and (iii) eosinophils recruited to inflammatory sites of *in vivo* mouse model of allergy (as approved by CEUA/FIOCRUZ L002/08). Biologically activity of eosinophil-derived PGD_2 was studied by employing inhibitors of PGD_2 synthesis and activity. **Results:** Cytoplasmic constitutive expression of h-PGDS was found within non-stimulated human blood eosinophils isolated from healthy donors. Acute stimulation of human eosinophils with a supraphysiological stimulus calcium ionophore A23187 (0.1 – 5 μM) evoked in dose-dependent manner PGD_2 synthesis (n = 3), which was located at eosinophil nuclear envelope, and inhibited by pre-treatment with HQL-79 (10 μM) − a specific inhibitor of h-PGDS (n = 3). Pre-stimulation of human eosinophils with physiological agonists, arachidonic acid (AA; 10 μM) and human eotaxin (100 ng/mL) were also capable to enhance HQL-79-sensitive synthesis of PGD_2, which displayed autocrine/paracrine ability to activate DP1-mediated lipid body biogenesis – a hallmark event of eosinophil activation. Similar to AA-stimulated human eosinophils, *in vitro*-differentiated mouse eosinophils also synthesized PGD_2 within lipid bodies in response to AA stimulation. Infiltrating eosinophils found at inflammatory site of allergic reaction were identified as the cell population responsible for PGD_2 production detected 24 h after allergic challenge (n = 3). **Discussion and Conclusion:** Altogether, our results reveal that eosinophils are indeed able to synthesize PGD_2, which functions as an autocrine signal for eosinophil activation through its DP1 receptor. Moreover, infiltrating eosinophils appear to contribute as cellular source of PGD_2 found at inflammatory sites of allergic reactions. **Financial support:** FIOCRUZ, CAPES, CNPq and FAPERJ.

04.054

Kinetics of tissue response to orthodontic forces in mice: mechanical stimulation leads to bone remodeling through differential expression of osteoclast and osteoblast related factors. Garlet TP[1], Tadei SR[2], Silva TA[3], Garlet GP[4], Cunha FQ[1] [1]FMRP-USP, [2]ICB-UFMG, [3]UFMG − Patologia, [4]FOB-USP

Introduction: The ability to remodel itself in response to mechanical stimuli is fundamental to bone tissue to maintain its integrity and structure. This characteristic allowed orthodontic tooth movement to be successfully achieved in clinical practice for a long time, but only lately the inflammatory mechanisms involved are being unraveled. These mechanisms include the RANK-RANKL-OPG axis, direct regulator of osteoclast activation, as well as inflammatory cytokines, directly or indirectly stimulating bone cells. Understanding how bone responds and remodels itself might allow pharmacological interventions in order to improve clinical treatment of different bone pathologies. **Methods:** In order to investigate the tissue response and remodeling, in this study we developed a mouse model of orthodontic tooth movement. The appliance is comprised by a NiTi open spring, installed between the incisors and the first maxillary molar in order to mesially displace the molar. The movement was measured in different time points (12, 24, 72 and 144h), and samples from Compression (C) and Tensions (T) sites were harvested separately to real-time PCR assays. This protocol has been approved by FMRP Commission on Ethics in Animal Research − 034/2008. **Results:** Our results show that tooth movement occurs after a quick lag phase: no increase in distance between the first and second molars was observed after 12h; after 24h a trend has been detected; after 72h and 144h a significant difference has been noticed. Along with tooth displacement, osteoclast activation markers have been analyzed. Samples from both T and C sides expressed higher levels of the osteoclastogenic signal RANKL when compared to control samples, indicating active bone remodeling in these areas. However, the expression of RANKL in C sides were higher than in T sides, reflecting the increased bone resorption required to displace the first molar in mesial direction. Similar results were observed when Cathepsin K, a mediator of osteoclast bone resorption, was analyzed. After 24h, both C and T sides expressed higher levels of this protease when compared to controls. However, the increase in Cathepsin K expression was markedly higher in C side when compared to T side. These results indicate moderate osteoclast activation on T side and high osteoclast activation on C side. Osteoblast activation was also assessed by means of its marker RUNX2. While the expression of RUNX2 in C side remained stable, with a trend towards declining, after 72h a marked increased in its expression was observed in T side. **Discussion:** Taken together, these results show how different responses from the periodontal tissue will determine bone remodeling and ultimately the outcome of orthodontic therapy. Compression stimuli increase the expression of osteoclastogenic molecules enhancing bone resorption, while tensile forces stimulate the differentiation and activation of osteoblasts, increasing bone deposition. These results also reinforce that our model resemble periodontal response to orthodontic forces in humans at tissue and molecular levels, and therefore future studies with pharmacological and genetic tools, targeting specific immune/inflammatory molecules, will certainly contribute to unravel the mechanisms involved. **Financial support**: FAPESP.

04.055

Signaling transduction pathway involved in LPS-induced suppression of melatonin production by rat pineal gland. Cruz-Machado SS[1], Pinato, L[2], Carvalho-Sousa CE[1], Tamura EK[1], Ferreira ZS[1], Markus RP[1] [1]IB-USP − Fisiologia, [2]UNESP − Fonoaudiologia

Introduction: The pineal gland produces melatonin at night due to norepinephrine (NE) stimulation of arylalkylamine-N-acetyltransferase (AA-NAT) transcription. This enzyme converts serotonin (5-HT) to N-acetylserotonin (NAS), which is methylated by the hydroxyindole-O-methyltransferase to melatonin. The suppression of nocturnal melatonin production was suggested to play a role in the mounting of an inflammatory response (Markus, Neuroimmunomodulation, 14: 126, 2007). Our aim was to uncover the mechanisms involved in the suppression of melatonin production induced by lipopolysaccharide (LPS), the pathogen-associated molecular pattern of gram-negative bacteria. **Methods:** Approved ethical committee (CEA/IB: 045/07). Cultured pineal glands from pre-pubertal Wistar rats were stimulated in the absence or presence of LPS. Toll-like receptor 4 (TLR4) and CD14 expression were evaluated by immunohistochemistry of frozen sections of rat pineal glands. The nuclear factor kappa B (NFKB) translocation was assayed in nuclear extracts by EMSA. The tumor necrosis factor (TNF) production by the pineal was measured in the medium by ELISA. Melatonin and precursors were measured in the medium by HPLC. **Results:** TLR4 and CD14-positive immunostaining were observed in the cytoplasm and membrane of pineal cells. In addition, TLR4 is expressed rhythmically in rat pineal, with maximal expression at late afternoon. LPS (1.0 µg/mL) induced NFKB activation in a rapid and transient profile with maximal nuclear detection at 15 min. The super-shift assay indicates presence of p50/p50 and p50/RelA NFKB dimers. Pineal glands incubated with LPS presented a time-dependent increase in the amount of TNF, with maximal production after 240 min (7.7 ± 3.3 and 8.6 ± 1.9 pg/well, LPS 0.1 and 1.0 µg/mL respectively, n=6). Blockage of nuclear NFKB translocation with N-acetyl-leu-leu-norleucinal (ALLN, 12.5 µM, 48 h) abolished LPS-induced TNF production. In addition, LPS (0.1 µg/mL, 240 min) up-regulates TNF receptor 1 (TNFR1) expression in pinealocytes. Finally, LPS (0.1 µg/mL, 13 h) significantly inhibited NE-induced melatonin (control: 91.6 ± 14.7 ng/well, n=10; LPS: 50.4 ± 6.9 ng/well, n=10) and NAS production (control: 15.2 ± 2.6 ng/well, n=5; LPS: 7.8 ± 1.4 ng/well, n=7), resulting in accumulation of 5-HT in the medium (control: 178.1 ± 29.3 ng/well, n=6; LPS: 242.8 ± 9.6 ng/well, n=7). **Discussion:** Here we show that the rat pineal gland is instrumented to recognize LPS through TLR4/CD14 receptors. The activation of NFKB pathway leads to the production of TNF, the up-regulation of TNFR1 expression in pinealocytes and the inhibition of melatonin biosynthetic pathway. In fact, TNF is known to inhibit melatonin synthesis due to *Aa-nat* suppression (Fernandes, J Pineal Res, 41: 344, 2006). Taking into account that nuclear translocation of the p50/Rel A and p50/p50 dimers leads, respectively, to activation and inhibition of gene transcription, our data support the hypothesis that the same transduction pathway is responsible for mediating the expression of TNF and the inhibition of melatonin synthesis. Collectively, our data indicates that LPS activates downstream signaling through TLR4/CD14 receptors, controls melatonin synthesis and the production of TNF by rat pineal gland. **Financial support:** FAPESP (2007/07871-6), CNPq (484206-2006-0), CAPES.

04.056

Enhanced airway smooth muscle reactivity to cholinergic provocation is associated to mast cells in A/J mice. Anjos-Valotta EA, Farias-Filho FA, Serra MF, Cordeiro RSB, Silva PMR, Martins MA FIOCRUZ − Inflamação

Background and aim: Prior studies show that genetic strain variations may exist with regard to respiratory mechanics and airway hyperresponsiveness in mice. It has been shown that A/J mice are more sensitive to cholinergic-mediated bronchial spasm as compared to Balb/c mice. The current study is undertaken in order to test the hypothesis that the number of mast cells in the target tissue underlies the relative hyperreactivity expressed by mice of the A-J strain. **Methods:** Age-matched mice of strains A/J and Balbc were employed as donors of tracheal and bronchial tissues for functional and histological approaches. Mast cell population was evaluated by histomorphometry in carnoy-fixed and paraffin-embebed sections stained with alcian blue-safranin. Tracheal and bronchial rings were mounted in isolated organ bath for assessment of carbachol response (0.01-100 μM) in presence or absence of sodium cromoglicate (SCG). **Results:** We found increased mast cell numbers in tracheal (16.2 ± 1.7 cells/section) and bronchial tissues (36.7 ± 4.1 cells/section) (mean ± SEM, n=5) recovered from naïve A/J mice as compared to those from BALB/c tracheal (9.8 ± 0.7 cells/section) and bronchial tissues (15.4 ± 3.7 cells/section). Both tracheal and bronchi rings obtained from A/J mice were clearly more responsive to carbachol-induced contraction, as compared to those obtained from BALB/c mice. Following 1 mM SCG treatment, there was a marked attenuation of the tracheal response to carbachol irrespective to the mouse strain; however a higher sensitivity of A/J mice tracheal rings to the mast cell stabilizer was noted. **Conclusions**: Our findings indicate that there are more mast cells in the airway smooth muscle tissue of A/J mice as compared to Balb/c mice and that this mast cell tissue enrichment may be critical in the airway hyperresponsiveness exhibited by A/J mice following cholinergic provocation. **Supported by**: FAPERJ and CNPq

04.057

Prior exposure to staphylococcal enterotoxin type B (SEB) potentiates the pulmonary eosinophil infiltration of allergic mice. Squebola Cola DM[1], Mello GC[1], Schenka A[2], Souza IA[1], Antunes E[1] [1]FCM-UNICAMP − Farmacologia, [2]FCM-UNICAMP − Patologia

Introduction: Asthma has a variety of causes, including exposure viral and microbial components. Clinical evidences have shown a strong association between *Staphylococcus aureus* and pathogenesis and/or exacerbation of bronchial asthma. *S aureus* secretes several heat-stable toxins (23 to 29 kDa) with superantigenic activities. We have previously shown that airways exposure to staphylococcal enterotoxin type A (SEA) potentiates the eosinophil influx into the bronchoalveolar lavage (BAL) fluid in allergic rats (Mariano et al., 2010). The enterotoxin type B (SEB) causes acute inflammatory lung injury characterized by an increased vascular permeability and granulocyte infiltration, but no study has evaluated whether it affects the cell pulmonary infiltration in allergic conditions. Therefore, this study aimed to investigate the effect of prior exposure to SEB on ovalbumin (OVA)-induced allergic pulmonary inflammation in BALB/c mice. The study was approved by the Ethical Committee of University of CAMPINAS – UNICAMP. (Protocol n° 1657-2). **Methods and Results:** Balb/c mice were immunized with a s.c injection of OVA (100 µg) dissolved in AL(OH)3. Two weeks thereafter, sensitized mice were intranasally exposed to SEB (1 µg) or sterile PBS (control group). Bronchoalveolar lavage (BAL), inflammatory cytokines and pulmonary histology were analyzed at 48 h after the first OVA challenge. At 4 h of SEB exposure, we observed an increased number of eosinophils in BAL (5.08 ± 0,61 X 10^6 cells) and levels of eotaxin (1598,86 ± 156,53pg/ml) in pulmonary homogenates of OVA-challenged mice compared with control group (2.85 ± 0,32 X 10^6 cells and 1067 ± 68,26 pg/ml, respectively; $P<0.05$). **Conclusions:** Our findings show that airways exposure to SEB exacerbates the allergic pulmonary eosinophilic inflammation, which is associated with increased eotaxin levels. **References:** Mariano N.S, et.al., *Int Immunopharmacol.* 10(1) 43-9; 2010. **Support:** FAPESP.

04.058

Role of mast cells on the production of CINC-2, migration of neutrophils and bone resorption in SHR animals submitted to periodontal disease. Belini L[1], Salzedas LMP[2], Oliveira SHP[1] [1]FO-UNESP-Araçatuba − Ciências Básicas, [2]UNESP-Araçatuba − Radiologia

The aim of this study was to evaluate levels of CINC-2 and neutrophil migration (NM) in the gingival tissue (GT) from spontaneously hypertensive rats (SHR) and Wistar (W) (180-200g) (Protocol. 52/06) submitted periodontal disease (PD) and the role of mast cells (MAST) on this process. Thus, a group of animals was depleted of MAST by treatment with compound 48/80 (48/80), ip, this being administered on day 1, two doses of 0.6 mg / kg, 2 and 3 days in two doses of 1.2 mg / kg and the 4th day in two doses of 2.4 mg / kg (range, 12 hours between doses). After 5 days was performed ligation of the first molars counterparts with silk thread to induce PD. The induction was also performed in animals of the groups that were not treated with the 48/80 and after 7 and 14 days, GT were collected for the determination of CINC-2 by ELISA, the MN was evaluated by the level of myeloperoxidase (MPO), and the mandibles were dissected and radiographed to evaluate the level of the bone resorption (BR) software Digora. We observed that the levels of CINC-2 tends to increase in W and SHR animals in the presence of PD. CINC-2 is increased in the absence of MAST in SHR with 7 days after induction of PD and tends to increase in animals W without MAST 7 days after induction of PD. In animals with 14 days of induction, the SHR with PD showed a significant increase over its control. (W without SD = 189.1 ± 120.1, W + DP (7 days) = 393.2 ± 343.7, W +48 / 80 (7 days) = 1167 ± 1053, W + DP (14 days) = 341, 5 ± 96.38; SHR without SD = 163.6 ± 49.17; SHR + DP (7 days) = 342.1 ± 232.5; SHR +48 / 80 (7 days) = 2083 ± 891.4; SHR + DP (14 days) = 558.6 ± 40.85; SHR +48 / 80 (14 days) = 812.7 ± 258.2, $p < 0.0001$). NM for the GT in SHR with PD is higher when compared with W animals with PD, both 7 and 14 days after disease induction. This migration was decreased in MAST-depleted SHR animals but not in animals W. Compared to BR, we note that the PD was effective, since we observed higher BR in all animals with PD. The presence of MAST increases BR only in SHR 14 days after induction of PD. (W without PD = 1.630 ± 0.097; W + PD (7 days) = 1.149 ± 0.294, W + PD (14 days) = 1.261 ± 0.212; SHR without PD = 1.724 ± 0.1241; SHR + PD (7 days) = 1.059 ± 0.1998; SHR + PD (14 days) = 0.9511 ± 0.1778; SHR +48 / 80 (14 days) = 1.232 ± 0.2514, $p < 0.05$). In conclusion, we observed that the presence of MAST enhances the inflammatory process in SHR and BR with PD but not in animals W. In contrast, the presence of MAST decreases the production of CINC-2 suggesting that this chemokine is not related to bone resorption and no MN in this model, especially in SHR animals.
Financial support: FAPESP Process: 2008/58061-7 and 2006/59794-2

04.059

Modulation of FCgR-mediated phagocytosis in macrophages by TLRS agonists: involvement of 5-LO products. Pinheiro CS[1], Monteiro APT[2], Benjamim CF[3], Canetti C[1]
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Introduction: Toll-like receptors (TLRs) are pattern recognition receptors which recognize molecules in microorganisms. Once recognized, microorganisms are engulfed trough a mechanism known as phagocytosis. Leukotrienes (LTs), lipid mediators produced from 5-lipoxygenase (5-LO) pathway, are able to increase phagocytosis in FcgR dependent process. They are produced from arachidonic acid metabolism in a two-step process to yield the epoxide intermediate, LTA_4. This step is dependent on the interaction of the 5-LO with a nuclear membrane protein, termed 5-LO activating protein. LTA_4 is then converted into either LTB_4 by an enzymatic reaction mediated by LTA_4 hydrolase or conjugated with glutathione by LTC_4 synthase to form the cysteinyl LTs (cys LTs). The aim of this study was to evaluate the participation of TLRs ligands on FcgR-mediated phagocytosis. **Methods**: Rat alveolar macrophages (AMs) or mouse (SV129 or 5-$LO^{-/-}$) peritoneal macrophages (PMs) were harvested, cultured, and treated with TLR ligands and then challenged with IgG opsonised sheep red blood cells (IgG-sRBCs). In some experiments, zileuton, a 5-LO inhibitor was incubated before TLR ligands addition. After 90 min, the phagocytic targets were removed and phagocytosis evaluated through a colorimetric assay. Lipid mediators (LTB_4 and cys LTs) were measured by EIA. Western blotting, was used to detect specific proteins in a given sample was also used to verify activation and expression of mitogen-activated protein kinase pathway. **Results:** Except for CpG, all TLR ligands tested (i.e ligands for TLR2, TLR3, and TLR4) were able to amplify phagocytosis of IgG-sRBC in AM. The phenomenon occurred in a concentration-dependent manner. Furthermore, phagocytosis amplification induced by TLR3 ligand was a time-dependent event, fact not observed for TLR2 and TLR4 ligands. AMs treatment with zileuton also impaired TLR4 ligand-induced phagocytosis amplification, suggesting the participation of 5-LO products in this phenomenon. To confirm LTs involvement in phagocytosis mediated by FcgR, PMs from 5-$LO^{-/-}$ mice did not presented any difference on phagocytosis when stimulated with TLR2, 3, and 4 ligands. LTB_4 production induced by IgG engagement was amplified by TLR2 and TLR4 ligands, but not by TLR3. Similar results were obtained for cys-LTs, whose production was increased by TLR2, TLR3, and TLR4. ERK1/2 and p38, mitogen-activated protein kinase (MAKs) *pathways were phosphorilated under TLRs agonists. This phenomenon was exacerbated under IgG-sRBC treatment.* **Discussion:** Our data suggest that TLR ligands amplify phagocytosis in a time and concentration dependent manner. Furthermore, 5-LO products pathway seems to be responsible for this phagocytosis amplification. ERK1/2 and p38 presented a greater activation when cells were incubated with TLRs agonists and challenged with IgG-sRBC, suggesting a possible interaction between FcgR and TLR signaling pathway. **Financial support**: Capes, CNPq, FAPERJ, FUJB. **Licença de autorização do Comitê de Ética Animal**: DFBCICB028

04.060

Odontoblasts stimulated by lipopolysaccharide express SCF and FGF-2 via p42/44, p38 and PI3K. Santos VAC[1], Oliveira SHP[2] [1]FOA-UNESP − Odontologia Social e Preventiva e Ciências Básicas, [2]FOA-UNESP − Ciências Básicas

The odontoblasts in the interface pulp and dentin are the first cells to find the bacteria and can play an important role in controlling the inflammatory process. The aim of this study was to evaluate whether LPS-stimulated odontoblasts cell line to express SCF and FGF-2, the mechanisms involved and the involvement of p42/44, p38 and PI3K pathways in the process. We used odontoblasts (OD) cell line MDPC-23 murine. Cells were stimulated with LPS (0.1, 1, 10 and 100 µg/mL) for 1, 6 and 24 hours. The SCF and FGF-2 expression in odontoblasts was evaluated by RT-PCR. The relationship between the expression of the area of the band for β-actin compared to SCF and FGF-2 was observed by densiometer. SCF production in the culture of the supernatant was analyzed by ELISA. To analyze whether SCF and FGF-2 expression is dependent on the cytokine and/or chemokine production and/or the 5-lypoxigenase production, cells were pretreated with Dexamethasone (DEXA) and MK886 (MK) for 30 minutes and then stimulated with LPS (0.1 µg/mL) for 1 hour. To evaluate the involvement of signal transduction pathways, cells were pretreated for 30 minutes with a specific inhibitor to p42/44 (PD98059, PD), p38 (SB203580, SB) and PI3K (wortmannin, Wort), soon after were stimulated with LPS (0.1 µg/mL) for 1 hour. The results showed that LPS-stimulated odontoblast to express SCF in a concentration of 0.1 µg/mL (SCF / β-actin =24.9) already in the 1st hour. The concentrations of 1µg/mL (SCF / β-actin = 13.01), 10 µg/mL (SCF / β-actin = 13.06) and 100 µg/mL (SCF / β-actin = 10.0) inhibited the expression. Six hours after stimulation LPS-induced SCF expression only at 10 ug/mL (SCF / β-actin = 19), but it was decreasing at the dose of 100 ug/mL of LPS (SCF / β-actin = 0, 10). After 24 hours we not observed LPS-induced SCF expression. Regarding the expression of FGF-2, we observed that LPS-stimulated odontoblasts induced FGF-2 expression at 0,1 µg/mL (FGF-2/β-actin = 8,04), 1 µg/mL (FGF-2/β-actin = 7,98) and 10 µg/mL (FGF-2/β-actin = 7,88) 1 hour after, however, we observed that 100 µg/mL (FGF-2/β-actin = 3,97) of LPS was able to inhibit the expression. We further note that 6 and 24 hours after LPS stimulation, no difference in the FGF-2 mRNA expression was observed compared to the control in any dose. The DEXA and MK were able to inhibit the SCF mRNA expression DEXA (SCF / β-actin = 0.5) and (FGF-2/ β-actin = 0.8). PD, SB and Wort inhibited the SCF mRNA expression (SCF / β-actin = 2) (SCF / β-actin = 4), (SCF/β-actin= 1) and FGF-2 mRNA expression (FGF-2/β-actin = 0.2) (FGF-2/β-actin = 0.2) (FGF-2/β-actin =0.2), respectively. These findings demonstrate that LPS-stimulated OD to express SCF and FGF-2 mRNA and this process is dependent on cytokine and/or chemokine production and also leukotriene, whereas DEXA and MK inhibited SCF and FGF-2 mRNA expression via activation of p42/44, p38 and PI3K. The data together suggest that SCF and FGF-2 released by the OD can act as modulators of immune response and tissue repair after inflammation and may therefore regulate neovascularization, leading to production of chemical mediators that participate in the formation of new tissue pulp. **Financial support**: FAPESP (process n^{o} 2008/51486-2)

04.061

Role of *GILZ (glucocorticoid-induced leucine zipper*) on resolution of inflammation. Nogueira CRC[1], Tavares LP[1], Silva JPV[1], Queiroz ALL[1], Silva DM[1], Soriani FM[1], Russo RC[1], Garcia CC[1], Lopes F[2], Pinho V[1], Teixeira MM[1], Sousa LP[3] [1]UFMG − Bioquímica e Imunologia, [2]UFMG − Morfologia, [3]UFMG − Patologia Clínica − COLTEC

Introduction: The apoptosis of neutrophils has been characterized as an important factor in the resolution of inflammation. Apoptotic neutrophils in tissues are recognized and phagocytosed by macrophages, which limit tissue injury caused by neutrophils at the sites of inflammation. In contrast, the permanence of neutrophils in tissues exacerbates inflammation by releasing proteases, reactive oxygen species, and pro-inflammatory mediators. Therefore, the induction of neutrophil apoptosis could be a potential benefit in the control of inflammatory diseases. Glucocorticoid (GC)-induced leucine zipper (GILZ) has been shown to mediate several GC functions, such as modulation of T-lymphocyte activation, apoptosis, anti-proliferative and anti-inflammatory activities. GILZ interacts with and inhibits the activities of the key inflammatory signaling mediators NF-kB and AP-1. In the present study, we investigated the role of GILZ on the spontaneous and pharmacologically-induced resolution of neutrophilic inflammation in the pleural cavity of LPS-challenged mice. **Methods:** BALB/C mice were challenged by i.pl. (intrapleural) administration of LPS (250 µg/cavity) or PBS. The PDE4 inhibitor rolipram (6 mg/kg) and the synthetic glucocorticoid dexamethasone (2 mg/kg) were administered systemically (i.p.). Wortmannin, a PI3K inhibitor, was given i.pl. (1 mg/kg). The cells present in the pleural cavity were harvested at different times of LPS challenge or after 4 h of treatment with drugs and processed for total and differential leukocyte counts and western blot analysis for GILZ, P-ERK1/2 and caspase-3 cleavage. This study was approved by the Ethics Committee in Animal Experimentation (CETEA/UFMG), protocol number 148/2006. **Results:** The injection of LPS induced a time-dependent influx of neutrophils into the pleural cavity of mice reaching maximally at 8-24 h. Neutrophils were diminished at 48 h, and complete resolution occurred only at 72h. The spontaneous resolution of neutrophilic inflammation was accompanied by an increase of mononuclear cells at the pleural cavity and was associated with an increase in the number of apoptotic cells, at times of 8-48 h that antecedes the complete resolution, as demonstrated by morphological criteria and caspase-3 cleavage. The GILZ expression was detected in PBS challenged mice and disappears during the peak of LPS-induced inflammation returning to basal levels on resolution phase (48-72 h). Pharmacological treatment of mice with drugs that cause the resolution of inflammation, such as rolipram, wortmannin and dexamethasone reduces the number of neutrophil into the pleural cavity and increase the levels of GILZ, that is associated with increased of caspase-3 cleavage and decrease of ERK1/2 phosphorylation. **Discussion:** Our results showed that GILZ is expressed during the natural resolution phase of LPS-induced pleurisy. Moreover, it is shown that pharmacological agents that induce resolution of inflammation increase GILZ expression. These studies show a positive correlation between expression of GILZ, decreased expression of survival pathways and resolution of inflammation, suggesting GILZ plays an important role in the signaling events leading to resolution of neutrophilic inflammation. **Financial support:** CNPq, PRPq-UFMG, FUNDEP/SANTANDER and FAPEMIG

04.062

Evaluation of the anti-inflammatory effect of mycofenolate mofetil in mice LPS-induced pleurisy. Beduschi MG[1], Darmarco ED[3], Frode TS[2], Guimarães CL[4] [1]FURB − Medicina, [2]UFSC − Análises Clínicas, [3]FURB − Farmácia, [4]FURB − Ciências Farmacêuticas

Introduction: Inflammation presents yet a huge collective impact and remains as an important target of therapeutic interest. Mycophenolate mofetil (MMF) is a pro-drug of mycophenolic acid (MPA), an immunosuppressant with high selective cytostatic effect on B and T lymphocytes has demonstrated efficacy in the treatment of various inflammatory diseases. The aim of this study was evaluate the acute anti-inflammatory effect of MMF in a murine pleurisy model induced by lipopolysaccharide (LPS). **Methods:** Male Swiss mice (35-40 g) were used. The experimental protocols were approved by the local Ethics Committee (CEUA 113/09 − FURB). The pleurisy was induced by intrapleural injection (100 µL/cavity) of LPS (2 µg/cavity – *E. coli* 0111:B4, Sigma, USA). After 24 h, the pleural cavity was washed with 2 mL of heparinized sterile saline (10 UI/mL). Samples of the pleural lavage fluid were used to perform total and differential leukocyte counts and myeloperoxidase (MPO), adenosine-deaminase (ADA) and nitrite and nitrate (NOx) activity quantification. Total leukocyte counts were performed in a Neubauer chamber, while cytospin preparations of pleural lavage fluid were stained by the May-Grünwald-Giemsa technique for the differential leukocyte counts, which were performed under an oil immersion objective. Different groups were pretreated with MMF (10–200 mg/kg, v.o.) or dexamethasone (Dexa, 4 mg/kg, i.p.) 30 min after LPS administration. Results were presented as mean ± S.E.M of at least 6 animals, and analyzed statistically by one-way ANOVA, and differences between groups were assessed using the Student-Newman-Keuls post-test (# $P < 0.05$). **Results and Discussion:** Dexa reduced the neutrophil migration ($0.59 \pm 0.21 \times 10^{6\#}$) compared to the control group ($1.37 \pm 0.25 \times 10^{6}$). MMF altered the total leukocyte migration at $3.25 \pm 0.4 \times 10^{6}$, $3.05 \pm 0.3 \times 10^{6}$, $2.47 \pm 0.17 \times 10^{6}$ and $2.10 \pm 0.10 \times 10^{6\#}$, at the doses of 10, 50, 100 e 200 mg/kg, respectively (Control: $2.98 \pm 0.47 \times 10^{6}$). The neutrophil infiltration was altered to $1.38 \pm 0.3 \times 10^{6}$, $1.53 \pm 0.15 \times 10^{6}$, $0.72 \pm 0.17 \times 10^{6\#}$ and $0.29 \pm 0.10 \times 10^{6\#}$ (Control: $1.35 \pm 0.27 \times 10^{6}$). Mononuclear cell migration remained unaltered. The pretreatment with MMF (10–200 mg/kg) altered MPO levels (mUI/L) in 200 ± 58, 139 ± 32, 231 ± 34 and 137 ± 42 (Control: 179 ± 63), ADA levels (UI/L) in $9.37 \pm 0.89^{\#}$, $7.59 \pm 0.78^{\#}$, 4.46 ± 0.89 and 3.12 ± 0.33 (control: 3.57 ± 1.78), and NOx levels (µM) in 10.40 ± 1.80, 7.35 ± 1.47, $3.23 \pm 0.73^{\#}$ and $1.62 \pm 0.44^{\#}$ (control: 10.88 ± 1.76). MMF reduced significantly neutrophil migration (100 and 200 mg/kg) and NOx levels (100 and 200 mg/kg). These preliminary results showed that MMF shows promising anti-inflammatory activity in this murine model of inflammation induced by LPS. However, further studies are necessary in order to evaluate the mechanism of anti-inflammatory action. **Financial support:** FURB.

