04. Inflammation and Immunopharmacology

04.001 Role of hyperglycemia, **inflammation and autophagy in bone marrow derived macrophages in Type 1 Diabetes**. Sousa ESA, Queiroz LAD, Martins JO USP

Introduction: Hyperglycemia causes damage to the immune system, providing diabetic individuals with greater susceptibility to infections when compared to non-diabetic individuals. The high susceptibility to infections is, at least in part, due to an inadequate immune response. Thus, the aim of this study was to evaluate the influence of hypoglycemic in the autophagy process and lipopolysaccharide (LPS)-induced response in bone marrow derived macrophages (BMDM) from diabetic and non-diabetic animals. **Methods**: T1DM was induced in mice by alloxan (60 mg/kg, i.p) [CEUA/FCF/USP nº570/2018]. BMDM from diabetic and non-diabetic C57BL/6 mice were used. The macrophages were maintained in culture medium with normal concentration (5.5 mM) and high glucose concentration (25 mM) and were stimulated or not with LPS at the concentration of 100 ng/mL at times of 24, 32, 48 and 72 hours. The autophagy activation pathway was evaluated by dosing the autophagic proteins LC3b and Beclin-1 by the Western Blotting technique in the 24-hour time. The anti-inflammatory cytokine IL-10 was also dosed by enzyme-linked immunosorbent assay (ELISA) at all times. **Results**: The LC3B protein showed an increase in expression in hyperglycemic medium with LPS stimulation and Beclin-1 protein had a decrease in its expression in hyperglycemic medium, with or without LPS stimulation. It was also possible to observe alterations in the secretion of the anti-inflammatory cytokine IL-10, at times of 24, 32, 48 and 72 hours, where BMDM of diabetic mice showed an increase in secretion of this cytokine compared to non-diabetic group. **Conclusions**: Alterations in autophagic proteins expression and IL-10 secretion may directly interfere in the inflammatory response of diabetic subjects, causing an imbalance in the response of these patients to infections, making them susceptible to develop more severe pictures of the disease. Therefore, we can suggest that hyperglycemia plays an important role in the inflammatory response of BMDM in diabetic mice, causing an imbalance in cellular homeostasis. **Acknowledgments**: (2017/11540-7) Sao Paulo Research Foundation (FAPESP); (301617/2016-3) National Council for Technological and Scientific Development (CNPq; PQ-1D) and Coordination of Superior Level Staff Improvement(*CAPES:*88882.327675/2019-01).

04.002 Diosmetin presents topical anti-inflammatory effect on an UVB radiation-induced skin inflammation model in mice. Camponogara C, Brusco I, Brum ES, Pegoraro NS, Oliveira SM UFSM

Introduction: The skin is constantly exposed to chemical and physical agents, including ultraviolet (UV) radiation, which can promote cutaneous pathological alterations causing an imbalance between defense mechanisms and deleterious effects, favoring the latter (Gegotek, A., Redox Biol, 12, 733, 2017). Current treatments include topical glucocorticoid, which induces adverse effects that limit its long-term use (Simpson, B.S., J Nat Prod, 77, 85, 2014). Therefore, new therapies are urgently needed. Thus, since transient receptor potential vanilloid type 1 (TRPV1) participate of cutaneous neurogenic inflammation and modulates the skin inflammatory process induced by UV radiation (Gouin, O., Protein Cell, 8, 644, 2017; Lee, Y., Arch Dermatol Res, 303, 727, 2011), the TRPV1 blockade may be an interesting pharmacological strategy to control the skin inflammatory processes induced by UV radiation. Consequently, we investigated the topical anti-inflammatory effect of a novel TRPV1 antagonist, the diosmetin, on a skin inflammation model induced by UVB radiation in mice. **Methods**: Skin inflammation model was induced by UVB radiation (0.5 J/cm^2) exposition in the mice ear (male adult Swiss) (Pegoraro, N.S., Colloids Surf. B, 150, 32, 2017). Procedures were approved by the Institutional Committee for Animal Care and Use of the Federal University of Santa Maria (number 7999090818/2018). Diosmetin (0.01-1%; TRPV1 antagonist) and dexamethasone (0.5%; positive control) or vehicle (Lanette base cream; 15 mg/ear) incorporated in semisolid formulations were topically applied after the UVB irradiation process. Inflammatory parameters [ear edema formation, enzymatic (myeloperoxidase activity) and histological parameters of inflammatory cells infiltration and inflammatory cytokines (IL-1β and MIP-2)] levels were evaluated at 24h after the UVB irradiation process. The results were analyzed by one-way analysis of variance followed by post hoc Tukey test. **Results**: The UVB radiation increased the ear edema, inflammatory cells infiltration and cytokines levels, when compared with naïve group. Diosmetin and dexamethasone in semisolid formulations inhibited the UVB radiation-induced ear edema, with a maximum inhibition (I_{max}) of 82±9% (at 1%) and 97±1% (at 0.5%), respectively. They also reduced the UVB radiation-induced inflammatory cells infiltration verified by the myeloperoxidase activity reduction [I_{max} of 59±10% to diosmetin (at 1%) and 93±2% to dexamethasone (at 0.5%)] and by the histological procedure. Diosmetin and dexamethasone incorporated in semisolid formulations also reduced the IL-1β and MIP-2 levels, with I_{max} of 40±12% and 54±7% to MIP-2 and 85±9% and 100% to IL-1β, respectively. **Conclusion**: Diosmetin presented an effective topical anti-inflammatory activity on skin inflammation model UVB radiation-induced. This effect demonstrates the potential of TRPV1 antagonists as new approaches in the drugs development for the treatment of inflammatory skin conditions induced by solar radiation.

Acknowledgment: CAPES, CNPq, FAPERGS

04.003 Leukotrienes and angiotensin in diabetes. Guimarães JPT[1], Martins JO[2], Jancar SJ[1] [1]ICB-USP, [2]USP

Introduction: Diabetes mellitus (DM) is a chronic metabolic disease that may be associated with physiological disorders such as hypertension. Studies have shown common pathways linking diabetes to insulin resistance and the renin-angiotensin system (RAS).Angiotensinogen (Agt) is the main precursor of RAS. Activation of Agt is often linked to dysfunctions in cellular processes, such as autophagy. In DM, the pro-inflammatory profile in adipose tissue leads to the production of adipokines and cytokines that promote glucose intolerance and insulin resistance. Leukotriene B4 (LTB4) plays a central role in the establishment of insulin resistance and development of DM in animal models. **Aim**: In the present study we investigated the involvement of LTB4 and RAS in insulin resistance in metabolically active tissues in a mouse model of diabetes (STZ-induced), focusing initially in muscles. **Methods**: T1D was induced by streptozootocin (60 mg/kg, i.p) in 129 SVE mice (WT) andmice knockout of 5-lipoxigenase ($5LO^{-/-}$), enzyme responsible for leukotrienes synthesis. [CEUA/ICB/USP nº8/2014]. Captopril (30mg/L) was given in drinking water during 4 weeks and replaced each day. Response to insulin was evaluated by Insulin Tolerance Test (ITT), insulin concentration by ELISA and phosphorylation of Aktby Western Blotting. Gene expression of insulin receptor, IL6, Stat1, MCP-1, Ym1 and Arg1was evaluated by qPCR and IL10 by ELISA. **Results**: We observed that after a single dose of insulin (NovolinR – 1UI/Kggiven i.p to T1D mice),the reduction of glycemia was more pronounced in $5LO^{-/-}$ mice compared to WT. In quadriceps and gastrocnemic muscles of $5LO^{-/-}$ diabetic mice, the expression of M2 macrophages markers Ym1 and Arg1 and phosphorylation of Akt was higher compared to the WT diabetics. The expression of insulin receptor gene in muscles was also higher in $5LO^{-/-}$ diabetic mice. High relative expression of Agt was found in the liver from diabetic WT mice and was reduced in diabetic $5LO^{-/-}$ mice, indicating that the expression of this RAS marker in liver could be dependent on leukotrienes produced in diabetes. We also noted that diabetic and diabetic 5LO-/- mice lost weight throughout the time of induction of the disease, but captopril-treated WT and 5LO mice gained more weight. **Conclusions**: These results suggest that LTshave impact on inflammatory response and on insulin receptor signaling pathway in muscles as well as in the relative expression of Agt in liver in mice with T1D. **Financial support**: FAPESP (2013/15719-0, 2017/11540-7, 2018/23266-0) and CNPq (302903/2016-0, 301617/2016-3).

04.004 Reduction of S-nitrosothiol levels improves inflammation in experimental pneumonia-induced sepsis in mice. Oliveira FRMB, Rosales TO, Assreuy J UFSC

Introduction: S-nitrosylation has emerged as an important mechanism in cardiovascular biology, with critical relevance in the onset of inflammation (reviewed in IUBMB Life, 65: 819, 2013). This structural modification is based on the ability of nitric oxide (NO) to react with sulfhydryl groups of cysteines (Chem. Res. Toxicol., 21: 2134, 2008). Previous studies of our laboratory demonstrated that proteins undergo through S-nitrosylation during sepsis, and this event could be related to systemic arterial hypotension and diminished blood perfusion, which are linked to disease severity. When the increased protein S-nitrosylation induced by sepsis was returned to the basal levels by administration of the compound 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) after sepsis onset, we found a substantial improvement in cardiovascular parameters of rats submitted to cecal ligation and puncture-induced sepsis (Benedet et al.,, BBA-Mol. Basis Dis., 1864: 307, 2018). Since sepsis also involves an overwhelming inflammatory response and since the consequences of interfering with S-nitrosylation in this response are not known, the present study aimed to investigate the consequences of denitrosylation on septic inflammatory parameters. **Methods**: Swiss female mice (35 - 40 g) were anesthetized with isoflurane. An incision was made in the skin of the neck, the trachea was identified and 50 µL of bacterial suspension (Klebsiella pneumoniae, 1 × 10^8 CFU) was injected with a sterile 30-gauge needle. Skin was sutured and warm PBS was administered (30 mL/kg). Animals were treated with DTNB (31,5 - 126 mg/kg, s.c.) 12 h after infection and animals were sacrificed 12 h later. Thirty minutes before sacrifice, Evans blue dye (EBD; 40 mg/kg), was injected via gingival vein. Lungs and bronchoalveolar lavage fluid (BALF) were collected for pulmonary vascular permeability assay, protein levels, leukocyte counts and cytokine assay. In addition, pulmonary nitrosothiols levels were quantified by the biotin switch assay. All results were expressed as mean ± standard error of the mean and analyzed using one-way variance analysis followed by Tukey's post-test. The difference between the means were significant when $p < 0.05$. All data were analyzed using GraphPad Prism® software version 5.01. **Results**: Sepsis increased vascular leakage in lung tissue (68.8 ± 7.5 µg EBD/g of wet tissue) which was reversed in animals treated with DTNB (17.2 ± 2.5 µg EBD/g wet tissue). In BALF, DTNB reduced exudate formation (1795 ± 131.1 vs 607.8 ± 232.5 µg protein/mL) and neutrophil migration (2.02 ± 0.36 vs 0.69 ± 0.19 x 10^6 cells/mL). Furthermore, IL-1β levels increased in BALF of septic animals (2280 ± 314.8 pg/mL vs 1238 ± 106 pg/mL in naïve group) but it was reduced in BALF from animals treated with DTNB (1511 ± 485.5 pg/mL). Finally, DTNB decreased nitrosothiol formation in lung parenchyma. **Conclusion**: Our results show that inducing denitrosylation by DTNB administration reduced markers of the inflammatory process associated with sepsis severity, as well as it was seen with cardiovascular parameters. These findings show that high NO levels produced during sepsis could be involved with endothelial protein nitrosylation that are critical for vascular permeability and leukocyte migration regulation. However, it remains to be seen whether the improvement of cardiovascular parameters is a consequence or a parallel condition of the improvement of inflammatory response of sepsis.

04.005 The effect of different prostaglandin F2α concentrations on mesenchymal stem cells. Santos ACA, Sartori T, Borelli P, Fock RA USP

Introduction: Studies have shown that Mesenchymal stem cells (MSCs) show the ability to differentiate into mesodermal cell types, such as osteoblasts, chondrocytes and adipocytes. In addition, more recent studies have shown that MSCs are capable of influencing the function of other cells through direct cell-cell interaction or by releasing a broad spectrum of bioactive factors, such as cytokines and growth factors. The prostaglandins (PGs) are arachidonic acid metabolites, physiologically produced through the action of cyclooxygenase-1 (COX-1) and COX-2 and exert a diverse range of biological activities through interaction with their receptors that are present in practically all tissues. Since MSCs have the ability to differentiate into adipocytes, as well as osteoblasts and act to form microenvironments with immunomodulatory capacity, or even to directly or indirectly modulate other cells, altering cell survival, proliferation and development, we chose to evaluate in this work important aspects of the $PGF_{2\alpha}$ influence on some mechanisms that control the main functions of MSCs. **Methodology**: Murine MSC line C3H10T½ were maintained in culture with DMEM culture medium, supplemented with 10% fetal bovine serum, at a concentration of $1x10^6$ cells/ml and with the $PGF_{2\alpha}$stimulus in the following concentrations: $2x10^{-13}$ M,$2x10^{-9}$ M, and $2x10^{-7}$ Mfor24 hours. MTT assay was performed to evaluate enzymatic activity. The viability and cell cycle were performed by flow cytometry technique while the IL1β, IL6, IL10 and TGFβ were measured by ELISA. **Results**: $PGF_{2\alpha}$at the $2x10^{-13}$ M and $2x10^{-9}$ M concentrations did not alter the cell viability of MSCs, while the $2x10^{-7}$ M concentration decrease the cell viability, increasing the percentage of cells in apoptosis. The different concentrations of $PGF_{2\alpha}$did not significant change the cell cycle in MSCs although it can be observed at$2x10^{-13}$ M and $2x10^{-9}$ M concentrations an increase of mitochondrial enzymatic activity when compared with the control group. None of the$PGF_{2\alpha}$ concentrations used in the culture medium was able to significantly modify the cytokines production (IL1β, IL6, IL10 and TGFβ). **Conclusion**: The higher $PGF_{2\alpha}$concentration ($2x10^{-7}$ M) tested increase the apoptosis in MSCs decreasing the viability of those cells in culture and $2x10^{-13}$ M and $2x10^{-9}$ M concentrations increase the mitochondrial enzymatic activity without alter the cell cycle. Furthermore, any used $PGF_{2\alpha}$ concentration was not able to modulate the IL1β, IL6, IL10 and TGFβproduction by MSCs. **Financial Support**: CAPES, CNPq Keywords: Mesenchymal stem cells; Prostaglandin $F_{2\alpha}$; Cell viability; Cell cycle; Cytokines.

04.006 Augmented Interleukin-8 (IL-8) correlates with classical cardiovascular risk factors in overweight children. Fonseca GAA[1], Alves JD[2], França ACH[2], Fagundes DL[2], Lobato NS[1], Lima VV[2], Giachini FR[2] [1]UFG, [2]UFMT

Introduction: The prognosis of some obesity-related comorbidities may be predicted by the circulating levels of some cytokines, since they areconsistently release due the low-grade inflammation process. The cytokine IL-8 stimulates the migration of macrophages into adipose tissue, thus increasing oxidative metabolism, contributing to the appearance of cardiovascular diseases. The aim of the study was to investigate possible correlations between interleukin (IL)-8 with anthropometric and biochemical parameters in overweight children. **Methods**: Children aged from 14 to 18 years, were classified according to their body mass index (BMI) at or above the 95th percentile for children and teens of the same age and sex in overweight(n=18) oreutrophic (n=10). IL-8 circulating levels, anthropometric and biochemical parameters were evaluated. The research was approved by Human Research Ethical Committee by the number (CAAE: 34229414.0.0000.5587). **Results**: Compared to eutrophic, overweight group displayed increased: BMI(27,49±3,94 vs 19,71±2,43); body fat percentage(25,8±7,3vs 12,7±5,9%); waist circumference(86,1±10,7vs 70,0±5,5cm); triglycerides (96,3±45,4vs 49,7 ±24,1mg/dL); total cholesterol (141,9±31,4 vs96,2 ±22,9mg/dL); VLDL cholesterol (22,4±5,2vs 15,0±3,4mg/dL);LDL cholesterol (79,5±32,8 vs 32,7±22,3mg/dL); Castelli I Index (3,4±1,13 vs 1,8 ±0,6) and IL-8 (3,2±1,7vs 1,5±0,6).Correlation between IL-8 and total cholesterol (r=0.443; p=0.026; R^2=0.197) or triglycerides (r=0.442; p=0.027; R^2=0.196) were found. Multiple backward linear regression revealed that cholesterol and triglycerides explained 10% of the variance in IL-8 levels. **Conclusion**: Increased levels of IL-8 observed in overweight teenagers may be an early risk factor for cardiovascular diseases. **References**: Adair L. The emergence of cardiometabolic disease risk in Chinese children and adults: Consequences of change in diet, physical activity, and obesity. Obes Rev. 2014; 49. **Financial support**: FAPEG; PPSUS 003/2018 FAPEMAT 0324552/18.

04.007 Effect of isopropyl galate on ifosfamide induced hemorrhagic cystite in mice. Bandeira SRM, Oliveira LSA, Gonçalves RLG, Rezende DC, Sousa IJO, Neto FPR, Trindade GNC, Oliveira FA UFPI

Introduction: Hemorrhagic cystitis is the main adverse effect associated with the clinical use of oxazaphosphorins, including ifosfamide (IFOS). This event occurs through the formation of acrolein, a toxic metabolite responsible for the urotoxicity of these drugs, resulting in increased oxidative stress and production of proinflammatory cytokines, culminating in the tissue degradation of the bladder tissue (AL-MALKI,2014).The literature demonstrates that gallic acid derivatives may exert an anti-inflammatory effect by reducing neutrophil migration and stabilizing mast cell degranulation (CORREIA, et al 2015), which indicates that isopropyl gallate (IPG) is potentially useful in the development of novel anti-inflammatory prototypes and agents that may counteract acute inflammatory reactions (BARBOSA, 2010). Therefore, the objective of this study was to analyze the protective effect of IPG against ifosfamide-induced hemorrhagic cystitis.

Methods: The hemorrhagic cystitis model was induced by a single dose of ifosfamide (400 mg/kg, i.p.) preceded by pretreatment with saline or IPG (6.25; 12.5; 25 e 50 mg/kg, v.o) in *Mus musculus*(CEUA nº 458/2017).In order to analyze the reduction of the damage, it was evaluated the wet mass of the bladder, hemoglobin content and the extravasation of the Evans blue dye in the bladder matrix and the analysis of the inflammatory damage by Protein C reactive (PCR) by immunoturbidimetric method. Inflammatory cytokines (TNF-α and IL-1β) were measured by ELISA immunoassay technique. Inhibition data were calculated by normalization in relation to the negative control (NC) and significance calculated considering $p<0.05$. **Results**: The results showed that pretreatment with IPG (12.5 and 25 mg/kg) significantly reduced (29.73% and 36.86%, respectively) the wet mass of the bladder and significantly attenuated the hemorrhage (30.1% and 54.55%, respectively). IPG (25 mg/kg) was also able to mitigate the vascular extravasation of proteins (42.94%), besides decreasing the PCR in 56.41%. In the evaluation of cytokines, pre-treatment of animals with IPG (25 mg/kg) resulted in a significant reduction of TNF-α levels (88.77%) and IL-1β levels (62.87%). **Conclusion**: The results indicate that IPG has a potential anti-inflammatory effect by reducing inflammatory parameters related to hemorrhagic cystitis induced by ifosfamide.

Acknowledgements: The authors are grateful to the Federal University of Piauí (UFPI) and National Council of Technological and Scientific Development, CNPq (Brazil). **References**:

AL-MALKI, A. Synergistic effect of lycopene and melatonin against the genesis ofoxidative stress induced by cyclophosphamide in rats. Toxicol Ind Health, 5705, 2014. CORREIA, L.B. Anti-inflammatory effect of methyl gallate on experimental arthritis: inhibition of neutrophil recruitment, production of inflammatory mediators, and activation of macrophages**.** J Nat Prod., 1, 2015. BARBOSA, V.F; Caracterização do perfil da ação do ácido gálico e seus derivados sobre processos oxidativos *in vitro* e *ex vivo*. 2010. Dissertação (Programa de Pós-Graduação em Biociências e Biotecnologia aplicada à Farmácia), – UNESP/Campus Araraquara, SP.

04.008 A novel platelet-activating factor and protease-activated receptor (PAR)-2 network in lung inflammation in mice. Silva IS, Almeida AD, Lima Filho ACM, Braga WF, Capettini LSA, Leite JIA, Leite MFL, Klein A UFMG

Introduction: PAF is a lipid inflammatory mediator playing an important role in asthma. PARs are GPCR activated via proteolytic cleavage of a specific sequence of amino acids in their N-terminal region. Studies carrying out PAR-2 blockade indicate a role for PAR-2 in the pathophysiology of allergic asthma and airway inflammation. Here, we investigated the role of PAR-2 blockade on PAF-mediated lung inflammation in mice. **Methods**: BALB/c mice were pretreated with intraperitoneal injection of PBS, PAF antagonist WEB2086, PAR-2 antagonist ENMD-1068 or protease inhibitor aprotinin 1h before intranasal (i.n.) instillation of C-PAF or PAR-2 agonist SLIGRL-NH_2, and 4, 24 or 48h, lungs and bronchoalveolar lavage (BAL)were obtained to analysis of leukocyte infiltrating, chemokines, NAG and MPO production, and perform histopathological analysis. RAW 264.7 cells were preincubated with ENMD-1068 (5μM)1h before C-PAF(100 nM) to analysis of Ca^{2+} signaling, PAR2/NF-ƘB (p65) immunofluorescence and mRNA expression of PAR2(qPCR) were performed. Immunoprecipitation and immunoblotting for PAF receptor (PAFR) and PAR2 were studied in RAW 264.7 cells stimulated with C-PAF (100 nM), SLIGRL-NH_2 (SLI, 50 μM) or both. Protocols approved by Local Ethics Committee CEUA-348/2014. **Results**: PAF i.n. increased the number of neutrophils recovered in BAL (C-PAF 10^{-8}M: 24.0±3.3; C-PAF 10^{-7}M: 35.0±4.1; C-PAF 10^{-6}M: 73.0±5.2, PBS: 0.3±0.1 x 10^4/mL), peaking 24 h after C-PAF 10^{-7}M (4h: PBS 0.1±0.8, PAF 16.2±1.9; 24h: PBS 0.6±0.2, C-PAF 28.8±2.4; 48h: PBS 1.0±0.6, C-PAF 11.5±2.6 x 10^4/mL), and WEB2086 (PBS: 1.2±0.3; C-PAF: 43.5±1.3; WEB 0.05 mg/Kg: 17.6±0.6; WEB 0.5 mg/Kg: 13.5±1.2, WEB 1.0 mg/Kg: 1.9±0.6 x 10^4/mL),ENMD1068(PBS 1.3±0.5, C-PAF: 60.8±4.6, ENMD 0.05 mg/Kg 5.6±0.6, ENMD 0.5 mg/Kg 10.6±1.9, ENMD 1.0 mg/Kg 10.3±2,2 x 10^4/mL) oraprotinin(PBS 15.4±0.8, C-PAF: 63.3±3.7,Aprot10ng 35.3±3.1,Aprot 30ng 27.5±3.9,Aprot 100ng 22.1±1.3 x 10^4/mL)reduced this effect, SLI increased the number of neutrophils in BAL (SLI 3ng: 20.8±3.9; SLI 10ng: 40.6±5.5; SLI 30ng: 59.1±6.2, PBS: 2.8±1.5 x 10^4/mL)and ENMD significantly reduced histopathological score, NAG and MPO levels in lungs obtained from C-PAF instilled mice as well as CXCL1 and CXCL2 releasing in BAL (CXCL1 1h: PBS 116.7±4.9; C-PAF:398.3±66.8; ENMD: 242.4±20.2; 4h: PBS 246.4±66.4, C-PAF: 487.5±29.5, ENMD: 307.9±3.0, 12h: PBS 46.8±9.2, C-PAF: 119.8±17.3, ENMD: 36.8±2.1 x picograms/mL; CXCL2 1h: PBS 144.6 ± 9.9, C-PAF: 274.5 ± 36.2, ENMD: 194.7 ± 8.1, 4h: PBS 113.7 ± 66.4, C-PAF: 487.5±29.5, ENMD: 308.0±3.0, 12h: PBS 46.8±9.2, C-PAF: 119.8±17.3, ENMD: 36.8±2.1 picogram/mL), amplitude and responsiveness of Ca^{2+} signaling and nuclear fluorescence NF-ƘB (p65) in C-PAF-stimulated RAW 268.7 cells. C-PAF induced PAR2 mRNA expression and co-immunoprecipitation studies showed that PAFR and PAR2 physically interacting macrophages. **Conclusion**: PAFR and PAR2 cooperate in a novel protease/lipid mediator network to lung inflammation downstream. A better understanding of this network could lead to novel therapeutic approaches in neutrophilic diseases. **Financial support**: CNPq and FAPEMIG/Brazil.

04.009 Synthesis, **structural characterization and cytotoxicity evaluation of new 4-Aminoquinoline derivatives**. Silva Neto GJ, Silva AE, Silva KCJ, Moreira MSA, Campesatto EA, Meneghetti MR UFAL

Introduction: In chronic inflammatory diseases, such as rheumatoid arthritis, the inflammatory process induced by a noxious stimulus cannot be repaired. The usual treatment consists in non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, as well as disease-modifying antirheumatic drugs (DMARDs), such as chloroquine – a 4-aminoquinoline derivative. In order to achieve a better treatment efficacy, chloroquine and NSAIDs are usually associated. The development of new drugs is often related to the synthesis of hybrid molecules, which may show an action synergism due to the presence of different pharmacophoric groups with similar activities. Thus, the aim of this study is to synthetize hybrid molecules of 4-aminoquinoline derivatives with ibuprofen and naproxen (NSAIDs) that may have an anti-inflammatory potential, followed by their structural characterization and cytotoxicity evaluation. **Methods**: the 4-aminoquinoline derivatives were synthetized through a one-pot methodology, in which 1 mmol of a NSAID (ibuprofen or naproxen) were added to 1 mmol of a 4-aminoquinoline derivative (*N*^{1}-(7-chloroquinolin-4-yl)ethane-1,2-diamine) and 3 mmol of triethylamine in dichloromethane, followed by the addition of 1 mmol of thionyl chloride. The mixture was stirred for 6 h at room temperature. After reaction, the products were characterized by ^{1}H and ^{13}C Nuclear Magnetic Resonance. After structural characterization, MTT cell viability assay was performed in order to evaluate the cytotoxicity of the new compounds. **Results**: the 4-aminoquinoline derivatives synthetized are new hybrid compounds, never reported before, which presented an easy route of synthesis through a one-pot methodology. Both compounds were completely well characterized by ^{1}H and ^{13}C Nuclear Magnetic Resonance, which validated the planned molecule structures. In the MTT cell viability assay, the new 4-aminoquinoline derivatives show no signs of cytotoxicity ($p > 0.05$) in concentrations below 10 µmol/mL, which is similar to chloroquine. When compared to the NSAIDs, the new compounds show signs of cytotoxicity ($p < 0,05$) only in high concentrations. **Conclusion**: the new 4-aminoquinoline derivatives are easy to synthetize and present a therapeutic potential since they are hybrid molecules of two commercial drugs. Even though they show a decrease in cell viability in high concentrations, they present similar results to chloroquine, which is no sign of cytotoxicity under 10 µM. Further studies, such as pre-clinic assays in animal models are necessary to evaluate the oral acute toxicity and anti-inflammatory potential of the new compounds. **Financial support**: CNPq, CAPES, and FAPEAL.

04.010 Anti-inflammatory activity of ethil fraction acetate and chemical determination of *Poincianella pyramidalis* (**Tul**.) **L**.**P**.**Queiroz**. Moraes SZC[1], Graça AS[1], Souza JB[1], Almeida SM[1], Mota DCS[1], Araújo BS[1], Shan AYKV[1], Quintans JSS[1], Quintans-Júnior LJ[1], Barreto E[2], Brandão GCB[3], Estevam CDS[1] [1]UFS, [2]UFAL, [3]UFOP

Introduction: *Poincianella pyramidalis*, popularly known as "Catingueira", is an endemic plant of the Caatinga biome, being traditionally used for the treatment of diseases, among them, pains and inflammations. The described pharmacological activities of the species are related to antioxidant compounds, especially phenolics, known to have anti-inflammatory characteristics. At the end of the inflammatory event the uncontrolled release of ROS can occur, which activate proinflammatory cytokines generating oxidative stress, which can advocate diseases that affect humans. Thus, this work verified the anti-inflammatory activity of the ethyl acetate fraction (EFA) obtained from the *P. pyramidalis* weeds as well as elucidated the possible bioactive compounds of the fraction. Method:The EAF was obtained after liquid-liquid extraction of the crude hydroalcoholic extract and subsequent fractionation by liquid-liquid extraction.Pharmacological tests for anti-inflammatory evaluation were formalin-induced licking of paw, mechanical hypernociception, and carrageenan-induced pleurisy, the EAF was used as pretreatment at concentrations 25, 50 and 100 $mg.Kg^{-1}$, in addition, inflammatory cytokines TNF-α and IL-1β were quantified 4 hours after the injection of carrageenan.LC-DAD-MS (Liquid chromatography with diode arrangement detector coupled to mass spectrometry) and LC-ESI-MS/MS were used to determine the chemical profile of EAF. The chromatography was coupled to mass spectrometry liquid chromatography with electrospray ionization coupled to a hybrid mass spectrometer). Values were expressed as mean ± SEM (standard error of the mean), one-way analysis of variance (ANOVA), followed by Tukey posttest, n = 8. Differences were considered significant with $p < 0.05$**. Results**: In the pre-clinical trials, in the first phase of the formalin test, no dose tested was responsive, but in the second phase, the group treated with EAF (100 $mg.kg^{-1}$;intraperitoneal) demonstrated a 50% reduction ($p < 0.01$) and 63% ($p < 0.001$), respectively, compared to control. In the mechanical hypernociception test, EAF (100 $mg.kg^{-1}$;o.v.) was effective until the third hour of evaluation with $p < 0.001$. Pretreatment with EAF (100 mg/kg i.p.) is associated with total cholesterol, leukocytes, neutrophils and mononuclear cells and reduced cytokines based on TNF-α and IL-1 proteins. The chemical analyzes identified 15 compounds among them: gallic acid, corilagin, ellagic acid, 3,3'-dimethoxyellagic acid-4'-*O-β*-D-glucopyranoside, gallic acid 3,4-dimethyl ether, ellagic acid 3,3'-dimethyl ether, tellimagrandin I**. Conclusion**: This study provided new evidence that *P. pyramidalis* possesses anti-inflammatory action probably due to the pool of identified phenolic compounds which can be exploited for pharmacological control of acute inflammation. **License number of ethics committee**: 29/2017**. Financial support**: CAPES, UFS, UFAL, UFOP for the support.

04.011 Hepatic microcirculation and metabolic effects of chronic physical exercise in obesity. Rodrigues KL, Silvares RR, Pereira ENGS, Flores EEI, Silva VVD, Daliry A Fiocruz
Introduction: Obesity is the main risk factor for the wide variety of chronic conditions observed in Metabolic Syndrome (MS). Studies suggest an important causal relationship between obesity and cardiovascular disease (CVD), and that obesity is a risk factor for microvascular diseases, involving structural and functional changes in the microcirculation, as well as being the main cause of death in obese patients. The liver microcirculation that is vital for the body also serves as a gateway for the entrance of the leukocytes into the hepaticparenchyma. Physical exercise improves the lipid and inflammatory profile, and reduces the risk of CVD. However, the mechanisms by which obesity triggers damage to hepatic microcirculation are not completely understood. This study aimed to elucidate metabolic and microcirculatory effects induced by obesity, focusing on liver function and microcirculation and evaluating whether physical exercise was able to modulate these alterations. **Methods**: In C57BL/6 mice, obesity was induced with a hyperlipidand hypercarbohydrate (HLHC) diet for 24 weeks. After that, mice were submitted to treadmill aerobic exercise of high intensity for 12 weeks. During 34 weeks the weight and glycaemia of the animals were monitored monthly, while the microcirculatory parameters were analyzed at the end of the protocol. Microcirculatory parameters included: rolling and adhesion of leukocytes evaluated by intravital microscopy and hepatic microvascular flux assessed by laser speckle contrast imaging (LSCI). **Results**: Mice fed HLHC diet showed an increase in body weight when compared to the group that received the control diet. In addition, the HLHC group showed increased content of epididymal and abdominal fat, brown adipose tissue, liver weight and fasting blood glycemia when compared to the control group. Regarding hepatic microcirculation, the HLHC group showed increased adhesion and rolling of leukocytes in the endothelium and a significant decrease in microvascular blood flow compared to the control group. Physical training was able to reverse body weight and the leukocyte recruitment to the microcirculatory observed in the HLHC group. **Conclusion**: Physical training was able to reduce the recruitment of leukocytes and to reverse the weight gain of obese mice, which may help in the understanding of the microvascular diseases developed in this pathology. Thus, our results indicate that physical training may be a potential non-pharmacological treatment for microcirculatory dysfunction and metabolic complications associated with obesity. **Financial Support**: CNPQ, FAPERJ e PAPES / FIOCRUZ. Todos os procedimentos experimentais foram conduzidos de acordo com os princípios internacionalmente aceitos para o Cuidado e Uso de Animais de Laboratório e foram aprovados pelo Comitê de Bem-Estar Animal da Fundação Oswaldo Cruz (Licença L-0012/2018 A1).

04.012 Therapeutic administration of gold nanoparticles (AuNPs) accelerates resolution of silica-induced lung fibrosis in mice. Ribeiro NBS[1], Capelozzi VL[2], Silva VM[2], Machado MP[1], Sa YAPJ[1], Arantes ACS[1], Martins PMRS[1], Martins MA[1] [1]Fiocruz, [2]USP
Introduction: Silicosis is an occupational lung disease developed as a result of crystalline silica particle inhalation. At the moment, there is no treatment for this disease. Evidence exists that gold nanoparticles (AuNPs) have a marked anti-inflammatory activity, which indicates them as a potential therapeutic option. This study was undertaken to investigate the effect of AuNPs on lung fibrosis experimental silicosis in mice. **Methodology**: Anesthetized male Swiss-Webster were intranasallly (i.n.) instilledwith silica particles (10 mg) or vehicle (saline). AuNPs (0.3 - 10.0 µg/Kg) were aerosolized on alternate days starting on day 21 up to day 27 post-silica and the analyses were performed at 24 h or 15 days after the last administration. The parameters included: i) lung function (resistance and elastance) and airways hyper-reactivity to methacholine by invasive plethysmography (Finepointe, Buxco System) and ii) morphology/morphometry analysed by light microscopy usinghematoxylin & eosin (HE), picrus sirius (PS) (collagen fibers) and resorcine-fucsine (elastic fibers), as well as naphthol-AS-D-chloroacetate esterase (NASD-CAE) (granulocytes). Transmission electron microscopywas also used. Iii) tissue collagen was quantified by Sircol technique. All experimental procedures were approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA L-001/19). **Results**: Therapeutic treatment with AuNPs, 24 h after the last administration, inhibited lung function decrease (resistance and elastance) as well as airways hyper-reactivity in silicotic mice. Total content of lung collagen showed similar levels in AuNP-treated and untreated silicotic mice. Interestingly, alterations in the lung tissue morphology was noted under condition of AuNP therapy, including changes in the granuloma pattern (disruption and disorganization) with adjacent mononuclear cells and granulocytes being detected. In parallel, an enlargement of the alveolar spaces and the presence of cellular plugs inside the bronchioles were detected, supporting the idea that AuNPs improve silicotic lung functionality and the clearance of inflammatory cells. Fifteen days after the last administration of AuNPs, silicotic mice still exhibited improvement of the lung function and granuloma deconstruction, though at this time point, the total content of lung collagen was at lower levels when compared to the untreated ones. Additionally, transmission electron microscopy revealed restoration of the ultrastructure of lung epithelial, endothelial cells and extracellular matrix, supporting the suppressive effect of AuNPs in the silicotic lungs. **Conclusion**: Altogether, our results show the therapeutic treatment with AuNPs has the ability to reverse the fibrotic response associated to silica particle inhalation in mice, in a time-dependent manner, indicating that this seems to be a promising approach in the discovering of an effective treatment to silicosis. Key words: Lung, Fibrosis, Silica, Therapy, Gold Nanoparticles

04.013 Development and challenges of topical mitochondrial target hydrogen sulphide based-nanocarrier system for burn skin wound. Cerqueira ARA[1], Matos JKRM[1], Spadari CC[1], Teixeira SA[1], Whiteman M[2], Muscará MN[1], Lopes L[1], Costa SKP[1] [1]ICB-USP, [2]University of Exeter

Introduction: Studies from our group and others have revealed a promising role for hydrogen sulfide (H_2S) in the treatment of sensitive (pain and pruritus) and inflammatory (acute / chronic) diseases in several tissues, including the skin [1]. However, the effects and action mechanisms of H_2S underlying inflammation and healing of cutaneous wounds resulting from thermal injury are scarce and controversial. Burn injuries are among the most devastating causes of death, standing out as the 4th largest cause of trauma. The extent of the lesion and the number of layers (depth) of affected skin classify the lesions in epidermal or deep lesion, which in turn can progress to a hypermetabolic syndrome in which obese individuals exhibit high risk factors and compromised sensitive and healing responses. The aim of this study is to test the hypothesis that the development of nanocarriers containing the H_2S mitochondrial donor, AP39, as a strategy to improve drug efficacy and enable its use for topical treatment of burn [2]. **Methods**: Three different carriers were tested as delivery system to AP39, (i) alginate nanoparticles, by mixing span 80 as surfactant, sunflower oil as oil phase, alginate solution as aqueous phase, and $CaCl_2$ solution, through an internal gelatination method (AP39, 100 nmols, was dissolved in the aqueous phase), nanoparticles obtained were frozen in liquid nitrogen and lyophilized, AP39 encapsulation was assured through indirect method; (ii) alginate beads, by dripping AP39 (100 nmol) diluted in alginate, in a $CaCl_2$ solution; and (iii) microemulsion, prepared with sunflower oil, phosphatidylcholine and monoolein, creating a structure forming nanocarrier in combination with water. Drug integrity after encapsulation was assured by HPLC, using a C18 column (acetonitrile in water; 1:1). Statistical analysis was performed on GraphPad PRISM 7.0**. Results**: AP39 molecule incorporation was successful for all tested carriers. Alginate nanoparticles displayed an average size of 279±20.3nm and negative zeta potential (-35.2 ± 4.3 mV). AP39 incorporation did not affect size or zeta potential, and drug encapsulation efficiency was 45 %. The Average size of Alginate Beads was 800 ± 120 µm, and swelling followed first order kinetics. When exposed to water, Microemulsion transformed into a lamellar structure phase gel following first order kinetics. **Conclusion**: In general, sulphide donors (slow releasing included) presents immediate reaction in aqueous medium. Investment in formulations that do not require water for storage might be the best solution for a promising clinical use. The effective skin penetration and irritation assay as well as the potential effects of topical AP39 in burn model are under investigation. **Acknowledgments**: The present work was performed under the Ethical Committee of Animal Experimentation number9319020818. **References:** 1 – RODRIGUES, Pharmacol Res. Vol.115:255; 2017. 2- AHMAD. Pharmacol Res. Vol.113:348; 2016. Inhibition of neutrophil extracellular traps improves experimental arthritis

04.014 Inhibition of neutrophil extracellular traps improves experimental arthritis. Schneider AH[1], Machado CCM[1], Maganin AGM[1], Barroso LC[2], Fukada Alves SY[3], Alves-Filho JCF[1], Cunha TM[1], Louzada-Júnior P[1], Silva TA[2], Cunha FQ[1] [1]FMRP-USP, [2]UFMG, [3]FCFRP-USP

Introduction: Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that affects synovium. It is characterized by signs such as pain, swelling of the joints, migration of neutrophils and cartilage damage. Recently, neutrophils have recognized as essential for the development of RA. In addition to producing important cytokines, it is known that these cells release the neutrophil extracellular traps (NETs), which have microbicidal capacity, but in autoimmune diseases they appear to be involved in lesions. Therefore, the aim of the present study is to evaluate the role of NETs in RA. **Methods**: The animals were divided into Naive, AIA (antigen-induced arthritis) and AIA + PLZ (AIA animals treated with Pulmozyme s.c, a DNase) and edema, neutrophil migration, IL-6 through ELISA, and pain were evaluated. Intra-articular NET concentration was assessed by PicoGreen kit dosing. Histological sections of the animals were made, and the histopathological score was evaluated and loss of proteoglycans were quantified. **Results**: Animals AIA has an increase of joint edema, migration of neutrophils to joint, pain and IL-6 joint. Treatment with Pulmozyme was able to reduce all these parameters, except neutrophil migration, demonstrating that its effect in reducing the experimental disease is not related to the migration of these cells. Animals AIA has an increase of NETs in the joint and the treatment with Pulmozyme leads to a reduction of these. The increased histopathological score in AIA animals is reduced with treatment. Animals AIA has greater loss of proteoglycan than NAIVE, and treatment reduces this loss. Discussion: These results suggest that the NETs may act worsening experimental arthritis and its inhibition causes an improvement of this condition.