04.063

CC chemokine receptors play different roles in the pathogenesis of dengue virus infection in mice. Guabiraba R[1], Pereira-Silva REM[1], Besnard AG[2], Souza DG[3], Ryffel B[2], Teixeira MM[1] [1]UFMG − Bioquímica e Imunologia, [2]CNRS-IEM, [3]UFMG − Microbiologia

Introduction: Dengue virus (DENV), a mosquito-borne flavivirus, is a public health problem in Brazil and other tropical regions. Infection is characterized by a systemic inflammatory response and hematological alterations that may evolve with shock and death in severe cases. Chemokines and their receptors are important molecules involved in leukocyte recruitment and activation in many viral infections. Recent clinical data have shown an association between components of the chemokine network and severity of Dengue. However, the function of the chemokine system in the context of dengue infection is not known. Here we evaluated the role of CC chemokine receptors CCR1, CCR2, CCR4 and CCR5 in an experimental model of DENV-2 infection using mice genetically deficient for each chemokine receptor. Chemokine receptor antagonists were used to confirm major findings and evaluate potential therapeutic benefit of targeting the system. **Methods**: C57/BL6 (WT) and gene deficient mice were inoculated i.p. with 1 or 10 LD_{50} of a mouse adapted DENV-2 strain. Survival, MPO (as a marker of neutrophil accumulation), cytokines, histological analysis and viral titration in spleen and liver were evaluated. Hematocrit, platelet numbers and levels of cytokines and transaminases (TGO/TGP) were also evaluated in blood. Protocol CETEA/UFMG: 113/2009. **Results:** WT animals infected with DENV-2 died around day 7 after infection. At day 6, there was evidence of clinical disease and tissue damage, as shown by thrombocytopenia, hemoconcentration, increased levels of transaminases, neutrophil accumulation in tissues and elevated levels of cytokines (TNF-α, IFN-γ, IL-6, IL-12p40, CXCL1/KC). CCR1-/- mice were similar to WT mice with similar disease pattern and mortality, but slightly increased neutrophil accumulation and levels of IL-6, IFN- γ and KC in spleen, liver or serum. Lethality was decreased in CCR2-/- mice (p=0.03), tissue injury was slightly increased and systemic parameters of inflammation were similar to those of infected WT mice, although IFN- γ and IL-6 are reduced in liver. Infection enhanced levels of CCL17/TARC, a ligand for CCR4. More importantly, lethality rate, tissue injury and systemic inflammation were decreased in CCR4-/- mice. The phenotype of CCR5-/- mice was qualitatively similar to that of CCR4-/- mice but protection was much more pronounced in the former animals. Indeed, infected CCR5-/- mice did not die after infection and showed no hematological alterations or evidence of tissue injury or systemic inflammation. Importantly, viral load was greatly reduced in CCR5-/- mice (< 3 Log reduction). Finally, treatment with a CCR1/5 antagonist, MetRANTES (10 µg/mouse/day), was associated with decreased clinical disease, systemic inflammation and lethality after DENV-2 infection. **Discussion:** This study shows that the chemokine system plays a major role in the context of experimental dengue infection. Although CCR1 appears to play a minor role, CCR2 seems to be important for liver-associated pathology and CCR4 and CCR5 contributed markedly to tissue and systemic inflammatory changes associated with the experimental infection. CC chemokine receptor blockade, especially CCR5, may represent an interesting therapeutic approach for the treatment of dengue infection. **Financial support**: CNPq, FAPEMIG

04.064

Inhibition of guanylyl cyclase restores neutrophil migration and maintains bactericidal activity increasing survival in sepsis. Amêndola R[1], Neto H[2], Souto FO[3], Alves-Filho JC[3], Spiller F[3], Freitas A[3], Cunha FQ[3], Barja Fidalgo TC[2] [1]UERJ − Farmacologia e Psicobiologia, [2]UERJ − Farmacologia, [3]USP − Farmacologia e Dor

Introduction*:* In patients and animal models of sepsis, there is a failure of neutrophil migration that correlates with the severity of clinical status. This state of hyporesponsiveness can be rescued by pretreatment of animals with pharmacological inhibitors of NO synthesis. Additionally the impaired migratory response of neutrophils isolated from septic patients is associated with increased iNOS expression, suggesting an important role of NO in this phenomenon. However, the mechanisms triggered by NO in neutrophils that lead to impaired migration is still unknown. **Objective***:* This study evaluates the molecular mechanisms involved in the failure of migratory response triggered by NO in neutrophils. **Method***:* We used models of neutrophils activation with LPS *in vitro* and an animal model of sepsis (protocol n.181/2008) to better understand the mechanisms that lead to impaired neutrophil migration in sepsis. **Results***:* LPS inhibited the migratory response of neutrophils to chemotactic stimuli. This phenomenon was dependent on NO synthesis and subsequent activation of the enzyme guanylyl cyclase (GC) and activation of cGMP-dependent proteins kinase (PKG). The inhibition of neutrophil migratory response seems to involve the internalization of G protein-coupled receptors (GPCRs) due to increased expression the GPCR kinase (GRK) 2, a process also dependent on the NO-GC-PKG pathway. Using an animal model of sepsis, we observed that inhibition of GC activity was able to restore the neutrophil migration response during sepsis, accompanied by efficient control of infectious and increased survival of animals. **Conclusion***:* Our data suggest that LPS is able to inhibit neutrophil migration response by induction of NO synthesis and activation of NO-GC-PKG pathway and emphasize the GC inhibition as a promising therapeutic approach in sepsis. **Financial support**: CAPES, CNPq, FAPERJ and FAPESP.

04.065

Anti-inflammatory effects of lovastatin on the tests of formalin and dextran-induced paw edema. Siqueira RMP[1], Gonçalves DO[1], Calou IBF[2], Olinda TM[1], Figueiredo IST[1], Pinheiro CN[4], Melo TS[1], Cavalcante, ALC[5], Viana GSB[1] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Análises Clínicas, [4]FATECI − Biomedicina, [5]UFC − Ciências Médicas

Introduction: Statins are among the most prescript drugs in recent clinical practice. They are also known for their pleiotropic actions, effects independent from their lipid-lowering properties. The objectives of this study were to evaluate lovastatin's (LOV) effects against dextran-induced paw edema in rats and in the formalin test in mice. **Methods:** The project was previously approved by the Animal´s Ethics Committee of the Faculty of Medicine of the Federal University of Ceará (protocol number: 2810). In the dextran model, groups of rats (n = 8) were treated with LOV (25 and 50 mg/kg, p.o.), dexametasone (10 mg/kg, p.o.), or vehicle (distilled water, p.o.). One hour after the treatments, each animal received a subcutaneous injection in the right paw of 0.1 mL dextran (1%). The left paw received the same volume of distilled water. The edema was measured as the difference between the paws, and was recorded after half, one, two, three and four hours after the dextran administration. The edema was expressed in milliliters, and was assessed by a plethysmometer (Ugo Basile, Italy). In the formalin test mice (8 per group) were treated with LOV (2, 5 and 10 mg/kg, i.p.), morphine (10 mg/kg, s.c.) or naloxone (3 mg/kg, s.c., 15 min prior to LOV or morphine administration). Thirty minutes later, each animal received a subcutaneous injection in the right paw of 20 µL formalin (1%). The time each mouse spent licking the injected paw was recorded in seconds at the first (0-5 min) and second (15-30 min) phases. **Results and Discussion:** LOV exhibited protective effect against the dextran-induced inflammation at the tested doses of 25 and 50 mg/kg. The extent of inhibitions at the end of the second hour were 31% and 34% for the above doses as compared to the vehicle group. Dexametasone, the positive control, offered as expected a significant protection (reduction of 52%) ($p < 0,01$). LOV at the doses of 2, 5 and 10 mg/kg (i.p.) efficiently reduced the time mice spent licking the injected paw, at both phases of the formalin test: 1st phase (51, 65 and 70%, respectively) and 2nd phase (73, 57 and 66%). Morphine (10 mg/kg, s.c.), used as positive control, also produced a significant reduction in both phases (76 and 69%) ($p < 0,01$). Naloxone reversed the antinociceptive effects of both drugs, morphine (26%) and LOV (14%) at the first phase of the test. The effects at the second phase were not statistically significant. The results of the present study indicate an anti-inflammatory effect of LOV on the paw edema induced by dextran and antinociceptive action on the formalin test. Nevertheless, other studies must be carried out to elucidate lovastatin's actions on inflammation. Support: CAPES e CNPq.

04.066

Effect of the crude extract and the aerial parts fractions of *Sesbania virgata* on the inflammatory response in animals. Arruda LLM[1], Bonfim, N. M.[2], Kummer R[1], Souza, M. C.[3], Sarragioto MH[2], Baroni S[1], Grespan R[1], Bersani-Amado CA[1] [1]UEM − Farmácia e Farmacologia, [2]UEM − Química, [3]UEM − Biologia

Introduction: There are few studies showing the pharmacologic effects of leaves from Sesbania virgata (Sv), mainly depressor effect of SNC, analgesic and anti-inflammatory properties. However, there are not studies investigating the anti-inflammatory activity of Sv fractions in acute experimental models. The aim of this study was to investigate the effect of the crude extract (CE) and the Sv fractions on the models of carrageenin-induced pleurisy and oil croton-induced ear edema. **Methods**: Aerial parts of Sv were extracted with methanol, and the CE was subjected to partition in hexane and ethyl acetate to afford the hexane (FH), ethyl acetate (FEA) and aqueous methanol (FAM) fractions. In the ear edema model, the CE, FEA and FAM were diluted in acetone 70% and the FH in chloroform 70%. After the application of croton oil (CO), groups received the CE, fractions from Sv (5.0 mg/ear) or indomethacin (1 mg/ear) in the left ear. In the right ear was applied vehicles (acetone 70% or chloroform 70%). After 6 hours, percentage of inhibition of edema was calculated though weigh (mg) of the ears that were sectioned in discs 6.0 mm in diameter. In the carrageenin-induced pleurisy model, the CE and FAM were diluted in water and the FEA and FH in tween 16%. The pleurisy was evaluated in rats treated orally with vehicle (water or tween 16%), CE and fractions of Sv (500 mg/kg) or with indomethacin (5 mg/kg). The animals received carrageenin (200 µg/animal) in the pleural cavity one hour after the treatments. After 4 hours, the total volume of the exudate was measured and a 50µl aliquot was used to determine the number of leukocytes in a Neubauer chamber. The protocol (number 021/2009) regarding this study was approved by the ethical commission of ethics in animal research. **Results and Discussion**: The application of CO in the mice´s left ear induced an evident inflammatory response in 6th hour. The FH and FEA showed significant inhibition of the edema intensity (FH=72.76% $P< 0.001$; FEA=43.13% $P< 0.05$) when compared with the mice group that received only CO. The application of the CE and FAM did not affect the development of the response. The treatment with indomethacin provoked a significant reduction of ear edema in mice. In the pleurisy model, the FEA and FH groups promoted a significant reduction in the volume of inflammatory exudate when compared with the control group: control (Tween 16%): 0.85 ± 0.07; FEA: 0.31 ± 0.08*; FH: 0.7 ± 0.08*. However, the FAM and CE did not inhibit the exudate volume: control (water): 0.77 ± 0.15; FAM: 0.66 ± 0.05; CE: 0.7 ± 0.07. In the counting of cells present in the inflammatory exudate was observed that FEA altered the number of migrated leukocytes (29.14 ± 2.39 cells x 103/mm3), when compared with control group (57.32 ± 2.58 cells x 103/mm3). These data suggest that the FEA and FH present an antiedematogenic effect; however, only FEA presented effect on the leukocytes recruitment. Supported by: CNPq/PIBIC-UEM. Acknowledgments: Jailson Araujo and Celia Regina Miranda.

04.067

Effects of resveratrol in the acute and chronic models of inflammation in rats. Silva RBM[1], Maciel IS[2], Souto AA[3], Morrone FB[4], Campos MM[5] [1]PUCRS − Farmacologia Aplicada, [2]PUCRS − Farmacologia, [3]PUCRS − Química, [4]PUCRS − Farmácia, [5]PUCRS − Cirurgia-Odontologia

Introduction: Extensive research in the last decade revealed that most chronic disorders such as cancer, arthritis, autoimmune and neurological diseases exhibit deregulation of multiple cell signaling pathways linked to inflammation (Kuzhuvelil et al., Landes Biosc Rev. Vol 7:8, 2008). Resveratrol (trans-3, 5, 4´-trihydroxystilbene), a component found in grapes, berries and peanuts, is a polyphenol that exert its effects through modulation of many different biological pathways (Martin et al., Br J. Pharmacol., 147:873, 2006). The present study aimed at evaluating the effects of resveratrol administered orally, in models of acute and chronic inflammation in rats. **Methods:** Male Wistar rats (n= 5; 180-200 g) were used. For induction of acute inflammation, animals received an intradermal (i.d.) injection of saline solution containing carrageenan (300 µg/paw, 100 µl) in the right hindpaw. The left paw received 100 µl of saline. Edema was determined with a plethysmometer (Ugo Basile), at several time points after carrageenan injection (30, 60, 120, 180 and 240 min), as the difference between the right and left paws. For this model, two distinct schedules of treatment have been adopted. In the prophylactic scheme, the animals were pretreated orally with resveratrol (100 and 200 mg/kg), 30 min before carrageenan injection. In the therapeutic scheme, rats received resveratrol at the same doses, 120 min after injection of carrageenan. The control group received saline at the same schedules of administration. In the sub-chronic model, animals received an i.d. injection of 100 µl of CFA (1 mg/ml heat-killed and dried Mycobacterium tuberculosis, each milliliter of vehicle containing 0.85 ml paraffin oil plus 0.15 ml mannide monooleate), which was suspended in a 1:1 oil/saline emulsion (in a total volume of 200 µl/paw). The left paw received 200 µl of saline. In this model, the rats were treated with resveratrol (100 mg/kg, p.o.), 2 h post-CFA injection, and once a day for 3 days. The control groups received saline at the same intervals of time. The edema was measured by using a plethysmometer at several time-points following CFA injection (2, 4, 6, 8, 24, 48 and 72 h). The experimental protocols were approved by the Local Ethics Committee (07/03611, PUCRS). **Results:** The results demonstrated that prophylactic administration of resveratrol (100 and 200 mg/kg) markedly inhibited the edema caused by carrageenan, when compared to the control group (31 ± 7% and 22 ± 4% of inhibition, respectively). In addition, the edema elicited by carrageenan was significantly reduced by the therapeutic administration of resveratrol (100 mg/kg), dosed 120 min after carrageenan, with inhibitions of 35 ± 8% and 45 ± 9%, at 180 and 240 min, respectively. Of interest, the oral treatment with resveratrol (100 mg/kg) in the sub-chronic model caused a significant inhibition of CFA-induced edema in 16 ± 4%, 22 ± 3, 19 ± 4% and 20 ± at 6, 24, 48 and 72 h, respectively. **Discussion:** The results indicate that resveratrol shows anti-inflammatory effects, which were more pronounced in therapeutic schedules of treatment. Resveratrol might be also effective in poliarthritis models. Further studies are under development for confirming this hypothesis. **Financial support**: CNPq, PUCRS.

04.068

Inflammatory response induced by carvacrol, a *Thymus vulgaris* essential oil constituent. Fachini FC, Kummer R, Ritter AMV, Anteguera AAC, Domiciano TP, Bersani-Amado CA, Cuman RKN UEM − Farmácia e Farmacologia

Introduction: *Thymus vulgaris* (Labiaceae) is an aromatic and medicinal plant commonly known in Brazil as tomilho. Thymus essential oil (TEO) has been used as condiment, in cosmetics and food industries. Thymus species is traditionally used for its antiseptic, antispasmodic and antitussive effects. The main constituents obtained from TEO are: thymol, carvacrol, p-cymene, limonene and γ-terpinene. Furthermore, TEO possesses antimicrobial, antifungical, antioxidative and antiviral activities. The aim of this study was to investigate the effect of the carvacrol (CVL) on the inflammatory response evaluated by ear edema mice method. **Methods:** The leaves of *Thymus vulgaris* were collected from the Herbarium of the State University of Maringá. The TEO was extracted by conventional steam distillation during 3 hours using a Clevenger-type apparatus. The CVL was cromatografic identified by CG-MS and RMN. The dexametasone (standard anti-inflammatory drug) was topically applied at mice ear 1h before inflammatory stimuli (croton oil). The animal's right ear was topically treated with CVL (10 mg/ear), croton oil (5% v/v) and dexametasone (DEX) (0,1mg/ear). All drugs were diluted or dissolved in acetone (vehicle). Eight animals were used for each group. Four hours after, the ear weight (edema volume) and the myeloperoxidase (MPO) activity were evaluated according to the technique of Bradley & Priebat (1982). MPO is an enzyme present in the intracellular granules of neutrophils, and can be used as a marker for the influx of polymorfonuclear leucocytes into inflamed tissues. The MPO activity was evaluated in the supernatant of homogenates of the ear sections. The ear tissue was placed in potassium phosphate buffer, ph 6.0 containing 0.5% hexadeciltrimethyl ammonium bromide in a homogenizer. The supernatant was added to a 96-wells microplate, followed by addition of a potassium phosphate buffer solution containing o-dianisidine dihydrochloride and 1% H2O2. The enzyme activity was determined by measuring the optical absorbance (460 nm) ELISA reader. The results were statistically analyzed using one-way ANOVA followed by Turkey test. The difference were considered significant at $P<0.05$*. The experimental protocol was approved by the ethical commission in animal research (041/2008/CEAE-UEM). **Results and Discussion:** After topically administration of croton oil and CVL, an incresead ear edema was observed when compared to control group (croton oil: 0.013 ± 0.004*, $p<0.001$; DEX: 0.015 ± 0.007*, $p<0.05$; control: 0.01 ± 0.003*, $p<0.001$). Mice ear edema was reduced for DEX treatment (0.01 ± 0.001*, $P<0.05$). An increased MPO activity was observed in CVL and croton oil groups (0.020 ± 0.01*, $p<0.01$;0.01 ± 0.013*, $p<0.01$, respectively) but not in the DEX treatment group (0.03 ± 0.005*,$p<0.01$). Data showed an important inflammatory response after CVL treatment, evaluated by edema formation and leucocyte chemotaxis stimuli. Conclusion: CVL showed a topically irritative effect on ear mice. **Supported by**: CNPq; UEM. Reference: Bradley PP, Priebat DA, Christensen RD, Rothtein G Measurement of cutaneous inflammation: stimulation of neutrophil content with a enzyme marker. *J. Invest Dermatol*, v.78, n.3, p206-209, 1982.

04.069

LTB4 as chemoattractant factor in the regulatory T cells migration. Pecli CP[1], Molinaro RC[2], Peters-Golden M[3], Kunkel SL[4], Canetti C[5], Benjamim CF[1] [1]ICB-UERJ, [2]IOC-FIOCRUZ, [3]University of Michigan − Pulmonary and Critical Care Medicine, [4]University of Michigan – Pathology [5]IBCCF-UFRJ

Introduction: The innate immune response is the host early defense, its afford protection against infection through recruitment and activation of phagocytic effector cells and through production of soluble mediators. The leukotrienes (LTs) are lipidic mediators, in which LTB4 plays a critical role in leukocyte recruitment and activation. LTB4 is derived from the metabolism of arachidonic acid by the enzyme 5-LO assisted by FLAP. It exerts its actions by ligating two G proteins-coupled receptors, BLT1 and BLT2. LTB4 chemoattractant function has also been shown in the adaptive immune cells, CD4+, CD8+ and γδ T cells. Another T cells subset are the regulatory T (Treg) cells. Treg are suppressive cells that modulate the immune system response. The aim of our study is evaluate if LTB4 has the ability to induce *in vitro* chemotaxis of Treg and if it's important to the migration of Treg to the lymphoid organs in a model of infection disease as sepsis. **Methods:** Male or female wild type (WT) of s.v.129 and knockout for the 5-lipoxygenase enzyme (5-LO-/-) mice from the same genetic background with 20-24 g and C57Bl/6 (B6) were raised in the animal facility of Pharmacodynamics Department in FIOCRUZ, RJ. Splenic cells from B6 were subjected to an EasySep Treg Kit (StemCell, USA), according to the manufacturer's instructions. Purified Treg was stained for anti-Foxp3 and anti-BLT1 and subjected to flow cytometry analysis. After this, purified Treg cells were placed in a Boyden chamber. Treg cells were added on the upper well. In the bottom wells, 0,1; 0,3; 1; 3 or 10 nM LTB4 diluted in medium was loaded. As negative control we use RPMI medium only. Migrated cells were collected after 1 or 2 h incubation at 37°C and counted by hemocytometer. To the *in vivo* experiments, s.v.129 WT and 5-LO-/- mice were subjected to cecal ligation and puncture (CLP) to induce an experimental polymicrobial sepsis. Animals were sacrified, their spleen and mesenteric lymph node were removed and analyzed to the presence of Treg cells. All experiments were conducted in accordance with the ethical guidelines of the Institutional Animal Care Committee-CEUA in UFRJ, Rio de Janeiro, RJ, protocol code: DFBCICB028. **Results and Discussion**: The analysis of Treg for the BLT1 expression was positive. LTB4 had a chemotatic effects on Treg cells, as this cells respond to the LTB4 stimulus. However, this response was only time dependent and not dose dependent. In the *in vivo* experiments we noted that most Treg from both Sham and CLP, WT and KO animals, were BLT1+. Our first data were not sufficient to confirm the LTB4 participation in the *in vivo* Treg cells recruitment. Data show that Treg express the BLT1 receptor and have a chemotatic response *in vitro* toward LTB4. It will be necessary more experiments to confirm the LTB4 effects *in vivo* in a sepsis model. Support: FAPERJ, CNPQ, CAPES References: Peters-Golden, M. Curr. Allergy Asthma Rep., v 8, p 367-373. 2008 Tager AM. et al. Prostaglandins *Leukot Essent Fatty Acids*, v 69, p 123-134. 2003 Tager, A.M. et al. *Nat Immunol*, v 4, p 982-990. 2003 Goodarzi K et al. *Nat Immun*ol v 4, p 965-973. 2003 Miyahara N et al. *J Immunol*, v 174, p 4979-4984. 2005 de Souza Costa, M.F.et al. *J Leukoc Biol*., v 87, p 323-332. 2009 Sakaguchi S et al. *J. Immunol*., v 155, p 1151-1164. 1995 Baker, C.C et al. *Surgery*, v 94, p 331-335. 1983

04.070

Ntipyretic effect of dipyrone is not related with the hypothalamic PGE2 synthesis inhibition in rats. Malvar DC, Figueiredo MM, Martins JM, Pessini AC, Soares DM, Souza GEP FCFRP-USP − Física e Química

Introduction: Dipyrone thought to display antipyretic properties unrelated to COX inhibition because it abolished the Tityus serrulatus venom (Tsv)-induced fever which does not depend on PG generation1. On the other hand, ample evidence suggests that PGE2 generated in the preoptic area of the anterior hypothalamus is the main mediator of fever induced by LPS22,3. Thus, in this study we have compared the effects of dipyrone and indomethacin on fever response and changes in PGE2 concentration in the CSF and the hypothalamus induced by LPS and Tsv. **Methods:** Male Wistar rats (200g) received vehicle, dipyrone (120mg kg-1) or indomethacin (2mg kg-1) i.p. 30 min before the i.p. injection (0.5 ml) of LPS (50mg kg-1), Tsv (150mg kg-1) or saline (control group) and the body temperature (oC) was measured every 30 minutes for up 1-3h by telemetry. Immediately after the last measurement, the animals were anesthetized with xylazine and ketamine and both CSF and hypothalamus was collected from each animal. PGE2 was measured by using ELISA kits. Ethical commission protocol nº 200/2008 – CETEA/FMRP-USP. **Results:** The fever induced by either LPS or Tsv injection was abolished by dipyrone. In contrast, indomethacin only reduced (47.0%) the LPS-induced fever at 3 h. Pre-treatment with indomethacin also reduced the increase of PGE2 concentration in the CSF (2h: 68.5% and 3h: 75.0%) and hypothalamus (2h: 79.8% and 3h: 74.2%) while dipyrone impeded the increase of PGE2 concentration in the CSF, without change the hypothalamic PGE2 levels after LPS injection. Interestingly, the basal CSF and hypothalamic PGE2 content were not altered after Tsv injection, but the basal hypothalamic PGE2 content was reduced by indomethacin (1 h: 97.8% and 2 h: 98.1%) and dipyrone (1 h: 86.8% and 2 h: 60.0%). Effect of indomethacin and dipyrone on fever and on CSF and hypothalamic PGE2 concentration after i.p. injection of LPS or Tsv. Treatment Stimuli Time (h) D rT (oC) PGE2 in CSF (pg ml-1) PGE2 in Hypothalamus (pg g-1 of tissue) n Vehicle Saline 1st 0.0 ± 0.1 nd 390 ± 42 5 2nd 0.0 ± 0.1 nd 310 ± 91 6 3rd 0.1 ± 0.1 nd 355 ± 71 6 LPS 2nd 1.2 ± 0.1* 1136 ± 223* 996 ± 69* 6 3rd 1.6 ± 0.1* 981 ± 216* 1033 ± 86* 6 Tsv 1st 1.4 ± 0.2* nd 405 ± 73 5 2nd 2.1 ± 0.3* nd 388 ± 31 5 Indo LPS 2nd 1.3 ± 0.1* 357 ± 70*# 201 ± 90# 6 3rd 0.8 ± 0.1*# 245 ± 63*# 256 ± 45# 6 Tsv 1st 1.3 ± 0.1* nd 8 ± 8*a 5 2nd 2.3 ± 0.1* nd 5 ± 5*a 5 Dipyrone LPS 2nd -0.1 ± 0.1a nd 871 ± 77* 6 3rd 0.1 ± 0.1a nd 1017 ± 88* 6 Tsv 1st -0.1 ± 0.1a nd 52 ± 30*a 5 2nd 0.1 ± 0.1a nd 103 ± 10*a 5 *, #, a $p<0.05$ when compared with Vehicle/Saline, Vehicle/LPS or Vehicle/Tsv, respectively; Indo= indomethacin; nd= not detected. **Discussion:** These results reinforces the assumption that PGE2 is not involved in the Tsv-induced fever1 and suggest that the antipyretic effect of dipyrone on LPS- and Tsv-induced fever also relies on the blockage of synthesis/effects of mediators others than prostaglandins. 1 Pessini et al. *Toxicon*. 48: 556, 2006. 2 Engblom et al. Nat Neurosci. 6: 1137, 2003. 3 Roth et al. *Immunol Allergy Clin North Am* 29: 229, 2009. Apoio Financeiro: CNPq, FAPESP.

04.071

The role of acid-induced laminin polymer in splenic dendritic cells. Ladislau L[1], Da-Fe AR[2], Coelho-Sampaio TL[3], Kunkel SL[4], Benjamim CF[5] [1]ICB-UFRJ, [2]UERJ − Farmacologia e Psicobiologia, [3]UFRJ − Histologia, [4]University of Michigan − Pathology, [5]UFRJ − Farmacologia Básica e Clínica

Introduction: The dendritic cells (DCs) are considered professional antigen-presenting cell (APCs). For APC function, DCs have to display foreign antigen by the major histocompability complex (MHC), express Toll-like receptors and co-stimulatory molecules on its surface and release cytokines. Laminin is a major protein, important and biologically active part of the basal lamina, which plays a role in the cell differentiation, migration, adhesion as well as phenotype and survival of several cells. Previous study had demonstrated that acid-induced laminin polymers are more efficient to induce cellular response. DCs express β1 integrin, laminin receptor, but the effect of laminin on DCs's phenotype and activation is poorly understood. Our aim is to characterize the effect of acid-induced laminin polymer on splenic DCs. **Methods**: C57BL/6 mice with 20-24g of both sexes were used. Mice were gently donated from National Institute of Cancer (INCA). The laminin was diluted in sodium acetate pH 4.0 or Tris pH 7.0, both with additional calcium chloride, and this laminin was kept overnight on round glass coverslips at 37oC. The laminin polymerization was observed by immunofluorescence microscopy. The round glass coverslips covered with laminin were kept overnight with rabbit anti-laminin at 4oC. After, it was added secondary Cy-3 anti-rabbit for one hour at room temperature. To analyze the phenotype profile of DCs, it was obtained from spleen with aseptic techniques and prepared. Splenic cells were subjected to an EasySep CD11c-PE positive selection (StemCell Technologies), according to the manufacturer's instructions. DCs purity, determined by flow cytometry of CD11c+ cells, was >95% after selection. These cells were cultured in 24-well plates with or without laminin and with a second hit of LPS (1 ug/mL). After 24 hours, the cells were subjected to flow cytometry analysis. This animal study was performed in accordance with Instructional guidelines, approved by Ethical committee of animal use, protocol number: DFBCIB 028. **Results and Discussion**: We observed that the acid-induced laminin polymer presented more organization, less interruption and regular topology, compared to pH 7,0 induced laminin polymer according to Barroso et al. Furthermore, calcium presence is very important for laminin polymerization as EDTA inhibits the laminin polymerization. We observed that DCs presented higher expression of all surface molecules analyzed (MHC, CD-80 and CD-40) when incubated with LPS and acid-induced laminin polymer. This result suggests that laminin seems to have an important role in immune response increasing expression of surface molecules. More investigations about cells profile, such as cytokine release, phagocytosis and migration needs to be made. **Supported by** FAPERJ, CNPQ and PIBIC/UFRJ.

04.072

Clinical evaluation of the anti-inflammatory effect of *Baccharis dracunculifolia* propolis gel (patent PI 0904121-4) on cervicitis. Paulino N[1], Scremin Paulino A[2], Marcucci MC[1], Vautier P[1] 1UNIBAN − Farmácia, [2]UFSC − Farmácia

Introduction: Propolis is a natural resin produced by bees and used in folk medicine to treat several diseases, including fungal and bacterial infections and inflammatory pathologies. The objective of this work is to evaluate the anti-inflammatory effect of standardized Brazilian propolis (G1) gel on cervicitis. **Methods:** The chemical standardization of Brazilian green propolis extract was made by mean of HPLC following the patent INPI PI006272-3A2 (Maria Cristina Marcucci, 2000). Gel containing standardized Brazilian propolis was produced by pharmaceutical technology following our patent PI 0904121-4 (Niraldo Paulino 2009). This study was performed in women with cervicitis and evaluated by the presence of neutrophils in cervico-vaginal material. These women received G1 and were instructed to use it for the next seven days (UNISUL Ethical Committee no. 251/2005). After this time, women were conducted to collect gynecological material. The results were presented as the average of neutrophils found in the collected materials and statistically assessed by Student's t test. **Results:** We have demonstrated that treatment for seven days resulted in a significant decrease in the number of neutrophils in the collected cervico-vaginal material. After seven and fourteen days, the level of inhibition in the vulva was 55 ± 5% for both, 38 ± 4% and 60 ± 5% in the vagina, and 39 ± 4% and 37 ± 5% in the cul-de-sac, respectively. In the colon (data after seven days), the inhibition was 55 ± 6%, and, in contrast, inhibition in the endocervix was 49 ± 5%. **Discussion:** We have shown in previous published results that Brazilian green propolis produced anti-inflammatory effect in preclinical assays. Taken together, our data led us to reinforce the hypothesis that the anti-inflammatory effect of Brazilian green propolis may be a due to inhibition of iNOS gene expression, through interference with NF-kappaB site in the iNOS promoter. These results suggest that G1, containing Brazilian green propolis may be an important new bioproduct to use during inflammatory chronic cervicitis in women. **Acknowledgements**: This study was supported by grants from the UNIBAN-BRASIL and UNISUL.

04.073

Activation of TLR9 in circulating neutrophils inhibits their migration to inflammatory site.

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Introduction: Neutrophils are major players in innate immunity and constitute the first line of host defense against invading bacteria and other pathogens. Chemotaxis is a crucial event in neutrophil migration and is controlled by several mediators, such as chemokines through activation of G proteins-coupled receptors (GPCRs). Furthermore, the functionality of these receptors is regulated by G protein-coupled receptor kinases (GRKs). It was shown that activation of Toll like receptor (TLR) 2 and TLR4 down-modulates chemotatic receptors expression on surface of neutrophils reducing their migration. However, the effect of other TLRs activation on neutrophils migration was not verified. Thus, the aim of this study was to verify whether the activation of TLR9 on circulating neutrophils could interfere with the migration of these cells to the inflammatory site. **Methods:** C57BL/6 wild type (WT), MyD88-/- and TLR9-/- mice were treated i.v. with type B CpG oligodeoxinucleotide (ODN), TLR9 agonist (n=5). The mice received thioglicolate i.p. 30 minutes after TLR9 agonist administration and, 4 hours later, neutrophil migration in peritoneal exudates was evaluated. Chemotaxis of blood neutrophils to CXCL2, CXCR2 expression and GRK2 induction on blood neutrophil were performed 2 hours after TLR9 agonist treatment. All experiments were developed in accordance with the ethical guidelines of the FMRP-USP, São Paulo, Brazil (protocol n°150/2009). **Results and Discussion**: Administration of the type B CpG ODN inhibited the neutrophil migration induced by thioglicolate i.p. in WT ($p<0.001$), but not in MyD88-/- and TLR9-/- mice. Investigating the mechanism by which activation of TLR9 inhibited neutrophil migration, it was observed that neutrophil derived from CpG ODN-treated mice presented a reduction in their ability to migrate *in vitro* (chemotaxis assay) toward CXCR2 ligands ($p<0.001$). Moreover, the inhibition of neutrophil chemotaxis caused by TLR9 activation was associated with a reduction in CXCR2 expression ($p<0.001$) on neutrophil surface and an increase in GRK2 expression ($p<0.01$). These results indicate that the presence of unmethylated DNA with CpG motif in the circulation impairs neutrophil migration to inflammatory focus by reducing chemotatic response, and this may be due to induction of GRK2. Although activation of TLRs is important to recognize pathogens and consequently control the infection, the localization where the recognition occurs seems to be essential to the establishment of an efficient immune response. For instance, activation of TLR9 in blood circulating neutrophils, similar to previously observed with TLR2 and TLR4, can be harmful to control the infection, because it impairs these cells to reach the infection focus.