04.015 Macrophage activation and antitumor effect of a sulfated polysaccharide fraction obtained from red seaweed *Gracilaria cornea*. Teles FB, Assef ANB, Monteiro VS, Holanda TBL, Alves APN, Benevides NMB, Wilke DV UFC

Introduction: The use of compounds from natural sources capable of altering the tumor-associated macrophages (TAMs) phenotype for antitumor profiles is a strategy that has received attention from researchers in recent years. These compounds if combined with chemotherapy may improve the antitumor effect with an added advantage of not exhibiting host toxicity. Sulfated polysaccharides (SP) from seaweeds is one of these compounds. This study aimed to evaluate the antitumor effect of SP obtained from red seaweed *Gracilaria cornea*. **Methods:** *G. cornea* was collected on the coastal zone of Flecheiras Beach-Trairi, Ceará. SP from *G. cornea* were obtained by enzymatic digestion. The fractionation was performed by ion exchange chromatography and the majority fraction obtained was denominated FI. The evaluation of macrophage activation to proinflammatory phenotypes *in vitro* by FI of *G. cornea* (Gco-FI) at 10, 100 and 250 µg/mL was performed using the Griess test using murine macrophages RAW264.7 (CTNBio approval number 50.026265/2018-96).Antiproliferative effect of Gco-FI against murine metastatic melanoma B16-F10 was performed by the SRB assay. Finally, antitumor effect was evaluated on mice bearing B16-F10 (CEUA approval number 50/17). Mice (C57BL/6) were treated intraperitoneally during 14 days with sterile saline or Gco-FI (10 or 25 mg/kg/day). A blood sample was used for biochemical and hematological analyses at day 15 post tumor implant. Mice were euthanized for excision and weighing of tumor, spleen, liver and kidneys (100g/body weight). After weighing, the organs were fixed in formalin for further histopathological analysis. **Results**: Gco-FIactivated RAW264.7. at 100 and 250 µg/mLbut showed none antiproliferative activity against B16-F10 cells. Regarding the antitumor assay, the group treated with Gco-FI 25 mg/Kg/day had a mean reduction of 50% on tumor weight when compared to the negative control. In addition, at the same dose, Gco-FI enhanced spleen and liver weight when compared to negative control group. Histopathological analysis showed no signs of organs toxicity at any of the doses tested. **Conclusion**: Gco-FI was able to activate macrophages in vitro without presenting antiproliferative effects against B16-F10 cells. In addition, Gco-FI showed antitumor activity against murine metastatic melanoma model. Studies are ongoing to investigate the correlation between macrophages activation by Gco-FI and tumor growth inhibition in the context of tumor microenvironment. Acknowledgments: CNPq, ProjetoCiências do Mar II/CAPES and INCT BioNat.

04.016 Association of early exposure to electrophilic pollutant in initiating non-alcoholic fatty liver disease and cardiovascular risk in APOE-/- mice. Marques CL, Soares AG, Teixeira SA, Feitosa KB, Araújo LCC, Carvalho CRO, Antunes VR, Muscará MN, Costa SKP USP

Introduction: Evidences show that exposure to ambient air pollution (AAP) is associated with increased hospitalizations, morbidity, and mortality, as a result of respiratory and cardiovascular diseases; interestingly, recent epidemiological studies have provided strong evidence that AAP acts to accelerate non-alcoholic fatty liver disease (NAFLD; steatosis) [1,2]. We previously shown that neonatal exposure of 1,2-naphthoquinone (1,2-NQ), one of the chemical contaminants of fine particulate matter [PM, 2.5 □m] released from diesel exhaust (DEP), exacerbates asthma and inducedfunctional changes in isolated atria and vessels [3,4]; however, there is still little understanding about the effects and mechanisms of 1,2-NQ-induced cardiac abnormalities and liver steatosis, especially when it is associated with malnutrition. This study was undertaken to simulate the early exposure to AAP (1,2-NQ) in susceptible life period, added to impact of malnutrition by the use of high-fat diet (HFD). **Methods**: Apolipoprotein deficient male mice ($ApoE^{-/-}$; 6, 8 and 10 days old), were inhalated with 1,2NQ (100 nM) for 15 min or its vehicle. After 21 days of life, the mice received a standard chow diet (SD) or HFD. Mice were weekly weighing and ECG analysis performed at 42 days old. Following euthanasia, blood, liver and right atria were removed. Atria was mounted on a wire myograph, and the atrial rate (bpm) was measured in response to increasing concentrations of adrenergic (norepinephrine, NE) or cholinergic (carbachol, CCh) stimuli. **Results**: Exposure of $ApoE^{-/-}$ mice to 1,2-NQ, regardless of HFD, induced greater weight gain compared to respective control group (vehicle). Mice exposed to 1,2-NQ and fed with HFD exhibited increased serum concentrations of total cholesterol and triglycerides, as well as increased serum activity of hepatic alanine aminotransferase (ALT) enzymes compared to control group. Exposure to 1,2-NQ *per se* caused moderate NAFLD and this condition was more evident in the respective HFD group. Atrial frequency (bpm) versus adrenergic stimulus, but not cholinergic, tends to increase the maximal response (Emax) in animals exposed to 1,2-NQ in the presence of HFD, besides the increase of sensitivity to NE in relation to the control group. **Conclusion**: These results show that acute exposure to the 1,2-NQ pollutant during the postnatal period, and independently of HFD, was able to promote a sympathovagal autonomic imbalance, as well as led to a moderate steatosis (NAFLD). The association between the malnutrition status of the offspring and the early exposure to 1,2-NQ account to increase the risk of atria dysfunction and development of NAFLD.We also suggest that 1,2-NQ or its metabolites may be translocated from lung to liver, thus contributing to liver diseases. **Acknowledgments**: CAPES, CNPq and FAPESP. CEUA: ICB/USP number 48/2016. References: 1. WHO, 2017. 2. Kim et al., Toxicol Res. 2014;30(2):65-70. 3.Santos et al., Arch Toxicol. 2014;88(8):1589-605 4. Soares et al..In: Pharmacology, 2016. Proceedings of the BPS, 2016.

04.017 Role of DNA-PK complex during Zika virus infection. Patricio DO, Mansur DM UFSC

Introduction: Infectious diseases caused by viruses are major public healthconcerns. In the last years, theZika virus (ZIKV) become responsible for many cases of neurodegenerative manifestations and microcephalyin newborns in Brazil (Zanotto PMA, Front Immunol., 9, 2018). Eukaryotic cells express sensors that detect viral nucleic acids (DNA and RNA). RNA sensors detect ZIKV genome and consequentially that activatepathwaysresulting in type one interferon (IFN-I) production.IFN-I are essential cytokinesfor cellular antiviral responses (Arimoto K, J. Leukoc. Biol., 2018). However, recent studies have shown that ZIKV, although an RNA virus, also activates DNA sensors (Zheng Y, EMBO J., 37, 2018). The DNA-dependent protein kinase (DNA-PK) complex is constituted by DNA-PKcs, Ku70,and Ku80 subunits. It is located in the nucleus acting on non-homologous and joining process during DNA double-strand breaks. During DNA virus infections, DNA-PK complex translocates to the cytoplasm and acquires a DNA sensor function (FERGUSON SHE, eLife, 8, 2012). The present study intends to investigate the role of DNA-PK complex during ZIKV infection. **Methods**: A549 cell line was chosen as a model of infection with ZIKV;viral quantification was made by titration with violet crystal; generation of A549 cell knockout for PRKDC gene (DNA-PKcs protein) by CRISPR; and Immunofluorescence of infected cells. **Results**: We generated A549 cells deficient for DNA-PKcs (A549-KO). Although A549-KO cells are still activated by RNA stimuli (Poly(I:C) and the infection), they are more susceptible to ZIKV when compared with wild type cells. This was shown by virustitration and analysis of virus spread in adjacent cells. Additional experiments are being done to determine the mechanisms surroundingDNA-PKcs mediated virus control. **Conclusion:** These results suggest the DNA-PKcs has a function on ZIKV restriction.

Financial Support: CNPq and CAPES

04.018 Comparison of *in vitro* models of airway epithelial cells for the secretion of mucus and inflammatory process. Lagente V, Bodin A, Victoni T, Gicquel T, Pons F University of Rennes

Chronic Obstructive pulmonary disease (COPD) is a lung disease characterized by chronic inflammation, remodeling excess of tissue, and mucus hypersecretion. Moreover, Inflammation process and remodeling tissue in COPD, is increased by involvement of matrix metalloproteinases. Currently, curative treatment does not exist, and some patients develop resistance to corticoid drugs. Gene therapy could be a novel approach to treat the disease and improve patients' quality of life. However, the effectiveness of gene vectors in the airways is decreased by the presence of mucus that acts as a barrier. The aim of this study is to develop on an in vitro model, to able to production cytokines and mucus secretion; in the perspective to testing new inhibitors of MMPs by gene therapy. We used three airway epithelial lines (A549, Calu-3 and NCI-H292) that were stimulated with different concentrations of CSE (Cigarette Smoke Extract) alone or in association with LPS (lipopolysaccharides), for 24 or 48 hours. NCI-H292 and Calu-3 cells are also co-cultured in the same conditions. Then, we evaluated MUC5AC, IL-8/CXCL8, GROα/CXCL1 and MCP-1/CCL2 gene expression by RT-qPCR and their production by ELISA. CSE alone or with LPS did not impact MUC5AC gene expression, in A549 cells. In contrast, LPS (0.1 µg/mL) increased IL-8/CXCL8, GROα/CXCL1 and MCP-1/CCL2 release. When CSE was associated with LPS, there was an additive effect on cytokine release. Regarding Calu-3 cells, treatment with CSE, LPS, or both, did not affect MUC5AC gene expression, neither cytokine secretions. For NCI-H292 cells, CSE alone increased MUC5AC gene expression and an additive effect was observed when CSE was associated with LPS. LPS at low concentrations triggered some IL-8/CXCL8 release, which was more important when LPS was associated with CSE; but not for GROα/CXCL1. NCI-H292 cells did not release MCP-1/CCL2. In the NCI-H292 and Calu-3 co-culture, CSE and LPS increased MUC5AC gene expression, but CSE did not affect cytokine secretion. LPS alone increased IL-8/CXCL8 secretion, but not GROα/CXCL1 or MCP-1/CCL2. Our results showed that NCI-H292 cells, alone or in co-culture with Calu-3, appear as the best model to evaluate the efficacy of gene vectors, as there are able to produce mucins and cytokines, after CSE and LPS exposure. Nonetheless, the co-culture could allow obtaining production of a greater panel of cytokines. Acknowledgments ANR. Project LUTHER ANR-17-CE18-0034-01.

04.019 Friedelin improves migration of thymocytes and inhibits their IL-2 production *in vitro*. Lins MP[1], Carmo JOS[1], Reis MDSR[1], Savino WS[2], Smaniotto SS[1], Barreto E[1] [1]UFAL, [2]Fiocruz

Introduction: Our previous studies have shown that friedelin, a natural pentacyclic triterpene, inhibited immune cells accumulation into lung tissue in the OVA-induced mouse asthma model. It is well known that CD4+T deviation is a well-established paradigm for asthma, and all peripheral CD4+T subsets (Th1, Th2, Th17, and Tregs) are derived from CD4+T thymopoiesis. Since cellular targets for friedelin remain largely unknown, we aimed to assess in vitro the effects of friedelin on migration and secretion of interleukin-2 (IL-2) by thymocytes. Additionally, we evaluated the thymotoxicity caused by short-term systemic treatment with friedelin in C57BL/6 mice. **Methods**: Female C57BL/6 mice were used (CEUA nº 085/15). Naive mice thymocytes were treated with friedelin (0.1-100 µM) for 6 hours, and then cellular viability, CXCL12-induced migration, and concanavalin A-induced IL-2 secretion was performed by MTT method, transwell assay and ELISA, respectively. In another set of experiments, mice were treated by intraperitoneal route with friedelin or dexamethasone (2.3 µmol/Kg of body weight per day) for 4 consecutive days, and after 24 h of the last injection, the weight and the total cellularity of the thymus were evaluated. The effect of treatments on relative content of T-cell subpopulations was measured by flow cytometry. Statistical analyses were performed using one-way ANOVA followed by Tukey post-test. **Results**: Treatment with friedelin at 0.1, 1, 10, 50 and 100 µM for 1 h or 6 h did not induce any significant changes in thymocytes viability. CXCL12-induced thymocytes migration was increased by 37%, 20% and 44% after treatment for 1 h with friedelin at 0.1 µM, 1 µM and 10 µM, respectively. Evaluating the migration patterns of thymocyte subpopulations, the treatment with 0.1 µM friedelin increased the migration of both CD4+CD8+ (double-positive) and CD4-CD8- (double-negative) cells, but decreased the migration of CD4+ (single-positive) cells. Migratory profile of CD8+ (single-positive) cells was unchanged after friedelin treatment. Concanavalin A-activated thymocytes showed a 3.5-fold increase in IL-2 production compared to unstimulated cells. Treatment with friedelin at concentration of 1 µM and 10 µM reduced conA-induced IL-2 production by 55% and 83%, respectively. Mice administered intraperitoneally with friedelin at 2.3 µmol/Kg daily for 4 days did not show any alteration in thymus weight, the total cellularity of the thymus, and relative content of T-cell subpopulations. In the opposite way, treatment with dexamethasone (reference drug) induced a reduction in all these parameters. **Conclusion**: Collectively, the data show that friedelin has a direct effect on thymocyte migration and production of IL-2 in stimulated cells. In addition, short-term systemic treatment with friedelin did not induce thymotoxic effects. **Financial support**: INCT-NIM. CNPq

04.020 A mimetic peptide derived from Annexin-1 (**AnxA1**), **a glucocorticoid-inducible protein**, **controls inflammation and remodeling induced by house dust mite** (**HDM**) **in mice**. Ferreira TPT[1], Arantes ACS[1], Flower RJ[2], Perretti M[2], Martins MA,[1] Martins PMRS[1] [1]Fiocruz, [2]The William Harvey Research Institute

Introduction: Asthma is an airway inflammatory response, driven by Th2 cells, marked by eosinophilic infiltration, bronchial hyper-reactivity, mucus exacerbation and peribronchiolar fibrosis. Endogenous glucocorticoid hormones are critical on their potent anti-inflammatory activity, a response partially dependent on the release of pro-resolving mediators such AnxA1. This protein is shown to be secreted in respiratory fluid and reported to be up-regulated in asthmatic bronchial lavage fluid. In many inflammatory and cellular settings, the anti-inflammatory activity of AnxA1 is reproduced by peptides Ac2-26, derived from the N-terminal region of the protein. In this study we investigated the therapeutic properties of the N-terminal AnxA1-derived peptide Ac2-26 on experimental model of asthma induced by HDM in mice. **Methods:** AnxA1 null and wild type littermate (Balb/c) mice were sensitized with intranasal instillation of house dust mite (HDM - 25 µg/25 µL), every other day, during 3 weeks. In another set of experiments, wild type littermates were treated therapeutically with intranasal peptide Ac2-26 (200 µg/mouse) or budesonide (10µg/mouse), 1 h before antigen, starting on the week 2 of sensitization. Twenty-four hours after the last challenge, lung function, inflammatory and fibrotic markers were measured. **Results**: We found that HDM led to increased airways hyper-reactivity to methacholine and intense infiltration of leukocytes in the BALF. A marked eosinophil accumulation was noted in the peribronchial area as well as an excessive deposition of extracellular matrix. Increased tissue generation of inflammatory and fibrotic cytokines (IL-4, TGF-beta, eotaxin -1 and -2 and MCP-1) was also detected. A clear exacerbation of these pathological changes was observed in AnxA1 null mice as compared to the wild type littermate controls. Intranasal peptide inhibited HDM-induced airway hyper-reactivity and accumulation of leukocytes in the BALF. Ac2-26 also prevented other pathophysiological changes triggered by HDM in lung tissue including peribronchial eosinophil and neutrophil infiltration, subepithelial fibrosis, increased content of mucus and levels of cytokines. Treatment with budesonide was able to afford an inhibitory effect of HDM-induced lung function and morphological alterations, though being less effective that the peptide ac2-26 in some parameters. **Conclusion**: Taken together, our findings show that AnxA1 null mice show an exacerbation of several aspects of asthma, indicating that AnxA1 plays a pivotal role in the negative regulation of features of severe asthma. In addition, The AnxA1-derived peptide Ac2-26 protects against several pathological changes associated with allergen provocation in wild-type mice, suggesting a pharmacological correlation that turns possible the development of a therapeutic agent for severe asthma. **Financial Support**: FIOCRUZ, CNPq, FAPERJ (BR) and European Community (UE FP7- 2007-2013 - n°HEALTH-F4-2011-281608).

04.021 Probiotics increased lymphocytes subpopulations of CD3+CD4+, CD45+CD25+CCR6-, and CD45+CD25-CCR6+ cells in irinotecan-induced experimental steatohepatitis. Aragão KS[1], Melo A[2], Wong D[2], Fernandes C[3], Gurgel D[2], Pereira M[2], Freitas JA[2], Almeida PRC[2], Lima-Júnior RCP[2] [1]Estácio, [2]UFC, [3]UECE

Introduction: Nonalcoholic steatohepatitis (NASH) is a new complication of irinotecan (IRI)-based anticancer regimens. NASH may complicate the clinical management course of patients submitted to hepatic resection because it decreases hepatic function reserve. Preliminary results suggest that the intestinal microbiota plays an important role in the development of this condition and the modulation with probiotics is able to significantly prevent its development in several models of NASH. The involvement of key lymphocyte population such as regulatory T lymphocytes (i.e., regulatory CD4+CD25+ cells), made be involved in the protect effect of probiotcs in NASH disease. In the present study, we aimed to evaluate the role of probiotics in lymphocytes subpopulations of CD3+CD4+, CD45+CD25+CCR6-, and CD45+CD25-CCR6+ spleens cells in irinotecan-induced experimental steatohepatitis model. **Methods**: C57BL/6 mice (25-30g) were divided into experimental groups (n = 8-10) and injected thrice a week, every other day, with saline (5 ml/kg, ip) or irinotecan (50 mg/kg, ip) alone or in combination with daily injection of probiotics suspension (Simfort®, which contains *Lactobacillus acidophilus, Lactobacillus casei, Lactococcus lactis, Bifidobacterium bifidum e Bifidobacteirium lactis;* $1x10^7$ CFU/mL, p.o.). At week 5, blood samples were collected for total leukocyte count and, following euthanasia, liver and intestinal samples were obtained for assessment of histopathology, inflammatory parameters (number of neutrophils/mg of tissue), *real time* PCR to evaluation of Toll-Like Receptor 4 (TLR4) and TNF-□ receptor (TNFR) expression and flow cytometry to quantification of lymphocyte subsets. One-Way ANOVA or Kruskal Wallis test was used. $P<0.05$ was accepted. **Results**: When compared to saline group, irinotecan caused a pronounced leukopenia (IRI: 3206 ± 398.6 *vs* SAL: 6400 ± 902.4) and intestinal damage [IRI: 10 (7-12) *vs* SAL: 4 (3-5)], which were significantly prevented by probiotics [6113 ± 683.5; 5 (3-8), respectively]. In addition, liver histopathological scores [IRI: 4.5 (2-6) *vs* SAL: 3 (2-3)] and the number of inflammatory foci (IRI: 7.6 ± 2.0 vs SAL: 0.6 ± 0.4) were increased in irinotecan group when compared to saline. These parameters were prevented in the animals treated with probiotics [3 (2-3); 2.7 ± 0.6, respectively]. Treatment with probiotics increased the % of lymphocytes subpopulations of $CD3^+CD4^+$ (40.09±1.18%), $CD45^+CD25^+CCR6^-$ (4.37±0.85%) and $CD45^+CD25^-CCR6^+$ (5.18± 0.63%) when compared to the irinotecan group (28.81±3.33%; 2.00±0.40% and 2.96±0.28%, respectively). In addition, irinotecan (39.67±2.40%) increased the number of $CD45^+CD25^+CCR6^+$ lymphocytes vs. saline (31.32 ± 1.20%) and probiotic groups (32.21 ± 1.85%). On the other hand, probiotics did not change TLR4 (2.72 ± 0.17) and TNF-α receptor expression (4.47 ± 0.71), which were increased in the irinotecan group (TLR4: 3.51 ± 0.75,; TNFR: 11.68 ± 3.49) when compared to the saline group (TLR4: 0.97 ± 0.03; TNFR: 1.93 ± 0.69). **Conclusion**: Probiotics prevented irinotecan-related NASH, in part by the modulation of systemic immune cells. **Financial support**: CAPES, CNPq and FUNCAP. **Keywords**: Steatohepatitis. Irinotecan. Intestinal microbiota. Probiotics.

04.022 Dsatinib, **a tyrosine kinase inhibitor**, **down-regulates airway inflammation and lung remodeling in a mouse model of glucocorticoid resistant asthma**. Santana ACC[1], Serra MF[1], Pimentel AS[1], Arantes ACS[1], Abreu SC[2], Xisto DG[2], Martins PMRS[1], Rocco PRM[2], Martins MA[1] [1]Fiocruz, [2]UFRJ

Introduction: Glucocorticoid (GC) insensitivity is a significant barrier to treat several chronic pulmonary diseases, including asthma. However, the molecular mechanisms involved in steroid resistance remains poorly understood. The development of new animal models that are refractory to GC and mimic hallmark features of asthma will be useful in the identification of associated mechanisms and novel therapies. Our group developed a new long-term mouse model of GC-insensitive asthma in which A/J mice exposed to 9 allergen provocations, once a week, for 9 consecutive weeks, reacted with airway hyperreactivity (AHR), lung inflammation and airway remodeling, all of which were clearly resistant to GC treatment. Previously, we have reported that the second-generation tyrosine kinase inhibitor dasatinib reduces lung inflammation and remodeling in a GC-sensitive murine model of allergic asthma. The current study investigates the effectiveness of the dasatinib interventional treatment on allergen-induced pathological changes in a long-term mouse model of GC-insensitive asthma. **Methods**: A/J mice were sensitized on days 0 and 7 by a suspension of Al(OH)3 and ovalbumin (OVA) given subcutaneously, and challenged intranasally, once a week, for 9 consecutive weeks, starting on the second-week post-sensitization. Dexamethasone (1mg/kg), dasatinib (10 mg/kg) or vehicle was given orally for 7 consecutive days only during the last week of OVA provocations. Airway hyper-reactivity (AHR), leukocyte infiltration on bronchoalveolar lavage (BAL), extracellular matrix deposition, mucus exacerbation and cytokines generation were evaluated 24 h after the last challenge. Western blotting was used to investigate GATA-3 expression in the lung tissue. The Committee on Use of Laboratory Animals of the Oswaldo Cruz Institute (license L-030/2015; Rio de Janeiro, Brazil) approved all protocols and experimental procedures involving animals. **Results**: We found that OVA-challenged mice developed marked lung eosinophil and neutrophil infiltrations, and increased peribronchial fibrosis as compared to sham-challenged mice. All these changes were sensitive to dasatinib, but not dexamethasone treatment. Similarly, increased lung tissue levels of IL-4, TNF-α, eotaxin-1 and -2, KC, TARC and MIP-1α, noted in asthmatic mice and were significantly inhibited only by dasatinib. Despite being both dasatinib and dexamethasone able to inhibit allergen-induced mucus exacerbation, none of them modified neither AHR nor the changes in lung tissue levels of catalase and TBARS in this model. Finally, we observed a decreased expression of GATA-3 in mice treated with dasatinib, but not dexamethasone. **Conclusion**: Our findings show that dasatinib, given orally, can reverse lung inflammation and tissue remodeling in a murine model of GC-insensitive asthma, without affecting the oxidative stress and AHR. This effect seems to be at least in part accounted for by down-regulation of GATA-3 expression and pro-inflammatory cytokine production.

Financial Support: CNPq, FAPERJ, MS/DECIT, TARKINAID, TIMER and CAPES.

04.023 Reduced macrophage P2X7 receptor function during schistosomiasis impairs host defense. Thorstenberg MLP, Monteiro MMLV, Martins M, Silva RC, Silva CLM UFRJ

Introduction and objective: Schistosomiasis is a debilitating intravascular parasitic disease related to chronic liver and mesenteric inflammation. Our group reported that the purinergic P2X7R expression is reduced in macrophages from *Schistosoma mansoni* infected mice (Oliveira et al.,2014,Mediators Inflamm.2014:1), and this finding was related with TGF-β production. Here we investigated if the disease-related reduction of P2X7R expression impairs the phagocytosis by peritoneal macrophages.

Methods: Swiss, C57BL/6(wild type, WT), and P2X7 knockout(P2X7$^{-/-}$) male mice were infected (CEUA048/2016). After 60 days the animals were euthanized and peritoneal macrophages were recovered and plated(2x10^5/ coverlip/well) for 24h. For phagocytosis assay, macrophages were exposed to *L. amazonensis*(*LA*) for 4h, fixed and stained with panoptic. The phagocytosis index(PHI) reflects the number of parasites inside macrophages. The E-NTPDase enzymatic assay was carried out in a reaction medium containing 1 mM ATP, ADP or AMP(30min), and the amount of the Pi released was determined by malachite green method. The number of peritoneal macrophages that expresses E-NTPDase was determined flow cytometry using (CD11bFITC,F4/80PE,and CD39alexafluor647). Data were expressed as mean and SEM of n individual experiments.

Results: The PHI of macrophages from infected mice(Swiss, 2.16±0.68 P=0.0005) and(WT,2.4±0.4 P=0.002) was smaller than in uninfected/control mice (Swiss 3.4±0.55 and WT 3.5± 0.6, N=5). Moreover, the PHI from the infected group was comparable to the index observed with macrophages from uninfected P2X7R$^{-/-}$(2.4±0.5), or uninfected Swiss mice treated with the selective P2X7R antagonist A740003(25nM;2.6±0.6), suggesting that the role of P2X7R to innate immunity is impaired by the disease. Accordingly, cell treatment of WT macrophages with 500 µM ATP(10min) increased the PHI only in the uninfected group. Moreover, macrophage pretreatment with apyrase, which degrades ATP(2U/L;2.17±0.9),TGF-β1 (10ng/ml;2.2±0.2) or adenosine(30µM;2.5±0.1) also reduced the PHI in the uninfected group, while the adenosine deaminase restored the PHI of infected macrophages(3.6±1.1P=0.008), suggesting that adenosine may be linked to the reduced macrophage function. The hydrolysis of ATP was higher in the cells from infected (20.7±2.6,N=6) as compared to control mice(9.4±2.8 nmol Pi/2x10^5 cells, N=3, P=0.03; Student´s t test). The same was observed for ADP(Inf: 18.5±2.6, N=6; Co: 5.9±2.5 nmol Pi/2x10^{5}cells,N=4,P=0.01) and AMP(Inf:6.1±0.8,N=5;Co:3.4±0.5nmolPi/2x10^{5}cells, N=5,P=0.02). Moreover, the frequency of the CD39$^+$F4/80$^+$ cells was higher in infected (10.2±5.1,N=5) than in control mice(3.5± 3.0,N=2). *In vivo*, mice paw edema was higher *S. mansoni*-infected(233±1.4) than in control mice(173±28.4), as well as the parasitic load of L.A. Altogether, these data suggest that schistosomiasis increases the macrophage adenosinergic signaling and reduces P2X7R signaling, favoring the occurrence of co-infections. **Conclusions**: Our findings suggest a reduced function of macrophages P2X7R to host defense during *S. mansoni* infection and may explain schistosomiasis morbidity. **Financial support**: CNPQ, FAPERJ, CAPES

04.024 Losartan modulates gene expression of renin-angiotensin system components, decreases inflammatory cytokines molecules and attenuates bone volume loss in rats with experimentally-induced periodontitis. Dionisio TJ, Souza GP, Colombini-Ishikiriama BL, Garbieri TF, Parisi VA, Oliveira GM, Santos CF USP

Introduction: The renin-angiotensin system (RAS) is known for its role in cardiovascular regulation, but it has been implicated as an important factor in inflammatory processes. The aim of this study was to evaluate the effect of AT1 receptor blockade on the progression of experimentally-induced periodontitis (EP) in rats, by evaluating gene expression (q-PCR) and bone loss analysis. In addition to the RAS-regulating genes, gene expression of inflammatory cytokines in the mandible around the first lower molar affected by EP was evaluated. **Methods**: Ethics Committee on Animal Research at Bauru School of Dentistry/USP (Permit Number: 020/2016) approved this study. After anesthesia, silk suture thread (4.0) was placed around the lower right first molar. This method is well established and is capable of causing alveolar bone loss in addition to compromising adjacent supporting tissues. Animals remained with the silk suture around the tooth for 1, 3, 7 and 14 days. For bone analysis, CT scans were performed on the mandibles. Bone volume, as well as the quantity, size and distance between the bone trabeculae were measured. Besides treatment with water and losartan (50 mg/kg/day) on the same day of EP induction, groups of animals were previously treated with the same drug and at the same dosage for 30 days. Therefore, the present study contained 4 groups with 5 animals in each group: G1 - control without EP; G2 - animals with EP and treated with water; G3 – losartan-treated animals (treatment started in the same day of EP induction) and G4 - animals previously treated with losartan for 30 days followed by induction of EP and continuity of treatment. **Results**: Tomographic analysis in animals' mandibles, affected by EP, revealed bone loss volume, increase number and thickness of bone trabeculae, and decrease separation of trabeculae, which characterized a fragile and porous bone with larger and near trabeculae and recurrent dental mobility. On the other hand, animals with EP pretreated with Losartan, showed significant improvements in these parameters, i.e., a more resistant bone with smaller trabeculae without dental mobility. Moreover, EP promoted AT1a receptor increased expression, but losartan treatment did not modulate this response. On the other hand, EP alone did not influence the expression of ECA-2 enzyme, however losartan treatment promoted an increase in MAS and AT2 receptors expression, which may explain the decrease of bone loss observed in the animals treated with this AT1 receptor antagonist, since ECA-2 is capable of cleaving Ang II into Ang 1-7, which in turn promotes anti-inflammatory actions when bound to MAS receptors. The same anti-inflammatory mechanism occurs when Ang II binds to AT2 receptor. Several inflammatory mediators presented upregulation after 7 days of EP such as TNF-α, IL-1β, IL-6, IL-17, MCP-1, MIP-1-α, IFNγ, COX-2, VEGF and VEGF receptor 1. These responses were abolished in both groups of Losartan-treated rats. **Conclusion**: The present results support the conclusion that AT1 receptor modulates EP progression. **Financial support**: São Paulo Research Foundation (FAPESP 2015/03965-2).

04.025 Evaluation of the leishmanicidal activity of *Allium sativum*, *Curcuma longa*, *Zingiber Officinale* **and** *Glycine max*. Ferreira SCA, Nunes ICM, Albuquerque LWN, Alves AA, Moreira MSA, Leite AB, Silva AE, Santos MS, Queiroz AC UFAL

Introduction: Leishmaniasis is an anthropozoonosis endemic in 100 countries caused by protozoan of the genus *Leishmania.* In general, this parasitosis is divided in two forms, cutaneous leishmaniasis and visceral leishmaniasis, both with specific pathogenesis, and continues to be one of the most neglected diseases in the world (WHO, 2019). Currently, the therapeutic arsenal for this disease is restricted and presents high toxicity. Therefore, use of plants with antiparasitic property is important in the search for active substances against the *Leishmania* parasite. Thus, the present work aims to evaluate the leishmanicidal activity of medicinal plants *Allium sativum*, *Curcuma longa*, *Zingiber officinale,* and *Glycine max* from the National Relation of Medicinal Plants of Interest of the Health Unic System of Brazil (RENISUS) and with antiparasitic potential described in scientific literature. **Methods**: Firstly, the cytotoxicity of the plants in macrophages of the J774.A1 cell line was investigated by the reduction test of MTT. Macrophages infected with the promastigotes of *Leishmania chagasi* or *Leishmania amazonensis* were treated with aqueous extractive solution of these plant species at 100, 10, 1, and 0.1 µg/mL and also standard drugs as pentamidine, meglumine antimoniate and miltefosine (100, 10, 1 and 0.1 µM).The treatment was carried out for 48 hours. Later, the nitric oxide (NO) production of the host cells infected was evaluated and the nitro compound LB1491 (100 µM) was used as positive control in this assay. The results analyze were performed in Prisma® software, evaluating the levels of significance between the experimental and control groups positive or negative (Student t test or ANOVA). The values will be considered significant when * $p < 0.05$, and expressed as mean ± standard error of the mean**. Results**: Plant species didn't present cytotoxic effects to the macrophages until the maximum concentration tested, 100 µg / mL. None of the plants induced a deleterious effect against amastigotes of *L. chagasi* or *L. amazonensis*. *Zingiber officinale* at 100 µg/mL (310.7%) and LB1491 (953.1%) also induced increase of NO production in macrophages infected with *L. amazonensi*s when compared to the negative control groups (medium or DMSO 0.1%, respectively). The standard drugs did not induce alterations in the NO production. In addition, the plants species tested did not express a significant effect on the increase of NO in macrophages infected with *L. chagasi* when compared to the negative control. **Conclusion**: The results suggest that the tested plants don't presented leishmanicidal activity, however, *Zingiber officinale*, *Curcuma longa* and *Allium sativum* increase the concentration of nitric oxide. Therefore, new studies must be carried out to better understand the pharmacological activities of these plants. **Reference**: OMS. Diponível em: <http://www.who.int/leishmaniasis/en/>. Acesso em: 07 de junho de 2019. **Funding**: CNPq , UFAL, INCT-INOFAR (573.564/2008-6), FAPEAL (PRONEM 20110722-006-0018-0010), Decit-SCTIE-MS/FAPEAL/SESAU-AL (PPSUS 60030 000820/2016).

04.026 Investigation of the antinociceptive effect and acute toxicity of stigmasterol. Morgan LV, Alves BO, Scatolin M, Zilli GAL, Volfe CRB, Daniel C, Guzatti JGG, Souza MA, Lopes MLLC, Oltramari AR, Zottis C, Müller LG, Scapinello J Unochapecó

Introduction: Stigmasterol is a phytosterol present in many medicinal plants. Its structure is similar to the human steroids, which present anti-inflammatory activity. Stigmasterol is a precursor in the synthesis of progesterone and vitamin D_3, and it's also an intermediate in the biosynthesis of androgens, estrogens and corticoids. It has been studied for its anti-hypercholesterolemic, anti-osteoarthritic, anti-tumor, hypoglycemic, antioxidant, antimutagenic, anti-inflammatory and central activities. Thus, this research aimed to investigate the activity of stigmasterol on different mice models of nociception as well as its effects on locomotor activity, motor coordination and the acute toxicity of this molecule in mice. **Methods:** Male Swiss mice (35-40 g) were used in the study – three doses of stigmasterol (10, 30 and 100 mg/kg, p.o.) were tested to standardize its lowest effective dose. The antinociceptive activity, by chemical nociception, was investigated by acetic acid-induced writhing test and formalin test. The motor coordination was assessed by rota-rod test and the locomotor activity, by the open field test. The acute toxicity of stigmasterolwas investigated using OECD 423 guideline – on this test, female mice were used. Indomethacin (10 mg/kg, p.o.) was the positive control. Groups treated with saline pluspolysorbate 80 at 1% (vehicle) were used as negative control. The results were evaluated by one-way or two-way (repeated measures) ANOVA post hoc Student-Newman-Keuls (Animal Research Ethical Committee - Unochapecó approval: 007/17; 006/19). **Results**: Stigmasterol (10 – 100 mg/kg) significantly ($p < 0.05$) reduced the number of abdominal writhes comparing to the vehicle-treated group. The animals treated with stigmasterol (10 mg/kg) also had a significantly lower nociception time (s) compared to the vehicle-treated group in the first ($p < 0.05$) and second ($p < 0.01$) phases of the formalin test. Stigmasterol-treated (10 mg/kg) mice presented a number of crossings significantly lower ($p < 0.01$) than the number of the ones that received the vehicle in the open field test. Nevertheless, the results of rota-rod test show that stigmasterol has no effects on mice motor coordination. The acute (2000 mg/kg, p.o.) administration of stigmasterol did not change the relative organs' weight, neither the body weight gain of mice. Stigmasterol-treated mice showed significant ($p < 0.01$) difference in the food ingestion only on the 12^{th} day, when compared to the vehicle-treated group. No mice mortality was observed. **Conclusion**: Stigmasterol present anti-inflammatory and antinociceptive (chemical nociception) activities and is devoid of acute toxicity (LD50 > 2000 mg/kg, p.o., OECD category 5). It also does not affect the motor coordination in mice but acts as a sedative. The mechanism of stigmasterol antinociceptive/anti-inflammatory activities will be further studied.

Acknowledgements: This work was supported by the Universidade Comunitária da Região de Chapecó and Programa de BolsasUniversitárias de Santa Catarina - Uniedu [Art. 170 and 171 CE].

04.027 Maternal glucocorticoids *in utero* **determines the production of corticosterone and reduces acute lung injury response in adult rats**. Severo PH, Gil NL[2], Balbino AM[1], Azevedo GA[1], Landgraf MA[1], Landgraf RG[1] [1]Unifesp-Diadema, [2]Unifesp

Introduction: Maternal food restriction could be considerate a gestational stress factor, increasing the maternal glucocorticoids, impairing the fetal development and allowing physiological disorders in adulthood. Previous data of our group showed that Wistar rats of caloric-protein restricted mothers presented low birth weight, and in adult life, this offspring presented reduced leukocytes transmigration and reduced allergic lung inflammatory response, besides increased corticosterone (CORT) basal levels. In this context, the aim of this study is investigating the influence of 50% caloric-protein restriction throughout pregnancy on CORT and acute lung injury (ALI) of adult offspring. **Methods**: Pregnant Wistar rats, on the first day of gestation were separated in: control mothers (CM), mothers who received 50% of food restriction in comparison of controls intake (RM) and mothers who in addition to food restriction were treated with metyrapone (MET – 0,5mg/mL) v.o. (RM+MET). The food restriction and the treatment were introduced only on the days of pregnancy. The offspring with 12 weeks old were induced to ALI by lipopolysaccharide (LPS) (750µg/mL) i.n., and 6h after, the blood, bronchoalveolar lavage fluid (BALF), bone marrow lavage fluid, lungs and hypothalamus were collected to analysis. **Results**: The corticosterone levels of MET treated mothers was similar to control mothers. Both the RM and RM+MET offspring, showed a low birth weight (LBW). Total and differential leukocyte count in BALF and histopathological analysis showed a decreased neutrophilic infiltration in RM offspring in comparison of RM+MET and MC offspring. Only the RM offspring showed an increase in basal CORT concentrations. RM and RM+MET offspring showed hypocellularity in bone marrow. The histopathological analysis showed a natural thickening alveolar wall in RM andRM+MET offspring. **Conclusion**: This study showed that overexposure of maternal glucocorticoids, even though do not interfere on the birth weight and lung morphology in this model of gestational food restriction, lead to high levels of circulating CORT and reduced inflammatory response to ALI in adult offspring. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, FAPESP (2017/02042-3 and 2019/05242-9) and CNPq.

04.028 Nerolidol in polymeric nanoparticles: **anti-inflammatory effect in arthritis model**.
Gomes MVLD[1], Souza EPBSS[1], Silva LAS[1], Souza EV[1], Santos WM[1], Araújo JMD[1], Soares RC[2], Almeida RG[1], Araújo AAS[1] [1]UFS, [2]UFU
Introduction: Rheumatoid arthritis is an autoimmune chronic disease characterized by inflammation of the synovial membrane and progressive degradation cartilages.Nerolidol is a sesquiterpene found naturally in essential oils of many plants, has several biological activities, highlighting the anti-inflammatory [1]; [3].However, has high volatility and low solubility. In this context, nanotechnology may be used as an alternative to improve stability. Therefore, the objective of study is to and evaluate Nerolidol-loaded nanocapsules activity on zymosan-induced arthritis model. **Methods:** Formation of NETs was quantified and evaluated using neutrophils from the marrow of female mice and the kit Quant-iT ™ PicoGreen® DNA (Invitrogen)[2](CEPA/UFS #22/2018 protocol number). The reading was done by fluorescence in the plate reader (Synergy HT®, Biotek) at 484 nm and excitation and emission wavelengths of 520 nm, respectively. In order to evaluate the proinflammatory cytokines IL1- β, IL-6 and TNF-α involved in the mechanism of action of nerolidol in synovial inflammation, by enzyme immunoadsorum assay (ELISA) [3];[4]. The histological sections was made in zymosan-induced arthritis model in mice[5]**. Results**: It was observed through fluorescence values, a high labeling DNA in the supernatant medium of cells treated with Phorbol 12-myristate 13-acetate (PMA) and reduced in 50% NETs formation in the wells treated with Nerolidol. The nerolidol loaded-nanocapsules showed a higher inhibition of the IL1-β and TNF-α cytokines when compared with the control group. Histopathological studies showed a considerable reduction of edema and cellular infiltration in the group of animals treated with free nerolidol and more expressively in the group treated with nanoencapsulated nerolidol**. Conclusion**:The results point to a significant inhibition of inflammation in the joint cavity in the arthritis model, by the free nerolidol, and improved by the polymer nanoparticles. Further studies may bring important contributions to the treatment of rheumatoid arthritis. References: [1] Chan et al. Molecules, 21 (5) 2016; [2] KHANDPUR et al Sci Transl Med 5(178) 2013; [3] FONSECA et al Fund Clin Pharm 30 (2016) 14–22; [4]YAMADA et al, Am J Chin Med 41(4) 913-926, 2013; [5] Hashimoto et al, Eur J Histochem. 62, 1, 2847. Acknowledgments: CAPES, CNPq, FINEP and FAPITEC/SE for the **Financial Support** and fellowships.