Key words: Neutrophil migration, CXCR2, GRK2, Toll like receptor 9. **Grant support**: FAPESP, CNPq and CAPES.

04.074

Effects of eotaxin in the peritoneal migration of eosinophils and neutrophils, dependent on 5-lipoxygenase products. Lages PM1, Arcanjo LCG[2], Lopes RS[1], Silva CLCA[1], Luz RA[3], Elsas PPX[4], Elsas MICG[5] [1]IMPPG-UFRJ, [2]IFF-FIOCRUZ, [3]IFF-FIOCRUZ − Pediatria, [4]UFRJ, [5]FIOCRUZ

Introduction: Prolonged administration of glucocorticoids (GC) or continuous production at high levels, are accompanied by neutrophilia. Our laboratory has demonstrated that GC also stimulates murine eosinophilpoesis (Xavier-Elsas et al., *Br J Pharmacol*; 143:541, 2004). This suggests that GC induces more than a granulocytic lineage, with the possible existence of positive cross-regulation and enhancer sharing mechanisms. A role for 5-Lipoxygenase (5-LO) products in regulating eosinophils and neutrophils is also suggested by recent experiments (Cheraim et al., Life Sciences; 83:214, 2008). Blockade of 5-LO and LTB4 receptors prevented eosinophil migration *in vivo* in response to i.p. administration of eotaxin, a potent chemotactic stimulus for eosinophils. The contribution of LTB4 to the migration of leukocytes *in vivo* may also include eosinophils and neutrophils. This observation also raises the question, still unresolved, as is maintained in the apparent selectivity of eotaxin for eosinophils, as its action is linked to the presence of a chemoatractor with powerful effects for neutrophils. Objectives: We investigated whether: a) the peritoneal recruitment of eosinophils induced by eotaxin is accompanied by recruitment of neutrophils and if this process is affected by inactivation of 5-LO, b) if there is an indirect mechanism, involving eosinophils, in the effects of eotaxin on the recruitment of neutrophils. **Methods:** 5-LO deficient (ALOX) and their wild type control (PAS), or eosinophil deficient (GATA-1 KO) and their wild type control (BALB/c), from CECAL-FIOCRUZ/RJ, was used following institutionally approved (CEUA: L-002/09). Animals received varying amounts of recombinant murine eotaxin or RPMI 1640, i.p. Mice were euthanized in a CO2 chamber at different times after inoculation, staring with 4 h. Peritoneal lavage were collected and the total leukocyte, neutrophil and eosinophil contents were determined, using Wright-Giemsa stain. **Results:** Eotaxin recruited in early time (4 h) a significant amount of eosinophils but also neutrophils in BALB/c animals. This effect is dose dependent, with an optimum at 50 ng/peritoneal cavity for the recruitment of neutrophils. The effect of eotaxin, on the cellularity of the peritoneal cavity at 4 h was significantly different from the effect of the vehicle in PAS mice. There were no significant difference between the effects of these two treatments in 5-LO deficient mice. The early recruitment of cells into the peritoneal was also dependent on GATA-1, suggesting that eotaxin-induced eosinophils and neutrophils recruitment is mediated by mechanisms dependent on 5-LO and GATA-1 gene. Conclusions: In wild type animals, eotaxin induces an early recruitment of eosinophils and neutrophils, which seems dependent on the 5-LO and GATA-1. This research was funded by Capes, Cnpq and FAPERJ.

04.075

Adenosine and adenosine-monophosphate present into the *Phlebotomus papatasi* saliva block dendritic cell function and ammeliorate collagen-induced arthritis. Carregaro V[1], Sá-Nunes, A[2], Cunha, TM[3], Grespan R[4], Oliveira CJ[1], Lima-Jr DS[5], Costa DL[1], Milanezi CM[1], Verri Jr WA[5], Valenzuela JG[6], Silva JS[1], Ribeiro JM[6], Cunha FQ[3] [1]FMRP-USP − Biochemistry and Immunology, [2]ICB-USP − Immunology, [3]FMRP-USP − Pharmacology, [4]UEM − Farmácia e Farmacologia, [5]UEL − Pathology and Pharmacology, [6]NIAID/NIH − Vector Biology

Introduction: Among several potent pharmacologic factors, phlebotomine saliva has anti-inflammatory activity that inhibits the neutrophil migration to inflammatory foci by dendritic cell-derived PGE2/IL-10 sequential pathway (Carregaro V et al., 2008, J Leuc. Biol., .). Such property protects the tissue from inflammatory insult, which means that salivary compounds could be a prototypal to treat several inflammatory chronic disorders. AIM: we evaluated the potential therapeutic role of salivary gland extract (SGE) of Phlebotomus papatasi in an experimental collagen-induced arthritis model (CIA) and we identified the salivary constituent. **Methods:** CI was elicited in DBA male mice by collagen-emulsion injection (200ug/id./mice) and then treated with *P. papatasii* SGE (1 gland/mice/i.v.route) daily during 14 days. Ribeirão Preto School of Medicine Ethics Committee (Protocol N° 059/2005). **Results:** SGE administration at the onset of disease attenuated the severity of CIA and reduced joint destruction. SGE treatment was associated with a marked decrease of pro-inflammatory cytokines whereas anti-inflammatory mediators such as PGE2 and IL-10 were enhanced. Furthermore, dendritic cells treated with SGE limited specific-CD4+ Th17 activation and proliferation. Using a combination of fractionation techniques (microcon filtration and reversed-phase HPLC), we identified adenosine and 5´AMP as the major salivary molecules responsible for such anti-inflammatory activities. Pharmacological inhibition of adenosine receptor or enzymatic catabolism of salivary nucleosides reversed the immunosuppressive effect of SGE. Moreover, both adenosine and adenosine monophosphate (5´AMP) commercial standards mimicked SGE-induced anti-inflammatory activity upon DC function *in vitro*. Importantly, adenosine up-regulated the expression of COX2 mRNA and PGE2 production in response to LPS. *In vivo*, adenosine and 5´AMP effectively attenuated the establishment of CIA. **Discussion:** all the results presented here indicate that ADO and 5´AMP present in the P. papatasi SGE, mediate, at least in part, the anti-inflammatory activity of saliva. These constituents act in the effector phase of the inflammatory process, inhibiting DCs ability to presenting antigen and thus leading to suppression of CD4+Th17-induced inflammatory immune response, indicating that this mechanism could be a major step in limiting the tissue damage observed in several diseases disorders, represented here by AR. **Financial support**: FAPESP and CNPq.

04.076

Anti-inflammatory activity of crude extract and of flowers fractions from *Palicourea rigida* in mice. Arruda LLM[1], Rosa EA[2], Oliveira CMA[3], Fachini RF[2], Silva CC[2], Baroni S[1], Grespan R[1], Bersani-Amado CA[1] [1]UEM − Farmácia e Farmacologia, [2]UEM Química, [3]UFGO − Química

Introduction: The aerial parts of plant species *Palicourea rigida* (Pr) are used in folk medicine for the treatment of urinary disorders. There are reports of some biological tests of Pr as the antimicrobial and cytotoxic activity. Emphasizing the latter, the crude and basic ethyl acetate fraction of Pr leaves exhibited significant activity against human melanoma cells SK MEL 37. Considering the pharmacological potential of this species, this study aims to evaluate the potential topic anti-inflammatory effect of the crude extract and fractions of the Pr flowers in mice. This study enriches the research with gender Palicourea, enhanced the understanding of a medicinal plant popular use in the Cerrado in Goiás and also consolidate the collaboration of research groups involved. **Methods**: The Pr flowers were collected, dried, ground and subjected to extraction in methanol, obtaining methanolic crude extract (CE) and hexane (HF), chloroform (ClF), ethyl acetate (EAF) and hydromethanolic (MF) fractions. The CE, EAF and MF were dissolved in acetone 70%, and HF and ClF were diluted in chloroform 70% immediately before use. The anti-inflammatory activity of CE, fractions (5.0 mg/ear) or indomethacin (1 mg/ear) was evaluated by inhibition of ear edema induced by croton oil. Briefly, in a group of Swiss mice was topically administered croton oil on the inner surface of the left ear. And as a control, the vehicles (solution of acetone 70% or chloroform 70%) were administered in the right ear. Immediately after applying the inflammatory agent, other groups received CE, Pr fractions or indomethacin on the inner surface of the left ear. After six hours, the animals were anesthetized, euthanized, the ears sectioned and weighed. The means of different treatments were compared by ANOVA, followed by Tukey's test ($P<0.05$). The protocol number 021/2009 regarding this study was approved by the Ethical Commission of Ethics in Animal Research). **Result and discussion**: The application of croton oil in the left ear of mice induced an inflammatory response very evident at the 6th h. As noted left ear weight increased by two times compared to the right ear (control, without application of croton oil). The CE, MF, HF ClF of Pr significantly inhibited the intensity of edema (CE = 43.74% $P<0.001$; MF = 42.6% $P<0.001$; HF = 50.43% $P<0.001$; ClF = 29.7% $P<0.01$) when compared with the group of animals who received only the croton oil. The EAF did not affect the development of the response (EAF = 22.25% $P> 0.05$). Treatment of animals with indomethacin, an anti-inflammatory drug reference, caused a pronounced reduction in ear edema. This way, our results showed the anti-inflammatory effect of crude extracts and Pr fractions. These data support the development of additional studies in order to identify the active constituents of the Pr fractions and to elucidate the mechanism of action. **Supported by**: CNPq/PIBIC-UEM. **Acknowledgments**: Jailson Araujo and Celia Regina Miranda for technical assistance.

04.077

Short chain-fatty acid effects on acute gout: importance in induction and resolution of inflammatory responses. Vieira AT[1], Shim D[2], De-Leon E[2], Schilter HC[2], Amaral FA[3], Arruda MCC[3], Maslowski KM[2], Fagundes CT[3], Nicoli JR[4], Teixeira MM[3], Mackay CR[2]
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Introduction: Gout is an inflammatory disease characterized by uric acid crystals release into the joint cavity. This event promotes neutrophil infiltration and activation that leads to tissue damage. Short-chain fatty acids (SCFAs) are produced by bacterial fermentation of dietary fibers and bind to G-protein coupled receptor 43 (GPR43), exerting modulator effects in inflammatory responses. Then, we decided to study the effect of acetate (the most abundance SCFAs in the body) in an acute gout experimental model. **Methods:** Gout was induced in wild type (WT) and GPR43-deficient (GPR43-/-) mice by intra-articular injection of Monosodium Uric Acid (MSU) crystals. Mice were kept in specific pathogen free as well as germ free environments. The periarticular tissue was monitored for neutrophil recruitment and activation as well as cytokine and chemokine production. Pleurisy model was induced by LPS injection into the pleura. To assess the effect of SCFA, mice were treated with acetate. All procedures performed in mice were approved by the Garvan Medical Research Institute Animal Studies Committee (08/25) and UFMG (165/2008) **Results and Discussion**: After MSU injection, WT mice showed elevated cytokine and chemokine production, diminished neutrophils infiltration into the synovial cavity as well as reduced myeloperoxidase activity in periarticular tissue. Interestingly, we observed that GPR43-/- mice did not present any alteration in inflammatory parameters after gout induction, when compared to MSU-injected WT mice. Germ-free (GF) mice, which are devoid of bacteria and express little or no SCFAs, showed hyporesponsiveness toward gout induction, presenting reduced cell recruitment to tissue after MSU injection. However, when these mice were treated with acetate before MSU injection, their ability to recruit neutrophils was restored and consequently these mice showed normal gout injury. These data suggested the ability of acetate to prime neutrophils and allow inflammatory cell recruitment during response to MSU injection. However, Acetate treatment before gout induction in WT mice abolished cell recruitment and tissue injury. Of note, Acetate-treated WT mice produced elevated IL-10 levels after MSU injection. Finally, acetate treatment 4 hours prior to LPS injection led to reduction in neutrophils counts in pleural cavity. This reduction in cell counts was accompanied by an increase of neutrophil apoptosis. SCFA present dual effects during neutrophilic inflammation: Microbiota-induced SCFA and GPR-43 activation are necessary for proper neutrophil recruitment. However, SCFA treatment during an established inflammatory response promoted resolution of the inflammatory response. This work suggests that endogenous microbiota shapes the host's ability to respond to inflammatory stimuli and the presence of SCFAs provides a molecular link between gastrointestinal bacterial metabolism and inflammatory responses. **Support**: CNPq, FAPEMIG, CRC.

04.078

In vivo hydroquinone exposure affects leukocyte recruitment and adhesion molecules expression on LPS inflamed lung. Ribeiro ALT[1], Shimada ALB[1], Hebeda CB[1], Bolonheis SM[1], Tavares de Lima W[2], Farsky S[1] [1]USP-Clínical and Toxicological Analyses, [2]ICB-USP − Pharmacology

Introduction: Hydroquinone (HQ) is a phenolic compound found in large quantities in cigarette, medicines and foods. Moreover, it is one of the most important toxic metabolites of benzene. Here we investigated the effect of HQ inhalation on recruitment of leukocytes into LPS inflamed lung and on adhesion molecules expression by circulating leukocytes and endothelium. **Methods:** Male Swiss mice were exposed to 12.5ppm, 25ppm or 50ppm of aerosolized HQ, 5 days, 1 hour, once a day. Control animals received the same amount of vehicle (Veh; saline with 5% ethanol). Lung inflammation was induced by inhalation of LPS (0.1 mg/mL; 10 min) one hour after the last exposure. Three hours later bronchoalveolar lavage (BAL) and blood were collected. Total and differential counts of leukocytes were determined in Neubauer chamber and in stained smears, respectively. Expressions of β3-Integrin, β2-integrin, L-selectin and PECAM-1 were performed on 1x105 circulating leukocytes obtained one hour after the last exposure and then were subsequently stimulated or not with fMLP (10-8 M; 10 or 30 min). Adhesion molecules expression on leukocytes was measured by flow cytometry and on endothelium by immunohistochemistry assay. The activity of myeloperoxidase (MPO) was measured by colorimetric assay. The experiments were conducted according to Ethics Committee in Animal Experiments n.53/2008 – Protocol n.196. **Results:** HQ exposure reduced the number of leukocytes in the BAL (12.5ppm= 43.6% vs. control; 25ppm= 53.1% vs. control; 50ppm= 61.7% vs. control), but the number of circulating leukocytes was not modified in all schedules of HQ exposure. HQ exposure enhanced the expression of β3-Integrin (111.59% vs cells from Veh) and PECAM-1 (252.24% vs cells from Veh) by circulating leukocytes, especially by polymorphonuclear cells In the other hand, after *in vitro* fMLP stimulation, the expression of β3-Integrin (50.61% vs cells from Veh) and L-selectin (73.95%) was reduced. The expression of PECAM-1, VCAM-1 and E-selectin on endothelium was no modified by the HQ exposure. The activity of myeloperoxidase (MPO) was higher in animals exposed to HQ (5,81 U/mg tissue) than in control ones (9,58 U/mg tissue). **Discussion:** Results presented show that inhaled HQ impairs LPS-induced neutrophil recruitment into lungs and alters the ability of polymorphonuclear cells to express adhesion molecules expression. The latter effects may contribute to the altered inflammatory response in HQ exposed animals. **Financial support**: FAPESP (Process nº08/55382-7 and 09/03964-5) and Capes.

04.079

Exogenous leptin modulates acute lung inflammation induced by LPS in mice. Landgraf MA[1], Silva RC[2], Hiyane M[3], Cunha CS[3], Vieira PMM[3], Cenedeze MA[2], Keller AC[4], Pacheco-Silva A[4], Araújo RC[6], Câmara NOS[1], Landgraf RG[4] [1]ICB-USP, [2]UNIFESP − Nefrologia, [3]ICB-USP − Imunologia, [4]UNIFESP-Diadema − Ciências Biológicas, [6]UNIFESP − Biofísica

Introduction: Leptin is an adipocyte-derived hormone that regulates food intake as well as metabolic and endocrine functions. It is known that this hormone can influence a multitude of physiological systems including immunity, inflammation, and hematopoiesis (Conus, JACI, 116:1228, 2005). However, the role of leptin in pulmonary inflammatory response is still unclear. Lipopolysaccharide (LPS), a major constituent of the outer membrane of gram negative bacteria, is an important factor in acute lung injury. Airway exposure to LPS in mice induces acute pulmonary inflammation with recruitment and activation of neutrophils, vascular leakage and bronchopulmonary hyperreactivity (Lefort, *Am J Respir Cell Mol Biol*, 24:345, 2001). In this work, we have investigated the role of exogenous leptin in acute lung inflammation induced by LPS. **Methods:** All procedures used in this study were approved and performed in accordance with guidelines established by the ethics committee of the Institute of Biomedical Sciences (CEEA – 83/2009), USP. Five or six male C57BL/6 mice at 8-9 wk of age were used for each group in this study. Control group was given saline intranasally (20 uL). Experimental groups were given leptin (1 ug/g/20 uL), LPS (1.5 ug/g/20 uL) or leptin (1 ug/g/20 uL) and LPS (1.5 ug/g/20 uL) intranasally. 24 h after instillation, the bronchoalveolar lavage fluid (BALF) was collected to evaluate cellular infiltration in lung. Blood was collected to measure serum cytokines/chemokines concentration. Lungs were harvested for measurement of the mRNA expression of keratinocyte chemoattractant (KC) by real-time PCR. Cells were isolated from lung tissue for analysis of T cell subsets by flow cytometry. **Results:** The intranasal administration of leptin into C57BL/6 did not alter any of the parameters evaluated, when compared to the control group; on the other hand, the intranasal administration of LPS significantly increased all of them. The intranasal administration of leptin 30 minutes prior to LPS administration significantly reduced inflammatory cell infiltration into airways (50% the total cells and 25% the neutrophils), level of KC mRNA expression (32%), lymphocytes subpopulations (52% of CD4, 50% of CD8, 60% of B lymphocytes), and serum cytokine/chemokine levels (50% − IL-17; 74% − IL-12; 83% − MIP-1a; 40% -MIP-1b), when compared to the group that received LPS alone. **Discussion:** These results indicate that exogenous leptin can modulate the LPS-induced acute lung inflammation in mice by down-regulation of proinflammatory cytokines/chemokines. **Financial support**: FAPESP, CNPq, and INCT Complex Fluids

04.080

Gastroprotection and healing activities of the seeds oil from *Carapa guianensis* Aubl. in mice. Gonçalves DO[1], Figueiredo IST[1], Osório CBH[1], Siqueira RMP[1], Oliveira RSB[2], Freitas LBN[1], Vieira IR[1], Pinheiro RSP[2], Vasconcelos MAM[3], Alencar NMN[1] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Bioquímica e Biologia Molecular, [3]EMBRAPA − Agroindústria

Introduction: The seed's oil from *Carapa guianensis*, popularly known as Andiroba, is widely used by its anti-inflammatory actions in order to treat several disorders such as skin diseases, arthritis, rheumatism, ear infections. The objective of this study was to evaluate the gastroprotection and healing effects of seed's oil from *Carapa guianen*sis (OCG) in mice. **Methods**: Gastroprotective action of OCG was evaluated in model of gastric ulcer by ethanol. Swiss mice (female, 25–30g) after food deprivation of 16 h were divided into groups (n=8) and treated with OCG (400 mg/kg, p.o.), N-acetilcysteine (750mg/kg, p.o.) or vehicle (tween 80 2%). After 1 h the animals received ethanol (0,2 mL, p.o). Thirty-five minutes later mice were sacrificed and had the stomach excised, opened by the great curvature and washed with saline 0,9%. The percentage of damaged area was determined through planimetry. To evaluate the healing activity of OCG, surgical wounds (1cm2) were made on the dorsal region of Swiss mice (female, 25-30g). After the surgery the animals were divided in 3 experimental groups: C (control), OCG 10 (*Carapa guianensis'* oil 10%) and OA (oil 20%). The topical treatment was undertaken after the wound induction and has lasted 11 days. The wounds were evaluated macroscopically during 12 days. At the days 2, 7 and 12 after the surgical procedure the animals were sacrificed (n=5/group) and wounds samples were collected to hystopathological analysis. Animal handling and experimental protocols were registered on the Institutional Ethics Committee (nº 24/09). **Results and Discussion**: The evaluation of gatroprotective effects of OCG (400mg/kg, p.o.) has demonstrated a significative reduction of damaged area (0.87 ± 0.4%, 94.13% of inhibition) when compared to the group that received only the ethanol (14.84 ± 0.92%) ($p<0,05$). N-acetilcysteine has reduced the damaged area too (0.4 ± 0.15%, 97.35%) and the group that was treated only with vehicle has not showed ulceration. The evaluation of healing activity has showed a higher frequency of edema and hyperemia on wounds of mice treated with OCG 20 (100%) when compared to the other groups OCG 10 (80%) and C (60%) ($p<0.05$). Treatment with OCG 10 and OCG 20 has induced contraction of the wounds in comparison to group C in a more evident way at the day 7 (59% − OCG 20; 31% − OCG 10; 13% − C) and day 8 (82% − OCG 20; 64% − OCG 10; 45% − C) ($p<0,05$). Besides that a higher percentage of re-epithelialization was observed at the days 10 and 12 in the groups OCG 10 e OCG 20 in comparison to group C ($p<0,05$). Hystopathological analysis of the wounds at the days 2 and 7 has demonstrated increased polymorphonuclear cell infiltration in the wounds treated with OCG 10 e OCG 20 when compared to group C ($p<0,05$). These results suggest that the oil from Carapa guianensis' seeds module the inflammatory phase of the healing process in surgical cutaneous wounds in mice contributing to tissue repair. In the model of gastric ulcer the OCG has demonstrated a significative gastroprotective effect. Nevertheless, several studies must be carried out to elucidate the mechanisms involved. **Support**: CAPES, CNPq.

04.081

ADP effect on skin wound healing on diabetic mice. Branco AMC[1], Brogliato AR[1], Figueiredo JB[2], Melo PA[3], Benjamim CF[3] [1]UFRJ − Farmacologia, [2]ICB-UFRJ, [3]UFRJ − Farmacologia Básica e Clínica

Introduction: Denmark and the United States estimate that 1 and 2% of population, respectively, had already experienced impaired wound healing (Gottrup, 2004). Wound difficult healing process is the one healing does not occur naturally. Patients who did not take special care, they suffer serious consequences such as amputation of limbs, generalized infections and severe pain. Patients with diabetes have impaired wound healing (Chettibi et al., 1999). This is an important public health problem, which treatment involves high costs and low efficiency. Topical application of adenosine A2A receptor agonists accelerate healing of dermal wounds in both healthy animals and diabetics rats with impaired wound healing (Montesinos et al.,2008). Take it into account, our aim is to investigate the role of ADP skin wound treatment in diabetics animals. **Methods:** Swiss mice with 25-30g of both sexes were used. Diabetes was induced with a single dose of alloxan (65 mg/ kg, i.v.). The wound (10mm diameter) was performed using a biopsy punch at the dorsum of the animal. Treatment with ADP (0.3 µg/site) is topically performed during the first five days after injury. Morphometric assessment of the skin wound was performed at days 0, 4, 7, 10 and 14. Skin samples were excised at day 7 for the determination of myeloperoxidase (an indirect method for neutrophils quantification) and for IL-6 quantification by EIA. This animal study was performed in accordance with Institutional guidelines, approved by Ethical committee of animal use, protocol number: DFBCIB 028. **Results and Discussion:** Treatment with ADP is able to accelerate the healing process in diabetic mice, compared to diabetic mice treated only with saline (control). The ADP-treated diabetic mice presented the same pattern compared to non-diabetic. ADP was able to reduce neutrophil infiltration at day 7 postinjury in both diabetic animals as in non-diabetics. We also intend to assess if ADP alters the cytokine (IL-6, TNF-a and TGF-b) release into the bed of the lesion, as well as evaluate the granulation tissue (histology) and collagen deposition after ADP treatment. Conclusion: ADP seems modulate the tissue repair process altering the immune cells such neutrophils. However, to complete the characterization of ADP action on wound healing more data is necessary. **Financial support**: CNPq,FAPERJ,LM FARMA.

04.082

Inflammasome activation and IL-1b and IL-18 production are essential for host resistance to dengue virus primary infection. Fagundes CT[1], Ávila TV[2], Costa VV[2], Silveira KD[3], Cisalpino D[2], Valadão DF[2], Tavares LD[2], Morcatty TQ[2], Santos AG[2], Souza RS[2], Vieira LQ[3], Zamboni DS[4], Souza DG[2] Teixeira MM[3] [1]UFMG − Bioquímica e Imunologia/Microbiologia, [2]UFMG – Microbiolo-gia, [3]UFMG − Bioquímica e Imunologia, [4]FMRP-USP − Biologia Celular, Molecular e Bioagentes Patogênicos

Introduction: Dengue virus (DENV) infection has emerged as the most important mosquito-borne disease on Earth. There are no treatments or vaccines available against Dengue disease and mechanisms involved in host response to infection are poorly understood. In the past few years, several studies have shown the importance of inflammasome complex activation during infectious processes. Then, we evaluated the role played by this complex during host response to DENV infection. **Methods:** To assess the role played by the inflammasome complex, ASC-deficient (ASC-/-), Caspase-1-deficient (Casp-1-/-), and their respective wild type (WT) control mice were infected with DENV-2 (10 PFU, i.p.). The role of IL-1 during infection was assessed by treatment of DENV-2-infected mice with IL-1ra (20 mg/kg, s.c., every 8 hours). Finally, IL-18-deficient (IL-18-/-) mice were infected with DENV-2 to study the role played by IL-18 during disease. Seven days after infection, caspase-1 cleavage, hematocrit and platelets number, neutrophil accumulation in spleen, AST and ALT activity in plasma and cytokines production in spleen and serum, as well as viral loads in spleen were evaluated. For lethality rates after infection, the same infected groups were monitored for 14 days. All procedures have been approved by local ethics committee (protocol 113/09). **Results and Discussion**: There was an increase in levels of cleaved Caspase-1 in spleen of DENV-2-infected mice. However, Casp-1 cleavage in spleen of DENV-2-infected ASC-/- mice was reduced. These animals, as well as Casp-1-/- mice, presented a more severe disease manifestation after DENV infection, showing increased thrombocytopenia, marked hemoconcentration, elevated plasmatic transaminases activity, enhanced neutrophil arrest and tissue injury in liver and elevated systemic production of TNF-a and IL-6. In addition, viral loads in spleen of ASC-/- and Casp-1-/--infected mice were markedly elevated and lethality rates after infection were strikingly higher. Of note, both groups showed reduced IL-1b and IL-18 production after DENV infection, when compared to WT-infected mice, suggesting these cytokines are of great importance during host response to DENV infection. Hence, IL-1ra-treated and IL-18-/- mice showed marked susceptibility to DENV-2 infection when compared with infected-WT mice, showing similar alterations to that seen in ASC-/- and Casp-1-/--infected mice. These data suggest that inflammasome activation is essential for host ability to deal with DENV infection, through promotion of IL-1b and IL-18 production and control of viral replication. Then, strategies that improve this pathway activation could be useful during the control of primary infection by the Dengue virus. Support: INCT em Dengue, CNPq and FAPEMIG

04.083

Membrane TNF-α is essential for the pathogeneses of gouty arthritis. Tavares LD[1], Amaral FA[1], Costa VV[1], Fagundes CT[2], Quesniaux V[3], Ryffel B[4], Teixeira MM[2], Souza DG[1] [1]UFMG − Microbiologia, [2]UFMG − Bioquímica e Imunologia, [3]CNRS − Molecular Immunology and Embryology, [4]IEM-CNRS

Introduction: Monosodium uric acid crystals (MSU) accumulation at articular joint results in intense local inflammatory response, but the precise mechanisms are not fully understood. Recently, the participation of inflammasome (NLPR3 prototype) in MSU-induced inflammation has been well characterized, with the important involvement of IL-1β cytokine. However, other mediators must be relevant in this context, like the cytokine TNF-α, since it is involved in the activation of innate immunity and is associated to various arthritic diseases. Thus, the aim of this study is evaluate the role of TNF-α in the development of gouty arthritis in mice. **Methods:** Wild type male C57/BL6, TNF-α KO (TNF-α-/-), TNF-α knock-in (TNF KI), TNF-α, Lymphotoxin-α, Lymphotoxin-β (TNFIII – triple KO), TNFRI-/-, TNFRII-/- and TNFRI/II-/- mice were used. MSU (100 µg/10 µL) were injected into the tibio-femoral joint and analyzes were evaluated 15 h later. Intra-articular lavage was performed for cellular count (Neubauer clamber and Cytospin3 centrifuge). Samples of periarticular tissue were removed for cytokines and chemokines (ELISA) analysis. Treatments were performed 40 minutes before the MSU injection − Infliximab (10 mg/kg), Enbrel (10 mg/kg) and Xencor (50 mg/kg). **Results:** Different doses of MSU induced the release of TNF-α in the periarticular tissue, indicating a possible involvement of this cytokine in this response. In accordance, mice lacking TNF-α had reduced inflammatory response evaluated by lower detection of IL-1β and CXCL1 in the periarticular tissue and neutrophils in the joint cavity. Still, mice lacking TNF-α receptors (TNFRI and TNFRII) had also a diminished inflammation. The treatment with whole TNF-α (soluble and membrane) inhibitors, Infliximab and Enbrel, were effective in reducing the inflammatory response. However, the treatment with Xencor, a compound that block only soluble TNF-α, had no effect in prevent inflammation. Confirming the important participation of membrane TNF-α in this model, mice treated with TACE (responsible for membrane TNF-α cleavage) had an intense articular inflammation, similar to the vehicle-treated animals after MSU injection. **Discussion:** Our data indicate that the cytokine TNF-α has an important participation on the inflammation induced by uric acid crystals injection since the treatment with TNF-α inhibitors shows efficiency in the reduction of this process. Moreover, membrane TNF-α seems to be essential for the development of MSU-induced inflammatory response. **Financial support**: FAPEMIG, CNPq

04.084

ATL-1, a synthetic analog of 15-Epi-lipoxin A4, promotes changes in dendritic cells phenotype and function. Da-Fe AR[1], Ladislau L[2], Kunkel SL[3], Benjamim CF[4], Fierro IM[5]
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Introduction: Dendritic cells (DCs) participate in the immune response and are originated from hematopoietic cells. After pathogen recognition, become efficient antigen presenting cells and activate the acquired immune response. However, the uncontrolled induction of the immune response during infectious events in an attempt to eliminate the pathogen can be lethal for the host due to high production of inflammatory mediators. Lipoxins (LXs) are metabolites of arachidonic acid with known anti-inflammatory and pro-resolution activities. In DCs, LXA4 activates two different receptors, the ALX, a transmembrane G protein coupled receptor and the aryl hydrocarbon (AhR), a nuclear receptor. Activation of ALX in DCs inhibits the expression of pro-inflammatory cytokines such as TNF-α, IL-6, IL-12 and IFN-g and reduces DCs migration. The molecular basis of these effects is not clear and there are no studies showing the role of LXs on DCs differentiation. The aim of this study is to investigate the role of ATL-1, a synthetic analog of 15-epi-lipoxin A4, on the regulation of the immune response by DCs. **Methods:** Bone marrow cells were isolated from C57Black/6 mice and cultured for 7 days in RPMI medium with 10% fetal bovine serum and in the presence of GM-CSF (25ng/mL on day zero and 15ng/mL on days 3 and 6) to stimulate the differentiation into DCs. On day 7, CD11c+ cells were selected by using Mouse CD11c Positive Selection Kit (Stemcell Technologies) and the purity was assessed by FACS. We evaluated whether treatment of bone marrow cells with ATL-1 would be interfering with the differentiation of DCs and with co-stimulatory molecules and MHCII expression. Bone marrow cells were pretreated with ATL-1 (1-100nM) or vehicle (ethanol 0.02%) for 30 minutes, cultured with GMCSF for 7 days (25ng/mL on day zero, 15ng/mL on day 3 and 10ng/mL on day 6) and the purity of DCs and CD80, CD86, CD40 and MHCII expression was evaluated by FACS. This animal study was performed in accordance with Institutional guidelines, approved by Ethical Committee of Animal Use, protocol number DFBCIB 028. **Results:** Our results show that the pretreatment of bone marrow cells with ATL-1 interferes with the differentiation into DCs stimulated by GM-CSF, increasing the purity of the cultures and promoting increase of CD80 and MHCII expression, in a concentration dependent manner. In contrast, CD86 and CD40 expression was not changed by the treatment of cells with the analog. The results are representative of two independent experiments. **Discussion:** ATL-1 positively regulated changes in the phenotype of DCs. This activity suggests that these lipids can modulate the immune response by regulating differentiation of DCs. **Financial support**: FAPERJ and CNPq.

04.085

Platelet-Activating Factor (PAF) contributes to the neuroinflammatory process involved in the *Plasmodium berghei* ANKA infection. Lacerda-Queiroz N[1], Rodrigues DH[1], Miranda AS[2], Vilela MC[3], Teixeira MM[4], Teixeira AL[5] [1]UFMG − Biologia Celular, [2]UFMG − Medicina Tropical, [3]UFMG − Neurociências, [4]UFMG, [5]UFMG − Medicina

Introduction: Cerebral malaria (CM) is a neuroinflammatory condition, characterized by a strong immune response and neurological syndromes. Some features have been registered on the immunopathogenesis of CM like circulating cytokines and chemokines and, recently, the leukocyte recruitment. Paralleling, platelet-activating factor (PAF) has been implied as an inflammatory mediator, involved in the leukocyte recruitment. Thus, the aim of this work was investigate the role of PAF in the outcome of Plasmodium berghei ANKA (PbA) infection and the relevance of this molecule for the inflammatory process involved in this model. Method: C57Bl/6 (WT) and PAFR knockout (KO) mice were infected with PbA, and parasitemia, survival and SHIRPA evaluation were monitored daily. Leukocyte recruitment in the cerebral microcirculation was evaluated by intravital microscopy and the histopathological analysis (H&E) were made in order to determinate morphological changes in the brain on the course of infection. The NAG activity (an index of macrophage activity) and the concentration of TNF-alpha and the chemokines CXCL1, CXCL9, CCL2, CCL3 and CCL5 were measured in brain tissue by ELISA on day 5 p.i. To prove the effect of the genetic depletion in the course of the infection, WT mice were treated with PAFR antagonist (UK-74505) and monitored. Experimental protocols were submitted to and approved by the Animal Ethics Committee of the Instituto de Ciências Biológicas, UFMG (UFMG), Brazil (application number 193/06). **Results:** Infected PAFR KO mice resisted to the malaria infection for a longer period (21 days) than WT (6 days), but parasitemia levels and behavioral performance showed no differences between groups until 6dpi. Additionally, on day 5 p.i., the leukocyte recruitment, NAG concentration and the cytokine levels had similar profile. The histopathology studies showed perivascular inflammatory infiltrates and parenquimal hemorrhage, but more intense in WT mice. The treatment with UK74505 share a similar profile of the PAFR KO infected mice. **Discussion:** These results suggest that PAFR contributes to the neuroinflammatory process involved in the Plasmodium berghei ANKA infection. Blockade of PAFR may prevent the development of CM. **Financial support**: CAPES, CNPq, FAPEMIG.

04.086

iNOS-derived nitric oxide modulates bone loss from ligature induced periodontitis by inhibiting osteoclast differentiation and activity. Herrera BS[1], Martins Porto R[2], Costa SKP[2], Spolidório LC[1], Van Dyke TE[3], Gyurko R[3], Muscará MN[2] [1]FOAR-UNESP − Physiology and Pathology, [2]ICB-USP − Pharmacology, [3]Boston University − Periodontology and Oral Biology

Introduction: Inflammatory stimuli activate inducible nitric oxide synthase (iNOS) in a variety of cell types, including macrophages, osteoclasts (OC) and osteoblasts (OB), resulting in a sustained release of nitric oxide (NO). The role NO in bone metabolism is not clear: large doses inhibit or kill osteoblasts and osteoclasts, but complete inactivation of iNOS genes in mouse knockouts result in impaired function in these cells. Objective: In this study we evaluated the extent of bone loss from rats with periodontitis (P) under chronic iNOS inhibition, and the differentiation and activity of osteoclasts from iNOS-knockout mice *in vitro*. Materials and **Methods:** All the experimental protocols were approved by the local Ethics Committee for Animal Experimentation (number 058/03). Periodontitis was induced in Wistar rats (male, 180-220 g) by placing a cotton ligature around the first lower molar tooth, sham animals (S) had the ligature immediately removed. Two weeks prior to P induction, and until the end of the experiments, half of the animals received Aminoguanidine (Ag; 200 mg/L in the drinking water) until being sacrificed, 3, 7, 14 days after the ligature procedure. Were collected, jaws for histological and radiographic analysis. The experiments *in vitro* were performed in OC cultures, OC differentiation was induced with M-CSF and RANKL in freshly isolated bone marrow cultures extracted from the femoral bone of iNOS KO and C57BL/6 mice (wild type, WT). OC were counted 6 days later following TRAP staining, TRAP-positive cells having three or more nuclei were considered OC and presented as the proportion of the culture dish area covered by OC (OC covered area). Bone resorption was assessed *in vitro* on dentine discs, bone marrow cells were differentiated on dentine discs for 14 days. At the end of the incubation period, the surfaces of dentine discs were processed for scanning electron microscopy and resorption pits were counted. **Results:** Rats with ligature showed progressive and significant alveolar bone loss when compared with the sham (day 7: $p<0.001$; day 14: $p<0.01$). Aminoguanidine treatment significantly inhibited ligature-induced bone loss at 7 and 14 days after the induction (day 7: $p<0.001$; day 14: $p<0.01$). n vitro, OCs from iNOSKO mice has less OC covered area in the culture dish ($p<0.01$) and less resorption pit counts to a comparable degree with decreased OC growth ($p<0.01$). Conclusions: In conclusion, our results demonstrate that NO from iNOS plays a crucial role in the pathogenesis of periodontitis, probably inhibiting osteoclasts differentiation and activity, and treatment with Ag prevented alveolar bone loss in a rat model of ligature-induced periodontitis. **Supported by** NIH/NIDCR DE14568, RR00533, FAPESP and CAPES

04.087

HQ impairs nitric oxide synthesis in neutrophils via post transcriptional modifications. Hebeda CB[1], Bolonheis SM[1], Pinedo F[1], Teixeira SA[2], Muscará MN[2], Farsky S[1] [1]USP − Análises Clínicas e Toxicológicas, [2]USP − Farmacologia

Introduction: Circulating neutrophils are sentry cells which defend the organism from invading agents phagocyting and releasing a powerful cocktail of reactive oxygen and nitrogen species. Nitric oxide (NO) is a signaling molecule that presents cytotoxic activities. Here, we investigate the effects of *in vitro* HQ exposure, an important environmental contaminant, on mechanisms involved on secretion of NO by neutrophils. **Methods:** Cells were obtained from male Wistar rats 4 hours after intraperitoneal injection of oyster glycogen (1%, 10 mL). Neutrophils were cultured (RPMI 1640 with 10% FBS) in absence (control) or presence of HQ (5 or 10 uM) during one hour and subsequently incubated with LPS (5ug/mL; 18 hours). Supernatant was used to quantify levels of NO by Griess reaction and cells were employed to quantify nitric oxide synthase (NOS) activities by enzymatic assay, protein expression of iNOS by Western blot as well as gene expression of iNOS and eNOS by RT-PCR. The experiments were conducted according to the Ethics Committee in Animal Experiments n.53/2008 – Number Protocol – 169. **Results:** Concentrations of HQ used in this study were based on absence of cytotoxicity. HQ inhibited LPS-induced NO production (53%), NOS activity (HQ 5uM = 48% and HQ 10uM = 90%) and iNOS protein expression (60%) but did not modify its mRNA levels. **Discussion:** Based on these findings, data presented show that HQ impairs secretion of NO dependent on post transcriptional modifications. These effects may be relevant for the actions of HQ on the host defense, as NO is a signaling molecule involved in the neutrophils triggering innate response. Further investigations will be achieved to confirm this hypothesis. **Financial support**: Capes; FAPESP (07/56299-3).

04.088

Melatonin inhibits adhesion of neutrophils induced by lipopolysaccharide (LPS) in endothelial cells culture. Abrantes-Lima KD, Tamura EK, Markus RP IB-USP − Fisiologia

Introduction: Melatonin is an indolamine synthesized by the pineal gland and released rhythmically in the circulation during the night. Besides the pineal gland, other tissues and cells also produce melatonin, which exerts paracrine functions. Melatonin decreases neutrophil-endothelium interaction induced by leukotriene B4 (LTB4) *in vivo*, resulting in reducing vascular permeability (Lotufo, *Eur J Pharmacol*, 430: 351, 2001). Melatonin also inhibits lipopolysaccharide (LPS)-induced nitric oxide production by endothelial cells (Tamura, *J Pineal Res*, 46:268, 2009). Here we evaluated the effect of melatonin in an *in vitro* model for studying neutrophil-endothelium interaction. In order to determine if endothelial cells are targets for melatonin, these cells were incubated in an acute or chronic manner with the indolamine. **Methods:** Approved by the ethical committe (CEA/IB: 080/2008). Endothelial cells were obtained from the cremaster muscle of rats maintained in 12/12h light/dark cycle, with food ad libitum. Endothelial cells were incubated with LPS (1 mg/mL, 2 h) in the presence or absence of melatonin (100 mM − acute treatment). Alternatively, endothelial cells were incubated for 20 days with melatonin (100 mM − chronic treatment) and at the last 2 hours LPS (1 mg/mL) was added. In both protocols medium with or without melatonin was changed each 48 hours. Neutrophils, obtained from rat aorta (Lotufo, *Eur J Pharmacol*, 534:258, 2006), were added to the endothelial cells to adhere for 30 minutes. Number of cells adhered was estimated by myeloperoxidase reaction and expressed as percentage of the color obtained from basal adherence (control 100%). **Results:** LPS treatment promotes an increase in the adhesion of neutrophils to endothelial cells (120.8% ± 4.136 n= 3). Acute melatonin treatment reduced neutrophils adherence induced by LPS in 99.97% (± 3.597 n= 3). The chronic treatment was not able to change LPS-induced increase in neutrophil adherence (123.2% ± 2.701 n= 3). **Discussion:** Our data indicate that acute melatonin inhibits neutrophil-endothelial interaction, while chronic treatment is unable to modify in this interaction. Considering that the effect of melatonin on the endothelium was proposed to be mediated by membrane receptors (Lotufo, *Eur J Pharmacol*, 430:351, 2001) and that these receptors are known to desensitizated after chronic exposition to melatonin (Hazlerigg, *Endocrinology*, 132:285, 1993; Mackenzie, *Biochem Pharmacol*, 63:587, 2002), we may suggest that desensitization of melatonin receptors mediates this effect. In conclusion, our data clearly shows that inhibition of neutrophil-endothelial interaction by melatonin is lost after chronic treatment, suggesting that this mechanism is not observed in animals that maintain high levels of melatonin along the 24 hours of the day. **Financial support**: FAPESP (2008/56391-0; 2007/07871-6), CNPq (472881/2009-4) and CAPES. Acknowledgments: The technical support of Débora A. Moura is gratefully acknowledged.

04.089

Interaction of the anti-inflammatory annexin A1 protein and tacrolimus immunosuppressant in the renal function of rats. Truzzi RR[1], Araújo LP[2], Oliani SM[1] [1]UNESP − Biology, [2]UNIFESP − Morphology

Introduction: The immunosuppressant tacrolimus (FK) is used in the transplant patients and in the treatment of autoimmune diseases. The most important side effect of FK is nephrotoxicity, which manifest through renal functional and/or structural alterations. This pathogenesis has been studied through an experimental rat model characterized by dietary sodium depletion that enhances FK nephrotoxicity and reproduces the renal alterations observed in humans (Andoh et al, Transplant., 57:483, 1994). The anti-inflammatory protein annexin A1 (ANXA1) is a potent mediator of inflammation resolution (Perretti M, D´Acquisto F. *Nat Rev Immunol*, 9:62, 2009). ANXA1 has an important role in the resolution of acute and chronic inflammation. Moreover, ANXA1 inhibits the innate immune system response, regulating neutrophil and monocyte adhesion and migration to inflamed tissue. We used an experimental model of acute FK nephrotoxicity to evaluate the effects of ANXA1 treatment in the pathogenesis of FK-induced renal functional and structural changes. We report the protective effect of ANXA1 treatment on FK-induced structural injury and macrophage infiltration in rats after seven days of treatment. **Material and Methods:** Animals and Experimental groups Adult male Munich-Wistar rats received a low salt diet, boiled rice supplemented with amino acids and vitamins (Aminomix®), prior seven days to treatment. Then, rats were randomly assigned to receive daily FK, ANXA1 mimetic peptide, FK+ANXA1 or identical volume of vehicles (VH) for seven days. After, groups were allocated for glomerular filtration rate assessment (06 rats) or renal blood flow measurement (06 rats). Renal tissue was collected for histological evaluation at the end of each experiment. Experiments were approved by the São José do Rio Preto Medical School Ethical Committee on Animal Experimentation (001-001082/2008). Analysis The analysis included renal function studies (assessment of sodium, potassium, creatinine, osmolality and glomerular filtration rate), hemodynamic studies (renal blood flow, mean arterial blood pressure, renal vascular resistance) and blood and urine analysis (urinary and serum inulin and creatinine, urinary and serum sodium and potassium concentrations, urinary osmolality and the fractional excretion of sodium and potassium). ELISA was used to determine FK and TGF-β levels and immunohistochemistry to proceed the quantification of macrophages in the renal cortical tubulointerstitium. Lastly, morphological analyses were performed to histopathological analysis. **Results and Discussion** In this study ANXA1 single administration did not cause any renal dysfunction, while animals receiving FK showed significant reduction in the glomerular filtration rate (70%), renal blood flow (55%) and increases in serum creatinine and renal vascular resistance, characteristics indicative of acute nephrotoxicity. ANXA1 administration did not alleviate the functional impairment caused by FK treatment. On the other hand, we observed a partial protective effect of ANXA1 on FK-induced structural injury and a reduction of macrophage influx after seven days of treatment. FK induced early macrophage infiltration, which was reduced by ANXA1 administration. Macrophages are known sources of cytokines, including TGF-β, which has important role in cellular proliferation and extracellular matrix deposition. In fact, we detected this molecule in the plasma of FK-treated rats, suggesting an early activation of this pathway by FK. Moreover, TGF-β is a well known renal fibrogenic growth factor and its accelerates matrix proteins production and inhibits matrix degradation in chronic FK nephrotoxicity. Our results suggest that ANXA1 might have an important anti-inflammatory role in FK nephrotoxicity. **Acknowledgments:** CNPq and FAPESP.

04.090

Crosstalk of TLR2/CD36 with PPARg in lipid metabolism and inflammatory response during infection by *Mycobacterium bovis* BCG: role of rafts. Almeida PE[1], Antunes KM[2], Maya-Monteiro CM[1], Almeida CJ[1], Silva AR[1], Castro-Faria-Neto HC[1], Bozza PT[1]
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Introduction: The nuclear receptor PPARg acts in a TLR2-dependent signaling pathway as a key modulator of lipid metabolism, inflammation and pathogenesis in BCG-infected macrophages (Almeida PE et al., 2009). However the molecular mechanisms involved in lipid metabolism and mycobacterial pathogenesis is not completely clear. TLR2 deficient mice are incapable of inducing PPARg expression and to form lipid bodies during BCG infection, suggesting a requisite role for TLR2 in this phenomenon. We have demonstrated that the activation of macrophages *in vitro* with the TLR2 agonists -zymosan or M. smegmatis- fail to induce PPARg expression, lipid body formation and PGE2 production, while producing TNFα synthesis. Thus, suggesting that TLR2 activation although essential for mycobacterial-induced lipid body formation is not sufficient to trigger pathways for lipid body formation. In this research, we studied the signaling of TLR2 and co-factors TLR6, CD36 and integrins (CD11b/CD18). As well, the involvement of lipid rafts in the molecular mechanism of lipid bodies formation, PGE2 production, cytokines synthesis and PPARg expression in macrophages during BCG infection. Methods and **Results:** Peritoneal macrophages were obtained of CD57bl6, KO-TLR2 and KO-TLR6 animals, performed under the approval of the Animal Ethical Committee-number 002/08. BCG induced lipid body formation and PGE2 production was similar in wild-type and TLR6 deficient animals, suggesting that TLR6 is not involved in this phenomenon. Increased expression of CD36 in macrophages *in vitro* was observed after BCG infection. Pretreatment with CD36 neutralizing antibodies significantly inhibited lipid body formation, PGE2 production and PPARg expression induced by BCG. Moreover, we observed that CD36 co-immunoprecipitate with TLR2 in BCG-infected macrophages suggesting the CD36 interaction with TLR2 in BCG signaling. Pretreatment with CD11b/CD18 neutralizing antibodies significantly inhibited BCG-induced lipid body formation and PGE2 although not TNFα synthesis. As CD36 and CD11b/CD18 might recruit TLR2 signaling to rafts, we then investigate the role of rafts in BCG-induced response. Disruption of rafts using filipin or methil-β-cyclodextrin, significantly inhibited lipid body formation and PGE2 synthesis, although not TNFα production after BCG infection. **Discussion:** Our results suggest that CD36 and CD11b/CD18 cooperating with TLR2 in lipid rafts are involved in the intracellular signaling during *Mycobacterium bovis* BCG infection which leads to increased PPARg expression and lipid body formation. Reference: Almeida PE, et al., J Immunol, 2009. 183(2):1337-45. Support: CNPq, FAPERJ, PAPES-FIOCRUZ.

04.091

Role of PPARγ in macrophage activation but not in neutrophil recruitment during *Mycobacterium bovis* BCG infection *in vivo*. Sette-Martins R, Almeida PE, Roque NR, Bozza PT IOC-FIOCRUZ − Imunofarmacologia

Introduction: Macrophages have important roles in both lipid metabolism and inflammation and are central to immunity to intracellular pathogens. Foamy-like -lipid laden- macrophages are present during the course of Mycobacteria infection and have been implied in mycobacterial pathogenesis. The peroxisome proliferator activated receptors (PPARs) act in several inflammatory processes and immunoregulation due to the property to regulate the expression of many genes, thus are implicated in the pathophysiology of atherosclerosis, inflammation, obesity, diabetes and immune response. We had demonstrated that PPARγ is highly expressed in macrophage-during *Mycobacterium bovis* BCG infection *in vitro* (Almeida et al., J Immunol, 2009. 183:1337-452009). In this study we investigated the role of PPARγ in cellular activation during BCG infection in mice. **Methods:** C57BL/6 mice were obtained from the FIOCRUZ, Rio de Janeiro, Brazil. This study was performed under the approval of the Animal Ethical Committee-number L002/08. Mice were intrapleurally injected with BCG (5 x 105 bacilli/cavity) in 100 µl of sterile PBS, control animals received an equal volume (100 µl) of sterile PBS. After different time intervals (1 and 24 h), the animals were killed by CO2 inhalation, and their thoracic cavities were washed with 1 mL of PBS. Cellular analysis was performed by light microscopy in smears prepared in a cytocentrifuge. Supernatants were collected and stored at –20 oC until the day of analysis. KC production was analyzed by ELISA. **Results and Discussion:** Our results demonstrated that pretreatment with the selective PPARγ antagonist, GW9662 and subsequently infection with BCG significantly inhibited the lipid body formation in macrophages after 1 h and 24 hours of *in vivo* infection. In contrast, the pretreatment with GW9662 failed to interfere with KC production or neutrophil recruitment into the pleural cavity. Thus, our results suggest that PPARγ activation is essential for lipid body formation in macrophages, but not for the chemokine production or cellular recruitment induced by *Mycobacterium bovis* BCG infection *in vivo*. This suggests that different signaling pathways of cellular activation might cooperate with the PPARγ signaling during mycobacterial infections. Supported by: CNPq, PAPES/FIOCRUZ and FAPERJ.

04.092

Role of substance P in different endogenous pyrogen-induced fever. Brito HO, Reis RC, Zampronio AR UFPR − Farmacologia

Introduction: Fever is an essential component of host defense against environmental aggressors such as Gram negative bacteria. During this event, various cell types, activated in response to lipopolyssacharide (LPS), release different cytokines, including interleukin(IL)-1β, IL-6, Tumor Necrosis Factor (TNF-α) and Macrophage Inflammatory Protein-1α (CCL3/MIP-1α) among others. These endogenous pyrogens act in the central nervous system to induce the release of central mediators such as prostaglandins and Corticotrophin-Releasing Factor (CRF). Substance P (SP) is an abundant neuropeptide restrained mainly in the nervous fibers, but also present in some immune cells such as macrophages and mast cells, which is released during several nociceptive and inflammatory processes. Its action is almost exclusively mediated by neurokinin (NK)-1 receptor. We showed previously that the neuropeptide SP is also involved in the LPS-induced fever by activating NK-1 receptors but does not participate in the febrile response induced by IL-1β . Based on that, the aim of this work was to investigate the effect of the NK1 receptor non-peptidic antagonist, SR140333, in the febrile response induced by TNF-α, IL-6, CCL3/MIP-1α and CRF. **Methods:** Male Wistar rats (200 g) were implanted with guide cannula in lateral ventricle and with data loggers for measurement of body temperature in the peritoneal cavity one week before the experiment under the same anesthesia. On the day of the experiment, animals were pre-treated with SR140333 (3.0 µg/2µl, i.c.v.) and after 30 min were also injected i.c.v. with the following mediators: TNF-α (250 ng/2µl), IL-6 (300 ng/2µl), CCL3/MIP-1α (500 ng/2µl) and CRF (2,5 µg/2µl). Control animals received vehicle (Tween 20 0.1%) and saline. Body temperature (◦C) was registered every 15 min. The room temperature was kept at 28˚C. All the methods were previously approved by Institutional Ethics Committee under protocol no. 384. Results : The administration of TNF-α and IL-6 induced an increase in body temperature which was reduced by SR140333 (TNF-α: 37.94 ± 0.1 ◦C ; SR140333 + TNF-α: 37.41 ± 0.1 ◦C at 2.5h and IL-6: 38.27 ± 0.1◦C ; SR140333 + IL-6: 37.81 ± 0.1 ◦C at 5h). On the other hand, the i.c.v. administration of the antagonist did not interfere with the febrile response induced by CCL3/MIP-1α or CRF. **Discussion:** The results show that SP is a central mediator of fever which is released after TNF-α and IL-6 but not after MIP-1α and CRF, similarly to what happened with IL-1β. However, complementary studies are necessary to establish the exact role of this neuropeptide in the pyrogenic mediator´s cascade induced by LPS. **Financial support**: CNPq, CAPES and Sanofi-Aventis.

04.093

Pineal gland is instrumented to be an integral player of innate immune response. Carvalho-Sousa CE[1], Cruz-Machado SS[1], Fernandes PACM[1], Tamura EK[1], Pinato, L[2], Petrilli CL[1], Markus RP[1] [1]IB-USP − Fisiologia, [2]UNESP − Fonoaudiologia

Introduction: Pineal gland is a neuroendocrine component of the circadian timing system, as it transduces light information into the nocturnal melatonin surge. Melatonin also plays a role as immunomodulator either in innate or acquired immune responses (Guerrero, Curr Top Med Chem, 2: 167, 2002). Pineal gland is outside of blood-brain barrier having access to circulating immunoregulatory factors that in turn modulate its neuroendocrine activities. According to this cross-talk between the circadian timing and immunological system, it was proposed the existence of an immune pineal-axis (Markus, *Neuroimmunomodulation*, 14: 126, 2007). In such context, during the mounting of an innate immune response there is a suppression of nocturnal melatonin surge. This is mediated by the pro-inflammatory cytokine, tumor necrosis factor (TNF), which inhibits melatonin biosynthetic pathway (Fernandes, J Pineal Res, 41: 34, 2006). In the present work, we aimed to investigate the cellular mechanisms involved on pineal gland response to TNF. **Methods:** Animal procedures were performed according to the institutional ethical committee approved protocols (CEA/IB 081/2008). Pinealocyte culture was obtained by the dissociation of pineal glands by trypsinization and dispersed in DMEM medium in an 8-well culture plate (0.5-1 x 105 cells/ well). Cells were incubated in the absence or presence of TNF (30 − 80 ng/mL, for 1 − 240 min, depending on the experiment), fixed and incubated overnight with primary antibodies anti-TNFR1, anti-TNFR2 or anti NFKBIA. Secondary antibodies were incubated at room temperature (1 h) and analyzed by confocal microscopy. The data are expressed as% relative to the non-stimulated group (100%). Rat pineal glands were cultivated for 48 h and then treated or not with TNF (30 ng/mL, 5 – 60 min). Nuclear factor kappa B (NFKB) nuclear translocation was assayed in the pineal nuclear extracts by Electromobility Shift Assay (EMSA) and its subunits were determined by Gel Supershift assay. Results Rat pineal glands express TNFR1 and TNFR2 on glia and pinealocytes. TNF (30 ng/mL, 5 min) induces a rapid and time-dependent translocation of p50/p50 and p50/RelA NFKB dimmers to the nucleus. This was accompanied by a rapid (10 min) and transient reduction on the expression of the inhibitory protein NFKBIA. In addition, the exposition to high concentrations of TNF (80 ng/mL, 1h) reduces the amount of TNFR1 receptors observed in glia cells and in pinealocytes. This culture has a low level of glia cells. Therefore, we only quantified the reduction of immune stained TNFR1 receptors in pinealocytes (56% ± 8 n= 4 cultures). All the concentrations of TNF used did not interfere with the viability of cell cultures. **Discussion:** The present work disclosed the molecular machinery and an intracellular mechanism by which TNF modulates pineal gland function. These data increase the knowledge concerning to the pineal gland as a relevant player of the defense system because its shows how the gland is instrumented and responds to an important cytokine of the immune response. **Financial support**: FAPESP, CNPq, CAPES.

04.094

The intestinal ischemia/reperfusion in rats promotes changes in the lung depending on the time of reperfusion. Vitoretti LB, Breithaupt-Faloppa AC, Gimenes-Junior JA, Domingos HV, Sudo-Hayashi LS, Oliveira-Filho RM, Tavares de Lima W ICB-USP − Pharmacology

Introduction: Experimental and clinical studies have reported that intestinal ischemia/reperfusion (I/R) induces acute lung inflammation (ALI), which can lead, in severe cases, to acute respiratory distress syndrome (ARDS). ALI is characterized by the release of many inflammatory mediators, neutrophil infiltration and increased vascular permeability. It is known that inflammatory mediators generated at the site of intestinal I/R are transported by the thoracic lymphatic system and, reaching the lungs, contributes to the ALI. Furthermore, data on late effects of intestinal I/R in ALI/ARDS are still fragmented. **Methods:** In the present study, male Wistar rats were subjected to 45 min of intestinal ischemia due to obstruction of the superior mesenteric artery and then to 24 h, 72 h or 120 h of reperfusion. After the desired time of reperfusion the activity of myeloperoxidase (MPO), the extravasation of Evans blue dye (EB), inflammatory mediators in the lymph and in lung explants were quantified. We also quantified the expression of endothelial molecules and the effect of treatment with a broad-spectrum antibiotic. **Results and Discussion**: The intestinal I/R increased the MPO activity and the EB extravasated in lung 120 h after reperfusion. Lymph collected from intestinal I/R animals contained significant amounts of IL-1b, IL-6, VEGF and LTB4. Cultured lung explants from 120 h-reperfused animals revealed increased amounts of VEGF and IL-1b, whereas IL-10 decreased. Our data also revealed decreased expression of vWf and increased expression of integrin b1, PECAM-1 and collagen type I and IV in the pulmonary endothelium of animals I/R 120 h. Treatment with antibiotic was more or less effective in reducing the MPO and the EB extravasation in the lung, 120 h after reperfusion, depending on the period of ischemia or reperfusion when the drug was administered. Our data indicate that the time of reperfusion mediates lung inflammation. Lymph and blood-borne inflammatory mediators participate in the onset/maintenance of pulmonary inflammation by altering the integrity of the pulmonary endothelium. It is possible that the regulation of endogenous control of inflammation is altered so that a bacterial infection may contribute to pulmonary inflammation observed after 120 h of reperfusion. CEEA/IBC-USP: nº 080, page 19, book 2. **Financial support**: FAPESP 07/56872-5; 5/02271-5 and CNPq 306526/2006-9

04.095

Saccharomyces boulardii prevents the inflammatory response in intestinal mucositis induced by 5-fluorouracil in mice. Justino PFC[1], Silva LMN[1], Melo LFM[1], Costa JVG[1], Nogueira AF[1], Ribeiro RA[1], Souza MHLP[1], Soares PMG[2] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Morfologia

Introduction: Intestinal mucositis is a frequent side-effect associated to 5-fluorouracil (5-FU) clinical use and results in inflammatory events. It is characterized by epithelial ulcerations in the mucosa and clinical manifestations of abdominal pain, nauseas and diarrhea. *Saccharomyces boulardii* is a probiotic yeast which has been shown to protect the gastrointestinal microflora from disequilibrium and from associated gastrointestinal disorders. Objective was to evaluate effect of *Saccharomyces boulardii* in inflammatory aspects of intestinal mucositis induced by 5-FU in mice. **Methods:** Swiss male mice (30-35g) were treated with 5-FU (450 mg/kg, i.p., only dose) or saline (control). The groups, mice were treated with *Saccharomyces boulardii* (SB; 800 mg/kg) daily for 3 and 6 days (3D and 6D). On the third day after administration of 5-FU or 5-FU + SB, mice were sacrificed and samples of jejunum (J) and ileum (I) were collected for assessment of histopathological scores, MPO activity and GSH concentration for spectrophotometry, nitrite dosage using the Griess method. All animal treatments and surgical procedures were approved by the local ethics committee (protocol 34/10). Significance statistics (tests ANOVA and Bonferroni), values considers with $p < 0.05$. **Results:** 5-FU induced significant histopathological alterations, such as villi shortening with vacuolized cells, crypts necrosis, inflammatory cell infiltration, vacuolization and edema. All of these alterations were reverted by the treatment with SB- 3D and 6D (Jejunum: C= 0 (0-1), 5-FU= 2,5 (1-3), 5-FU + SB (6D)= 1 (1-2), 5-FU + SB (3D)= 1 (0-2) / Ileum: C= 0 (0-1), 5-FU= 2,5 (1-3), 5-FU + SB (6D)= 1 (1-2), 5-FU + SB (3D)= 0 (0-1). 5-FU induced an increase on the MPO activity (nº neutrophil/mg of tissue) (jejunum: C= 1,73 ± 0,37, 5- FU= 7,37 ± 1,77/ ileum: C= 1,69 ± 0,23, 5-FU= 13,05 ± 2,79), and nitrite concentration (jejunum: C= 37,00 ± 2,39, 5-FU= 59,04 ± 11,41/ ileum: C= 26,03 ± 1,51, 5-FU= 41,97 ± 5,21), a decrease on the GSH concentration (jejunum: C= 477,60 ± 25,25, 5-FU= 270,90 ± 38,50/ Ileum: C= 186,80 ± 22,84, 5-FU= 46,01 ± 6,37). SB (3D and 6D) reduced the increase on the MPO activity (jejunum: SB (6D)= 3,58 ± 0,70, SB (3D)=4,15 ± 0,73 / ileum: SB (6D)= 6,71 ± 1,20, SB (3D)= 6,51 ± 1,63), reduced the increase of Nitrite concentrations (jejunum: SB (6D)= 27,86 ± 4,55, SB (3D)=37,90 ± 5,78/ ileum: SB (6D)= 22,27 ± 2,91, SB (3D)= 43,64 ± 5,60), reverted the GSH concentrations (jejunum: SB (6D)= 505,60 ± 37,69, SB (3D)= 514,00 ± 38,64/ ileum: SB (6D)= 143,70 ± 18,68, SB (3D)= 124,70 ± 18,19) **Discussion:** Our results suggest that the treatment with *Saccharomyces boulardii* reverted the inflammatory events on the intestinal mucositis induced by 5-FU in mice. **Financial support**: CNPq.