04.029 Mechanism of chronic anti-inflammatory action of the essential oil of the leaves of *Lantana montevidensis* (**Spreng**) **Briq**. **evaluated by granulomatous tissue formation-MCAIAEOLLMEGTF**. Pessoa RT[1], Oliveira MRC[1], Oliveira-Tintino CDM[2], Silva MGL[1], Magalhães FEA[3], Martins AOPBB[1], Menezes IRA[1] [1]URCA, [2]UFPE, [3]UECE
Introduction: The *Lantana montevidensis* (Spreng) Briq. is popularly known as "chumbinho", and is commonly used in folk medicine to treat rheumatism, bronchitis and gastric disorders. Although some biological activities of this species as, antiinflammatory activity, have already been reported, however, the chronic anti-inflammatory property of its essential oil still needs to be investigated. The present work evaluated the mechanism of chronic anti-inflammatory action of the essential oil of the leaves of *Lantana montevidensis* (Spreng) Briq. evaluated by granulomatous tissue formation. **Methods**: Four cotton pellets were placed in the dorsal region of mice, each pellet weighing 0.01g (anesthesia: with 80mg/kg ketamine and 20 mg/kg xylazine). The treatment groups included saline 0.9%, dexamethasone 5 mg/kg, OEFLM (50 mg/kg). The treatment lasted ten days, and on the tenth day, the euthanasia was performed. Two pellets were collected, dried for 24h at 37 °C and weighed, and two other pellets were homogenated in saline and utilized for determination of total protein dosage by spectroscopy method using biuret assay at 550 nm. **Results**: The dry mass obtained from the cotton pellets in treatment with OEFLM (50 mg / kg) and dexamethasone (5 mg / kg) showed significate reduction with 65.42% and 88.55%, respectively when compared with the negative control group. The other result of the absorbance demonstrates that significate reduction of protein level in 76.36% and 82.56%, respectively to OEFLM and dexamethasone in comparison to the negative control, corroborating with results of dry mass. **Conclusion:** The OEFLM showed significant anti-inflammatory potential in chronic inflammation, suggesting that the oil acts in the proliferative phase, evidenced by the reduction of the total proteins that infiltrate the granuloma. **Financial Support**: CNPq, Capes, FUNCAP **Acknowledgments**: Regional University of Cariri, CNPq, Capes, FUNCAP.

04.030 Comparative study of T lymphocyte activity in different murine models of experimental Type 1 Diabetes mellitus. Queiroz LAD, Guimarães JPT, Assis JB, Sousa ESA, Martins JO, Sá-Nunes AD USP

Introduction: Type 1 Diabetes mellitus (T1DM) is characterized by a persistent hyperglycemia mainly caused by the destruction of pancreaticβ cells. Brazil is the third country with the highest number of cases diagnosed of T1DM in the world (1). Alloxan (ALX) and streptozotocin (STZ), two of the most widely used diabetogenic agents in experimental modelsinduce necrosis pancreatic β cells. However, little is known on the effects of these agents in the adaptive immunity of treated animals. The goal of the present project is to evaluate whether ALX and STZ affect parameters associated with the biology of T cells *in vitro* and *in vivo.* **Methods**: T1DM was induced in C57BL/6J mice by ALX (a single dose of 60 mg/kg, i.v) or STZ (five daily doses of 65 mg/kg, i.p) [CEUA/FCF/USP nº 388]. Hematological parameter of control and treated mice were evaluated by a hemocytometer. The levels of IFN-γ, TNF-α,IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70 and IL-17 were evaluated in spleen and pancreas homogenates by ELISA. The proliferative response of lymphocytes was evaluated in spleen cell cultures stimulated with concanavalin A. *In vivo* lymphocyte response was assessed in OVA-immunized mice by antigen-specific migration test. **Results**: There was a reduction in the number of total leukocytes only in ALOX-induced T1DM compared to the control group. There was no change in the baseline levels of the evaluated cytokines in the pancreas and spleen homogenates. Also, there was no difference in the lymphocyte proliferationamong the groups. In the evaluation of the in vivo response, OVA-induced edema was highest in the STZ group compared to the ALX group. **Conclusions**: The results suggest that ALX and STZ induce differential changes on hematological parameters, but not in baseline serum cytokines or in the spleen T cell proliferative response. **Acknowledgments:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – grant # 2017/11540-7); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – grant # 163410/2018-6 and 301617/2016-3). **References**: 1- International Diabetes Federation. ***IDF Diabetes Atlas, 8th edn.*** Brussels. Belgium.International Diabetes Federation, pag 17-62, 2017.

04.031 Oleic acid into semisolid dosage forms reduces UVB-induced skin inflammation via glucocorticoid receptors. Pegoraro NS, Camponogara C, Cruz L, Oliveira SM UFSM
Introduction: Currently, skin diseases affect million people around the world; between these are the skin inflammatory conditions (Hay et al., 2014). The therapeutic strategies available nowadays to treat these cutaneous disorders include the use of topical anti-inflammatory drugs and glucocorticoids (Barnes et al., 2015). Despite their efficacy, these medicines cause several adverse effects that limit their use (Coondoo et al., 2014). In this sense, efforts are necessary for the discovery of potential therapeutic alternatives with a lower incidence of adverse effects. Oleic acid (OA) is a natural compound found in vegetable oils like olive oil and foods like fish and oilseeds (Roncero et al., 2016) that exhibits benefits on the resolution of inflammatory processes (Sales-Campos et al., 2013). We developed semisolids based on Pemulen®TR2 and Lanette® containing OA (0.3%; 1% and 3%) and investigated if this compound presents *in vivo* anti-inflammatory effect employing a UVB-induced skin inflammation model. **Methods:** Male *Swiss* mice were employed (25-30g). Initially, the mice right ear thickness was evaluated using a digital micrometer (basal measure). After the anesthetic procedure, skin inflammation was induced by the irradiation of the animal right ear (UVB lamp; a peak of emission at 313 nm; employed dose: 0.5 J/cm^2). The effect of OA into semisolid dosage forms was verified after single (day 1) or repeated (days 1, 2, and 3) treatment of the mice ear following the UVB. The OA action on glucocorticoid receptors was also investigated. All experiments were approved by CEUA-UFSM (protocol number 2320290518/2018). **Results**: Pemulen® 3% OA inhibited the ear edema with superior efficacy than Lanette® 3% OA and dexamethasone after the single treatment (I_{max}=92.58±2.58%, 79.36±7.47% and 77.74±2.69%, respectively). Pemulen® 3% OA and dexamethasone, but not Lanette® 3% OA, also reduced inflammatory cells infiltration on damaged tissue (I_{max}=46.73±4.07% and 46.54±3.12%, respectively). After repeated treatments, Pemulen® 3% OA decreased the ear edema with Imax= 69.88±2.31%; 60.95±5.70% e 29.89±6.40% at 24 h, 48 h and 72 h after UVB. The pre-treatment with the glucocorticoid agonist mifepristone was able to prevent the antiedematogenic effect presented by Pemulen® 3% OA and dexamethasone acetate by 92.25±6.03 and 87.36±4.78. **Conclusion**: OA into semisolids, especially that based on Pemulen® TR2, presented greater anti-inflammatory activity, which seems to occur via glucocorticoid receptors. The natural compound OA could represent a promising alternative to those available to treat cutaneous inflammatory disorders. **References:** Barnes, L. et al. Drug Saf, v. 38(5), p. 493, 2015. Coondoo, A. et al. Indian Dermatol Online J, v. 5(4), p. 416, 2014. Hay, R. J. et al. J Invest Dermatol, v. 134, p. 1527, 2014. Roncero, J. M. et al. Grasas y aceites, v. 67, 2016. Sales-Campos, H. et al. Mini Rev Med Chem, v. 13, p. 201, 2013. **Funding sources:** This study was supported by the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul - FAPERGS, Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

04.032 Uvaol attenuates inflammatory functions of macrophages by inhibits M1-like phenotype and NF-Kb signaling. Cavalcante-Araújo PM[1], Lagente V[2], Barreto E[1] [1]UFAL, [2]University of Rennes

Introduction: Uvaol is a pentacyclic triterpenoid, especially abundant in olive oil, with antioxidant and anti-inflammatory properties. It is acknowledged that macrophages to have a critical role in the pathophysiology of asthma, so much that M1-polarized macrophages express proinflammatory cytokines and induce lung inflammation and tissue damage. Thus, compounds able to suppress the functions of activated macrophages may to have potential to the treatment of inflammatory diseases. The aim of the present study was to examine the anti-inflammatory activities of uvaol on macrophage activation *in vitro* and indicate the underlying mechanism. **Methods**: Macrophage cell line J774 was cultured in DMEM medium supplemented (10% fetal bovine serum, 2 mM L-Glutamine, 0.02% penicillin/streptomycin) at 37 °C in an incubator 5% CO_2. The cells were seeded onto 24-well plate culture dishes at a density of 2×10^5 cell/well and treated with uvaol (0.1, 1 and 10 µM) for 1h. After this period the supernatant was substituted for DMEM medium containing LPS (100 ng/mL) and uvaol.The cells that did not receive LPS served as control. After 24 h, cell-free supernatant was collected for the analysis of cytokine production by ELISA, and cellular viability was evaluated according to the MTT method. Expression of M1 polarization marker (CD86) was detected by flow cytometry.We also evaluated the effect of uvaol on phagocytic activity of macrophage using zymosan particles. Phagocytosis was assessed as phagocytic index (PI) being determined as PI = PP×MNP, where PP represents percentage of phagocytic cells and MNP the mean number of particles per cell. Nuclear translocation of NFkB was determined by immunofluorescence assay. Statistical analyses were performed using one-way ANOVA followed by Tukey post-test, and difference between means was considered significant when $p<0.05$**. Results**: Exposure of macrophages to uvaolfor 24 h did not affect the cell viability. Stimulation of macrophages with LPS induced an increase in production of IL-1β (of 79.5 ± 7.9 pg/mL to 170.0 ± 8.0 pg/mL) and TNF-α (of 9.6 ± 1.8 pg/mL to 53.9 ± 5.2 pg/mL).Treatment with uvaol at concentration of 0.1 µM, 1 µM and 10 µM reduced the IL-1β production in 60%, 62% and 68%, and the TNF-α production in 31%, 47% and 53%, respectively. In LPS-activated macrophages, increased levels of the M1-specific marker, CD86, were slightly inhibited by treatment with uvaol at 0.1 µM, 1 µM and 10 µM in 9.5%, 15% and 16%, respectively. In another set of experiments, uvaol at 0.1, 1 and 10 µM also increased the phagocytic activity of macrophages in 1.4-fold, 1.7-fold and 2.2-fold, respectively. LPS-stimulated macrophages showed an increase in the translocation of cytosolic NF-κB into the cell nucleus (1.5 fold higher compared to the control), event that was inhibited in 50% by 10 µM uvaol**. Conclusion**: Our results demonstrated that uvaol play an anti-inflammatory effect in macrophages by decreases cytokine secretion and increases phagocytic activity, phenomena that appear to be related to attenuation in M1 macrophage polarization and the reduction in nuclear translocation of NF-kB.

Financial Support: CNPq, CAPES and FAPEAL.

04.033 ERK5 mediates TGF-β signaling and shapes autoimmune inflammation. Prado DS, Damasceno LEA, Ferreira RG, Rosa MH, Cunha TM, Cunha FQ, Ryffel B, Waisman A, Alves-Filho JC FMRP-USP

Introduction: ERK5 is an atypical member of MAPK family that also exerts noncanonical functions such as act as a scaffold protein or co-transcription factor. It has been shown that TGF-βsignaling in fibroblasts, hepatocytes and epithelial cells leads to ERK5 phosphorylation, which induces ERK5 activation. In line, it is very well established that TGF-β is critical for Treg and Th17 cell differentiation, being important to control autoimmune response. Thus, we hypothesized that ERK5 could modulate Treg and Th17 cell differentiation, playing a key role in the fate of autoimmunity. **Aim**: to evaluate the role of ERK5 on CD4 T cell differentiation and experimental autoimmune encephalomyelitis (EAE) development. **Methods**: CD4naïve T cells purified from C57BL/6, $ERK5^{flox/flox}$ or $CD4^{cre}ERK5^{flox/flox}$ mice were cultured under Treg- or Th17-cell polarizing conditions (TGF-β and TGF-β+IL-6, respectively) and then their differentiation was analyzed by flow cytometry, ELISA or qPCR. First, we checked pERK5 expression in Treg and Th17 in relation a control group without TGF-β.In order to check the role of ERK5 in Treg and Th17 differentiation, ERK5 pathway was blocked with different inhibitors, such as XMD 8-92 or ERK5-IN-1 (0,3, 1 or 3 µM; 0,1, 0,3 or 1 µM, respectively; ERK5 inhibitors). The contribution of ERK5 in the pathogenesis of autoimmune diseases was investigated by inducing a model of multiple sclerosis, called experimental autoimmune encephalomyelitis (EAE), which is induced by injection of MOG_{35-55} peptide subcutaneously. **Results**: we found that ERK5 phosphorylation is increased in Treg and Th17 cells when compared with control group (Th0, no cytokines), suggesting that TGF-β can increase pERK5 expression. Then, we showed that pharmacological inhibition or genetic deficiency of ERK5 in CD4 T cells decreased Treg cell differentiation, whereas Th17 polarization was enhanced. Interestingly, ERK5 inhibition or genetic deletion do not modulate Th17 differentiation when the cells are cultured in conditions without TGF-β, suggesting its role in ERK5 activation. In line, ERK5 phosphorylation leads to ERK5 nuclear translocation. Thus, we analyzed ERK5 expression in the nucleus of Treg and Th17 and we found the both cell types express ERK5 in the nucleus, while Th0 lacks ERK5 in the nucleus, suggesting a pivotal role of TGF-β in ERK5 nuclear translocation into the nucleus. Moreover, $CD4^{cre}ERK5^{flox/flox}$ mice developed more severe EAE than control $ERK5^{flox/flox}$ mice, characterized by an increase of clinical score and Th17 response by recall assay, as well as a reduction of Treg cells in draining lymph nodes. **Conclusion**: our study reveals a novel role of ERK5 in modulating Treg/Th17 cell differentiation and attenuating the severity of EAE. Therefore, ERK5 could be a potential therapeutic target for autoimmune diseases.

04.034 Purinergic signaling converts N-acetylserotonin into the pineal darkness hormone. Sousa KS, Quiles CL, Ferreira ZFS, Markus RP USP

Introduction: The pineal gland is an integral part of the acute immune response. Danger- or pathogen-associated molecular patterns (DAMPs & PAMPs) activate the immune-pineal axis switching melatonin synthesis from the pineal gland to activated macrophages/microglia. The transcription of the gene and the activation of the enzyme that converts serotonin to N-acetylserotonin (NAS) is blocked in pinealocytes and activated in macrophages/microglia (Markus et al., Brit J Pharmacol 175: 3239, 2018). Although high ATP concentration also signalizes cell death (necrosis or necroptosis), activation of P2X7 receptors (P2X7R) in cultured pineal glands leads to an increase in NAS and reduction in melatonin due to the inhibition of the transcription of the enzyme ASMT (acetyl-serotonin methyltransferase), that converts NAS into melatonin. Here we tested whether increasing intracerebral content of ATP "in vivo" could activate the immune-pineal axis and if the activation of P2X7R could impair the synthesis of melatonin in the dark, but not the synthesis of NAS, transforming this precursor of melatonin in the darkness hormone. **Methods**: ATP (0.3 - 3.0 µg/ 5µL) or the selective P2X7R agonist benzoyl-ATP (BzATP; 1.5 ng/ 5µL) were injected in the lateral ventricle (i.c.v.) of Wistar rats (n=38) 30 min before darkness (ZT11.5). Animals were euthanized 6 hours later (ZT 18). Plasma melatonin was determined by ELISA, while pineal NAS and melatonin were measured by HPLC-EC. **Results**: ATP (3.0 µg/5µL) induced a higher increase in NAS (3.8 ± 0.3 times) than melatonin (2.1 ± 0.2 times; p=0.0004; n=7) in comparison to vehicle, suggesting that the conversion of NAS into melatonin was inhibited. The participation of P2X7R in this modulation was confirmed by BzATP (i.c.v.), which decreased melatonin levels (2.1 ± 0.2 times) in the pineal at the same time as it leads to increased NAS (2.5 ± 0.4 times). Lower concentrations of ATP (0.3 and 1.0 µg/ 5µl) promoted the increase in NAS (2.0 ± 0.3 and 3.7 ± 0.5 times) and melatonin (1.8 ± 0.2 and 3.1 ± 0.3 times), as expected when only P2Y1 receptors are activated. **Conclusion**: Our results confirm previous *in vitro* data indicating that ATP concentrations compatible with sympathetic co-transmission potentiate melatonin synthesis, while high concentrations, which signalize danger reduces melatonin pineal output. However, for the best of our knowledge, this is the first time that is shown an increase of NAS at night, dissociated from a melatonin increase, opening the possibility that NAS plays the role of the darkness hormone. Considering that NAS activates TrkB receptors (Jang, SW et al. Proc. Nat. Acad Sci, 107: 3876, 2010) here we open a new avenue for understanding pineal gland neuroprotective mechanisms.