04.096

Effect of protease-activated receptor-2 activating peptide on B1 cell spreading and its modulation by the C-terminus of the calcium binding protein S100A9. Moraes NF[1], Sampaio SC[1], Freitas JD[1], Pagano RL[2], Giorgi R[1] [1]IBu − Fisiopatologia, [2]IEP − Neuromodulação e Dor Experimental

Introduction: B1 cells, found in the peritoneal cavity of mice, are capable to differentiate in phagocytes. They show similar functions to macrophages, suggesting their importance as a new mononuclear phagocyte in the inflammatory process (Lopes and Mariano, An Acad Bras Cienc, 81:489, 2009). A number of serine proteases display diverse extracellular and intracellular functions during inflammation via protease-activated receptors (PARs). We have shown that PAR-1 and -2 influence the spreading and phagocytosis by adherent peritoneal cells in mice, and that the C-terminal peptide from murine S100A9 (mS100A9p) inhibits the increment induced by PAR-1 activating peptide in these events (Pagano, et al., *Eur J Pharmacol*, 628:240, 2010). To date, however, the participation of PARs and mS100A9p has not been studied in B1 cell function. In this work, we evaluated the ability of synthetic PAR-1 (PAR1-AP) or PAR-2 (PAR2AP) activating peptides to interfere in B1 spreading, and the putative modulatory effect of mS100A9p on this phenomenon. **Methods:** B1 cells from the peritoneal cavity of Swiss mice (protocol 674/09, approved by the Ethical Committee, Institute Butantan) were cultivated as described elsewhere (Almeida et al., *Int Immunol*, 13:1193, 2001). B1 cells obtained from stationary cultures were plated in glass coverslips (2x105 cells/coverslip) in 0.5 mL of R10 medium/well (control) in order to undertake spreading assays. In addition, B1 cells were incubated in R10 medium containing PAR1AP (20 or 40 mM/well) or PAR2AP (5, 10, 20 or 40 mM/well). The effect of the reverse PAR2AP peptide (5 or 10 mM/well) was also evaluated in the presence or not of PAR2AP. In addition, B1 cells were treated with mS100A9p (1.17 or 2.35 mM/well) during the spreading period (24 h) in the presence or not of PAR2AP (5 mM/well). Data were generated by evaluating cells by phase contrast microscopy. **Results:** The results demonstrated that only PAR2AP, in all concentrations tested, increased the spreading ability of B1 cells, and that this effect was blocked by its reverse peptide, suggesting the specificity of this receptor on B1 cell spreading. The reverse peptide of PAR2AP per se did not interfere in B1 spreading. However, mS100A9p inhibited not only cell spreading in the control group, but also the increase in spreading induced by PAR2AP. **Discussion:** These findings demonstrate for the first time that B1 cells respond to the PAR-2 agonist, and that this effect is modulated by mS100A9p. The model used here, in conjunction with the data shown, may be used as a tool for providing a better understanding of the involvement of PARs and B1 cells in the pathophysiology of inflammatory process, as well as for using mS100A9p as a modulatory molecule to control the function of inflammatory cells. **Financial support**: FAPESP and Fundação Butantan

04.097

Effects of the treatment with an inhibitor of CCL2 synthesis, in acute diet-induced adiposity in mice. Lima RL[1], Menezes Z[1], Santos MCC[1], Guglielmotti A[2], Teixeira MM[3], Ferreira AVM[4], Souza DG[1] [1]UFMG − Microbiologia, [2]ACRAF − Pharmacology, [3]UFMG − Imunofarmacologia, [4]UFMG − Enfermagem Básica

Introduction: Obesity is associated with chronic inflammation and is a major risk factor for diabetes and metabolic syndrome. CC chemokine ligand 2 (CCL2) has a central role in inducing insulin resistance and monocyte recruitment into adipose tissue (AT) that contributes to the beginning and maintenance of the inflammatory process in AT. **Objective**: Here, our aim was study the role of CCL2 in adiposity and inflammatory response associated with consumption of palatable diet, through the treatment with Bindarit. **Methods:** C57BL/6 mice were divided in three groups: fed with control diet (C), palatable diet (PD) or PD associated with Bindarit treatment (PDB). First, a dose/response curve of 10 (PDB10), 30 (PDB30) and 100mg (PDB100) of Bindarit by 1kg of body weight twice a day was performed. The bindarit was given orally during five days, starting the administration two days before the animals feed a PD. After the third day, mice were killed by exsanguination and plasma concentrations of cholesterol, triglycerides and glucose were determined biochemically. The epididymal (EAT), retroperitoneal (RAT) and mesenteric (MAT) adipose tissue were weighted and frozen for future evaluation of the concentration of IL-6, TNF-a and CCL2. **Results:** The adiposity, evaluated by weight of EAT, RAT and MAT, increased in the group PD compared to control mice (0,26 ± 0,03; 0,25 ± 0,03 and 0,41 ± 0,031 g/100g body weight vs 0,17 ± 0,02033 EAT; 0,13 ± 0,01949 RAT and 0,26 ± 0,04029 MAT g/100g body weight) . The mice fed PD increased the production of TNF-a (1394 ± 101,7 vs 1016 ± 89,57) and CCL-2 (174,1 ± 13,99 vs 244,1 ± 7,61) compared to control mice. The Bindarit treatment inhibited the production of CCL2 at all doses tested (244,1 ± 7,615 vs 158,7 ± 4,741 (PDB10); 150,8 ± 17,97 (PDB30) and 166,1 ± 12,28 (PDB100) without change the TNF-a production. There were no change in IL-6 AT concentration as well as in the serum metabolic parameters evaluated (cholesterol, triglycerides and glucose) in all groups. **Discussion:** Three days of PD intake dit not cause metabolic alterations although were sufficient to determine an increase in adiposity and in adipose tissue concentration of CCL2 and TNF-a. All Bindarit doses tested were able to decrease the production of CCL2 in AT, however, this effect did not interfere with the increased of adiposity induced by PD. License number of the Ethics Committee: 264/08 **Supported by**: FAPEMIG, CAPES and CNPq.

04.098

Hydrogen sulfide and antioxidant enzyme activities in allergic mice lungs. Campos D[1], Benetti LR[1], Nogueira JS[1], Gurgueira SA[2], Vercesi AE[3], Ferreira HHA[1] [1]USF − Inflamação, [2]FCM-UNICAMP − Bioenergética, [3]FCM-UNICAMP − Patologia Clínica

Introduction: A variety of inflammatory mediators result in the typical pathophysiologic changes observed in asthma. An imbalance between reactive oxygen species (ROS) production and antioxidant enzyme activities, such as those of manganese-superoxide dismutase (MnSOD), glutathione peroxidase (GPx) and catalase (CAT), contribute to the chronic inflammation process that characterizes asthma. Recent studies have shown that endogenous hydrogen sulfide (H2S) may be involved in the pathogenesis of airway inflammation (Gadala and Snyder, *J. Neurochem* 113:14, 2010). H2S is a highly reactive molecule and may react with other compounds, such as reactive oxygen and nitrogen species. Objective: We investigated whether endogenous H2S may affect antioxidant enzyme activities in airway inflammation in a mice model of asthma. Materials and **Methods:** All experiments were approved by the animal ethics committee of USF (protocol 0021108). Balb/c mice were sensitized at days 0 and 7 with a subcutaneous injection of ovalbumin (OVA). Intranasal OVA challenge was performed after 1 week, twice a day (OVA group). The H2S donor, sodium hydrosulfide (NaHS; 7-28 μmol/kg), was given intraperitoneally 30 min before OVA challenge (NaHS/OVA-treated group). At 48 and 96 hours after OVA-challenge, the mice were sacrificed and the lungs were removed, flash frozen in liquid nitrogen and stored at -80°C. Lung tissue was homogenized in buffer containing a protease inhibitor cocktail (Sigma) and then centrifuged at 800 x g for 10 min at 4°C. The supernatant was used to analyze the oxidative stress by the activities of fumarase, an essential tricarboxylic acid (TCA) cycle enzyme resistant to oxidants, and antioxidant enzyme activities. **Results:** Treatment of allergic mice with NaHS (7 μmol/kg) resulted in a reduction (63%) in lung CAT activity, whereas a dose-dependent decrease was seen in GPx activity (52, 40 and 35% with NaHS 7, 14 and 28 μmol/kg treatment, respectively), compared to the OVA group at 48h after allergen challenge. A reduction in antioxidant enzyme activity was also detected at 96h in the NaHS/OVA-treated group, but only with the administration of 28 μmol/kg of NaHS (CAT 47%; GPx 38%), as compared with the OVA group. Fumarase activity was not affected, indicating that differences in the activities of antioxidant enzymes is a specific regulatory effect and not to a global effect. Conclusions: H2S donor treatment decreases antioxidant enzyme activities, suggesting that at these times of exposure and concentrations, endogenous H2S did not recover antioxidant enzyme activities. The reduction in antioxidant enzyme activities could be the consequence of electron transport chain inhibition by H2S, resulting in decreased oxidative stress and also in antioxidant enzyme activities. **Financial support**: FAPESP and CNPq

04.099

Expression of adhesion molecules in vessels of the microcirculation affected by different metalloproteases isolated from *Bothrops* venoms. Zychar BC[1], Baldo C[2], Clissa, PB[2], Alves AS[3], Britto LRG[3] [1]IBu − Fisiopatologia, [2]IBu − Imunopatologia, [3]USP − Fisiologia e Biofísica

Introduction: Snake venom metalloproteinases (SVMP) are major toxins involved in inflammatory reactions observed at the site of the bite in human envenoming. Depending on the domains compositions, SVMP can be classified in P1 to P4. In this study, the participation of different domains of SVMP on alterations of leukocyte-endothelium interactions (LEI) at the microcirculation of the cremaster muscle of mice was evaluated and also the expression of the adhesion molecules ICAM-1 (CD54) and PECAM-1 (CD31), responsible for the adhesion and cell migration, respectively. Three toxins were used: Jararhagin (JAR) and JAR-C, isolated from B. jararaca venom, and BnP1, isolated from B. neuwiedi venom. JAR, a P3 SVMP with a strong hemorrhagic activity, comprises catalytic, disintegrin-like, and cysteine-rich domains. JAR-C is a degraded form of JAR devoid only of the catalytic domain, with no hemorrhagic activity. BnP1, a weakly hemorrhagic P1 SVMP, has only the catalytic domain. **Methods:** JAR, JAR-C or BnP1 (0.5µg) or PBS (100µL) were injected into the scrotal bag of mice, and the microcirculation of cremaster was analyzed by intravital microscopy 2 or 24h after the injections. Ten min after the exposition, a 100 µm segment of a post-capillary venule (20-40 µm) was observed during 5 min, and leukocytes rolling, firmly adhered and emigrated were counted. Together, it was also evaluated the expression of adhesion molecules by immunofluorescence against CD54 and CD31, in the microcirculation of cremaster muscles collected 2, 4 ou 24h after the toxins injections, and analyzed by confocal microscopy. **Results:** In all groups injected with SVMPs, adhered and emigrated leukocytes were significantly increased in all times studied. Adhered cells were diminished after 24h of the toxins injection when compared to 2h, but emigrated cells were significantly increased. There was a significant increase in expression of CD54 in samples analyzed 2 and 4 h, and a decrease 24 h after the toxins injection. Related to the immunostaining index for CD31, it was observed a progressive time-dependent increase, when compared to the control or naive groups. **Discussion:** Despite differences in hemorrhagic activities and domain compositions of the three toxins used, the dose of toxins used induced alterations on leukocyte-endothelium interaction of the similar magnitude. It is suggested that alterations on leukocyte-endothelial interactions observed in the microcirculation after SVMPs injections occur by the expression of adhesion molecules ICAM-1 (CD54) and PECAM-1 (CD31). In conclusion, our results suggest that catalytic, disintegrin-like, and cysteine-rich domains of these Bothrops SVMP can induce alterations of leukocyte-endothelium interactions depends on ICAM-1 (CD54) and PECAM-1 (CD31) expression. Under a protocol approved by the Ethical Committee for Animal Research of Butantan Institute n° 466/08 **Supported by**: FAPESP

04.100

Effects of the cystein proteinase obtained from *C. candamarcensis* P1G10-treatment on eosinophil recruitment in allergic mice pleurisy. Miwa MY[1], Lopes MTP[1], Ferreira RG[2], Gomides LF[1], Salas CE[3], Klein A[2] [1]UFMG − Farmacologia, [2]UFMG − Fisiologia/Farmacologia, [3]UFMG − Bioquímica e Imunologia

Introduction: P1G10 is a proteolytic fraction obtained from the *C. candamarcensis* latex, and their anti-inflammatory properties have been studied by our laboratories, showing evidences for their healing activities on experimental ulcer models as well as their ability to inhibit carrageen-induced edema and neutrophil migration in experimental pleurisy induced by carrageen. Here, we evaluated the effects of P1G10 administration on the eosinophil recruitment in response to antigen challenge or eosinophil chemoattractants. **Methods:** BALB/c mice were immunized with ovalbumin (OVA) in aluminum hydroxide, and fifteen days after the first immunization challenged through the intra pleural (i.pl.) injection of OVA (1 µg) 1 h after subcutaneous (s.c.) administration of P1G10. Naive BALB/c mice were pretreated s.c. with P1G10 1 h before the i.pl. injection of leukotriene (LT)B4 (500 ng/cavity) or eotaxin-1 (100 ng/cavity). Eosinophil migration was assessed after 48h. (UFMG Animal Ethics Committee Certificate number 146/2009). **Results:** P1G10 injection did not inhibit the eosinophil recruitment induced by i.pl. OVA (PBS+PBS, 0.1 ± 0.06; PBS + OVA, 6.4 ± 1.1; P1G10 + OVA, 6.7 ± 1.4; dexamethasone + OVA, 1.4 ± 0.5 .105 eosinophils/cavity, ANOVA followed by Tukey test), or LTB4 (PBS + PBS, 0.6 ± 0.2; PBS + LTB4, 1.8 ± 0.4; P1G10 + LTB4, 1.5 ± 0.3. 105 eosinophils/cavity, ANOVA followed by Tukey test). However, P1G10 pretreatment was able to inhibit the eotaxin-1-induced eosinophil migration (PBS + PBS, 1.2 ± 0.5; PBS + eotaxin-1, 7.0 ± 1.7; *P1G10 + eotaxin-1, 2.5 ± 0.5 . 105 eosinophils/cavity, *$p<0.01$ ANOVA followed by Tukey test).**Discussion:** There are several inflammatory mediators released in response to allergen exposition, including LTB4 and the C-C chemokine eotaxin-1. In spite of the high production of others, these mediators have been described as important to activate eosinophils *in vitro* and induce their migration *in vivo*. The results show that P1G10 is unable to affect the eosinophil influx in response to both an allergen challenge or LTB4 injection, while P1G10 administration just inhibited the eotaxin-1-induced eosinophil recruitment, suggesting a selective P1G10 activity to the eotaxin-1-mediated effects. **Financial support**: FAPEMIG, CNPq.

04.101

Aprotinin potentiates carrageneen edema formation: evidences for prostaglandin participation. Ferreira RG[1], Godin AM[2], Matsui TC[3], Coelho MM[4], Klein A[3] [1]UFMG − Fisiologia/Farmacologia, [2]FF-UFMG − Produtos Farmacêuticos, [3]UFMG − Farmacologia, [4]UFMG

Introduction: Aprotinin is a potent *in vitro* and *in vivo* proteinase inhibitor known to inhibit trypsin and kallikrein. Here, we evaluated their effects on the carrageneen-induced edema, and the role of prostanoids on these effects. **Methods:** Edema was induced in the hind paw of Swiss mice (25-30 g), and it was measured 1, 2, 4, and 6 h after the carrageneen (Cg) or aprotinin (APR) intraplantar (i.pl.) injection. Animals were pretreated i.pl. with APR at different doses 30 min before carrageneen challenge, or with intraperitoneal (i.p.) injection of indomethacin (INDO) 1h before carrageneen administration (UFMG Animal Ethics Committee Protocol number 104/10). **Results:** intraplantar APR injection failure to induce edema formation in all doses and time studied (ANOVA followed by Newman-Keuls), while i.pl. APRO injection potentiated carrageneen-induced edema at a dose and time-dependent manner in all times studied ($p<0.01$ and $p<0.05$, ANOVA followed by Newman-Keuls). This effect was inhibited by INDO pretreatment (*$p<0.05$ ANOVA followed by Newman-Keuls). **Discussion:** The role of proteinases to the carrageneen-edema induced has been well established. Our results surprisingly show an unexpected effect of aprotinin in order to potentiate the carrageen-induced edema. This effect was abolished by INDO treatment, suggesting that prostaglandins could be involved in the process. Taken together our findings suggest that proteinases such as trypsin or kalikrein could be involved in the regulation of prostaglandins release. **Financial support**: FAPEMIG.

04.102

Methotrexate effects on systemic and adipose tissue alterations induced by obesity in mice. De Oliveira CC, Acedo SC, Gotardo EMF, Gambero A USF − Farmacologia e Gastroenterologia

Introduction: The proinflammatory cytokine TNF-α has been demonstrated to mediate insulin resistance as a result of obesity in many rodent models. TNF-α is overexpressed in white adipose tissue (WAT) in obese and insulin-resistant states as well as others proinflammatory cytokines (IL-6, IL-1β). WAT also produce anti-inflammatory substances, but their production is reduced during obesity. Macrophage infiltration in WAT is also increased during obesity, suggesting that the deleterious alterations associated to obesity are due to inflammation in adipose tissue. methotrexate (MTX) is an immunosuppressant drug with several interesting actions, such as anti-TNF-α activity. Drugs able to modify the inflammatory alterations associated to obesity could be useful tools to understand this process. Thus, the aim of this project was to evaluate MTX effects in systemic and adipose tissue alterations induced by obesity. **Methods:** Swiss mice were feed with commercial chow (CN) or high fat diet (HFD). Two protocols were used: MTX 1 mg/kg/week during 8 weeks of HFD (MTX1) and MTX 2 or 4 mg/ kg/weed in the last two weeks of HFD (MTX2 and MTX4, respectively). Body weight, food intake, glucose blood levels and insulin tolerance test (ITT) were evaluated. Adipose tissue depots were quantified and biopsies were obtained for protein expression analysis by Western blot and adipocytokines production by ELISA. Basal lipolysis was also evaluated in supernatant of short-term adipose tissue cultures. (CEA/USF Protocol 1/2008). **Results:** There was no reduction in body weight, food intake or adiposity in animals after treatment with MTX. No changes in glucose blood levels and ITT were observed in MTX1 mice. However, glucose blood levels were reduced in MTX2 and MTX4 (197.89 ± 11.55, 285.78 ± 16.34, 239.38 ± 19.04 and 249.25 ± 11.83 mg/dl for CN, HFD, MTX2 and MTX4, respectively; $p<0.05$). ITT showed that MTX2 and MTX4 mice are sensible to insulin (2.90 ± 0.36, 1.24 ± 0.27, 3.79 ± 0.74 and 2.95 ± 0.58 kITT for CN, HFD, MTX2 and MTX4, respectively; $p<0.05$). The basal lipolysis was reduced in adipose tissue from MTX4 mice (0.70 ± 0.15, 1.37 ± 0.16 and 0.83 ± 0.17 mg/dl of glycerol for CN, HFD and MTX4, respectively; $p<0.05$). Adipose tissue adiponectin and IL-10 production was increased after treatment (1311 ± 190 and 2328 ± 378 ng/mg tissue of adiponectin; 40.4 ± 11.2 and 77.3 ± 11.9 pg/mg tissue of IL-10 for HFD and MTX4, respectively; $p<0.05$). Leptin and TNF-α levels were reduced after MTX treatment (15623 ± 2401 and 6340 ± 1956 pg/mg tissue of leptin; 23.5 ± 2.3 and 12.8 ± 5.4 pg/mg tissue of TNF-α for HFD and MTX4, respectively; $p<0.05$). The MTX treatment also reduced the protein expression of iNOS and F4/80 (a macrophage marker), but not the expression of MCP-1. The level of phosphorylation of JNK was also reduced in the MTX4. **Discussion:** Our results demonstrate that methotrexate improves glycemic control and insulin resistance in obese mice. This improvement seems to be associated with a reduced production of proinflammatory and increased production of anti-inflammatory adipocytokines by adipose tissue. The macrophage infiltration and other inflammatory parameters were also reduced, suggesting that the inflammatory control in obesity could result in a better metabolic control. **Financial support**: Fapesp

04.103

Crohn´s experimental model decreased the mechanical inflammatory hypernoception in rats- role of NO/cGMP/ KATP pathway. Barbosa ALR[1], Sousa RB[2], Torres JNL[2], Lucetti LT[2], Cunha, TM[3], Cunha FQ[3], Ribeiro RA[2], Vale ML[2], Souza MHLP[2] [1]UFPI − Fisiologia e Farmacologia / UFC, [2]UFC − Fisiologia e Farmacologia, [3]FMRP-USP

Introduction: Patients with Crohn´s disease showed a reduction in the acute inflammatory response, with a decrease in neutrophil infiltration (Marks DJ et al. Lancet. 2006 25; 367-9511:668 -78). It was also demonstrated that during the inflammatory process, the migrating neutrophils participate in the cascade of events leading to mechanical hypernociception (Cunha et al., J Leukoc Biol. 2008 Apr; 83(4):824-32). Our hypothesis was that in Crohn´s experimental model induced by trinitrobenzene sulfonic acid (TNBS) injection, there was a decrease in the mechanical inflammatory hypernoception in rats. **Methods:** Colitis was induced in the male Wistar rats (200-250) by intracolonic administration of 20 mg of 2,4,6-trinitrobenzene sulfonic acid (TNBS) in 50% ethanol (n = 8), or an equivalent volume of saline (C; n = 8). Three days after the colitis induction, the rats received PGE2 (100ng/paw) or carrageenan (CG; 500 µg/paw). Four hours after carrageenan administration, animals were sacrificed and the whole plantar region right paw skin was harvested to measure myeloperoxidase activity (Neutrophils/mg of the tissue). The mechanical behavioral tests were performed by measuring the force in grams (g) applied through a digital analgesymeter (Insight). In order to investigate the involvement of the NO/cGMP/ KATP pathway in this event. There were used L-NOARG (100ng/paw), L-NAME (90mg/kg), ODQ (8µg/paw), L-Arg (200mg/kg), Glibenclamide (160µg/paw).**Results:** Rats with colitis induced by TNBS showed an increased nociceptive threshold (D force in grams) when induced by CG and PGE2 (3rd hour; C + CG = 33,24g ± 2.912; CG + TNBS = 5,2g ± 1.689; C + PGE2 = 31.08 g ± 2.514; PGE2 + TNBS = 5.0 g ± 1.579), but it was not observed statistical differences in myeloperoxidase assay (C + CG = 13956 ± 440.5; CG + TNBS = 14303 ± 793.3). Treatment with ODQ, glibenclamide decreased the nociceptive threshold when compared with TNBS colitis (3rdhour; CG + TNBS = 3.929 ± 1.524, CG + TNBS + ODQ = 39.14g ± 3.144; CG + TNBS + Glibenclamide = 30.87g ± 2,684/3rdhour; PGE2 + TNBS = 4.44 ± 1.28; PGE2 + TNBS + ODQ = 21,14g ± 2.49; PGE2 + TNBS + Glibenclamida = 25.80 g ± 2.79). L-arginine reversed effect of TNBS colitis to decrease the nociceptive threshold after treatment with L-NAME or L-NOARG in these animals (3rd hour; CG + TNBS + L-NAME = 33.02 ± 2 28; TNBS + CG + L-NAME + L-Arg = 1.44 ± 0.88 / 3rd hour; PGE2 + TNBS + L-NOARG = 22.72 ± 2 58; PGE2 + TNBS + L-NOARG + L-Arg = 1.37 ± 2.90, 97). **Discussion:** Our results suggest that in antinociceptive effect of the experimental model of Crohn´s disease induced by TNBS, there was an increase in the nociceptive threshold by activation of NO/ cGMP / K + ATP pathway, but not dependent on the neutrophil infiltration.

04.104

Effects of hydrogen sulfide on leukocyte migration and protein tyrosine nitration in airways of allergic mice. Benetti LR[1], Teixeira SA[2], Campos D[3], Silva AA[3], Costa SKP[2], Muscará MN[2], Ferreira HHA[3] [1]USF − Farmacologia, [2]USP − Farmacologia, [3]USF − Inflamação

Introduction: Recent studies have shown that endogenous hydrogen sulfide (H2S), a water and industrial air pollutant, along with nitric oxide and carbon monoxide, may be an endogenous signaling gasotransmitter and act as a vasodilator and neutransmissor. Endogenous H2S may have an antiinflammatory role in the pathogenesis of airway inflammation in chronic obstructive pulmonary disease (COPD), as well as in asthma (Chen, Chest 128:3205, 2005; Cytokine 45:117, 2009). Although the underlying mechanisms are incompletely understood, a H2S donor has been shown to diminish aspirin-induced leukocyte adhesion to the rat mesenteric venule endothelium (Zanardo, FASEB J. 20:2118, 2006). In addition, H2S may react with reactive oxygen and/or nitrogen species produced under inflammatory conditions, thus limiting their toxic effects (Lowicka and Bełtowski, Pharmacol Rep. 59:4, 2007). The aim of the present study was to investigate the effect of a H2S donor, sodium hydrosulfide (NaHS), on leukocyte infiltration and protein nitrotyrosine (NT) residues in the airways of allergic mice. **Methods:** All the experiments were approved by the AEC/USF (protocol 002.11.08). BALB/c mice previously sensitized with ovalbumin (OVA) were treated with NaHS (7, 14 or 28 µmol/kg, i.p.), 30 min before OVA challenge. Forty-eight hours after antigen challenge, the mice were killed; bronchoalveolar lavage (BAL) was performed and fluid samples were collected and analysed. Lungs were removed and homogenized in 50 mM Tris/HCl (pH 7.4) containing 0.5 M PMSF and a protease inhibitor cocktail (Sigma) for analysis of NT contents by slot-blot. Differential cell counts in BAL fluids were performed using cytospin preparations stained with Diff-Quick. **Results:** Pre-treatment of allergic mice with NaHS (7-28 µmol/kg) resulted in a significant reduction of the number of total leukocytes (~52%), eosinophils (~58%) and almost abolished neutrophil infiltration (95% reduction) in BAL fluid, as compared with untreated OVA-sensitized animals. In addition, NaHS (at 14 and 28 µmol/kg doses) significantly increased lung NT contents by 22 and 51%, respectively, as shown by slot-blot analysis. **Discussion:** These preliminary results show that the exogenous administration of NaHS to mice significantly attenuated OVA-induced allergic airway inflammation. On the other hand, pre-treatment with the H2S-donor led to increased protein tyrosine nitration in lung tissues, thus suggesting that the beneficial effects of H2S on eosinophil and/or neutrophil migration to lungs during the allergic response is independent of oxidative protein modifications. **Financial support**: FAPESP and CNPq

04.105

Characterization of allergic lung inflammation in genetically obese mice. Lintomen L, Calixto MC, Schenka A, Antunes E UNICAMP − Farmacologia

Introduction: Obesity is a risk factor for the development of asthma. However, the mechanistic basis for this relationship is unclear. Studies have reported innate airway hyperresponsiveness in obese mice that were obese as result of genetic leptin deficiency (ob/ob mice; Shore et al., 2003; Rivera-Sanchez et al., 2004). Obese mice also exhibit enhanced airway responsiveness to methacholine, and augmented IgE production following allergen sensitization and challenge (Johnston et al, 2007). Therefore, the aim of this study was to investigate the effect of ovalbumin (OVA) sensitization and challenge on EO lineage in ob/ob mice. Methods and results: Wild-type (WT) and ob-ob mice were sensitized with two s.c. OVA injections mixed with Al(OH)3 at 7-day intervals. One week after the second injection, mice were intranasally challenged with OVA twice a day. Mice were killed at 24-96 h after the first challenge. EO counts were carried out in blood, bronchoalveolar lavage fluid (BALF) and bone marrow (BM). Lungs were collected for histological analysis and Th2 cytokine levels were measured in BALF. In WT mice, intranasal challenge with OVA significantly increased ($p<0.05$) the EO counts in BALF post-OVA challenge (at 72 h: 0.54 ± 0.1 x106/ml, respectively; n=7) compared with PBS-challenged mice (at 72 h: 0.01 ± 0.01 x106/ml, respectively; n=5). In ob/ob mice, intranasal challenge with OVA also significantly increased the EO counts in BALF in all studied time, particularly post-OVA compared with PBS-challenged mice (72 h: 0.47 ± 0.04 and 0.01 ± 0.0 x106/ml, OVA and PBS, respectively). The histological data showed a 4.4-fold increase in EO counts in lung tissue of ob/ob mice challenged with PBS compared with WT mice ($p<0.05$; n=6). At 72 h post-OVA challenge, EO counts in lung tissue in ob/ob mice were significantly increased (8.0 ± 0.3 eosinophils/μm2) compared with either PBS-challenged mice (1.6 ± 0.05 eosinophils/μm2) or WT OVA- challenged mice (4.2 ± 0.15 eosinophils/μm2). The EO counts in blood and BM of ob/ob mice were also significantly increased in sensitized mice at 48 h and 72 h after OVA challenge compared with PBS-challenged mice. **Discussion:** Our study shows that obesity per se increases EO counts in lung tissue, and potentiates the EO influx to lung tissue in obese mice challenged with OVA. **References**: Shore et al. 2003. J Appl Physiol. 95, 938–945 Rivera-Sanchez et al. 2004. J Appl Physiol. 96, 2200–2206. Johnston et al. 2007. Am J Respir Crit Care Med. 176, 650–658. **Support**: Fapesp

04.106

Mycobacterium bovis BCG infection activates a rapamycin-sensitive mTOR pathway: involvement in the lipid body formation and inflammatory response. D´Ávila H[1], Lage SL[2], Roque NR[3], Maya-Monteiro CM[3], Almeida PE[3], Melo RCN[4], Castro-Faria-Neto HC[3], Bozza PT[3] [1]UFJF − Biologia Celular, [2]USP − Imunologia, [3]FIOCRUZ − Imunofarmacologia, [4]FIOCRUZ − Biologia Celular

Introduction: During the infection, mycobacteria are recognized by macrophages through Toll-Like receptor-2 (TLR2) and triggers several intracellular signaling cascades. The differentiation of macrophages into foamy macrophages is a common pathological observation in tuberculous granulomas both in experimental settings as well as in clinical conditions. Our group demonstrated that BCG infection induced lipid body formation via TLR-2, however, the mechanisms that regulate intracellular lipid accumulation in the course of mycobacterial infection and their significance to pathophysiology of tuberculosis are not completely understood. PI3k/mTOR pathway is a master regulator of cell metabolism. Recently, we described a role of leptin in the lipid body biogenesis in a mechanism dependent of mTOR in murine macrophages. Objective: Here we investigated the role of mTOR pathway in the biogenesis of lipid bodies and the inflammatory mediator production during experimental infection by *Mycobacterium bovis* BCG in mice. **Methods:** Susceptible C57Bl/6 mice were Intraperitoneally (i.p.) infected with BCG for 24h. Treated animals received three (i.p.) injections of Rapamycin (15 mg/kg) or vehicle. Protocols were approved by the FIOCRUZ animal welfare committee (L-002/08). **Results and Discussion:** We observed that the *in vitro* BCG infection in macrophages induced the phosphorilation of p70S6K protein and the treatment of Rapamycin significantly inhibited this phenomenon at 1h of infection.BCG infection induced significant cell recruitment to the peritoneal cavity at 24h of BCG infection *in vivo*, with or without the Rapamycin treatment. However, eosinophil migration, but not mononuclear and neutrophil recruitment, was partially inhibited by Rapamycin treatment. Also, we observed a significant lipid body formation, correlated with an increased generation of PGE2 at 24h of BCG infection *in vivo*. The treatment with Rapamycin partially inhibited the lipid body formation (40.69%) induced by BCG, as well as PGE2 production (31.54%); indicating a role of mTOR in the formation of lipid bodies and eicosanoid synthesis derivated from these organelles. In addition, we observed an increase of TGF-b1, IL-10, TNF- a, IL1-b, IL-6, KC e MCP-1 in the inflammatory fluid during BCG infection. Rapamycin inhibited the anti-inflammatory cytokine production (IL-10 and TGF-b1), but not the pro-inflammatory cytokine production during infection. By electron microscopy analysis, we observed that Rapamycin enhanced the number and size of phagolysosomes. Also, inhibition of mTOR pathway by rapamycin increased the BCG killing during *in vivo* infection. These results suggest a role of mTOR through activation of the p70S6K protein in the mechanism of eosinophil recruitment and lipid body biogenesis. mTOR pathway appears to be involved on the synthesis of anti-inflammatory mediators, such as, PGE2, IL-10 and TGF-b, also the inhibition of mTOR signalling improved the BCG killing on inflammatory cells during experimental infection. Support: CNPq, Faperj, PAPES-FIOCRUZ.

04.107

Contribution of reactive-oxygen species to the enhancement of platelet aggregation in high-fat fed rats. Monteiro PF[1], Prada Morganti R[1], Delbin MA[2], Pires MEL[1], Priviero FBM[1], Marcondes S[1], Zanesco A[2], Antunes E[1] [1]UNICAMP − Farmacologia, [2]UNESP − Educação Física

Introduction: Obesity is often accompanied by cellular damage associated with decreased nitric oxide (NO) and increased reactive-oxygen species (ROS) production, leading to oxidative stress. We aimed to investigate whether obesity affect the *in vitro* platelet reactivity associated with increased oxidative stress. **Methods:** Male Wistar rats (230-280 g) received high-fat diet for 10 weeks. Arterial blood was collected from abdominal aorta. Washed platelets (1.2x108 platelets/mL) were used in our assays. Adhesion assays were carried out in 96-well plates coated with fibrinogen (50 mg/mL), whereas aggregation was performed using an aggregometer Chrono-Log. For aggregation assays, platelets were activated with either thrombin (100 mU/mL) or ADP (50 μM). In some protocols, platelets were pre-treated with either N-acetylcysteine (NAC; 1 mM) or PEG-Catalase (1000 U/mL) before adding agonists. ROS production was stimulated with ADP (10 μM) and measured by using DCFH-DA (5 μM), and determined by flow cytometry. **Results:** The high-fat fed rats exhibited significant increase in body weight and epididymal fat mass (551 ± 17.2 g and 17.9 ± 1.51 g, respectively) compared with control animals (457 ± 7.7 g and 7.6 ± 0.6 g, respectively). Glucose levels were also 42% higher ($P<0.05$) in high-fat fed rats compared with controls. Washed platelet aggregation in response to either ADP or thrombin was significantly higher in high-fat fed rats (70.7 ± 4.9% and 77.8 ± 4.8, respectively; $P<0.05$) compared with control animals (54.2 ± 2.9% and 63.5 ± 1.5%, respectively). Pretreatment of platelets with NAC or PEG-Catalase prevented the increased platelet aggregation induced by ADP or thrombin. In the adhesion assays, no differences in spontaneous adhesion and thrombin-induced adhesion were detected between high-fat fed rats and control groups either in absence or in the presence of NAC (and PEG-catalase).**Discussion:** Our data showed an increased platelet aggregation in high-fat fed rats, which was prevented by addition of antioxidant agents. These findings suggest that increased ROS production is involved in the augmented responses to platelets in conditions of obesity.References:Feuers, RJ., The effects of dietary restriction on mitochondrial dysfunction in aging. 1998; Jequier E. Pathways to obesity. 2002

04.108

Staphylococcal enterotoxin A (SEA) inhibits human eosinophil migration *in vitro*. Mello GC, Squebola Cola DM, Souza IA, Antunes E FCM-UNICAMP − Farmacologia

Introduction: *Staphylococcus aureus* is a common human pathogen, which is often found as part of the normal microflora in the nasal cavity. Evidences indicate for a putative effect of Staphylococcal enterotoxin type A (SEA) in allergic diseases such as asthma. We have previously shown that rat airways exposure to SEA significantly potentiate the eosinophil influx in bronchoalveolar lavage (BAL) fluid of allergic rats (Mariano et al., 2010). Eosinophil release of toxic granule proteins, reactive-oxygen species, cytokines, and lipid mediators is proposed to contribute to tissue injury during asthma. This study aimed to investigate the effects of SEA in RANTES-, eotaxin- and PAF-induced human eosinophil chemotaxis *in vitro*. **Methods and Results:** This study was approved by Ethical Committee in Research − CEP/FCM of UNICAMP (Protocol n° 472/2008). Eosinophil cell were obtained from health donors. Eosinophils were separated by Percoll gradient centrifugation, and isolated with a immunomagnetic cell separator. Isolated eosinophils was incubated with SEA (3 ng/ml) for 30 min, 2 h and 4 h. Thereafter, eosinophils (50 µl – 4x106 cells/ml) were placed in the upper compartment of chemotaxis chamber (Boyden chamber), and were allowed to migrate through 5-µm pore size polycarbonate filters for 1 h at 37°C. The lower compartment contained MEM, eotaxin (300 ng/ml), RANTES (100 ng/ml) or PAF (10 mM). After 1 h incubation, the membrane was subjected to Diff-Quick staining, and the number of migrated eosinophils was counted in 5 high-power field (HPF) per sample. Our data showed that migrated eosinophils in response to eotaxin were significantly decreased ($P<0.05$) the migrated eosinophils incubated with SEA at 30 min, 2 h and 4 h (33.6 ± 6.0, 28.7 ± 4.3 and 30.1 ± 10.7 HPF, respectively) compared with untreated eosinophils (57.9 ± 11.3 HPF). Chemotactic responses to RANTES were also reduced by incubation with SEA at at 30 min, 2 h and 4 h incubation (21.7 ± 4.8, 26.9 ± 6.8 and 28.9 ± 5.6 HPF, respectively) compared with untreated cells (42.3 ± 13.0 HPF). Conclusions: Our findings show SEA down-regulates the chemotactic mechanisms for eotaxin and RANTES in human eosinophils. References: Mariano N.S, et.al., *Int Immunopharmacol*. 10(1) 43-9; 2010. Support: FAPESP.

04.109

Effect of a combination of medium chain triglycerides, linoleic acid, soy lecithin and vitamins A and E on wound healing in rats. Magalhães MS[1], Moraes MEA[1], Fechine FV[1], Nascimento DF[1], Macedo RN[1], Monteiro DLS[2], Oliveira CC[1], Linhares JH[3], Leite ALAS[1], Moraes MO[1] [1]UFC − Fisiologia e Farmacologia, [2]FAMED-UFC − Fisiologia e Farmacologia, [3]UFC − Cirurgia

Introduction: The wound can be defined as any alteration in the anatomic integrity of the skin, resulting from any type of trauma, where it can even be classified as intentional (surgical incisions) or accidental. Wound healing by secondary union are submitted to the influence of various factors that can contribute to the delay of wound healing, resulting in the majority of cases in inflammation, edema and hypertrophic and unesthetic scars. All wounds, regardless of their etiology, are a disruption of continuity in the tissue, which results in the interruption of blood flow, in the perturbation of sensitivity, in the accumulation of dead cell debris and in a variable degree of contamination (with or without infection). With the aim of restoring the integrity of the skin, the organism utilizes a complex mechanism called wound healing.The aim of the study was to determine the effect of a combination of medium chain triglycerides (caprylic, capric, caproic and lauric acids), linoleic acid (essential fatty acid), vitamins A and E and soy lecithin, through a morphometric study, on the wound healing kinetics of experimental cutaneous ulcers. **Methods:** A total of 45 male Wistar rats were used, in which a skin flap of total thickness with an area of 4 cm^2 was removed. The animals were divided randomly into 3 groups of 15 rats each, Control, Reference and Test groups, which were treated topically with 0.9% NaCl, a preparation of clostebol combined with neomycin sulfate and the test formulation, respectively. The wound areas were measured by digital planimetry at days zero, 3, 7 and 12 postoperative. Based on the wound area, we determined the degree of tissue repair and mean rate of repair at different time intervals. The research project and the experimental protocol were submitted to the Research Ethics Committee of the Federal University of Ceará, which approved the protocol of nº 31/05. **Results and Discussion:** At day 3, an expansion of the wound area was observed in the Reference group and slight contraction in the Control and Test groups. On the subsequent days, the healing process, according to the degree of repair, proceeded in a linear manner, such that, at day 12, the healed area reached 77.95% of the initial ulcerated region in the Control group, 78.40% in the Reference group and 83.49% in the Test group, showing no significant differences. The overall mean rate of repair was equally similar at 12 days of treatment: 25.79 mm2/dia in the Control group, 25.42 mm^2/dia in the Reference group and 27.38 mm^2/dia in the Test group. The test preparation, applied topically on the experimentally induced cutaneous ulcers in rats, did not accelerate the process of tissue repair by secondary union. **Financial support**: CNPq, CAPES, FUNCAP, FINEP, MS-RNPC-UNIFAC-HM, Instituto Claude Bernard.

04.110

Leukotriene B4 mediates γδ T lymphocyte migration in response to diverse stimuli. Souza-Martins R[1], Costa MFS[1], Souza M[1], Piva B[1], Diaz BL[3], Peters-Golden M[4], Henriques MGMO[1], Canetti C[1], Penido C[1] [1]Farmanguinhos-FIOCRUZ − Farmacologia Aplicada, [2]IBCCF-UFRJ, [3]IBCCF-UFRJ − Imunobiologia, [4]University of Michigan − Pulmonary and Critical Care Medicine

Introduction: γδ T lymphocytes are unconventional T cells that comprise a minor subset of T cells in lymphoid organs, which are preferentially distributed in peripheral tissues, including lung and pleura. These cells recognize a broad spectrum of non-peptide antigens and play important roles in lung infections, exerting an early pro-inflammatory role followed by a subsequent regulatory role in an attempt to restrain the inflammatory response. γδ T lymphocytes increase in number at inflammatory sites during infection and allergy, a phenomenon mediated via migration towards chemotactic factors and/or local proliferation. OBJECTIVE: In this context, we investigated the involvement of the 5-lipoxygenase (5-LO)-derived lipid mediator leukotriene (LT)B4 in γδ T cell migration. **Methods:** & **Results:** Pleurisy was induced by an intrapleural (i.pl.) injection of LTB4 (0.5 µg/cavity), LPS (250 ng/cavity), OVA (12.5 µg/cavity), or *Mycobacterium bovis* BCG (4x105 CFU/cavity). OVA challenge was induced in mice 14 days after prior sensitization by a subcutaneous injection of 200 µl of a mixture of OVA (50 µg) and aluminum hydroxide (5 mg). Pleural cells were recovered from thoracic cavities after washing with 500 µl of PBS containing EDTA (10 mM, pH 7.4; License L-0004/08, CEUA). The i.pl. injection of LPS triggered increased levels of LTB4 in C57BL/6 mouse pleural cavities. The *in vivo* inhibition of 5-lipoxygenase (5-LO) activity by zileuton, or of 5-LO activating protein (FLAP) by MK886 impaired LPS-induced γδ T cell accumulation into pleural cavities. Accordingly, 5-LO knockout (5-LO-/-) mice failed to recruit γδ T cells into the inflammatory site after LPS i.pl. stimulation. The antagonism of high affinity LTB4 receptor, BLT1, by CP105,696 or LY292476 was also capable to impair γδ T cell accumulation in mouse pleural cavities induced by LPS. We found that BLT1 was also required to induce γδ T lymphocyte *in vitro* chemotaxis towards pleural washes obtained from LPS-simulated mice. Moreover, LTB4/BLT1 also accounted for γδ T cell migration induced by BCG or during antigen challenge in sensitized mice. Confirming that γδ T lymphocytes can directly respond to LTB4, we demonstrate that naïve resident γδ T lymphocytes, as well as LPS recruited γδ T cells express BLT1 receptor. In addition, isolated γδ T cells were found to undergo actin cytoskeleton reorganization when incubated with LTB4 *in vitro*. **Conclusion**: In conclusion, these data show that γδ T cell migration into the pleural cavity of mice during different inflammatory conditions is dependent on LTB4/BLT1. **Financial support**: CNPq, FAPERJ, FIOCRUZ.

04.111

Adhesion molecules and chemokines receptor expression in bone marrow eosinophils of obese mice. Calixto MC[1], Lintomen L[1], Thomé R[2], Tamashiro WMSC[2], Antunes E[2] [1]UNICAMP − Farmacologia, [2]UNICAMP − Imunologia e Microbiologia

Introduction: Eosinophils are derived in the bone marrow from myeloid precursors in response to cytokine activation, and, following antigen challenge, they are released into the circulation and recruited to tissues. Adherence of eosinophils to vascular endothelial cells via adhesion molecules is considered to be an initial event in eosinophilic allergic inflammation. Eotaxin stimulates the migration of EO from bone marrow into tissue via the chemokine receptor CCR-3. Recent study have shown that diet-induced obesity stimulated mice eosinophlipoiesis and enhanced EO trafficking from bone marrow to lung tissues, and delayed their transit through the airway epithelium into the airway lumen (Calixto et al., 2010). Communications between lung and bone marrow play an important role in the pathogenesis of allergen-induced asthmatic responses. Therefore, this study was aimed to investigate the influence of obesity on BM cytokines and chemokines levels, and the expression of adhesion molecules and chemokine receptor on BM eosinophils Methods and **Results:** The experimental protocols were approved by the Ethics Committee of University of Campinas (UNICAMP). Male C57bl6/J mice (initial weight 14.5 ± 0.9 g) received a high-fat diet for 10 weeks. On the eighth week, animals were sensitized with a s.c. injection of OVA (100 µg dissolved in Al(OH)3). Two weeks thereafter, mice were intranasally challenged with OVA (10 µg), after which eosinophil counts in bone marrow, the adhesion assays and flow cytometry were evaluated. Our data showed that intranasal challenge with OVA in previously sensitized mice largely increased the EO counts in bone marrow at 48 h post-challenge (2.2 ± 0.4 x106/ml; $P<0.05$) compared with PBS group (0.43 ± 0.21 x106/ml). The eotaxin level was elevated in BM of sensitized obese mice at an early time (12 h) compared with lean mice (24.3 ± 1.13 and 9.1 ± 2.1 pg/ml, respectively). Sensitized obese mice also showed diminished expression of CCR3 on BM eosinophils at 48 h when compared with lean mice. Eosinophils from obese BM had a low expression of MAC-1 and VLA-4 on their surface when compared with sensitized lean mice, at 48 h post-challenge. There was no difference in sensitized obese and lean EO bone marrow adhesion on plate coated with ICAM-1 (5 µg/ml) and VCAM-1 (2.5 µg/ml). Conclusion: We have observed an increase in eotaxin levels in BM of obese OVA-challenged mice that may down-regulates the expression of adhesion molecules and eotaxin receptor. Other studies have been currently performed to further elucidate the mechanisms determining the influence of obesity on eosinophil recruitment from BM to allergic lung. **Financial support**: Fapesp

04.112

Antinociceptive and anti-inflammatory effect of heme oxygenase-1 / biliverdin / carbon monoxide pathways in temporomandibular joint arthritis induced by zymosan. Chaves HV[1], Filgueira AA[2], Ribeiro KA[3], Silva AAR[2], Souza MHLP[4], Ribeiro RA[4], Bezerra MM[5], Brito GAC[6] [1]UFC − Ciências Médicas, [2]UFC-Sobral − Odontologia, [3]UVA − Biologia, [4]UFC − Fisiologia e Farmacologia, [5]UFC-Sobral − Medicina, [6]UFC − Morfologia

Introduction: Temporomandibular joint´s (TMJ) inflammation and pain are important clinical entities, although their mechanisms are not completely understood. Heme oxygenase-1 (HO-1) is induced in a variety of cells including endothelial cells, monocytes/macrophages and neutrophils by heme, endotoxins, cytokines, nitric oxide and other mediators produced during inflammatory responses. The purpose of the study is to investigate the effect of heme oxygenase-1/biliverdin/carbon monoxide pathway on the antinociceptive and antiinflammatory effects in TMJ´s arthritis. Experiments were approved by the Institutional Animal Care and Use Committee of the Federal University of Ceará (UFC), Fortaleza, Brazil (PROTOCOL NUMBER: 26/08). **Methods:** Male Wistar rats (160-220 g) were pretreated with HO-1/BVD/CO pathway modulators, knowingly: Hemin (substrate of HO-1/BVD/CO pathway; 0,1; 0,3; or 1mg/kg; s.c), DMDC (CO donor; 0,025; 0,25 or 2,5µMol/kg; s.c), ZnPP-IX (specific HO-1 inhibitor; 1, 3 or 9mg/kg; s.c) or Biliverdin (product of HO-1 pathway; 1, 3 or 9mg/kg; s.c) 60 min before TMJ´s artrhitis induced by 40 mL zymosan injected into left TMJ (i.a). Control group (C) received saline into left TMJ (i.a), not treated group (NT) received saline (s.c) 60 min before TMJ´s arthritis (Zy: 2 mg or 1 mg), and Indometacin (5 mg/kg) was used as a positive control group. Von Frey test was used to assess mechanical hipernociception (4th hour) and the animals were sacrificed 6h after Zy administration. Leucocyte influx count and myeloperoxidase (MPO) activity in the TMJ lavage and histopatological analysis of TMJ were utilized as parameters. **Results and Discussion:** Hemin 0,3 mg/kg (4050 ± 987, 77.2 ± 28.6), DMDC 2,5µMol/kg (2146 ± 1261, 44.7 ± 11.1) or Biliverdin 9 mg/kg (3567 ± 554, 43.72 ± 18.9) reduced ($P<0.05$) leucocyte influx count and neutrophil migration detected by MPO assay in the TMJ lavage compared to NT (32690 ± 6532, 307.3 ± 64.3), respectively. Hemin 0,3 mg/kg (119 ± 10), DMDC 2,5µMol/kg (115.5 ± 5.9) or Biliverdin 9 mg/kg (73 ± 1) increased ($P<0.05$) the head-withdrawal force threshold compared to NT (43 ± 3.7). On the other hand, ZnPP-IX 3 mg/kg (27936 ± 5356, 77.2 ± 13.4) potentiated ($P<0.05$) the leucocyte influx count and MPO activity in the TMJ lavage, as compared to NT Zy: 1mg (11156 ± 1166, 39.9 ± 13.4), respectively. ZnPP-IX 3 (57 ± 0.8) reduced ($P<0.05$) the head-withdrawal force threshold compared to NT (90.4 ± 8.2). Histopatological analysis of TMJ of Zy injected animals showed inflammatory cell infiltration in synovial membrane (SM), in conective periarticular tissue and in squeletic muscle tissue in 6 h after TMJ arthritis. Hemin 0,3 mg/kg (1.8 ± 0.3) and DMDC 2,5µMol/kg (1 ± 0.2) reduced ($P<0.05$) the histopatological alterations compared to NT (3.5 ± 0.3), and ZnPP-IX 3 mg/kg did not alter these parameters. These results suggest the participation of the HO-1/BVD/CO pathway in the patophysiological mechanisms of inflammation and nociception in TMJ arthritis. Acknowledgments: Funcap and CNPq.

04.113

Antipruritic activity of the ethanol extract from *Lecythis pisonis* Camb. (Lecythidaceae) leaves in mice. Silva LL[1], Gomes BS[1], Silva AMS[1], Oliveira JPC[2], Chaves MH[2], Oliveira FA[1] [1]UFPI − NPPM, [2]UFPI − Química

Introduction: Pruritus (itch) is an unpleasant cutaneous sensation that appears in many skin diseases such as atopic dermatitis and urticaria. *Lecythis pisonis* Camb. (Lecythidaceae), popularly known as “Sapucaia” is a tree that grows in Amazon region, occurring from Ceará to Rio de Janeiro. In traditional medicine, leaves are used for the treatment of itching (pruritus) (FRANCO, EAP., Rev. Bras. Plant. Méd., v.8, p.78, 2006.). The aim of this study was to assess the antipruritic activity of extract from leaves of L. pisonis (LP-EtOH) in model of pruritus induced by compound 48/80 (C48/80) in mice. **Methods:** Male Swiss mice (25-30 g, n=5-10/group), deprived of food for 18h, were treated orally with vehicle (0.9% saline 10 mL/kg), LP-EtOH (50, 100, 200 mg/kg) or cyproheptadine (10 mg/kg). After 60 min received a subcutaneous injection of (C48/80) (100µg/100µL) into the rostral part of the back. Control mice received a similar quantity of normal saline injection instead. Immediately after the injection, mice were observed and the scratching behavior was observed for 20 min and expressed as the time in seconds. Only scratching of nose by fore-or hind paws and the injection site by hind paw was counted (KURAISHI, Y., Eur. J. Pharmacol. v.275 p. 229, 1995.). In order to verify the possible role of endogenous opioids in the suppressive effect of LP-EtOH groups of mice (5-7/group) were pretreated with vehicle (po), naloxone (2 mg/kg ip), morphine (5 mg/ kg sc) or LP-EtOH (200 mg/kg po) alone or in their combinations with naloxone prior to the injection of (C48/80) (100µg/100µL sc). While LP-EtOH was administered 1 h before, naloxone and morphine were given 30 min prior to pruritogen. All the experimental protocols were approved by the local Ethics Committee for Animal Experimentation (number 009/09). **Results and Discussion:** Mice that received LP-EtOH (100 and 200 mg/kg) demonstrated a potent inhibition of C48/80-induced scratching (45.88 ± 10.74 and 31.13 ± 6.44, respectively) in comparison with the vehicle control group (84.67 ± 5.24).($p<0.05$). Cyproheptadine (10 mg/kg po) also caused marked inhibition of scratching responses (30.33 ± 3.42) ($p<0.05$). Morphine (5 mg/kg sc) and LP-EtOH (200 mg/kg po) pretreatments resulted in significant suppression of scratching behavior. Although naloxone alone showed no significant influence, it could reverse the morphine effect on induced scratching. The suppressive effect of LP-EtOH was antagonized by naloxone. The results showed that LP-EtOH inhibits scratching in Swiss mice induced by C48/80. Mast cells-derived mediators may play an essential role in this response. The m-opioid receptor antagonist naloxone produced no discernible effect on C48/80-induced scratching behavior. However, naloxone was found to antagonize the suppressive effect of morphine and of LP-EtOH the scratching. This suggests that naloxone-sensitive endogenous opioids are at least in part involved in the suppressive effect of LP-EtOH on C48/80-induced scratching in Swiss mice. SUPPORT: UFPI/CNPq/CAPES.

04.114

Effects of acute treatment with 1-(3-chlorophenyl) piperazine (mCPP) in the leukocyte traffic in rats. Lombardi L, Hebeda CB, Farsky S, Moreau RLM USP − Análises Clínicas e Toxicológicas

Introduction: 1-(3-chlorophenyl)piperazine (mCPP) is a new synthetic drug that has been seized recently in the recreative drug market because it has been used instead of MDMA as a abuse drug. However, there is no data concerning its actions in the immune system. Therefore, the aim of this study was to investigate the effects of acute treatment with mCPP in the leukocyte traffic into different body compartments. **Methods:** Male Wistar rats (180 – 220g) were exposed to mCPP (1mg/mL; gavage) or vehicle (saline 0.9%) and after 1 hour they received or not (basal group) intraperitoneal injection of LPS (E. coli, sorotype 026:B6; 1 mg/mL). Basal and LPS-inflamed groups were anesthetized (ketamine/xylazine; 100 mg/kg and 20mg/kg, respectively) and euthanized 1 hour and 5 hours after the vehicle or mCPP exposure. Circulating and bone marrow leukocytes as well as migrated leukocytes to the peritoneal cavity 4 hours after LPS local injection were collected and quantified in a Neubauer chamber. Leukocyte differential count was performed in staining smears (Rosenfeld staining). The experiments were in agreement with Colégio Brasileiro de Experimentação Animal (COBEA), protocol number 233. **Results:** The mCPP exposure did not modify the number of circulating and bone marrow leukocytes in basal condition. Four hours after LPS stimulation, mCPP evoked a marked increment on the number of circulating leukocytes (199%), mainly on polymorphonuclear cells (PMN; 116%) and migrated leukocytes into the peritoneal cavity (260%). This effect was accompanied by reduction on the number of cells in the bone marrow (61%).**Discussion:** Based on these findings, data obtained show that mCPP augments the leukocyte traffic in the body compartments during an inflammatory response. These data suggest that mCPP may strongly amplify the inflammatory reaction. Further investigations will achieved to elucidate the mechanisms involved in this process. **Financial support**: Capes.

04.115

Lipoxin A4 plays a protective role in experimental dengue disease. Cisalpino D[1], Fagundes CT[2], Costa VV[2], Guabiraba R[2], Souza DG[1], Teixeira MM[2] [1]UFMG − Microbiologia, [2]UFMG − Bioquímica e Imunologia

Introduction: Dengue virus (DV) has emerged as the most relevant arthropod-borne viral disease in tropical areas, reaching millions of cases worldwide each year. During the viral infection, there is a characteristic development of vascular instability, as increased vascular permeability, elevated haemoconcentration and thrombocytopenia are observed. The five-lipoxygenase (5-LO) pathway, is an important axis of balance for the inflammatory processes. The 5-LO pathway is associated with production of both leukotrienes and Lipoxins. Lipoxin is a molecule that has been described with important anti-inflammatory effects and its signaling is associated with SOCS-2 activation. Here, our aim was to evaluate the importance of 5-LO and SOCS-2 in an experimental model of Dengue virus serotype 3 (DEN-3) infection. **Methods:** C57 BL/6 wild type, 5-LO deficient (5-LO-/-) and SOCS-2 deficient (SOCS-2-/-) mice were infected intra-peritoneally with 10 PFU of DEN-3. Non-infected mice were used as controls. After discreet intervals (3rd, 5th and 7th day of infection for inflammatory parameters), hematocrit and platelet number, viral titers, cytokine/chemokine production and neutrophil influx were evaluated. **Results and Discussion:** Our model of DEN-3 infection is followed by great inflammatory response as assessed by cytokine/chemokine production and neutrophil recruitment and severe circulatory dysfunction. On another hand, 5-LO-/- and SOCS-2-/- mice showed an altered inflammatory response, with elevated haemoconcentration and reduced platelet count when compared to the wild type control, indicating a severe response to the infection. They also displayed elevated splenic, hepatic and pulmonary neutrophil influx, and accordingly higher levels of CXCL1 in the liver, spleen, lungs and serum. Furthermore, 5-LO-/- and SOCS-2-/- showed higher levels of splenic and hepatic IL-6 and TNFa, despite lower levels of IFNg in both organs. SOCS-2-/- mice also displayed greatly elevated splenic viral titers. Then, our results suggest that lipoxin play a protective role in our dengue infection experimental model and that the blockade in both lipoxin production or lipoxin signaling is associated with more severe disease, at least in part, because of the loss of virus load control.

04.116

Lipopolysaccharide *in vivo* increases the production of reactive oxygen species in rat platelet mainly through activation of NADPH-oxidase system. Pires MEL, Cardelli NJA, Anhê GF, Antunes E, Marcondes S UNICAMP − Farmacologia

Introduction: The most severe septic responses can be reproduced by LPS injection such as decrease of circulating leukocytes and platelets, along with increased production of reactive oxygen species (ROS). Different sources may generate O2.- like the electron transport chain, NADPH-oxidase, xanthine oxidase and cyclooxygenase. Many articles have described the effect of LPS on platelets; however, no study has evaluated the effect of *in vivo* LPS on ROS production in platelets. Therefore, in this study we decide to investigate the sources involved in the increased production of ROS in platelets of LPS-treated rats. **Methods:** The present study was approved by the Committee for Ethics in Animal Research (State University of Campinas – UNICAMP, protocol number 2097-1). Wistar rats (250-320 g) were injected i.p. with saline or LPS (from E. coli, 1 mg/kg) and after 8h arterial blood was collected in ACD-C (9:1 v/v). Production of ROS in washed platelets was measured by flow cytometry using DCFH-DA (5 μM). The platelets were activated with ADP (20 μM) in absence or presence of the inhibitors of cyclooxygenase (acetylsalicylic acid, ASA), xanthine oxidase (allopurinol), NADPH-oxidase (DPI) and electron transport chain (rotenone). **Results:** The ROS production in non-stimulated platelets was similar in control or LPS groups. However, generation of ROS in ADP-stimulated platelets in LPS group was significantly higher than control animals (increase of 2-fold 8 h after LPS injection). Incubation of platelets from LPS-treated rats with DPI (5 mM) significantly reduced the ROS generation in ADP-stimulated platelets (37% reduction at 8h after LPS treatment). Similarly, the increased ROS production in platelets of LPS-treated rats was diminished by 20% in presence of rotenone (100 μM) or ASA (100 μM). Allopurinol (100 μM) did not affect the generation of ROS in platelets of LPS group. On the other hand, ROS production in ADP-stimulated platelets of saline-treated rats was not significantly modified by DPI, rotenone, ASA or allopurinol. **Conclusion**: Our data indicate that NADPH-oxidase is the main source of ROS formation in ADP-stimulated platelets of LPS-treated rats, but the cyclooxygenase enzyme and the electron transport chain also contribute to this effect. The mechanisms involved in the increase of the NADPH-oxidase activity in platelets after *in vivo* treatment of rats with LPS are under current investigation. Supported by: CAPES

04.117

Synthesis and evaluation of activity of 4-antihipernociceptiva aminochalconas. Sonza DR, Buzzi FC, Rodrigues C, Corrêa R, Souza PS, Quintão NLM [1]NIQFAR-UNIVALI – Farmacêuticas

Introduction: The chalcones consist of an important family of organic compounds with proven therapeutic activity. Numerous studies have shown that several chalcones and their derivatives have wide biological applicability showing anti-inflammatory, antinociceptive, antitumor, antiviral and anti-leishmania properties. This study aims to prepare two 4-aminochalcones derivatives in order to obtain biologically active compounds. **Methods:** The synthesis was made according to the derivation of the general method of Claisen-Schmidt aldol condensation using equimolar amounts of 4-nitrobenzaldehyde and different acetophenones in the presence of sodium hydroxide 50% (w/v) and ethanol. After synthesizing and purifying the 4-nitrochalcones, they were refluxed with a nitrogen atmosphere, being added tin chloride (II) dihydrate, hydrochloric acid and acetic acid in order to reduce the nitro group to amino in the B ring of chalcone obtaining a series of 4-aminochalcones. All syntheses were monitored by thin layer chromatography (TLC) and at the end of the reactions the compounds were isolated by column chromatography and recrystallized in ethanol. The compounds were characterized by melting point, infrared spectroscopic (FT-IR), UV/Vis and proton nuclear magnetic resonance (1H NMR) and carbon (13C NMR); the Sn was determined colorimetrically by reaction with quercetin (absorbance at 425 nm). It was obtained the compounds named 2E-3-4-aminophenyl-1-fenilprop-2-en-1-one (D1) and 2E-1-4-morfolinofenil)-3-(4-aminophenyl)prop-2-en-1-one (D8). Male and females Swiss mice were used in the pharmacological tests (20 to 35g, n = 6-8). The models of hypernociception induced by carrageenan (300 mg/paw) and PGE2 were carried out to evaluate the activity of the compounds. The mechanical sensitivity was assessed using Von Frey filament (0.6 g). For the evaluation of anti-inflammatory activity was used the model of paw edema induced by carrageenan (300 mg/paw) and, the difference in volume of left and right paws of the animals checked by the pletismometer, was taken as an index of edema. All the procedures used in the present study were approved by the Animal Ethics Committee of UNIVALI (Protocol numbers 212/07). **Results:** The pretreatments with the D1 and D8 Chalcones were effective, significantly reducing the mechanical sensitization induced by both carrageenan. The mechanical sensitivity using Von Frey filament showed results for chalcone D1 57,5%; 41,25%; 32,2% when administred 0,03;01;0,3m mol / kg respectively. As by PGE2 chalcone D8 0,1m mol / kg reduce 57,17%, and 0,3m mol / kg reduce 52,9%. Chalcone D1 able to significantly inhibit the paw edema when administreted 0,1; 0,3mol / kg induced by carrageenan with inhibition under 63,8%, 59,4% respectively. **Discussion:** These results demonstrate that the use of chalcones was able to both interfere with the formation of edema and inflamation, as with the mechanical sensitization induced by carrageenan. **Financial support**: ProPPEC/ UNIVALI

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Mechanisms of the inflammatory response induced by topical application of cinnamaldehyde in mice. Silva CR[1], Oliveira SM[1], Rossato MF[1], Guerra GP[1], Dalmolin GD[2], Prudente AS[3], Cabrini DA[3], Otuki MF[3], Ferreira J[1] [1]UFSM − Química, [2]UFMG − Farmacologia, [3]UFPR − Farmacologia

Introduction: Cinnamaldehyde (Cin, an agonist of Transient Potential Receptor subfamily Ankyrin 1, TRPA1), a natural compound frequently present in cosmetic formulations, induces skin irritation when topically applied by mechanisms still unclear. Thus, the aim of this study is to study some mechanisms involved in the inflammatory process induced by topical application of Cin in mice ear. **Methods:** To induce inflammation, Female Swiss mice (25-35g) were topically administered on the ear with Cin (4 µg/ear) or vehicle (acetone, 20 µl/ear). All protocols employed have been approved by the Local Ethics Committee (process number: 23081.001086/2009-87). Some animals were pre-treated topically with different TRP or NK1 antagonists 15 min prior to the Cin application. The edema was expressed as the increase in ear thickness in response to Cin using a digital micrometer. The activity of the myeloperoxidase (MPO) was evaluated in ear samples as a leukocyte infiltration marker. To verify the occurrence of crossed sensitization/desensitization between Cin and capsaicin (Cap, an agonist of TRP subfamily vanilloid 1, TRPV1) in edematogenic effect, the animals were treated with Cin or capsaicin in day 1, 3 and 7. On day 8, animals of each group were challenged with Cin (4 µg/ear), Cap (200 µg/ear) or acetone and edema and the expression of TRPA1 and TRPV1 receptors by Western blot analysis was assessed. **Results and Discussion:** Topical application of Cin induced ear edema reaching maximum effect of 0.18 ± 0.02 mm at 30 min and with a ED50 value of 2.0 (1.1- 3.4) µg/ear. The non-selective TRP antagonist ruthenium red (RR; 24-240 µg/ear), the TRPA1 antagonists camphor (4.5-152 µg/ear) and HC030031 (10-100 µg/ear) and the tachykinin NK1 antagonist aprepitant (16-160 µg/ear) inhibited the edema formation (maximal inhibitions of 55 ± 10, 68 ± 15, 65 ± 12 and 43 ± 15, respectively). RR, camphor and HC030031 were also able to prevent the raise in the MPO activity caused by Cin in the mouse ear (47 ± 13%, 27 ± 23% and 56 ± 25% of reduction, respectively). In the ears pre-treated with Cin (days 1, 3 and 7), no alteration in the edematogenic response of Cin (day 8) was detected, but a partial reduction in capsaicin-response (41 ± 5%) was observed. Conversely, the pre-treatment with Cap not only abrogated the edema evoked by Cap treatment, but also significantly decreased Cin-induced edema (89 ± 3% and 61 ± 11% of reduction, respectively). We also verified that the TRPA1 or TRPV1 expression was not altered in the mice ears treated previously with Cin, capsaicin or vehicle. Taken together, the topical application of Cin in mouse produces an acute inflammatory response that is mediated by the stimulation of TRPA1 and NK1 receptor. Moreover, Cin did not produce homologous desensitization on TRPA1, but presented cross-desensitization with the TRPV1 agonist Cap. Our findings suggest a possible role of TRPA1 in inflammatory skin reactions. **Support**: CAPES, CNPq, CCNE/UFSM.