04.035 A hydrogen sulfide (H_2S)-releasing dexamethasone derivative with antioxidant activity attenuates the development of atopic dermatitis in mice. Coavoy Sánchez SA[1], Teixeira SA[1], Cerqueira ARA[1], Santagada V[2], Caliendo G[2], Costa SKP[1], Severino B[2], Muscará MN[1] [1]USP, [2]Università degli Studi di Napoli Federico II

Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by pruritus and eczematous skin lesions, associated with enhanced T-helper2 lymphocyte response, which results in elevated serum immunoglobulin E (IgE) concentrations. It affects children and adults with a high prevalence. H_2S is a gaseous mediator with physiological and pathophysiological functions in many organs, it is also produced in the skin and participates in the regulation of inflammation, pruritus, cytoprotection, scarring and angiogenesis[1]. Since the therapeutical potential of hybrid H_2S-donors on AD has not been studied to date, we decided to compare the effects of dexamethasone and two H_2S-releasing derivatives using a murine AD model. **Methods**: The experimental protocol was approved by the local Ethics Committee for Animal Experimentation (CEUA-ICB/USP; n° 129/2016). Female BALB/c mice (6-8 week-old) had the dorsal hair shaved and 200 μl of 0.5% 2,4-dinitrochlorobenzene (DNCB) in acetone/olive oil (3:1) were topically applied on days 1-3. On days 15, 17, 19 and 22, the mice were topically challenged with 200 μl of 0.2% DNCB on the dorsal skin and 20 μl on the right ear. On days 19-23 after sensitization, mice were topically treated with 250 nmol/mice of either dexamethasone (Dexa), dexamethasone-TBZ (Dexa-TBZ) or dexamethasone-ADT (Dexa-ADT), and 1000 nmol/mice of the H_2S-releasing moiety, thiobenzamide (TBZ) or anethole dithiolethione (ADT). **Results**: Topical DNCB induced AD-like skin lesions, scratching behavior, ear edema and eosinophilia. Topical treatment with 250 nmol/mice Dexa-TBZ or Dexa-ADT significantly reduced the skin severity score (27.9% and 31.8% respectively; $P<0.001$), scratching behavior (88.8% and 65.2% respectively; $P<0.001$), ear edema (98.8 and 100.0 % respectively; $P<0.05$), and decreased the number of eosinophils below the values observed in the animals without AD, Dexa-treated animals exhibited similar responses. In the analysis of antioxidant enzymes, superoxide dismutase (SOD), catalase and glutathione S-transferase (GST) was significantly reduced in animals with AD, and treatment with Dexa-TBZ increased the activity of the enzyme glutathione peroxidase (GPx) in animals with experimental AD, this effect was significantly different (22.3%; $P<0,05$) from that observed in the Dexa-treated group. On the other hand, treatment with the TBZ or ADT only decreased DNCB-induced eosinophilia (54.5% and 92.7% respectively; $P <0.001$). **Conclusions**: The presence of the H_2S-releasing moiety (TBZ or ADT) in the dexamethasone molecule does not interfere with the beneficial effects of this corticosteroid, and presents an advance on the parental molecule and may even stimulate the activity of antioxidant defenses in the treatment of AD, suggesting that this association has great therapeutic potential to be used in clinical practice. **Financial Support**: FAPESP (2017/16409-6), CNPq and CAPES. **References**:
[1]Coavoy-Sánchez et al., Br J Pharmacol. 2019 May 3. doi: 10.1111/bph.14699

04.036 Methyl cinnamate attenuates inflammatory and pathophysiological parameters in elastase-induced emphysema in mice. Carmo JOS[1], Nascimento LMPS[1], Correia ACC[2], Cartaxo TN[1], Ferro JNS[1], Barreto E[1] [1]UFAL, [2]UPE

Introduction: Pulmonary emphysema is a major pathological feature of chronic obstructive pulmonary disease and is characterized by proteolytic destruction of the alveolar structure and subsequent inflammation of the respiratory tract. The methyl cinnamate is a methyl ester of cinnamic acid found in the essential oil of several plant that possession of antinociceptive and antispasmodic effects. However, until now, no studies have demonstrated its potential effects on pulmonary emphysema. Here, we aimed to evaluate the effects of methyl cinnamate in both pulmonary inflammation and changes in lung structure on elastase-induced emphysema. **Methods**: C57BL/6 mice were anesthetized and received a tracheal instillation of porcine pancreatic elastase (2 IU, 50 µL) or vehicle (saline). Treatment with methyl cinnamate (MC, 1, 10 and 50 µmol/Kg) was performed once daily between days 16 and 20post-stimulus. Twenty-four hour after last treatment, the leukocyte infiltrate in bronchoalveolar lavage fluid (BAL), lung histological changes (hematoxylin-eosin staining) and cytokines (ELISA assay) were evaluated. Statistical analyses were performed using one-way ANOVA followed by Tukey post-test.All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Federal University of Alagoas (85/15). **Results**: We found that in the elastase-injected mice the bronchoalveolar lavage fluid exhibited marked leukocyte infiltration characterized by an increase in the counts of monocytes(of 0.96 ± 0.02 for 4.45 ± 1.02 $\times 10^4$cell/BAL),lymphocytes(of 0.05 ± 0.03 for 0.12 ± 0.03 $\times 10^4$ cell/BAL) and neutrophils (of 0.08 ± 0.02 for 0.39 ± 0.21 $\times 10^4$ cell/BAL), and increase in IL-6 levels (of 10.12 ± 2.0 for 404.9 ± 39.22 pg/mL).Treatment with 1, 10 and 50 µmol/Kg MC reduced the counts of monocytes (by 57%, 69% and 73% respectively), lymphocytes (by 57%, 77% and 60% respectively) and neutrophils (by 92%, 89% and 94% respectively).Histopathologic analysis of lungs from elastase-injected mice revealed evident airspace enlargement and widened alveolar septa due to inflammatory cell infiltration. These emphysematous changes were accompanied by increase in lung tissue of IL-1β (17.5-fold increase), TNF-α (2.6-fold increase), and IL-10 (5.7-fold increase).All histopathologic alterations induced by elastase were markedly decreased in MC-treated mice at the two higher doses. Methyl cinnamate at doses of 10 and 50 µmol/Kg significantly reduced lung concentration of IL-1β (by 31% and 43%, respectively) and TNF-α (by 35% and 37%%, respectively), but did not induce changes in IL-10 levels. These same treatments also decreased the levels of IL-6 at 65% and 73% in BAL from emphysematous mice. **Conclusion**: These results demonstrate that methyl cinnamate inhibited the airway inflammation and ameliorated elastase-induced lung injury, which shows its potential to be a therapeutic drug for emphysema. **Financial Support**: CNPq, CAPES.

04.037 Role of estradiol and progesterone on allergic lung inflammation previously induced in female obese mice. Umana ERP[1], Oliveira MA[1], Ribeiro MR[1], Moriya HT[2], Alves VF[1], Rigonati CA[1], Gama P[1], Lima LS[1], Scavone C[1], Oliveira-Filho RM[1], Vasquez YR[3], Lima WT, Prado CM[2] [1]ICB-USP, [2]USP, [3]King´s College

Rational: Obesity and sex hormones fluctuations modulate asthma symptoms. Clinical evidence show that post-menopause may account for worse asthma symptoms. However, experimental data investigating the role of reduction of female sex hormones in asthma models in obesity conditions are not totally established. **Methods**: Obesity was determined in female Balb/c mice, upon high fed diet for 10 weeks or with conventional diet. Only obese mice were rendered allergic by sensitization with Ovalbumin (OVA) + Alumen and challenged 3 weeks later (OVA, 3 consecutive days). Elapsed 7 days of the last OVA-challenge, the ovariectomy (OVx) was performed and the allergic obese mice were OVA-rechallenged 10 days later. After 24 h of last rechallenge, the magnitude of cell migration into the lung was measured in bronchoalveolar lavage (BAL x 10^4cells/ml); FlexiVent was used to quantify airways (Rn) and parenchyma (G) resistance caused by methacholine (Mch) x cm/H_20/ml. (by intravenous route; Serum levels of IL4 and IL10 were quantified (Milliplex® MAP kit (Luminex) x ng/Ml., and lung tissue was prepared to histological analyses. Estradiol (OVx+E, 280µg/kg) or Progesterone (OVx+P, 200µg/kg) were given to obese OVx allergic mice 4 h before each OVA-rechallenge. As control were also used Sham-OVx allergic mice (Sham). Data are expressed as mean ± SEM, and were analyzed by two–way ANOVA followed by Bonferroni post-test (Graphpad Prism 7.0; n=9 *p<0.05). **Results**: BAL of OVx allergic obese mice increased the total cells relative to Sham (Sham: 73.67±13.1 vs OVx:149.8±13.03*); Eosinophils (Sham:43.05±11.24 vs OVx:115.7±12.3*). Estradiol and Progesterone treatments of OVx allergic obese mice reduced the number of total cells (OVx: 115.7±12.3 vs OVx+E:51.7±10.6* vs OVx+P:79.9±7.8*) and those of eosinophils (OVx: 115.7±12.3 vs OVx+E:22.59±5.1* vs OVx+P: 42.47±7.2*) recovered in the BAL. Relative to Sham OVx, the lung mechanics of OVx allergic obese mice increased the airways (Rn) (Sham:2.6±0.2 vs OVx:3.8±0.4*) and parenchyma resistance (G) (Sham:10.24±1.1vs OVx:15.8±1.4*). Estradiol and progesterone decreased the Rn and G (Ovx:3.8±0.4 vs OVx+E:2.97±0.4* vs OVx+P:2.0±0.3*) and (Ovx:15.8±1.4 vs OVx+E:12.13±1.5* vs OVx+P:10.4±1.5*). Histological and morphometric analysis of the lung indicated that OVx allergic obese mice increased the mucus secretion (Sham:56.7±1.9 vs OVx:71.2±2.9*); bronchial smooth muscle (Sham:31.2±11.7; vs OVx:41.8±4.3*) collagen deposition (Sham:52.7±4.9 vs OVx:72.4±2.9*). Estradiol and progesterone treatments reduced mucus (OVx:71.2±2.9 vs OVx+E:51.6±2.7* vs OVx+P:44.7±2.6*) bronchial smooth muscle (OVx:41.8±4.3 vs OVx+E:10.7±1.0* vs OVx+P:6.98±1.2*) collagen deposition(OVx:72.4±2.9 vs OVx+E:42.9±3.6* vs OVx+P:26.8±3.3*). Increased serum levels of IL-4 and IL-10 but not IL-5 were found in OVx allergic obese mice relative to Sham OVx allergic obese mice (IL-4: Sham:3.01±0.54 vs OVx:8.01±1.8*) and IL-10: Sham:5.07±0.77vs OVx:25.13±5.1*). IL-4 levels were reduced by estradiol (OVx+E:1.7±0.3*) and progesterone (OVx+P:1.9±0.1); IL-10 levels were also modified by estradiol (OVx+E:11.44±3.6*) and progesterone (OVx+P:7.09±0.8*)**. Conclusion**: Our data may suggest that the ovaries removal modulate the allergic lung inflammation previously established in obese mice. Estradiol and progesterone exert protective effects on the lung repercussions observed in experimental asthma. **Financial Support**: *FAPESP, CAPES, CNPq.*

04.038 The effect of obesity and female sex hormones on the experimental asthma model with neutrophil predominance. Ribeiro MR[1], Oliveira MA[1], Umaña ER[1], Alves VF[1], Moriya HT[2], Sá-Lima LS[1], Scavone C[1], Riffo-Vasquez YR[1], Oliveira-Filho RM[1], Lima WT[1]
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Rational: In addition to presence of eosinophils, a significant proportion of asthmatic patients reveal a lung inflammatory phenotype where the neutrophils are also detected. Besides, obesity and asthma are related conditions and data of literature suggest that obesity account for neutrophilic asthma. Female sex hormones are involved in asthma induction and post-menopausal women are prone to develop obesity and neutrophilic inflammation. **Objectives**: In this study we have investigated the interaction between obesity and sex hormones on the modulation of an experimental model of neutrophilic asthma in mice. **Methods**: To characterize the obesity, female Balb/c mice, were fed with hyperlipidic diet (HFD) for 10 weeks or with conventional diet. Only obese mice were sensitized with Ovalbumin (OVA) + Complete Freund's adjuvant (sc, Day: 0).OVA challenge (25 µg/i.n.) was conducted at days 21, 22 and 23 after sensitization. Experiments were performed 24 h after the last challenge. Non-sensitized mice were challenged with PBS (Control group). To investigate the role of female sex hormones, challenged mice (1 cycle of challenge) were submitted to ovariectomy (OVx) and re-challenged 7 days later (days: 40, 41, and 42 post sensitization). Intact mice (Non OVx) were used as control and sham-OVx mice were also submitted to re-challenge. Lung inflammation was determined by the quantification of cells recovered in the bronchoalveolar lavage (BAL). Lung mechanics was determined using the FlexiVent equipment and analysed by the measurement of airways resistance (Rn), parenchyma resistance (G) and elastance (H) after methacholine (Mch) intravenous administration. Serum levels of IgE, IgG, estradiol, were determined using Milliplex® MAP kit (Luminex). Data are expressed as mean ± SEM, and were analysed by t Student test or two–way ANOVA followed by Bonferroni post-test (Graphpad Prism 7.0; *p<0.05). **Results**: BAL of OVA-challenged obese mice revealed a significantly increase of total cells (PBS: 33.81±4.27 n=4 vs OVA: 73.53±6.69* n=8 x10^4 cells/ml), neutrophils (PBS: 2.81±0.67 n=4 vs OVA: 33.76±3.15* n=8 x10^4 cells/ml) and eosinophils (PBS: 0.42±0.33 n=4 versus OVA: 20.19±2.35* n=8 x10^4 cells/ml). We also observed a significant increase in serum levels of IgG1 (PBS: 54.97±6.37 n=5 vs OVA: 375.2±36.32* n=10 ng/mL) and IgE (PBS: 9.46±0.82 n=5 vs OVA: 92.46±19.36* n=9 ng/mL). In addition, an increase of Rn to MCh was observed (PBS: 1.40±0.09 n=5 vs OVA: 2.29±0.28* n=8 cm/H_2O.s/mL), whereas G and H did not change. Nevertheless OVx reduced the serum levels of estradiol and the uterus weight, re-challenged OVx and Sham-OVx obese mice did not modify the BAL and lung mechanics parameters here investigated (Sham-OVx 1.52±0.26 n=11 vs OVx 0.74±0.15* n=9 ng/mL) and in the weight (g) of the uterus (Sham-OVx 0.06±0.006 n=8 vs OVx 0.01±0.001* n=5 g). In contrast, obese re-challenged mice exacerbated the lung inflammation and Rn relative those mice submitted to 1 cycle of challenge. **Conclusion**: Our data may suggest that the long-lasting exposure to antigen but not female sex hormones modulate the magnitude of asthma with neutrophil predominance in obese mice. **Financial Support**: FAPESP,CAPES, CNPq.

04.039 Effects of treatment with the PGD2 receptor antagonist TM30089 on mouse allergic inflammation. Tavares EBG, Andre DM, Medeiros ML, Oliveira MG, Antunes E Unicamp

Introduction: Prostaglandin D2 (PGD2) is the main lipid mediator resulting from the actions of cyclooxigenase-2 (COX-2) and PGD2 synthase (PGDS) on arachidonic acid. PGD2 is synthesized primarily in mast cells, but can also be found in Th2 cells and macrophages after antigen exposure. Current studies have linked PGD2 to bronchoconstriction and eosinophilia in asthma (Fajt ML, et al., 2013) via its interaction with CRTH2 receptors (Farne et al., 2016). In this study wefurther explored the effectsof selective CRTH2 antagonism byTM30089 in ovalbumin (OVA)-induced airways inflammation. Specifically,we evaluatedthe cell infiltration in mice treated with the CRTH antagonist TM30089. **Methodology**: C57BL/6 male mice aged 12 were used. Briefly, mice were sensitized with OVA (100 µg, s.c) mixed Al(OH)3 in 0.9% NaCl, at 7 day intervals. Two weeks thereafter, mice were intranasally challenged with OVA (10 µg) for two days. Groups ofchallenged mice were treated twice daily with TM30089 (5 mg/kg, orally, concomitant with the 4-days OVA challenge). At 48 h after the first challenge, the bronchoalveolar lavage fluid (BALF) was performed, and lungs were collected for morphological studies and cytokine measurements. **Results**: In OVA-challenged mice, TM30089 significantly decreased the infiltration of eosinophils in the peribronchiolar compartments by about of 60% ($p<0,05$), and reduced the eosinophil peroxidase (EPO) activity in the lung tissues. Treatment with TM30089 reduced by 50% ($p<0.05$) the levels of IL-5 in BALF without affecting the eotaxin levels. **Conclusion**: We show here that TM30089 display an anti-inflammatory effect in airways of allergic mice. **Financial Support**: CNPqThe experimental protocols have been approved by the Ethics Committee of UNICAMP (No. 4630-1). **References**: 1. Fajt ML, J Allergy Clin Immunol. 131:1504, 2013, 2. FarneH, ExpertOpinEmerg Drugs; 21: 359, 2016. 3. Mosen DM, J Allergy Clin Immunol. ;122: 507, 2008.

04.040 Protease-activated receptor (PAR)2 mediates LPS-induced pro-inflammatory repertoire in murine macrophages. Barra A, Brasil AF, Florentino RM, Leite MFL, Capettini LSA, Klein A UFMG

Introduction: PAR2 is a GPCR activated through the proteolytic cleavage of its extracellular N terminus by trypsin, tryptase and other proteases, and it is expressed on the surface of inflammatory cells. It has been suggested PAR2 plays a role in innate immune response. However, the role for PAR2 in macrophage activation is not fully understood. Here, we investigated the impact of PAR2 activation on pro-inflammatory repertoire of murine macrophages. **Methods**: Thioglycollate-elicited peritoneal macrophages from C57BL/6 mice were co-incubated with lipopolysaccharide (LPS,100ng/mL) and/or PAR2 agonist SLIGRL-NH_2 (SLI, 30µM). In some assays the cells were preincubated with PAR2 antagonist ENMD1068 (ENMD,1µM) 1 hour prior to lipopolysaccharide (LPS,100ng/mL) stimulation. For phagocytosis assay was also performed the incubation of zymosan (Zy, 10 particles/cell) for 1 hour. The results were assessed as percentage of phagocytic cells (PP), mean number of particles per cell (MNP) and as phagocytic index (PI) determined as PI = PP x MNP. Nitric oxide (NO) was measured in supernatants through Griess Method and reactive species oxygen (ROS)measured bychemiluminescence.IL-12 and CXCL2 were measured in supernatants through ELISA. Analysis of intracellular Ca^{2+} were obtained by using Fluo-4AM probe. The TLR4 and PAR2 expression was determined by immunofluorescence. Protocols were approved by Local Ethics Committee CEUA-150/2017. **Results**: ENMD impaired PI in LPS-stimulated macrophages (DMEM, 1.4±0.1; LPS, 1.9±0.1; ENMD + LPS, 0.9**±0.07), LPS increased SLI-induced PI(DMEN, 0.2±0.06; SLI, $1.1^{\&\&}$±0.05; LPS, $1.2^{\&\&}$±0.05; LPS+SLI, 1.6***±0.1), and SLI potentiates both NO production (DMEM, 1.8±0.06; SLI, 1.5±0.2; LPS, $6.5^{\&\&}$±0.28; LPS + SLI, 9.0***±0.6), and ROS in LPS-stimulated macrophages (DMEM, 1.5±0.04; SLI, 1.6±0.04; LPS, $2.0^{\&}$±0.03; LPS + SLI, 2.6*±0.2, RLU x 10^4). PAR2/TLR4 co-stimulation increased intracellular Ca^{2+} releasing, and PAR2 blockade prevented IL-12 production 4h after LPS(DMEM, 13.0±1.9; LPS, $30.6^{\&\&}$±1.4; ENMD + LPS, 17.4**±2.4x ng/mL),CXCL2 production 24h after LPS (DMEM, 4.3±0.1; LPS, $59.0^{\&\&\&}$± 0.2; ENMD + LPS, 3.0***±0.1xng/mL), SLI or LPS increased PAR2 expression (SLI, $330.6^{\&\&\&}$±20.2; LPS, $328.6^{\&\&\&}$±20.8; LPS + SLI, 171***±16.2, % of DMEM), andSLI increased TLR4 expression (SLI, $260.7^{\&\&\&}$± 18.9; LPS, 124.8±11.7; LPS + SLI, 19.1***±3.4, % of DMEM). $^{\&}p<0.05$, $^{\&\&}p<0.01$ and $^{\&\&\&}p<0.001$ when compared to DMEM and $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ when compared to LPS. Statistical analyses were performed using one-way ANOVA followed by Tukey post-test. **Discussion**: Our data demonstrate that PAR2 potentiates the activation of macrophages mediated by LPS, playingan relevant role on important hallmarks of inflammation such as macrophage phagocytosis, NO, ROS, pro-inflammatory cytokines production and cellular activation. **Conclusion**: PAR2 plays a pivotal role on pro-inflammatory repertoire of macrophages, suggesting that pharmacological blockade of PAR2 may bea new approach to the treatment of inflammatory diseases where macrophage activation is crucial to the progress of these diseases.

04.041 Inflammatory profile of first-episode psychosis: **Preliminary findings on the interaction between cannabis consumption and childhood maltreatment**. [1]Corsi-Zuelli F, [1]Marques L, [1]Roza DL, [1]Shuhama R, [1]Loureiro C, Menezes PR[2], [1]Louzada-Júnior P, [1]Del-Ben CM [1]FMRP-USP, [2]FM-USP

Introduction: Cannabis consumption and childhood maltreatment arethe main environmentalrisk factorsassociated with an increased incidence of psychosis. We have previously shown an association between childhood maltreatment and increased levels ofTGF-β in the blood of first-episode psychosis (FEP) patients(Corsi-Zuelli *et al.* 2019; *Psych Med, ahead of print*); nonetheless, no association was found for other cytokines. We now investigate: a) the synergistic effect between cannabis consumptionand childhood maltreatment on plasma cytokines (IL-1β, IL-6, TNF-α, IL-4, IL-10, TGF-β)for increased risk of psychosis, while adjusting for potential confounders (gender, age, ethnicity, years of study, body mass index, tobacco smoking,other recreational drugs); b) the interaction between frequency of cannabis (every day, less than every day, non-user), number of childhood maltreatment (one, two or more, none) and inflammatory pattern (high, low) on cytokines mean levels. **Methods:** This study is part of the STREAM study, conducted in Ribeirão Preto/SP (Brazil), which is part of the multicentre international consortium named EU-GEI. We included 153 patients and 256 community-based controls. Plasma cytokines (pg/mL) were measured using Multiplex and all participants answered the Cannabis Experience Questionnaire and the Childhood Trauma Questionnaire. To investigate the synergistic effects between the cannabis and childhood maltreatment on the increased risk of psychosis, the sample was divided into high- (upper quartiles) and low- (lower quartiles) inflammation, and the odds ratios (OR) and 95%CIs were calculated through logistic regression. Interaction between frequency of cannabis, number of childhood maltreatment and inflammatory pattern was analysed by adjusted ANCOVA**. Results:** A synergistic effect between cannabis consumption and childhood maltreatment was observed for IL-10 and TGF-β. Patients in the upper quartile for IL-10 and TGF-β had almost 6-times and 4-times, respectively, increased odds for psychosis (IL-10 adj: OR=5.9;95%CI=1.7-21.0; TGF-β adj:OR=4.2;95%CI=1.0-17.8). We also observed a significant interaction between frequency of cannabis, number of childhood maltreatment and inflammatory pattern for patient's TGF-β mean blood levels (adj. $F_{(11,129)} = 2.265$; $p = 0.015$)**. Conclusion:** Our results extend our previous findings, suggesting thatchildhood maltreatment interact synergistically with cannabis consumption to increase risk of psychosis**.**

Financial support: FAPESP (2012/05178-0; 2016/12195-9; 2017/17480-6); CNPq (476945/2012-7); and CRID (2013/08216-2). **Corsi-Zuelli F et al** (2019). Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood maltreatment. *Psychological Medicine*, 1–9.