04.119

IFN-g limits antigen-induced arthritis severity by inducing IDO/GCN2 kinase pathway. Lemos HP[1], Mellor AL[2], Chandler PR[3], Vieira SM[4], Grespan R[5], Cunha FQ[1] [1]FMRP-USP − Farmacologia, [2]Medical College of Georgia − Molecular Medicine and Genetics, [3]Medical College of Georgia − Immunotherapy, [4]COPE-INPA, [5]UEM − Farmácia e Farmacologia

Introduction: Several studies have shown a downregulatory role for indoleamine 2,3-dioxygenase (IDO) in several diseases, including rheumatoid arthritis (RA). However, recent studies verified certain controversies concerning the role of IDO in RA, evidencing the importance to study more profoundly the IDO pathways in the context of this disease. Herein, it was evaluated the relationship between IFN-g, a well-known IDO inducer, and IDO/GCN2 (general control non-depressing 2, GCN2) protein kinase/CHOP pathway in antigen-induced arthritis (AIA). **Methods:** AIA was induced by immunization and joint (tibio-femoral) challenge with methylated bovine serum albumin (mBSA). The joints were washed 24 h after mBSA injection for migrated neutrophil counts. Joint swelling was evaluated daily for 7 days after challenge. IL-17 levels were measured in supernatants from cultured draining lymph node cells (DLN) by Luminex TM. Proliferation of lymphcytes were evaluated by [3H]thymidine incorporation, IDO expression by immunohistochemistry and IgG titer by ELISA. All the experimental protocols were approved by the local Ethics Committee for Animal Experimentation (number 171\2008). **Results:** It was observed an increased joint swelling, neutrophil migration to the knee, proliferation of lymphocytes and IL-17 and IFN-g production by DLN cells with the treatment with 1-methyl Tryptophan or in IDO, GCN2, CHOP and IFNyR knockout (KO) mice when compared to not treated wild type mice. The IDO expression in DLNs and spleen was greatly decreased in IFNyR KO mice. No differences in IgG titers were observed among these groups. Conclusion: Altogether, the impaired IDO expression in the absence of IFN-g signaling, no differences in IgG leves and the increased inflammatory and T cell response observed in the absence of IDO/GCN2/CHOP pathway despite the increased IFN-g production suggest that IFN-g restrains arthritis severity by limiting T cells, but not humoral responses, through the upregulation of IDO/GCN2/CHOP pathway. Supported by FAPESP

04.120

IL-1 receptor antagonist (IL-1RA) Prevents hemorrhage, inflammation, nociception and bladder dysfunction in ifosfamide-induced hemorrhagic cystitis. Leite CAVG[1], Alencar VTL[1], Lima-Júnior RCP[1], Mourão LTC[1], Wong DVT[1], Melo DLR[1], Magalhães PJC[1], Santos AA[1], Brito GAC[2], Cunha FQ[3], Ribeiro RA[1] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Morfologia, [3]FMRP-USP

Introduction: Ifosfamide (IFO) is an alkylating agent with a broad spectrum of antineoplastic activity. Hemorrhagic cystitis (HC) is a side-effect which is attributable to treatment with IFO. Despite the effective use of Mesna on preventing HC, a complete protection is not always achieved (Lima, Cancer Chemot Pharm 59, 643, 2007). The participation of a cascade of cytokines in the pathogenesis of HC, previously determined by our group, provided potential therapeutic targets for treating this disease. The aim of this study was to investigate the protective effect of IL-1Ra upon inflammatory and nociceptive response, and parameters of bladder function in a mice model of IFO-induced HC. **Methods:** Swiss male mice (n=6, 25-30 g) were given Saline (1 mL/kg, i.p) or IL-1Ra (25, 50, 100 or 200 mg/kg, i.p) 1h previously the i.p. injection of saline (1 mL/kg) or IFO (400 mg/kg). Visceral nociception were assessed through von Frey test previously and 11h later IFO injection by the stimulation of abdominal with a pressure meter. The results were obtained in grams (T0-T1). The animals were then sacrificed 12h following IFO injection to determine bladder wet weight (BWW, mg), vascular permeability (VP, ug/mg tissue), macroscopic and microscopic analysis through scores to edema and hemorrhage according to Grey's criteria (J Urol 133, 497, 1986), Myeloperoxidase assay (MPO, U/L), *in vitro* contractility to Carbachol (CCh:KCl 60mM% ratio), and cystometrography (CMG, micturition interval [min] and amplitude of contraction [cmH2O]). Statistical analysis was performed with ANOVA/Student Newman Keul or Kruskal Wallis/Dunn as appropriate. $p < 0.05$ was accepted. The experimental protocols were approved by the Ethics Committee on Animal Research of the Department of Physiology and Pharmacology, UFC (protocol 09/06). **Results:** IFO induced significant nociceptive responses (6.756 ± 1.103 g) in comparison to saline treated group (1.014 ± 0.727). In addition to that, IL-1Ra inhibited (3.211 ± 1.051) in a significant manner ($p<0.05$) the nociceptive response when compared to IFO. Furthermore, IFO induced significant ($p<0.05$) increasing of BWW (39.11 ± 2.435), VP (28.81 ± 8.470) edema (2[1-3]), hemorrhage (3[1-3]), and microscopic (2[2-2]) scores, MPO (2.838 ± 0.298); decreasing of the *in vitro* contractility of bladder (165.9 ± 23.16), micturition interval (0.943 ± 0.100), and absence in the amplitude of contraction when compared with saline treated group (12.32 ± 0.729; 2.002 ± 0.470; 0[0-0]; 0[0-0]; 0[0-0]; 0.115 ± 0.115; 285.621 ± 23.164, 30.69 ± 3.045 respectively). These effects were prevented ($p< 0.05$) with IL-1Ra (100 mg/kg) treatment (21.48 ± 1.605; 7.657 ± 0.379; 0[0-2]; 0[0-2]; 1[1-2]; 1.539 ± 0.447; and 304.772 ± 47.875, 13.78 ± 1.470, respectively). **Discussion:** This study shows for the first time that the pharmacological target to IL-1 is effective in controlling visceral nociception, inflammatory, and dysfunctional disorders of HC and strongly provides perspective for the effective management of this pathological condition. Support: CAPES/CNPq.

04.121

Involvement of nitric oxide on the pathogenesis of irinotecan-induced intestinal mucositis: role of cytokines on inducible nitric oxide synthase activation. Leite CAVG[1], Lima-Júnior RCP[1], Wong DVT[1], Oriá RB[2], Brito GAC[2], Souza MHLP[1], Cunha FQ[3], Ribeiro RA[1] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Morfologia, [3]FMRP-USP

Introduction: Intestinal mucositis (IM) and the diarrhea associated are common costly side effects (15-25%) of colorectal anticancer therapy with Irinotecan. Several experimental models that mimic anticancer drugs toxicity in humans have implicated the detrimental role of nitric oxide (NO) in their pathogenesis. Additionally, we have previously demonstrated that cytokine modulators are able to attenuate IM. Then, we aimed to investigate the role of NO on the pathogenesis of IM, and the participation of cytokines on iNOS (inducible nitric oxide synthase) activation. **Methods:** iNOS knockout (iNOS-/-) and C57BL/6 animals (n=5-6) were given either saline or irinotecan (60 mg/kg/4 days), with/without aminoguanidine (50 mg/kg), thalidomide (60 mg/kg), or pentoxifylline (1.7 mg/kg) daily. On day 5, diarrhea was assessed, and following sacrifice, duodenal samples were obtained for iNOS activity (citrulline pM/h/mg of tissue), myeloperoxydase assay (MPO, neutrophils/mg tissue), morphometric analyses (villus height, μm), western blot (iNOS/β-actin ratio), immunohistochemistry to iNOS, and for *in vitro* contractility of duodenum (Acetylcholine/KCl60mM% ratio). Data were analyzed with ANOVA/Student Newman Keul or Kruskal Wallis/Dunn's test. $p<0.05$ was accepted. (CEPA: protocol 02/04). **Results:** It was verified that Irinotecan injection caused a significant increase in iNOS activity (3.34 ± 0.56) in comparison with saline treated mice (0.88 ± 0.60). Additionally, Irinotecan induced severe diarrhea (3[3-3]), intestinal smooth muscle over-contractility (176.1 ± 54.7), morphometric changes (156 ± 14), and increased MPO activity (3319 ± 407) compared with saline group (0[0-0], 96.4 ± 10.9, 382.2 ± 8.3, 3012 ± 618, respectively). These effects were abrogated in aminoguanidine-treated (1.5[1-2], 89.3 ± 9.6, 284 ± 8, 1814 ± 327, respectively) and iNOS-/- mice (1[1-1], 69.4 ± 7.1, 241 ± 19, 231 ± 103 respectively) in comparison with irinotecan group. Moreover, pentoxifylline, but thalidomide did not, inhibited iNOS expression (0.4 ± 0.1) and immunostaining (1.5[1-2]), in comparison with irinotecan (1.3 ± 0.4, 4[3-4]). **Discussion:** This study suggests the pivotal role of NO in the pathogenesis of irinotecan-induced IM since animals with genetic deletion to iNOS and the pharmacological modulation of this enzyme were able to prevent IM development. It also provides evidence for the participation of cytokines on activation of iNOS. **Financial support**: CNPq/CAPES

04.122

IL-6/ IL-23/ IL-17/ IL-22 axis mediates the inflammatory response in antigen-induced arthritis in mice. Pinto LG[1], Talbot J[1], Vieira SM[2], Verri Jr WA[3], Cunha, TM[1], Cunha FQ[1], Ferreira SH[1] [1]FMRP-USP − Farmacologia, [2]COPE-INPA, [3]UEL − Pathology and Pharmacology

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by an increase in the infiltrate of neutrophils, in the sensitivity to joint pain (hyperalgesia) and progressive destruction of cartilage and bone. IL-23 is a pro-inflammatory cytokine that contributes to the expansion and maintenance of a newly described subset of memory T cells, known as Th17 cells which, once activated, release the cytokines as IL-17, IL-22 and IL-6 which plays a pivotal role in the physiopathology of RA. The role of IL-17 in the genesis of nociception was demonstrated recently. In this study, we evaluated the role of IL-6/ IL-23/ IL-17/ IL-22 axis in the pathogenesis of RA in a model of antigen (mBSA)-induced arthritis. **Methods**: Arthritis was induced in mice, by subcutaneous (s.c.) injection of methylated bovine serum albumin (mBSA 500 µg/100 µl of saline) mixed with 100 µl of complete Freud's adjuvant. The procedure was repeated seven days after. Twenty-one days after the initial injection, arthritis was induced by intra-articular (i.a.) injection of mBSA or IL-23 dissolved in saline. Articular hypernociception was evaluated using an electronic version of the von Frey test. Neutrophil recruitment was assessed directly in knee joint exudate. Proteoglycan content of cartilage was measured by dimethylmethylene blue assay of papain digests. This study was approved by Animal Ethics Committee of FMRP/USP (nº 173/2008). **Results:** The challenge with IL-23 in the femur-tibial joint of mBSA-immunized mice induced a dose- and time-dependent mechanical hypernociception. In addition, the intra-articular injection of IL-23 increased the neutrophil migration 7 h after challenge. In agreement, we found that intra-articular injection of mBSA induced hypernociception and neutrophil recruitment which was reduced in IL-23-/- mice. Furthermore, the hypernociception and neutrophil migration induced by mBSA was also reduced in IL-6 KO, IL-22 KO and IL-17R KO. According these findings, we found that proteoglycan loss induced by administration of mBSA in the femur-tibial joint was inhibited in IL-6 KO and IL-17R KO mice. **Discussion:** These results suggest that IL-6/ IL-23/ IL-17/ IL-22 axis modulates the inflammatory response in antigen-induced arthritis. Therefore, it is reasonable to propose Th17 axis as a novel therapeutic target to development of new drugs to control RA. **Financial support**: CAPES, CNPq, FAPESP.

04.123

Nitric oxide, carbon moxide and guanylate cyclase modulate remote ischemic preconditioning: participation of adhesion molecules in inhibition of neutrophils migration.

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Introduction: The involvement of adhesion molecules in the migration of inflammatory cells is a key component which leads the leukocytes complete the transmigration and the arrival at the focus of inflammation. Currently, anti-inflammatory action of remote ischemic preconditioning (RIPC) is already well established. However, its mechanism of action is still under debate. Studies have shown a possible interference of RIPC at some stages of the inflammatory process, among them the migration of leukocytes. In this study, we investigated the inhibition of the action of endothelial adhesion (ICAM-1/CD54) and leukocyte (B2-integrin/CD11B-CD18) molecules on the anti-inflammatory activity of RIPC and participation of Nitric Oxide (NO), Carbon Monoxide (CO) and Guanylate-Cyclase (GC) in this process. **Methods:** Participation of ICAM-1/CD54 and B2-integrina/CD11B-CD18 were investigated using deficient mice for the genes for these same molecules: ICAM-1-/- and B2-integrin-/- mice. RIPC model was set up on tourniquet in right hind limb of a mouse for 10 minutes followed by 30 of reperfusion. An inflammatory reaction was induced by intraperitoneal administration of Carragenin (500 µg/cav). Four hours after, the peritoneal cavities were washed with saline and number of migrated leukocytes were evaluated on microscope. Participation of NO, CO and GC were investigated using inhibitors of iNOS (1400 W; 3 mg/kg or Aminoguanide; 50 mg/kg, sc), HO-1 (ZnPPIX;10 mg/kg, sc) and ODQ (5 µmol / kg, ip) as pre-treatment 30 minutes before RIPC. Afterwards, inflammatory reaction was induced by intraperitoneal administration of Carragenin (500 µg/cav). Upon four hours, the mice blood was harvested and the expression of CD11B and CD18 in neutrophils was determined by flow cytometry using specific antibodies for them and the expression of ICAM-1 was determined by immunofluorescence with peritoneal tissue. **Results:** Cg-induced neutrophils migration into peritoneal cavity of preconditioned ICAM-1 deficient animals occured, also with the wild-type(WT) (WT:1.03 PCI(ICAM-/-):0.75 $p>0.05$) , which was different on WT Sham mice (WT:0.82 PCI:0.30 $p<0.05$). Otherwise, upon preconditioned B2-integrin animals (WT:0.68 PCI(B2-int-/-):0.27), the neutrophils migration did not migrate as wild-type animals (WT:0.59 PCI:0.21 $p<0.05$). Furthermore, neutrophils obtained from preconditioned animals presented reduced CD11 expression in flow cytometry and sustained or raised with pre-treated mice (WT:4076 PCI:2493 PCI(1400W):3476 PCI(ZnPPIX):4830 PCI(ODQ):4078 $p<0.05$), besides different findings that CD18 expression was held the same on PCI and WT animals.(WT:304 PCI:267 $p>0.05$) and the decrease in ICAM-1 expression was observed by different intensity fluorecense. **Discussion:** The neutrophils migration occured with preconditioned ICAM-1-/- mice because that adhesion molecule is fundamental to perform the adhesion and transmigration of the cells. On the other hand, the preconditioned b2-integrin-/- mice did not complete the neutrophils migration. There are b-integrins on the leucocytes, moreover the b2-integrin molecule has two binding sites (CD18/CD11), these features can lead to partial inhibition of b2-integrin expression. In addition, the expression of CD18 was not significant but CD11 expression was it, which step up the incomplete response of integrins to RIPC. The ICAM-1 expression came to reinforce the central role of this molecule upon neutrophils migration.

04.124

Lipid droplet in pulmonary dendritic cells during severe sepsis. Molinaro RC[1], Vieira-de-Abreu A[1], Silva AR[1], Castro-Faria-Neto HC[1], Benjamim CF[2], Bozza PT[1] [1]DFF-FIOCRUZ, [2]UFRJ − Farmacologia Básica e Clínica

Introduction: Sepsis is a main cause of death in intensive care unit. Today, there is no therapeutic approach able to improve sepsis survival. Furthermore, post-septic patients can develop an immunosuppression after sepsis episode. Recent studies demonstrated that dendritic cells (DC) play an important role in immunosuppression sepsis-induced against secondary infection. Many inflammatory mediators such as cytokines and lipid mediators participate during sepsis and the immunosuppression stage. Lipid droplets (LD) are inflammatory organelles with multifunctional proprieties. Our group demonstrated that lipid droplet numbers were increased in leukocytes from septic patients compared with healthy patients. LD has a highly regulated biogenesis and within LD have cytokines, lipid mediator synthetic enzymes and cell-signaling proteins. Inflammatory cells including neutrophils and macrophages have increased numbers and/or size of LD under inflammatory conditions. OBJECTIVE: Our aim is to investigate the role of LD in DC during sepsis and in the development of immunosuppression state. **Methods:** Mice were subjected to cecum ligation and puncture (CLP) or Sham and 6 hours after, all groups were treated with antibiotics. Bronchoalveolar lavage (BAL), total lung cells and splenocytes from septic or sham group were collected and cellular suspensions were stained with tetroxide osmium and analyzed by light microscopy. To investigate the presence of LD in pulmonary DC, lungs were collected in different times (from 6, 24 and 48 hours) after surgery. Half of lung was used to obtain mRNA to evaluate ADRP or PPAR-g expression by real time PCR (qPCR) or digested with colagenase IV, isolated to CD11c+ cells by immunomagnetic positive selection. To generate murine bone marrow dendritic cells (BMDC), femurs and tibias were obtained after surgery and cells were plated with GM-CSF for 8 days as described Lutz et al., 1999. BMDC from septic or sham group were stimulated with LPS. After 24 hours, cells were analyzed to LD formation by osmium stain and ADRP, CD1d and CD36 relative expression by qPCR. **Results:** After 24 hours, BAL and pulmonary cells from septic group showed increased LD numbers as compared to Sham group. Although total lung cells from septic mice did not show difference on expression of ADFP, CD36 and CD1d, pulmonary DC from CLP group showed increased LD numbers as compared to pulmonary DC from Sham group. BMDC from septic group showed increased ADRP and PPAR-g relative expression and more responsiveness to LPS in LD formation when compared to BMDC from sham group. CONCLUSION: These results demonstrate LD biogenesis during acute sepsis in distal tissues as lung. Increased numbers of LD was also demonstrated in lung DCs and BMDC. Moreover, increased PPAR-g expression in BMDC from septic mice showed the suggestive involvement of PPAR-g in DC regulation during sepsis. Further experiments will be necessary to characterize the role of PPAR-g and LD in immunosuppression. **Financial Support**: CNPq; FAPERJ, PAPES-FIOCRUZ.

04.125

Intraperitonial injection of *S. aureus* induces fever accompanied by an increase of prostaglandin E2 (PGE2) in the CSF and hypothalamus in rats. Martins JM[1], Soares DM[2], Malvar DC[1], Figueiredo MJ[1], Souza GEP[2] [1]FMRP-USP − Farmacologia, [2]FCFRP-USP − Física e Química

Introduction: It has been shown that live S. aureus induces febrile response in rats [1] which is reduced by the treatment with antipyretic drugs[2]. The central COX-2 -derived PGE2 synthesis is considered a key step for fever induction[3]. In this study we investigated if *S. aureus*-induced fever is accompanied by an increase on PGE2 content either on CSF or hypothalamus and if celecoxib (CELE) or dipyrone (DIP) alters both fever and the PGE2 concentrations in rats. **Methods:** Male Wistar rats (200g) received CELE (2.5 mg/kg, p.o.) or DIP (120 mg/kg, i.p.) 30 min before the i.p. injection (2 ml) of S. aureus (1010 UFC/cavity) and body temperature (bT, oC) was measured for up to 12h by radio-telemetry system. For PGE2 evaluation, cisternal CSF and hypothalamus were collected 3h after injection of *S. aureus*. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations in conscious animals set by the Ethical Committee, protocol number 06.1.1281.53.1 – CEUA-Ribeirão Preto/USP. **Results:** As showed in the table intraperitoneal injection of S. aureus induced fever and increased the PGE2 concentration in the CSF and hypothalamus. S. aureus. CELE blocked the fever and reduced PGE2 content in the CSF and hypothalamus while DIP reduced both fever and PGE2 increase in the CSF but not in the hypothalamus. Effect of celecoxib (CELE) and dipyrone (DIP) on fever and on CSF and hypothalamic PGE2 concentration 3h after i.p. injection of S. aureus. Treatment Stimuli bT (oC) PGE2 in CSF (pg ml-1) PGE2 in Hypothalamus (pg g-1 of tissue) n Vehicle Saline 37.2 ± 0.06 nd 125.5 ± 36.47 5 S. aureus 38.8 ± 0.06* 862.4 ± 188.5* 535.9 ± 39.57* 5 CELE S. aureus 37.5 ± 0.15# 77.5 ± 19.25# 218.9 ± 56.44# 5 DIP S. aureus 37.4 ± 0.13# 124.3 ± 59.17# 521.3 ± 95.33 5 *, # $p<0.05$ when compared with Vehicle/Saline or Vehicle/ S. aureus, respectively; nd = not detected. **Discussion:** These data shown that S. aureus induces febrile response accompanied by an increase of PGE2 concentration in the CSF and hypothalamus which explain its sensitivity to celecoxib and suggest the involvement of PG-dependent pathways in this response. The effect of dipyrone on S. aureus-induced fever suggest that despite capable of reducing PGE2 in the CSF during S. aureus-induced fever, dipyrone promotes antipyresis by mechanisms distinct of hypothalamic PGE2 inhibition. [1] Longhi et al. Livro de Resumos, SBFTE, p. 41, 2006. [2] Martins et al. Livro de Resumos, SBFTE, p. 26, 2008. [3] Roth et al*., Immunol Allergy Clin North Am*, v. 29, p. 229, 2009 **Support**: FAPESP

04.126

Reduction of the expression of membrane CXCR2 and BLT1 receptors on neutrophils related to increased mortality of septic patients in emergency department. Sousa RB[1], Souto FO[2], Spiller F[2], Turato W[3], Lobo RR[1], Mendonça PR[1], Cunha FQ[2], Pazin A[1] [1]FMRP-USP − Clínica Médica, [2]FMRP-USP − Pharmacology, [3]FCFRP-USP − Análises Clínicas, Toxicológicas e Bromatológicas

Introduction: Two major impediments to the effectiveness of sepsis treatment strategies are a failure to recognize the early stages of the disease and underestimation of its severity. Aims: Determine the expression of CXCR2 chemokine and BLT1 receptors on neutrophil surfaces from patients with severe sepsis and their association with disease severity, APACHE II, and mortality. **Methods:** Prospective cohort clinical study conducted at emergency department (ED) in a tertiary referral teaching hospital. Flow cytometric measurements of these receptors surface expression of patients with severe sepsis(GI;n=8) and septic shock (GII;n=8) were studied in the first 24 hours at admission, and compared with healthy controls (n=16). Our study was approved by the Institutional Review Board (number 293/2009) and informed consent was obtained from subjects or surrogates. **Results:** The overall mean age was 68.1 (± SD 16.4) yrs, 50% were male and the most common source of infection was pneumonia (68,7%). CXCR2 and BLT1 were expressed in relation to healthy controls. CXCR2 (GI 1.04 ± 0.57 x GII 0.33 ± 0.16) and BLT1 (GI 1.17 ± 0.58 x GII 0.60 ± 0.30) were significantly reduced in the septic shock group compared with severe sepsis ($p < 0,05$). The reduction of the expression of the CXCR2 receptor correlated with both APACHE II($r=-0.65$) and SOFA($r=-0.76$) scores ($p < 0,01$). Moreover, the expression of both receptors were extremely lower in the patients that underwent to death ($p < 0,05$). Conclusions: Surface expression of CXCR2 and BLT1 receptors were reduced on neutrophils isolated from patients with septic shock compared with severe sepsis and healthy controls. The reduction of the receptors expressions correlated with scores of disease severity and related to mortality. **Financial support**: FAPESP.

04.127

JM 25-1, a non anesthetic lidocaine derivative, decreased the expression of transcription factor GATA-3 in a murine model of acute allergic inflammation. Couto GC[1], Serra MF[1], Cotias AC[1], Anjos-Valotta EA[2], Jurgilas PB[1], Ferreira TPT[1], Costa JCS[1], Pires ALA[1], Cordeiro RSB[1], Silva PMR[1], Martins MA[1] [1]IOC-FIOCRUZ − Fisiologia e Farmacodinâmica, [2]ICB-USP

Introduction: Antigen inhalation in asthmatic results in an early response, accompanied by airway hyperresponsiveness (AHR) and inflammatory cell infiltration into the lungs that is mediated via expression of Th2 cytokines including IL-4, IL-5 and IL-13, which are regulated predominantly by transcription factor GATA-3. Local anesthetics, particularly lidocaine, have received interest as possible new class of pharmacological agents for the treatment of asthma. Recently, we have demonstrated that JM25-1, non-anesthetic lidocaine analogue, was effective in inhibiting allergen-evoked lung eosinofilic inflammation and airways hyperreactivity. We sought to investigated here the effectiveness of the nebulized JM 25-1 therapy on Th2 cytokines and GATA-3 expression in a short-term A/J murine model of asthma. **Methods:** Mice of strain A/J (License number: L-034/09) were subcutaneously sensitized on days 0 and 14 by a mixture of Al(OH)3 and ovalbumin (OVA) and challenged on days 19 and 20 by 25 µg OVA (25 µl, intranasal instillations i.n.). Nebulization with JM25-1 (1%, 30 min) was performed immediately after ovalbumin provocations. Lung levels of IL-4, IL-5, eotaxin-1 and eotaxin-2 were quantified by ELISA, 24 h after the last provocation. The expression of GATA-3 in lung was detected using Western Blotting. Subepithelial airway fibrosis, mucus production and peribronchial eosinophil infiltration were measured by histomorphometry and total lung collagen was also determined by sircol collagen assay. **Results and Discussion:** Histological analysis of the airways revealed that OVA-challenged mice developed a peribronchial fibrosis, marked goblet cell hyperplasia within the bronchi in the lung and peribronchial eosinophil cellular infiltration as compared to vehicle-treated mice. Morphometric analysis showed that JM25-1 significantly attenuated peribronchial fibrosis (2.81 ± 0.73 to 1.02 ± 0,14 x 104 extracellular matrix deposition area per µm2 (Mean ± SEM, n=5), the total mucosubstance area per µm2 (0.07 ± 0.15 to 0.01 ± 0,00 x 104 per µm2 (Mean ± SEM, n=5) and peribronchial eosinophil numbers (11.01 ± 2.36 to 4.98 ± 1,62 x 104 eosinophils/unit area per µm2 (Mean ± SEM, n=5). Interestingly, we also demonstrated that JM25-1 inhibited increased levels of IL-4, IL-5, eotaxin-1 and eotaxin-2, content of collagen and GATA-3 expression in the lung tissue following OVA provocations. Our findings show that aerosolized JM25-1 inhibits peribronchial fibrosis, goblet cell hyperplasia and peribronquial eosinophil infiltration in a murine model of asthma. These effects seem to be close-related to the blockade of generation of Th2 cytokines and chemokines that play a pivotal role in the pathophysiology of asthma and the expression of GATA-3. These results reinforce the interpretation that the mechanism of GATA-3 inhibition may have relevant implications in understanding and modulating the anti-asthma effect of JM25-1. Taken together, these results provide evidence that JM25-1 should be further investigated as a putative alternative for asthma therapy. **Financial support**: FAPERJ, CNPq, PDTIS and CAPES

04.128

Lidocaine inhibits airway inflammation, peribronchial fibrosis and mucus production in a murine model of asthma. Serra MF[1], Cotias AC[1], Anjos-Valotta EA[2], Couto GC[1], Pão CRR[1], Jurgilas PB[1], Ferreira TPT[1], Pires ALA[1], Arantes ACS[1], Cordeiro RSB[1], Silva PMR[1], Martins MA[1] FIOCRUZ − Fisiologia e Farmacodinâmica, [2]ICB-USP

Introduction: Asthma is an increasingly common immune-mediated disease that remains difficult to manage. Airway hyperreactivity, chronic eosinophilic inflammation and lung remodeling are important components in the pathogenesis of asthma. Evidence suggests that nebulized lidocaine is beneficial in asthma therapy, but the mechanisms underlying this effect remains poorly understood. The aim of this study was to assess the impact of lidocaine treatment in the context of a murine model of allergic asthma marked by inflammation, mucus production and peribronchial fibrosis. **Methods:** A/J mice systemically sensitized to ovalbumin (OVA) were challenged with antigen intranasal instillations by 2 days and treated with nebulizated Lidocaine, or vehicle, immediately after OVA provocations (License number − CEUA L034/09). Airway responsiveness (AHR) was measured by recording the Penh values evoked by inhaled metacholine, using whole-body plethysmography and total and differential leucocytes were evaluated in samples of bronchoalveolar fluid (BALF,) 24 h post-challenge. Subepithelial airway fibrosis, mucus production and peribronchial eosinophils infiltration were measured by histomorphometry in formalin-fixed and paraffin-embedded lung sections stained with Trichrome Gomori, Periodic acid-Schiff (PAS) or Hematoxilin and Eosin (H & E). Total lung collagen, metalloproteinase (MMP)-9 activity and GATA-3 expression were determined by sircol collagen assay, zimography and western blotting, respectively. Lung levels of IL-4, IL-5, eotaxin-1 and eotaxin-2 were quantified by ELISA. **Results:** Lidocaine (0.25-1% M/V) dose-dependently inhibited ovalbumin-induced neutrophil and eosinophil accumulation in the BAL fluid, and airway hyperresponsivess to methacholine. Administration of lidocaine also prevented other pathophysiological changes triggered by ovalbumin in the lung tissue, including peribronchial eosinophilia and fibrosis, increased content of collagen and mucus, MMP-9 activity, GATA-3 expression, and increased levels of IL-4, IL-5, eotaxin-1 and eotaxin-2. **Conclusion**: Inhaled lidocaine prevents eosinophilic inflammation, overproduction of mucus and peribronchial fibrosis in a murine model of asthma, and impaired airway hyperresponsiveness, possibly by suppressing the upregulation of pro-inflammatory cytokines and chemokines. These data further support the interpretation that lidocaine should be considered as a molecular template in drug development for asthma therapy. **Financial support**: FAPERJ, CNPq and PDTIS.