04.042 Paraprobiotics prevent the development of irinotecan-induced intestinal mucositis in mice. Nobre LMS, Cajado AG, Lopes MHS, Ribeiro LR, Geraix J, Wong DVT, Lima-Palheta Júnior RC UFC

Introduction Intestinal Mucositis (IM) is a common side effect of irinotecan, a drug used in the first-line treatment regimens for colorectal cancer. Over the last decades, many pathogenic mechanisms of IM have been described, such as the activation of Toll-like receptors. However, no treatment used in the clinic prevents or effectively reduces such side effect. This study aimed to investigate the effect of bacterial lysates (paraprobiotics) during the development of IMand compare with the effect of a probiotic, since they are the most used by patients. **Methods** C57BL/6 female mice (18-22g) were divided into groups (n = 6-8) and pretreated with paraprobiotic Amazon® (Enterococcus faecalis, $30x10^6$ UFC/mice) or probiotic Med Lan® (Enterococcus faecalis and Bifidobacterium $30x10^6$ UFC/mice) 7 days before the first dose of irinotecan (75 mg/kg, i.p. for 4 days)or saline (5 mL/kg, i.p.). The animals were analyzed daily for diarrhea, survival and were euthanized on day 7. Blood samples were collected for the total leukocyte count. Following animal euthanasia, ileum samples were harvested for myeloperoxidase assay (MPO), histopathology, cytokine levels (pg/mg tissue) and qPCR. ANOVA/Bonferroni, Kruskal-Wallis/Dunn's or Log-rank tests were used for the statistical analysis. $P<0.05$ was accepted (CEUA: 4115280219). **Results** Irinotecan induced a pronounced leukopenia (1.907,0 ± 209,7 leukocytes x 10^3/µL), intestinal damage detected by reduced villus/crypt ratio (1.470 ± 0.053) and inflammatory response (5.699 ± 1.534 neutrophils/mg of tissue) versus saline group (4.125,0 ± 634,3 leukocytes x 10^3/µL; 2.176 ± 0.077; 1.987,0 ± 703,8 neutrophils/mg of tissue, respectively). Besides, both para- and probiotics significantly reduced the neutrophil infiltration (868 ± 333 and 1.235 ± 212 neutrophils/mg tissue, $P<0,05$) and enhanced the villi/crypt ratio (2.233 ± 0.069; 2.136 ± 0.072, $P<0,05$), when compared with irinotecan. Levels of anti-inflammatory cytokine IL-10 were decreased by irinotecan (15.5 ± 2.03 versus 46.8 ± 10 pg/mg of tissue from saline group) and sustained by para- and probiotics (20.7 ± 2,14; 24.5 ± 3.8 pg/mg of tissue). Irinotecan also increased the expression of IL-18 and TLR-4 (1.004 ± 0.15 and 1.9 ± 0.32, respectively), versus saline (0.1088 ± 0.106 and 0.999 ± 0.161, respectively), which were significantly reduced by paraprobiotics (0.474 ± 0.188; 1.04 ± 0,07; $P<0,05$). **Conclusion** Pretreatment with paraprobiotics a meliorates irinotecan-induced intestinal mucositis in mice with an similar effect to treatment with probiotic. **Financial Support**: CNPq, Capes and Funcap.

04.043 Antitumor effects of macrophages activated by non-cytotoxic sulfated polysaccharides from brown algae *Dictyota caribaea*. Assef ANB[1], Celestino RCA[2], Santos GRC[1], Mourão PAS[2], Cinelli LP[2], Wilke DV[1], Teles FB[1] [1]UFC, [2]UFRJ

Introduction: The tumor microenvironment (TME) adds another layer of complexity for the research of neoplasias. TME is composed by neoplastic and normal cells, plus a miriad of cytokines, that favors tumor growth and increases the aggressiveness of the disease. Among the non-tumor cells of TME, macrophages (MΦ) are very important ones and depict high phenotypic plasticity. Depending on the stimuli, they can play anti-tumor or pro-tumor roles. The classical activated MΦ (M1) depict anti-tumor capabilities. Modulation of naive MΦ (M0) to M1 is interesting strategie to improve polychemotherapy of malignant tumors. Sulfated polysaccharides of Dictyota caribaea were able to inhibit tumor growth in vivo and can activated immune responses. This work aimed to evaluate the stimulatory and antitumor potential of macrophages stimulated with sulfated polysaccharides from brown algae Dictyota caribaea in vitro. **Methods:** Crude extract of SP from D. caribaea was obtained by proteolytic digestion and the supernatant was precipitated with increasing concentrations of ethanol yielding 5 fractions named DCA. The screening of M1 polarization induced by DCA fractions was performed by indirect NO releasing through Griess assay after treatments of 24 or 48h. The antiproliferative effect of SP-DCA fractions and supernatants (Sn) of RAW 264.7, a murine MΦ cell line stimulated with saline (SnC-), LPS (SnC+) or DCA (SnDCA) was, initially evaluated against a metastatic melanoma (B16-F10 cell line) by the SRB assay after 48h incubation. The production of proinflammatory cytokines by stimulated RAW264.7 was evaluated after 48h of treatment. The morphology and integrity parameters of B16-F10 treated with the stimulated macrophage supernatants were also analyzed. **Results**: None of the SP-DCA inhibited melanoma proliferation until 250 ug/mL, nevertheless all SP-DCA activated the MΦ and the Sn of MΦ treated with SP-DCA inhibited B16-F10 growth. DCA F9 fraction was chosen for further studies, among other DCAs, due to its highest yield and better antiproliferative effect of Sn. Treatment of MΦ with DCA F9 increased TNF-α releasing measured by ELISA. Treatment of B16-F10 cells with SnDCA F9 decreased cell counting and caused morphological changes as cell shrinkage and increasing of granulosity without plasma membrane damage. Since we also tested the nitrite antiproliferative effect and it did not inhibit the B16-F10 cells growth, the antiproliferative effects of SnDCA F9 could be due to other cytotoxic substances produced by RAW264.7 cells, as TNF-α. **Conclusion**: In summary, we demonstrated that SP-DCA induced antitumor MΦ activation. Studies are ongoing to further characterize the phenotype of MΦ as well the mechanism of antitumor effect of SnDCA F9.Acknowledgments: INCT BioNat/CNPq, CAPES and FUNCAP.

04.044 Role of toll-like receptor (TLR)3 in lung fibrosis triggered by silica particles in mice. Sa YAPJ[1], Ferreira TPT[1], Ribeiro NBS[1], Correa AMC[1], Oliveira TAL[1], Alves-Filho JCF[2], Hogaboam C[3], Martins MA[1], Martins PMRS[1] [1]Fiocruz, [2]FMRP-USP, [3]Cedars-Sinai
Introduction: Silicosis is an occupational disease, associated with overexposure to inhaled crystalline silica particles. It is characterized by a marked tissue granulomatous fibrotic response, which paralleled to decreased lung function. There is no effective treatment to silicosis and identification of therapeutic targets is urgently needed. Toll like receptors (TLRs) are shown to exert a pronounced effect on fibrosis. TLR3 has been shown to act as a sensor of cell death by means of intracellular dsRNA. As silica particles can induce cell death during inflammation, this study was undertaken to investigate the role of TLR3 in the lung fibrosis triggered by silica particles in mice. **Methods**: Silica particles (10 mg/50 µL) or saline (control) were instilled by intranasal route into C57BL/6 ($TLR3^{+/+}$) and knockout mice ($TLR3^{-/-}$), and the analyses performed at 28 (late phase) days post-stimulation. The parameters included: lung function and airways hyper-reactivity (AHR) to methacholine (invasive whole-body-plethysmography and morphology/morphometry by classical hematoxylin & eosin and picrus sirius staining, respectively). Cytokines were quantitated by ELISA. The reactivity of lung fibroblasts was evaluated *in vitro,* when the cells were stimulated with IL-13 (40 ng/mL) or Poly(I:C) (10 µg/mL), a synthetic TLR3 agonist. Proliferation was evaluated by BrdU incorporation. All experimental procedures were approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA L-057/14). **Results**: We showed that $TLR3^{+/+}$ silicotic mice developed a marked inflammatory response, with granulomatous formation and fibrotic tissue, at 28 days post-silica. Basal levels of airway resistance and dynamic elastance were increased in the silicotics compared to control mice. Methacholine inhalation led to exacerbation of airway resistance and elastance indicating a condition of AHR. $TLR3^{-/-}$ mice showed restoration of normal lung function (resistance and elastance) and reduction of AHR. Lower levels of profibrotic cytokines (MCP-1 and IL-13) was also noted in $TLR3^{-/-}$ mice. Polarized light microscopy revealed a decrease in the number of silica particles in the lungs of $TLR3^{-/-}$ mice, suggesting draining of the particles to lymphatic system. In another set of experiments, cultured lung fibroblasts showed an increase of MCP-1 levels after stimulation with IL-13 (40 ng/mL) and Poly(I:C) (10 µg/mL). IL-13 also induced fibroblast proliferation, although no effect was noted in the case of Poly(I:C). **Conclusion**: Our data show the refractoriness of $TLR3^{-/-}$ mice to silica-induced inflammation and fibrosis, including AHR, suggesting a causative link among these events in experimental silicosis. In addition, TLR3 might be considered as a potential therapeutic target in the case of fibrotic diseases such as silicosis. More experiments are needed to clarify better the mechanism associated with TLR3 involvement in fibrosis. **Financial Support**: FIOCRUZ, CAPES, CNPq, FAPERJ.

04.045 The H3/H4 receptor antagonist LINS01007 attenuates the effects of lung allergic response in murine model. Balbino AM, Fernandes JPSF, Corrêa MF, Lippi BK, Fernandes GAB, Negreiros NGS Unifesp-Diadema

Introduction: H_3 and H_4 receptors are found in CNS and immune cells, respectively. H_4R seems involved with pulmonary allergic inflammation. 5-Substituted 1-[(2,3-dihydro-1-benzofuran-2-yl)methyl]piperazines (LINS01 series) molecules were synthesized. LINS01007 (5-chlorinated *N*-methyl compound) showed increased affinity for H_4R (pKi 6.06) and showed antagonistic activity (*Corrêa MF, Front. Pharmacol. 8:825, 2017*). In this study, the anti-inflammatory effects of histamine H3R/H4R antagonists were compared to dexamethasone, in a murine asthma model. **Methods**: Dexamethasone (5mg/Kg) and histamine H_3R/H_4R antagonists LINS1007 (1mg/Kg, 3 mg/kg and 5 mg/kg) were given, i.p., to ovalbumin sensitized mice 30min. before antigen challenge. After 24 h, bronchoalveolar lavage was performed for cell analysis; the blood was collected for IgE production analysis and the lungs were removed for histological study and evaluation of cytokines production, in Balb/C male mice, at 12 weeks old. Animal Research Ethical Committee: CEUA Nº 7601230317. **Results**: Treatment with LINS1007, in dose of 3m/Kg, decreased total cells (107±16.5 to 20.05±6.5 $x10^4$/ml, p <0.05) and eosinophils (66.38± 20.7 to 18.25±5.2 $x10^4$/ml, p <0.05) similarly to dexamethasone. In addition, this treatment decreased IgE production, inflammatory cytokines (IL5, IL6, TNF□□□IL13) and *RANTES,* collagen and mucus production. **Conclusion**: Our results showed that LINS1007 attenuated the effects of lung allergic response similarly to observed in dexamethasone treatment. **Financial support**: FAPESP (2017/05441-6, 2017/02042-3, 2019/05242-9) and CNPq.

04.046 Insulin modulates pulmonary allergic inflammation and increases pulmonary resistance in diabetic mice. Martins JO[1], Ferreira SS[1], Oliveira MA[2], Tsujita M[1], Casagrande FB[1], Gomes E[2], Russo M[2], Lima WT[2], Nunes FPB[1] [1]FCF-USP, [2]ICB-USP

Introduction: Reports have shown that the onset of diabetes mellitus (DM) in patients previously diagnosed with asthma decreases asthmatic symptoms, whereas insulin aggravates asthma. The present study evaluated the modulatory effect of insulin on the development of allergic airway inflammation in diabetic mice. **Methods**: To evaluate the effects of relative insulin deficiency, an experimental model of diabetes was induced by a single dose of alloxan (50 mg/kg, i.v.)[CEUA/FCF/USP 490/20154].After 10 days, the mice were sensitized with ovalbumin [OVA, 20 µg and 2 mg of $Al(OH)_3$, i.p.]. A booster immunization was performed 6 days after the first sensitization [20 µg of OVA and 2 mg of $Al(OH)_3$, i.p.]. The OVA challenge (1 mg/mL) was performed by daily nebulization for 7 days. Diabetic animals were treated with multiple doses of neutral protamine Hagedorn (NPH) before each challenge with OVA. The following parameters were measured 24 h after the last challenge: a) the levels of p38 MAP kinase, ERK 1/2 MAP kinases, JNK, STAT 3 and STAT 6 in lung homogenates; b) the serum profiles of immunoglobulins IgE and IgG1; c) the concentrations of cytokines (IL-4, IL-5, IL-10, IL-13, TNF-α, VEGF, TGF-β and IFN-γ) in lung homogenates; d) cells recovered from the bronchoalveolar lavage fluid (BALF); e) the profiles of immune cells in the bone marrow, lung, thymus, and spleen; and f) pulmonary mechanics using invasive (FlexiVent) and non-invasive (BUXCO) methods. **Results**: Compared to nondiabetic OVA-challenged mice, OVA-challenged diabetic animals showed decreases in ERK 1 (2-fold), ERK 2 (7-fold), JNK (phosphor-54) (3-fold), JNK/SAPK (9-fold), STAT3 (4-fold), the levels of immunoglobulins, including IgE (1-fold) and IgG1 (3-fold), cytokines, including Th2 profile cytokines such as IL-4 (2-fold), IL-5 (2-fold), IL-13 (4-fold), TNF-α (2-fold), VEGF (2-fold), and TGF-β (2-fold), inflammatory infiltrates (14-fold), T cells, NK cells, B cells and eosinophils in the bone marrow, lung, thymus and spleen, and airway hyperreactivity. STAT6 was absent, and no eosinophilia was observed in BALF. Insulin treatment restored all parameters. **Conclusion:** The data suggested that insulin modulates immune cell phenotypes and bronchial hyperresponsiveness in the development of allergic airway inflammation in diabetic mice. **Acknowledgments:** (2017/11540-7) Sao Paulo Research Foundation (FAPESP); (301617/2016-3) National Council for Technological and Scientific Development (CNPq; PQ-1D).

04.047 Experimental model of zymosan induced-arthritis without anesthesia in mice.
Soares DM[1], Gomes RO[1], Melo MCC[2], Santana Júnior JCV[1] [1]UFBA, [2]USP
Introduction: Arthritis is a clinical manifestation present in chronic inflammation diseases, as autoimmune profile, as well as in Rheumatoid Arthritis (RA). That is very relevant because its progression leads to loss of tissue function and bone destruction. The animal model of zymosan induced arthritis (ZIA) is known to involve production of cytokines, chemokines, lipid metabolites and reactive oxygen species (ROS), besides indirect activation of the complement and Fc receptors by the complement proteins and opsonization of the antibody, respectively. Isoflurane (ISO) exerts anti-inflammatory in multiple physiological mechanisms. Inhalation of ISO elicits protective effects during zymosan-induced lung injury, previous studies have revealed that ISO reducing cytokine release from alveolar macrophages, neutrophil recruitment and microvascular protein leakage1. However, the protective effects of ISO in ZIA remain unclear. Objective: Considering the use of anesthesia as a strategy to apply the intra-articular injection technique, we sought to standardize a new model of animal immobilization for the ZIA model and assessment the use of isoflurane through inflammatory markers. Materials and **Methods:** For the experiments, male mice of the Swiss lineage were used. (approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine of UFBA, under protocol n°. 58/2017). The animals were submitted to anesthetic and immobilization before induction of arthritis with zymosan (intra-articular injection) and naïve group received saline injection. The swelling of the knee joint was assessment by measuring the transverse diameter of the left knee using a digital caliper, two, four and six hours after ZIA. The leukocyte migratory profile was evaluated by cellular lavage with counting in the Neubauer chamber. The data were treated using the ANOVA methodology. **Results**: In this study, ISO did not reduce neutrophil migration but significantly reduced ($p < 0.05$) joint edema characteristic of excessive cytokine and ROS production.The variation of the joint diameter in the groups submitted to different agents (zymosan/saline) was significantly different from animals submitted to immobilization/anesthesia (ISO) (zymosan: 1,25 /0,68) and (saline: 0,53/0,29). It was not observed in the leukocyte migration (zymosan:127,6/124,3. 109) and (saline: 6/3,3. 109)mean values +/- EPM**. Conclusion**: These preliminary results suggest that ISO reduced the clinical manifestations caused by ZIA. This may affect the inflammatory/anti-inflammatory response of experimental models used to identify both the inflammatory mediators involved in the symptoms of RA and the search for new drugs. The reduction of joint edema and leukocyte migration is observed as important characteristic for the improvement of signs and symptoms/resolution of inflammation, respectively.
Reference: 1. Wang et al. Mediators of Inflammation. 1-14 (2013).
Support: CNPq e FAPESB.

04.048 AT1 Receptor blockade attenuates tissue destruction and bone volume loss in rats with experimentally-induced periodontitis. Ferreira C[1], Dionisio TJ[1], Souza GP[1], Colombini-Ishikiriama BL[1], Garbieri TF[1], Parisi VA[1], Oliveira GM[1], Oliveira SHP[2] [1]USP, [2]FOA-Unesp

Introduction: The renin-angiotensin system (RAS) is known for its role in cardiovascular regulation, but it has been implicated as an important factor in inflammatory processes. The aim of this study was to evaluate the effect of AT1 receptor blockade on the progression of experimentally-induced periodontitis (EP) in rats, by histological evaluation and bone loss analysis. **Methods**: Ethics Committee on Animal Research at Bauru School of Dentistry/USP (Permit Number: 020/2016) approved this study. After anesthesia, silk suture thread (4.0) was placed around the lower right first molar. This method is well established and is capable of causing alveolar bone loss in addition to compromising adjacent supporting tissues. Animals remained with the silk suture around the tooth for 1, 3, 7 and 14 days. For bone analysis, CT scans were performed on the mandibles. Bone volume, as well as the quantity, size and distance between the bone trabeculae were measured. Besides treatment with water and losartan (50 mg/kg/day) on the same day of EP induction, groups of animals were previously treated with the same drug and at the same dosage for 30 days. Therefore, the present study contained 4 groups with 5 animals in each group: G1 - control without EP; G2 - animals with EP and treated with water; G3 – losartan-treated animals (treatment started in the same day of EP induction) and G4 - animals previously treated with losartan for 30 days followed by induction of EP and continuity of treatment. **Results**: Histological analysis revealed that EP favors infiltration of inflammatory cells and destruction of junctional epithelium, cementum and alveolar bone crest. Abundant presence of polymorphonuclear leukocytes, alveolar bone crest resorption and apical migration of junctional epithelium characterizing tissue destruction were detected. On the other hand, animals pretreated with Losartan (G4) showed significantly lower scores, similar to control samples, which indicates improvements in evaluated parameters, i.e., tissue integrity maintenance. Tomographic analysis in animals' mandibles, affected by EP, revealed bone loss volume, increase number and thickness of bone trabeculae, and decrease separation of trabeculae, which characterized a fragile and porous bone with larger and near trabeculae and recurrent dental mobility. On the other hand, animals with EP pretreated with Losartan, showed significant improvements in these parameters, i.e., a more resistant bone with smaller trabeculae without dental mobility. **Conclusion**: The present results support the conclusion that AT1 receptor modulates EP progression. **Financial support**: São Paulo Research Foundation (FAPESP 2015/03965-2).