04.129

Role of arylhidrocarbon Receptor (AhR) in antigen-induced arthritis. Talbot J[1], Pinto LG[1], Alves-Filho JC[2], Vieira SM[3], Ferreira SH[1], Cunha TM[1], Louzada Jr P[4], Cunha FQ[1] [1]FMRP-USP − Farmacologia, [2]University of Glasgow − Immunology, Infection and Inflammation, [3]COPE-INPA, [4]FMRP-USP − Clínica Médica

Introduction: Rheumatoid arthritis (RA) is an auto-immune disease that affects almost 1% of the world population and it is characterized by a chronic inflammation of the joints. The inflammation pathogenesis is related to an enhanced Th17 immune response. The etiology of RA is unknown but studies have demonstrated the higher influence of environment factors in its development, mainly cigarette smoke. Recently studies have showed that activation of the aryl hydrocarbon receptor (AhR) by a cigarette smoke compound (TCDD) may influence the development of experimental autoimmune encephalopathy altering the Th17 immune response. The objective of our study was to analyze the role of AhR activation in experimental arthritis development, using the model of antigen induced arthritis (AIA). **Methods:** C57BL/6 mice were immunized to mBSA antigen by subcutaneous (s.c.) injection of methylated bovine serum albumin (mBSA 500 µg/100 µl of saline) mixed with 100 µl of complete Freud's adjuvant (CFA). The procedure was repeated seven days after. In some groups, animals were treated twelve and seventeen days after the initial injection with Vehicle (DMSO 1%) or with the AhR agonist (FICZ − 10, 30 or 90ug/kg). Twenty-one days after the initial injection, arthritis was induced by intra-articular (i.a.) injection of mBSA (10ug/i.a or 30ug/i.a.), and evaluated this parameters: a) Fêmur-tibial articular hypernociception, using an electronic version of the von Frey test; b) Neutrophil recruitment directly in knee joint exudates; c) To detect cartilage loss-induced inflammation, proteoglycan content of cartilage was measured by dimethylmethylene blue assay of papain digests; d) Histology to detect articular alterations was evaluated by Hematoxylin and Eosin stain, and Safranin Stain; e) The expression of AhR, CYP1a1 (activity marker of AhR) and ROR-yt (Th17 marker) mRNA was measured in mice lymph nodes in different times by Real-Time PCR. This study was approved by Animal Ethics Committee of FMRP/USP (n 038/2009). **Results and Discussion:** We demonstrated that immunization with mBSA induced enhancement of AhR mRNA expression and its activation (CYP1a1 mRNA) in mice lymphnodes that did not occurs in False-Immunized mice (only CFA s.c. injection). Furthermore, we accessed if the activation of AhR may influence arthritis development in AIA model. We detected that AhR activation with FICZ induced enhancement of mechanical articular hypernociception and neutrophil recruitment to articular cavity in a dose-dependent manner after injection of mBSA 10ug/i.a. In mice treated with FICZ at 90ug/kg, the hypernociception and neutrophil recruitment was similar to immunized mice treated with vehicle and mBSA injection of 30ug/i.a. We also found that AhR activation (FICZ 90ug/kg) enhances articular cartilage loss and lesion. These results suggest that AhR activation is involved with RA development and can be the link between smoking status and severity of arthritis described in literature. Acknowledge: CNPq, FAPESP, CAPES and FAEPA.

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Chemokine decoy receptor D6 deficiency protects against bleomycin-induced pulmonary inflammation and fibrosis in mice. Russo RC[1], Savino B[1], Mirolo M[1], Buracchi C[2], Anselmo A[2], Zammataro L[2], Pasqualini F[2], Germano G[2], Nebuloni M[3], Mantovani A[3], Teixeira MM[1], Locati M[2] [1]UFMG − Bioquímica e Imunologia, [2]Istituto Clinico Humanitas, Leukocyte Biology, [3]University of Milan − Pathology

Introduction: Pulmonary fibrosis, a chronic disease characterized by interstitial collagen deposition, is related to acute leukocyte influx induced by CC chemokines that perpetuate the initial lung injury, contributing to damage and scaring. Bleomycin-induced lung fibrosis is the main model used to study molecular and pathological mechanisms involved in pulmonary fibrogenesis. D6 decoy receptor, a chemokine scavenger that binds, internalizes and degrades the CC chemokines without G protein signaling, decreases its levels during pathologies. Our aim was to study the role of D6 receptor using the bleomycin-induced lung fibrosis model in D6 deficient (D6KO) mice. **Methods:** WT (C57BL6j) or D6KO mice received Bleomycin (BLEO) intra-tracheal (3.75U/kg), CETEA/UFMG (Protocol 146/06), and were culled at 2, 4, 8, 12, 16 and 22 days after. In each time-point we analyzed airway leukocytes by BAL and FACS, real-time qPCR, chemokine and cytokine levels by Bioplex and fibrosis by Sircoll collagen quantification. IL-17A blockade and γδ T cell depletion were done by monoclonal antibodies anti-IL-17A and anti-γδ TCR. The role of D6 on hematopoietic or non-hematopoietic tissues was studied using chimerical bone marrow constructs, donor->recipient: D6KO->WT or WT->D6KO. **Results:** BLEO-induced early lung D6 mRNA up-regulation (days 2-8) in WT. D6KO had reduced lethality, 15% vs 65% in WT, reduced weight loss and lung fibrosis 22 days post BLEO. D6KO showed a blunted lung expression of Arginase-1/2, TIMP-1, MMP3/9, von Willebrand factor, Neutrophil Elastase, CXCR1/2, CD11b, CCL24 and IL-25 mRNA post BLEO, but not WT. There were reduced PMN (days 8-16) and eosinophil (days 12-16) counts in lungs and BAL from D6KO when compared to WT post BLEO. Low levels of CCL2, CCL3, CCL11, CXCL2, CXCL9, IL-1β, IL-6 and IL-13 were observed in D6KO in contrast to WT post BLEO. However, increased CCL5, CCL12, CCL17, IL-17A and IFN-γ levels were found only in D6KO 2 days post BLEO unlike WT. Lung FACS analysis at day 2 post BLEO showed that γδ T cells are the major source of IL-17A in D6KO, compared to NK cells. D6KO γδ T cell depletion, but not IL-17A blockade, increased loss weight, lethality and fibrosis post BLEO. Chimerical constructs had increased lethality and fibrosis in D6KO->WT opposed to WT->D6KO post BLEO. **Discussion:** Chemokines are critical regulators of pulmonary inflammation and fibrosis. D6KO mice are protected against lung fibrosis by reduced granulocyte and early increase of γδ T cell influx in lungs induced by Bleomycin. We showed that lung injury and fibrosis depends on D6 expression in pulmonary resident cells (non-hematopoietic), but not on leukocytes. Mechanistically, the absence of D6 decoy receptor could protect mice against Bleomycin-induced lung fibrosis probably due to a differential chemokine microenvironment regulation via CCR1/5:CCL5, CCR2:CCL12 and CCR4:CCL17 enhancement and lung γδ T cell recruitment. Support: CNPq, CAPES, INNOCHEM, FAPEMIG.

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Early-lifetime exposure to 1,2-naphthoquinone (1,2-NQ) interact to increase asthma susceptibility and behavior changes in juvenile mice. Florenzano J, Santos KT, Teixeira SA, Barreto MAA, Muscará MN, Camarini R, Costa SKP ICB-USP − Farmacologia

Introduction: We recently showed that neonate male mice exposed to 1,2-naphthoquinone (1,2-NQ), one of diesel exhaust particles (DEP) contaminants, developed intense airway allergic inflammation (Santos et al. 41º SBFTE: Eventus. p.17: 2009). By comparison, epidemiological study has documented that children who live close to high density traffic areas present increased susceptibility to respiratory disease and also impaired cognitive function (Suglia et al. *Am. J. Epidemiol*. 2008 167(3):280–286). This study was undertaken to evaluate whether early-lifetime exposure of male and female mice to 1,2-NQ accounts to exacerbate asthma symptoms and behavioral parameters at a juvenile period. **Methods:** Neonate male and female C57Bl/6 mice (2-5 g) were used, under a protocol approved by our Institutional Ethics Committee (113/07/CEEA). Animals were nebulized with 1,2-NQ (100 nM; 10 mL) or corresponding vehicle (PBS:Tween 80:DMSO) during 3 days for 15 min. Seventeen days later, mice exposed to 1,2-NQ or its vehicle were sensitized by OVA (10 µg/0.2 ml PBS, s.c.) or vehicle (1.6 mg Al(OH)3/0,2 ml PBS) for 2 days. After 7 days, they were stimulated with OVA 1% or vehicle. Following 24 h, the non-invasive lung function was performed to examine the airway hyperresponsiveness. The quantification of inflammatory biomarkers was assessed in the bronchoalveolar lavage fluid (BALF), blood and bone marrow (BM). The motor activity was analysed in an open-field test for 5 min, after 24 h challenging with OVA or vehicle. Data are presented as mean ± SEM. Stats were performed by ANOVA followed by Bonferroni's t-test. $P<0.05$ was taken as significant. **Results:** The Penh values in allergic juvenile female (91 ± 16 AUC) and male (51 ± 9 AUC) mice previously submitted to 1,2-NQ (1,2-NQ + OVA) were not significantly different as compared with the pollutant vehicle and OVA group (111 ± 23 AUC and 55 ± 11 AUC, respectively). The number of neutrophils in 1,2-NQ-treated female group was significantly higher as compared with vehicle group. Both neutrophil and eosinophil numbers were significantly increased in the BALF of 1,2-NQ + OVA male mice. In contrast, the leukocyte number in the BALF of 1,2-NQ + OVA female group did not differ as compared with female vehicle + OVA. The total and differential blood cells (lymphocytes and eosinophils) in 1,2-NQ + OVA-treated male mice were significantly increased as compared with male vehicle + OVA group. The BM of 1,2-NQ + OVA-treated male group, the total and mononuclear cells was higher as compared to OVA male group. The number of mature neutrophils in 1,2-NQ-treated female mice was significantly increased as compared with vehicle group. In the BALF of 1,2-NQ + OVA-treated male mice (but not female mice), the concentrations of Th1/Th2 cytokines (eg. IL-4, IL-5, IL-13 and IFN-γ) were significantly higher as compared to its respective allergic group. The motor activity was significantly increased in 1,2-NQ + OVA-treated male mice when compared to vehicle 1,2-NQ + OVA. **Discussion:** Our results indicate a close link between increased susceptibility of juvenile male (but not female) mice to airway allergic inflammation and behavioural disturbances following early exposure of air pollution such as 1,2-NQ. **Acknowledgements**: Fapesp, CNPq and CAPES for **Financial support**.

04.132

Pharmacological blockade of the CXCL-ELR+ chemokine / receptor cxcr2 axis accelerates wound closure in mice. Castro TBR[1], Canesso MCC[1], Almeida BG[1], Colotta F[2], Bertini R[2], Proudfoot AEI[3], Andrade SP[1], Teixeira MM[4], Barcelos L[1] [1]ICB-UFMG − Physiology and Biophysics, [2]Dompé Research and Development, [3]Serono Pharmaceutical Research Institute, [4]ICB-UFMG − Biochemistry and Immunology

Introduction: Chemokines are important regulatory molecules of inflammatory and angiogenic processes in the pathophysiology of wound healing, representing, therefore, possible targets for new therapeutic strategies. CXC-ELR+ family members exert their effects on recruitment of polymorphonuclear leukocytes and on angiogenesis. The receptor CXCR2 is considered the main receptor through which the CXC ELR+ chemokines, such as CXCL1/KC and CXCL2/MIP-2, exert their biological functions. The aim of this study was to evaluate the kinetics of skin wound healing in C57BL/6J mice and the effects of Evasin-3, a CXC-ELR+ binding protein isolated from salivary gland of the tick Rhipicephalus sanguineus, and Meraxin (DF2156A), a non-competitive allosteric inhibitor of CXCR2, on skin wound healing in mice. **Methods:** Excisional wounds were created on the dorsum with the aid of a circular punch, removing the entire thickness of the skin. The area of the wounds was measured with the aid of a digital caliper for monitoring the closure of wounds. The activity of the enzymes myeloperoxidase and N-acetyl-glucosaminidase was used to indicate the accumulation of neutrophils and macrophages, respectively. Chemokine levels were quantified by ELISA assay. Animals received daily i.p. injection of Evasin-3 at doses of 40, 4 and 0.4 ug / kg or Meraxin (DF2156A) at doses 10, 1, 0.1 mg / kg and the control group received PBS vehicle. All treatment schedule started just after wounding and last until day 3 post surgery. The animals were sacrificed at different time points and their wounds collected for posterior analysis. All procedures described here had prior approval from the local animal ethics committee (CETEA-UFMG, license 254/08). **Results:** In control animals, we observed peak of neutrophils at 12 hours after surgery, while the peak of macrophages occurred on day 3. These returned to baseline after days 3 and 7 respectively. The groups treated with Evasin-3 showed faster closure of wounds in all doses evaluated when compared with the control group. However, we did not observe any difference in neutrophil content or CXCL2 levels after the treatment with Evasin-3. Furthermore, animals treated with Meraxin also displayed faster closure of wounds when compared to control group, although only at lower doses. **Discussion:** These data suggest that Evasin-3 accelerates wound closure in mice without affect neutrophil content or CXCL2 level. Likewise, Meraxin accelerates wound closure in mice. Experiments are being made in order to keep investigating the mechanisms responsible for these effects. In conclusion, pharmacological blockade of the CXCL-ELR+ chemokine / receptor CXCR2 axis accelerates wound closure in mice and may have therapeutical potential to treat non-healing wounds. Supported by CNPq and FAPEMIG.

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Absence of $P2X_7$ purinergic receptors in the hemorrhagic cystitis induced by cyclophosphamide in mice. Martins JP[1], Silva RBM[2], Santos Jr AA[3], Coutinho R[4], Battastini AMO[5], Santos DS[6], Morrone FB[6], Campos MM[7] [1]PUCRS − Medicina, [2]PUCRS − Farmacologia Aplicada, [3]INCTb-PUCRS − Biologia Molecular e Funcional, [4]IBCCF-UFRJ, [5]UFRGS − Bioquímica, [6]PUCRS − Farmácia, [7]PUCRS − Cirurgia-Odontologia

Introduction: Extracellular nucleotides are important signaling molecules that mediate many biological effects, through the purinergic receptors activation (Ralevic et al., Pharmacol. Rev., 50, 413, 1998). ATP is generated in response to cellular damage, and the P2X7 receptors have an essential role in the onset and maintenance of pathological changes (Chesselli et al., *Pain*, 114, 386, 2005). The hemorrhagic cystitis (HC) is a well known adverse effect of therapy with cyclophosphamide (CYP) used in patients in the treatment of many solid tumors (Mosque et al. 2007). These urotoxic effects are attributed to the toxic metabolic of the CYP, named acrolein, which can be partially prevented by 2-mercaptoetanosulfonato of sodium (Mesna) (Katz et al., J Cancer Res. Clin. Oncol., 121, 128, 1995). The present study aimed to determine the role of P2X7 receptors in the model of hemorrhagic cystitis induced by CYP in mice. **Methods:** Male C57/BL6 mice and mice of same lineage, knockout for P2X7 receptor (n= 4; 25-30 g) were used. HC was induced by a single administration of CYP (300 mg/kg, i.p.). Immediately after the i.p. injection of CYP, mice were housed in individual plastic cages to observe the spontaneous behavior for 4 h, for 2 min every half-hour. Three behavioral parameters were considered: (i) activity; (ii) immobility; and (iii) indicatives of visceral pain behavior ('crises'). In addition, the spontaneous behavior of mice was also scored according to the scale described by Olivar et al, 1999 (Olivar et al., Eur. J. Pain., 3, 141, 1999). We have also performed the gross examination of bladders at 6 h, in order to determine the presence of edema and hemorrhage. The wet weight of bladders (g per 100 g of body weight) was also registered at this time-point (Gray et al., *J Urol*, 136, 497, 1986). Control animals received saline at the same intervals of time. All the experimental procedures were approved by the Local Ethics Committee (08/00074, CEUA, PUCRS). **Results:** The results of the present study show that knockout mice for P2X7 receptor inhibited the nociceptive behavior score induced by CYP (18 ± 6%). In addition, present a reduction of both edema and hemorrhage indexes (55 ± 25% 33 ± 38% respectively) in the gross evaluation. Of note, markedly reduced the wet weight of bladders (36 ± 7%). **Discussion:** In the recent years, the interest in the therapeutical potential of purinergic receptors has dramatically increased (Burnstock et al., Pharmacol. Rev., 58, 58, 2006). Our study revealed the importance of P2X7 receptors in the HC induced by CYP. These results confirm what has been found in previous study using the A438079 the selective P2X7 receptor antagonist, suggesting that pharmacological inhibition of these receptors might represent a new therapeutical alternative for this pathological condition. **Financial support**: CNPq, PROBOLSAS-PUCRS.

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Characterization of the inflammatory process in epididymal fat tissues in mice submitted to a palatable diet. Bernardes PTT[1], Rezende B[2], Castor MGM[3], Ferreira AVM[4], Teixeira MM[5], Pinho V[5] [1]UFMG − Morfologia e Bioquímica, [2]UFMG − Bioquímica e Imunologia e Morfologia, [3]UFMG − Fisiologia e Farmacologia, [4]UFMG − Fisiologia e Biofísica, [5]UFMG − Bioquímica e Imunologia

Número CTEA: 264-08 Adipose tissue is an endocrine organ responsible for energy storage, heat production, metabolic control and secretion of adipokines, which are important in the inflammatory process. Previous studies have shown that mice fed with a diet rich in carbohydrates had increased fat tissue and intensified inflammatory response. Thus, we aimed to establish a temporal relationship between increased epididymal adipose tissue (EAT) and the recruitment of inflammatory cells to this organ after high-carbohydrate diet. **Methods:** C57BL / 6 mice were divided into two groups: one fed with regular diet (control diet; CD) and one fed with a regular diet loaded with condensed milk, called as palatable diet (PD), during 1, 3, 14, 28 and 56 days. Indirect quantification of neutrophils and macrophages (myeloperoxidase _MPO_ and N-acetyl-glucosaminidase _NAG activities) were performed in EAT over this time period. In addition, intravital microscopy of EAT microvasculature was performed. **Results and Discussion**: Animals fed with PD showed an significant increase in adiposity from day 3. Furthermore, it was observed an increase in rolling and adhesion in EAT microvasculature from PD group and increased neutrophils number in adipose tissue. The number of macrophages in this organ was decreased in all days analyzed. **Conclusion**: A diet rich in carbohydrates can trigger an inflammatory response in adipose tissue, even in animals subjected to only one day of PD. The PD group showed a significant increase in weight of EAT and an enhanced inflammatory response, characterized by higher recruitment and neutrophil accumulation in tissue. Considering this, the characterization of the inflammatory response in adipose tissue may provide future strategies to control inflammation in obesity-related diseases. **Financial support**: FAPEMIG e CNPQ

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In vitro-differentiated mouse eosinophils as a new tool for the study of eosinophil biology.

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Introduction: Eosinophils are recognized as key players of inflammatory diseases, like asthma and helminthic infections. Further understanding eosinophil biology may impact current comprehension of these disorders and contribute to development of new therapies. However, few experimental approaches allow proper study of eosinophil biology. Here, we described a new method to achieve a large number of mouse eosinophils and demonstrated that these *in vitro*-differentiated bone marrow-derived eosinophils (bmEos) are functionally active cells capable of responding to *in vitro* stimulation with rapid eicosanoid synthesis and lipid body biogenesis. **Methods:** To achieve a mouse eosinophil population with satisfactory purity and size, modifications on a mouse bone marrow cell differentiation methodology reported elsewhere (Dyer JI, 181:4004-4009, 2008) were made. Briefly, total bone marrows recovered from Balb/c mice (IBCCF065) were cultured for 4 days with RPMI-1640 containing 20% fetal bovine serum (FBS) and supplemented with rmFLT3-L (100 ng/mL) and rmSCF (100 ng/mL). On day 4, cell supernatant was replaced with medium containing rmIL-5 only (10 ng/mL). From day 8, at every other day, half of the medium was replaced with fresh medium with IL-5. On day 14, cells were collected and stained with May-Grünwald/Giemsa dye to evaluation of cell purity (by light microscopy). By employing such method, large numbers (~3x107 cells) of *in vitro*-differentiated bone marrow-derived eosinophils (bmEos) with purity ≥ 75% were obtained and used in functional assays for further eosinophil characterization. As eosinophil activation parameters, production of eicosanoids was evaluated by EicosaCell technique, and lipid body biogenesis was studied in osmium-stained cells. For comparison, human blood eosinophils isolated from healthy donors were used (licensed by Committee of Human Ethics/UFRJ – HUCFF 052/09). **Results:** To verify whether bmEos display functional features similar to those well-established ones described for human eosinophils, we initially studied bmEos-produced eicosanoid profile. As immunodeteced by EicosaCell, bmEos stimulated *in vitro* for 15 min with A23187 (0.1 mM) synthesized prostaglandins (PG) D2 and PGE2, as well as LTC4. As well-defined for eosinophils, bmEos were also unable to synthesize LTB4. As described for human eosinophils, *in vitro* stimulation with arachidonic acid (AA; 10 mM), PGD2 (25 nM), PGE2 (1 nM), bradykinin (BK; 10 nM), PAF (10 mM) or substance P (SP; 1 nM) triggered rapid assembly of cytoplasmic lipid bodies within bmEos (within 1h; n ≥ 3). Again similar to human eosinophils, pre-treatment of bmEos with PKA inhibitors while blocking AA-, PGD2-, PGE2-, BK- or SP-triggered lipid body biogenesis, failed to affect PAF-induced effect. Conclusion: Altogether, our findings show that, indeed, we have established a methodology that supplies mouse eosinophils with sufficient number and proper purity to study eosinophil biology. Such *in vitro*-differentiated eosinophils are biologically active cells with functionality and receptor-driven intracellular signaling comparable to human eosinophils. An advantage of these cells over human eosinophils is the opportunity to use eosinophils differentiated from genetically modified mice. **Financial support:** CAPES, CNPq and FAPERJ

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Behavioral changes are associated with central nervous system inflammation in an experimental model of malaria. Miranda AS[1], Lacerda-Queiroz N[2], Vilela MC[3], Rodrigues DH[2], Reis HJ[4], Teixeira MM[1], Rachid M[6], Teixeira, AL[7] [1]UFMG − Bioquímica e Imunologia, [2]UFMG − Biologia Celular, [3]UFMG − Neurociências, [4]UFMG − Farmacologia, [6]UFMG − Patologia, [7]UFMG − Clínica Médica

Introduction: Cerebral malaria (CM) is a severe complication resulting from Plasmodium falciparum infection. This condition has been associated with cognitive, behavioral and motor dysfunctions, seizures and coma. The underlying mechanisms of CM remains incompletely understood. The release of inflammatory mediators has been implicated in its pathogenesis. The aim of this study was to investigate behavioral changes and neuroinflammatory process in a CM model using Plasmodium berghei ANKA (PbA). Method: Six week-old, female C57Bl/6 WT mice were used in this study. Mice were infected with PbA (106 parasitized erythrocytes) by the intraperitoneal route (i.p). Memory and learning paradigms were assessed using the step-down inhibitory avoidance task and the object recognition test. Anxiety was evaluated by the elevated plus maze test. Locomotor and exploratory activities were assessed using the open field test. The concentration of cytokines (TNF-α, IL-1β) and chemokines (Kc, MIP-1α, IL-10, Rantes) in the brain tissue and serum of control and infected mice on day 3 and 6 post-infection (p.i.) were assessed by ELISA. The leukocytes adhesion and rolling in the brain microvasculature were evaluated using intravital microscopy. The histopatological changes in brain tissue were assessed by H&E staining. This study was submitted to ethical approval by CETEA/UFMG, protocol number 105/09. **Results:** Infected mice presented higher levels of anxiety on day 5 p.i. in comparison with controls ($p<0.05$). Infected mice also showed a significant decrease of locomotor and exploratory activities on day 6 p.i. when compared to controls ($p<0.01$). No significant differences were found in memory and learning tests. Sequestration of leukocytes in the brain tissue was found in the histological analysis on day 6 p.i. CM mice also presented higher brain levels of IL-1β, MIP-1α, Rantes and TNF-α, higher serum levels of IL-10, Kc and Rantes ($p<0.05$) and increased leukocyte rolling and adhesion ($p<0.05$) after six days of infection. Conclusions: In this study, we found that inflammatory changes are associated with anxiety and locomotor dysfunction in CM. These findings suggest a role of these cytokines and chemokines in CM pathogenesis. **Financial support**: CAPES, CNPq, FAPEMIG

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Hydrogen Peroxide is associated to neutrophil clearance in model of arthritis induced in mice. Lopes F[1], Sousa LP[2], Coelho FM[3], Costa VV[3], Gonçalves W[1], Teixeira MM[3], Pinho V[3] [1]UFMG − Morfologia, [2]UFMG − Patologia Clínica e Bioquímica e Imunologia, [3]UFMG − Bioquímica e Imunologia

Introduction: Neutrophils are critical effector cells in innate immune responses and are the most abundant of all leukocytes in the joints of mice with antigen-induced arthritis (AIA). The neutrophil accumulation in tissue depends not only on the number of cells being recruited but also on the number of cells being cleared (by apoptosis) or leaving the tissue by transmigration. Many substances orchestrate the survival/death of leukocytes in inflammatory sites, such as growth factors (i.e. G-CSF), virulence factors and pro-inflammatory cytokines. Among these substances, reactive oxygen species (ROS) are able to induce pro-inflammatory responses and are important regulators of cell survival. In the present study, we examined the ability of ROS, mainly hydrogen peroxide, to resolve the inflammation associated to arthritis induced by antigen in mice. **Methods:** AIA was induced by the injection of methylated bovine serum albumin (mBSA) into the knee joint of pre-immunized mice. We investigated the kinetics of neutrophil accumulation in joint cavity and periarticular tissue, as well as the production of hydrogen peroxide. The role of ROS was investigated, by the administration of NADPH oxidase inhibitors (apocinin 1mg/kg) and by the kinetics of AIA in the gp91phox-/- mice. The role of hydrogen peroxide was investigated, by the administration of catalase (1.2mg/kg) and SOD (0.3mg/kg), as so as hydrogen peroxide administration (0,5 M). The chemokine levels were evaluated by ELISA. The articular tissues were evaluated by histopathology. Neutrophil apoptosis was evaluated by flow cytometry, morphological aspects and activation of caspase 3, bax and p65 by western blot (WB). The role of NFkB was investigated by the administration of inhibitors of NFkB (SN-50 1µg/knee). All drugs were administered 12 hours after challenge with mBSA. The knee cavity was washed and the periarticular tissues removed for the evaluation of the aforementioned parameters. Hypernociception related to joint inflammation was evaluated by pressure test on paw (All procedures were approved by the Ethics Committee – CETEA 166/06 – UFMG). **Results:** The peak of neutrophil accumulation was observed at 24 hours and returned to baseline levels by 48 hours after challenge. Coincidently, it was observed a peak of hydrogen peroxide production in the AIA 24 hours after the challenge, paralleling with the beginning of the natural resolution of this model. In gp91phox-/- mice, or after apocinin/catalase treatment, the resolution was delayed. The administration of SOD reduced the amount of neutrophils in the WT mice and the levels of CXCL1, CCL2 and CCL5, wich were associated with less articular tissues damage, a common phenomenon in arthritis. The administration of hydrogen peroxide reduced neutrophil accumulation in WT and gp91phox-/- mice. The clearance of neutrophils after SOD and hydrogen peroxide was associated with the increase of apoptotic cell numbers and Bax and caspase-3 activation. The apoptosis induced by SOD administration was associated with inhibition of the translocation of NFkB to the nucleus. Despite the inflammatory resolution observed after administration of SOD, there was no decrease in the hypernociception associated to arthritis. **Discussion:** We propose here that the natural resolution in the AIA is ROS dependent. SOD and hydrogen peroxide administration cause: (a) reduction of neutrophils in the inflammatory site, (b) increase of apoptosis and decrease of antiapoptotic factors and (c) reduction of pro-inflammatory mediators. These events provide a positive control of the response in the model, contrary to the negative array of cellular and molecular events associated with the loss of function that is characteristic of arthritis. The hyperplasia of the synovial membrane may later lead to the erosion of cartilage and bone, and SOD administration may importantly preserve the integrity of that body. Furthermore, the maintenance of hypernociception may ensure the reduction of movement, important in the moment of recovery, ensuring a better outfit for the ongoing resolution process. This work was sponsored by CNPq and FAPEMIG.

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Role of transient receptor potential vanilloid 4 (TRPV4) in joint inflammation. Denadai-Souza A[1], Vergnolle N[2], Martin L[2], Muscará MN[1], Cenac N[2] [1]ICB-USP − Pharmacology, [2]INSERM

Introduction: We assessed the hypothesis that the Transient Receptor Potential Vanilloid 4 (TRPV4) activation induces an inflammatory reaction in the Temporomandibular Joint (TMJ). In second time we evaluate its sensitization by a pro-inflammatory mediator: PAR2 (Protease Activated Receptor 2). **Methods:** All experimental procedures have been accepted by the local ethics committees (08U563-NV18). TRPV4 and PAR2 expression in the rat TMJ and trigeminal ganglion (TG) was evaluated 4 h after an intra-articular injection of carrageenan by both real-time RT-PCR and immunofluorescence. The functionality of TRPV4 and its sensitization by PAR2 was analysed by [Ca2+]i measurements in dissociated TMJ fibroblast-like synovial cells or TG neurons. Plasma extravasation, myeloperoxidase (MPO) activity and head-withdrawal force threshold (index of mechanical allodynia) were evaluated after an intra-articular injection of the selective TRPV4 agonist 4aPDD, either alone or co-injected with the PAR2 activating peptide (PAR2-AP). **Results:** TRPV4 agonist 4αPDD specifically activated a calcium influx in TMJ fibroblast-like synovial cells or TG neurons. *In vivo*, 4aPDD triggered dose-dependent increases in plasma extravasation, MPO activity and mechanical allodynia. In the TMJ, TRPV4 and PAR2 expression were up regulated in response to carrageenan. In synovial cells or TG neurons pre-treatment with PAR2-AP potentiated 4aPDD-induced increase in [Ca2+]i. *In vivo*, 4aPDD-induced inflammation was potentiated by PAR2-AP activation. **Discussion:** TRPV4 is expressed and functional in TG neurons and in synovial cells. *In vivo* activation of TRPV4 causes an inflammatory reaction in the TMJ. TRPV4 activity is sensitized by pro-inflammatory mediator such as PAR2 activation. These results suggest a key role for TRPV4 in joint inflammation. Funded by: Institut UPSA pour la douleur, INSERM Avenir, ERC (European Research Council) and ANR (Agence Nationale pour la Recherche), CAPES, CNPq and FAPESP (São Paulo Research Foundation).