04.049 Effect of Patchouli (*Pogostemon cablin*) **essential oil on the zymosan-induced arthritis model**. Silva-Filho SE[1], Maranhão IF[1], Hamaji MP[1], Cardia GF[2], Wiirzler LA[2], Silva-Comar FM[2], Bersani-Amado CA[2], Cuman RKN[2] [1]UFMS, [2]UEM

Introduction: The events associated with the inflammatory response are complex and involve a number of factors that lead to signs such as: pain, heat, edema, flushing and loss of function. Chronic inflammation is characterized by being persistent, whether for weeks or even years. The Pachouli essential oil (PEO) is obtained from the leaves of *Pogostemoncablin*. Some works with extracts of the plant and some of the isolated constituents of the PEO presented activity on the inflammatory response. In this work, we investigated the PEO effect in experimental arthritis model in mice. **Methods**: Briefly, 200 µg/cavity of zymosan in 10 µl sterile saline was prepared. The right knee joints of the animals were then intra-articularly injected with zymosan, and the contralateral knee joint was injected with an equal volume of saline. The migrated leukocytes and differential counts were performed 7 days after zymosan-induced arthritis. For this, the animals were treated before zymosan injection and once a day, for 7 days, with vehicle, PEO at different doses (50, 100, or 200 mg/kg) or dexamethasone (reference drug) dissolved in vehicle. Histological analysis also was performed. For this, the right knee joint was demineralized, subjected to dehydration process, diaphanized, and embedded in paraffin. The samples were then serially sectioned using a rotary microtome (Leica RM2245). All of the sections were stained with Harris' hematoxylin and eosin and examined under a microscope (Olympus BX41; original magnification, 400x). The histological sections from each group were examined using a gradient scale of 0-3 according to the proportion of infiltrated cells: 0 (infiltrated cells equivalent to normal), 1 (poorly infiltrated cells), 2 (moderately infiltrated cells), 3 (densely infiltrated cells)**. Results**: The daily treatment with PEO at doses of 100 and 200 mg/kg reduced the influx of leukocytes into articular cavity (28.7 and 34.5%, respectively) when compared the animals treated with only vehicle, a similar effect was also observed with dexamethasone treatment. The decrease in the number of leukocytes was mainly attributable to a reduction of the number ofpolymorphonuclear leukocytes. In histological analysis, an increase in cell infiltration in the synovial membrane was observed in the arthritic animals compared with control animals. Furthermore, oral treatment with PEO (100 mg/kg) for 7 days in arthritic animals reduced leukocyte migration in the synovial membrane**. Conclusion**: PEO reduces the leukocyte migration to the articular cavity during a inflammatory process. Further studies are needed to elucidate the mechanism of the action of PEO.**License number of ethics committee**: CEAE/UEM 009/2015. **Financial support**: CAPES and CNPq.

04.050 The effects of aging on the production of hydrogen sulfide (H_2S) in the murine skin. Teixeira SA[1], Gomes GL[1], Rodrigues L[1], Cerqueira ARA[1], Alves KB[1], Oliveira L[1], Nascimento N[1], Silva GB[1], Akamine E[1], Whiteman M[2], Muscará MN[1], Costa SKP[1] [1]USP, [2]University of Exeter

Introduction: Previous evidences from the group (and others) suggest a potential anti-nociceptive, anti-pruritic, and anti-inflammatory employment for hydrogen sulfide (H_2S), a recently described endogenous mediator. The endogenous synthesis of H_2S mainly involves enzymatic pathways depend on cystathionine-gamma-lyase, cystathionine-beta-synthase (CBS) and 3 mercaptopyruvate sulfurtransferase (3MST). H_2S syntesis through CBS and CSE are dependent on co-factor pyrodoxal phosphate (vitamin B6). 3MST enzyme along with cysteine aminotransferase (CAT) uses 3-mercaptopyruvate (3MP) as substrate. Non-enzymatic pathways also participate in H_2S generation, such as the reduction of sulfur to H_2S. However, the amount of H_2S produced by this pathway is smaller when compared to the total H_2S (Wang R, Physiol Rev 92, 791, 2012). On the other hand, the characterization of H_2S production and enzymes involved in peripheral organs (eg.: skin) and other tissues of aging rodents is still unknown. Thus, the objective of this study was to evaluate the production of H_2S and protein expression (CBS, CSE and 3MST) in mice skin of a senescence accelerated model (SAMP8) and resistant (SAMR1; Takeda T, J Am Geriatr Soc 39, 911, 1997). **Methods:** The experimental protocol was approved by the local Ethics Committee for Animal Experimentation (CEUA, ICB/USP; nº 67/2017). H_2S production was measured in skin samples obtained from SAMP8 and SAMR1 at 2, 3, 4, 6 and 10 months was determined by lead acetate method (Hine C, Cell 160, 132, 2015) and protein expression from animals of 3 and 6 months was processed by western blotting. Antioxidant enzyme activities for superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), reductase (GR) and S-transferase (GST) were measured in skin of 6 months. **Results**: A significant increase in H_2S generation was observed in the skin of SAMP8 group when compared to SAMR1 group, at 6 months (0.307 ± 0.017 vs. 0.245 ± 0.006, p□0.001) and 10 months (0.359 ± 0.056 vs. 0.208 ± 0.008, p□0.001), and results were expressed in nmol/min/mg protein. The expression of CBS and CSE enzymes of these animals was not observed to be significant. Interestingly, we found a decrease in 3-MST expression in SAMP8 skin at 6 months (~25%) and antioxidant enzyme activity of catalase, when compared with its respective SAMR1 group (17.1 ± 0.9 vs. 27.2 ± 2.5 Ucat/mg prot, p□0.01). **Conclusion:** The increase in H_2S generation observed in skin of animals with accelerated aging is not accompanied by an increase in the protein expression of enzymes producing H_2S (CBS, CSE and 3MST) and nonenzymatic pathways might be involved and must be further investigated. **Financial support**: FAPESP, CNPq.

04.051 Mitochondrial pyruvate carrier regulates inflammation and fever in rats. Souza FHV, Guimarães NC, Alves DS, Gomes BRB, Vilela WR, Sousa MV, Bem AF UnB

Introduction: Fever is the increase in body temperature regulated by the central nervous system, which occurs as part of the acute phase reaction, in response to infectious and inflammatory processes. The preoptic area (POA) of the anterior hypothalamus is considered the main region responsible for the control of thermoregulatory mechanisms associated with maintenance of body temperature and changes that occur during febrile response. Data from neuroproteomic analysis obtained in our laboratory demonstrated that there is a significant increase in the mitochondrial pyruvate carrier (MPC)-1 abundance in the hypothalamic tissue of animals with lipopolysaccharide (LPS)-induced fever in relation to control animals (Firmino et al, 2018).The MPC-1 is an inner-membrane transporter that facilitates pyruvate uptake from the cytoplasm into mitochondria and UK 5099, the MPC inhibitor, is capable of decreasing pyruvate transport by causing structural modifications in the carrier protein (Corbet, 2018).We therefore investigated the effect of UK 5099in a rat model of fever, on neuroinflammation in LPS-stimulated POA cells, and on mitochondrial function. **Methods**: Female and male *Wistar* rats received intracerebroventricular administration of theUK 5099 (1µg) or vehicle 30 min prior to intravenous injection of LPS (5 µg/kg) or saline and the temperature was monitored during 5 h. We also investigated the effect of the treatment with UK 5099(10, 20, 40 and 80 µM) in POA primary cultures stimulated with LPS (10 µg/ml) for 4h. PBS was used as negative control. After this time, the supernatants were collected for cytokines (TNFα and IL-6)measurements. For the analysis of respiratory capacity of the hypothalamic homogenate, rats received intravenous injection of LPS (5 µg/kg) or vehicle 2,5 h prior the euthanasia and the hypothalamus was dissected. Oxygen consumption was measured byhigh-resolution respirometry using an Oxygraph-2k system, at 37 °C**. Results**: UK 5099 reduced fever in treated animals, in the first peak, around 2,5h after LPS injection.UK 5099 also attenuates secretion of proinflammatory cytokines, TNF-α and interleukin-6 in POA primary cell cultures. All UK 5099 concentrations significantly reduced TNF-α and IL-6 concentrations, with the exception of UK 5099 10 µM that was not able to significantly reduce TNF-α levels. In high-resolution respirometry, the oxygen flux per mass was higher in the hypothalamic homogenate of LPS-treated animals in relation to the control animals, mainly respiration dependent on oxidative phosphorylation and when the mitochondria were uncoupled, both linked to Complex I. This activation in mitochondrial energetic activity is attenuated when the hypothalamic homogenate was preincubated with UK 5099 1 µM for 5 min at room temperature. **Conclusion**: These results suggest that limiting mitochondrial pyruvate metabolism might have an important anti-inflammatory effect, suggesting that MPC may be a useful therapeutic target in neuroinflammation and fever. **References**: Corbet, Front Pharmacol., v. 8, p. 958, 2018. Firmino et al, J Proteomics, v. 187, p. 182, 2018**. Financial support**: FAP/DF; CNPq.

04.052 *Platymiscium floribundum Vog* **decreases bone degradation and modulates the levels of inflammatory mediators during periodontitis in rats**. Freire JMO[1], Chaves HVC[1], Sousa NA[2], Teixeira AHT[1], Sousa LH[1], Pinto IR[1], Nascimento Júnior MV[1], Costa JJN[1], Portela LR[1], Lima MAS[1], Pimenta ATA[1], Bezerra MMB[1] [1]UFC-Sobral, [2]UFPI

Background: Periodontitis is a chronic inflammatory process that affects the supporting tissues of teeth characterized by extensive alveolar bone resorption. *Platymiscium floribundum* Vog is a common plant in the Brazilian northeast and popularly known as sacambu or jacaranda from the coast and popularly used as anti-inflammatory. This study aimed to evaluate the effectiveness of *Platymiscium floribundum* Vog on periodontitis in rats, investigating the involvement of inflammatory mediators, and the safety of this treatment. **Methods**: The experimental protocol 05/2015 was approved by CEUA-UFC-Sobral.Cytotoxicity analysis was performed in culture of normal fibroblast cells (MRC-5) and human keratinocytes (HACAT) by MTT and clonogenic colorimetric assays. Rats were treated with *P. floribundum* (0.1, 1 or 10 mg/kg) or vehicle 1h before periodontitis-challenge and once daily during 11 days. On the 11th day, rats were euthanized, under anesthesia, and it was harvested the maxillae - for morphometric (ImageJ® software), histopathological (H&E), and Scanning Electron Microscopic analysis (SEM) and by ELISA assay to determine the levels of cytokine and gene expression analysis (qRT-PCR). **Results**: The extract of the *P. floribundum* Vog did not induce cytotoxic activity in the mouse fibroblast (L929) line (IC50 greater than 100.00 µg/ml) and in the keratinocyte line (IC50 greater than 50.00 µg/ml). *Platymiscium floribundum Vog*(10 mg/kg) reduced ($P < 0.001$) alveolar bone resorption(2.80± 0.34 mm^2)compared to the vehicle group (4.63± 0.24 mm^2).These data were confirmed by histopathology analysis of *Platymiscium floribundum* Vog (10 mg/kg) ($p < 0.001$) that showed discrete cell influx, reduction in osteoclast number, cementum and alveolar process well preserved, maintaining regular bone topography, compared to the vehicle group. Further, *Platymiscium floribundum* Vog (10 mg/kg) decreased ($P < 0.001$) TNF-α levels in gingival tissues (3,74±0,42), compared to the vehicle group (6,83 ± 0,36), as well as levels of IL-1β ($p < 0.0000001$), IL-8 ($p < 0.05$) and PGE2 ($p < 0.004$) also in the gingival tissue (4.41 ± 0.29; 185.8 ± 22.9; 2728 ± 938.5, respectively) when compared to the untreated group (9.43 ± 0.42; 342 ± 58.50; 6522 ± 769.8, respectively). Treatment with *P. floribundum* (10mg / kg) increased IL-10 levels ($p<0.02$) (8794 ± 1382) when compared to the vehicle-treated group (3364 ± 1163). *P. floribundum* (10 mg / kg) decreased TNF-alpha, IL-1 beta, COX2, RANK, and RANKLmRNA levels in the gingival tissues when compared to the vehicle group**. Conclusion**: These results suggest that *P. floribundum* may be an effective treatment by reducing the inflammatory process in experimental periodontitis in rats**. Financial Support**: CAPES, CNPq, FUNCAP, INCT-IBISAB, UFC. **References:** 1. BEZERRA et al. Selective cyclooxygenase-2 inhibition prevents alveolar bone loss in experimental periodontitis in rats. J Periodontol. 71:1009, 2000. 2. FALCAO et al., Cytotoxic flavonoids from *Platymisciumfloribundum.* J. Nat. Prod. 68, 423, 2005. 3. LIMA et al., Effects of TNF-α inhibitors pentoxifylline and thalidomide on alveolar bone loss in short-term experimental periodontal disease in rats. J Periodontol. 75(1): 156, 2004. 4. Ribeiro et al.,Tocoyenasellowiana extract decreases bone loss in an experimental model of periodontitis in rats: Putative role for cyclooxygenase-2 and IL-1beta inhibition. Biomed Pharmacother. 2018. 5. Goes et al., Low-dose combination of alendronate and atorvastatin reduces ligature-induced alveolar bone loss in rats. J Periodontal Res 49:45, 2014

04.053 Chitosan-based biomaterials benefit healing of lesions in rats. Pires CS, Souza AH, Gaissler V, Scholl S ULBRA

The healing of chronic wounds is a therapeutic challenge, developing skin ulcers that require a long time of treatment, resulting in high cost with medical care. The use of natural resources is a worldwide trend in the attempt to mimic an original cutaneous matrix that allows the migration, proliferation and cellular organization. The biomembrane formed by chitosan has shown to be promising.With the properties of accelerating the healing process by activating macrophages, increasing the number of fibroblasts, stimulating cell differentiation and skin reepithelialization. The objective of this study was to evaluate and compare the efficacy of different types of Brazilian chitosan biomembranes in the cicatricial process of skin lesions. Wistar rats were submitted to surgical excision under anesthesia (CEUA 2016/132).Tissue repair of a 2 x 2 cm2 wound on the back was measured and evaluated morphologically on days 0, 3, 7, 10 and 14 days. The animals received different treatments: physiological saline, collagenase, biomembrane chitosan salt, acetic nanochitosanbiomembrane and hydrochloric nanochitosanbiomembrane. Macroscopically, contraction, presence of exudate, wound appearance, inflammatory type analysis, neocolagenesiswere evaluated and histological studies were performed. The animals treated with chitosan biomembrane presented better appearance, being significantly better in the biomembrane group of hydrochloric nanochitosan throughout the period. The formation of exudate and the contraction of the wound end were similar in all groups. The evaluation of the type of acute inflammation of the groups with chitosan biomembrane showed better results despite the formation of crust under the membrane, which configured a sero-hematic aspect; despite this alteration, there was no alteration of the cicatricial process. Histological analysis demonstrated that from third day, the group with chitosan biomembrane presented more favorable histological aspects – especiallythe nano-hydrochloric acid group – which prepared and stimulated the subsequent healing phases. Analysis of inflammatory infiltrate demonstrated a significant early migration of polymorphonuclear leukocytes in the chitosan groups, being most important in the hydrochloric nanochitosan biomembrane group. The present study demonstrated that the treatment with chitosan biomembrane – especially the one with nano-hydrochloric acid – stimulated the subsequent phases of healing, making its histological arrangement denser and more consistent. The present study was effective and indicated for traumatic surgical injuries.

References: ABREU-VELEZ, A.M.; HOWARD, M.S. Collagen IV in normal skin and in pathological processes. North American Journal of Medici Science, n, 4, p.1–8, 2012. ADAMS, P.D. Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Molecular Cell, n. 36, p. 2–14, 2009. BELLO, Y. M.; PHILLIPS, T. J. Recent advances in wound healing. Journal of the American Medical Association, v. 283, n. 6, p. 716!718, 2000. BIONDO-SIMÕES, M.L.P.; BARETA, V.C.J.; FERREIRA, L.F.; et al. Efeito do açúcar na cicatrização por segunda intenção: estudo experimental em ratos. Acta Cirurgia Brasileira, v. 61, n. 5, 1991. BOGLIOLO, L. Patologia Geral. Rio de Janeiro: Guanabara Koogan, 2006. CAIADO F.; CARVALHO T.; SILVA F.; et al. The role of fibrin E on the modulation of endothelial progenitors adhesion, differentiation and angiogenic growth factor production and the promotion of wound healing. Biomaterial, n. 32, p. 7096–05,2013. CLARK, R. A. F.; GHOSH, K.; TONNESEN, M. G. Tissue engineering for cutaneous wounds. Journal ofI nvestigative Dermatology, v. 127, p. 1018–1029, 2007. CUNHA, P.; SILVA, A.; ROQUE, O.; et al. Plantas e produtos vegetais em cosmética e dermatologia. Lisboa: Fundação Calouste Gulbenkian, 2004. DE ANGELIS, B.; GENTILE, P.; AGOVINO, A.; et al. Chronic ulcers: MATRIDERM(®) system in smoker, cardiopathic, and diabetic patients. Journal of Tissue Engineering, p. 26-34, 2013. DIEGELMANN, R. F.; DUNN, J. D., LINDBALD, W. J.; et al. Analysis of the effects of chitosan on inflammation, angiogenesis, fibroplasia, and collagen deposition in polyvinyl alcohol sponge implants in rat wounds. Wound repair andregeneration, v. 4, n. 1, p. 48-52, 1996. EHRENREICH, M.; RUSZCZAK, Z. Update on Tissue!Engineered

Biological Dressings. Tissue Engineering, v. 12, n. 9, p. 2407!2424, 2006. GARY!SIBBALD, R.; WOO, K. The biology of chronic foot ulcers in persons with diabetes. Diabetes Metabolism Research and Reviews, v. 24, n. 1, p. 25!30, 2008. HILMI A.B.M.; HALIM A. S.; ASIAH A.B.; et al. Chitosan dermal substitute and chitosan skin substitute contribute to accelerated full-thickness wound healing in irradiated rats. BioMed Research International, v. 2013, 2013. KIM, C.H.;LEE, J.H.; WON, J.H.; et al. Mesenchymal stem cells improve wound healing in vivo via early activation of matrix metalloproteinase-9 and vascular endothelial growth factor. Journal of Korean Medical Science, v. 26, n. 6, p. 726-33, 2011. WALTER, M.N.M.; WRIGHT, K.T.; FULLER, H.R.; et al. Mesenchymal stem cellconditionedmedium accelerates skin wound healing: An in vitro study of fibroblast and keratinocyte scratch assays. Experimental Cell Research, v. 316, n. 7, p. 1271-81, 2010. WANG, L.; KHOR, E.; LIM, L. Y. Chitosan!alginate!CaCl2 system for membrane coatapplication. Journal of Pharmaceutical Sciences, v. 90, n. 8, p. 1134!1142,2001. ZIMMERMANN, M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, n.16, p.109-10, 1983.

04.054 Down regulation of macrophages might contribute to the refractoriness of ACKR2 knockout mice to silica-induced chronic lung inflammation. Correa AMC[1], Dias DF[1], Oliveira TAL[1], Sa YAPJ[1], Simões RL[2], Barja-Fidalgo TC[2], Cyrino FZGA[2], Bouskela E[2], Martins MA[1], Martins PMRS[1] [1]Fiocruz, [2]UERJ

Introduction: Chemokines are principal regulators of leukocyte activation and migration, being considered as important mediators in inflammatory processes. Were ported that atypical chemokine receptor 2 gene (ACKR2$^{-/-}$) deficient mice showed reduction of silica-induced chronic lung inflammation and granuloma. **Aim**: This study was undertaken in order to investigate potential target cells involved in the refractoriness of ACKR2 knockout mice, mainly focusing on macrophages. **Methods**: Both sexes C57BL/6 (ACKR2$^{+/+}$) and ACKR2 knockout mice (ACKR2$^{-/-}$) were used. Were anesthetized and then instilled intranasally with crystalline silica particles (10 mg/50 µL) or saline (control), and the analyzes made 28 days after silica provocation. The parameters included lung tissue morphology/morphometry, total and differential leukocytes of peripheral blood and bone marrow were evaluated in Neubauer chamber and cytocentrifuged smears stained by May-Grunwald-Giemsa, respectively. For *in vitro* systems, peritoneal macrophages or bone marrow derived macrophages (BMDM), stimulated with lypopolissacharide (LPS – 0.1 and 0.5 µg/mL) or silica (12.5 and 125 µg/mL) particles, were used and the generation of cytokine/ chemokine being measured by ELISA. Markers used to identify classically (M1) and alternatively activated (M2) macrophages included TNF-αand CCL17/TARC, respectively. All procedures were approved by the Ethics Committee on Animal Use (CEUA) of Fiocruz in the LW57/14 license**. Results**: We showed that there was no difference in the total and differential leukocyte numbers in peripheral blood and bone marrow, as well as M1 and M2 macrophage subtypes in both compartments when compared wild type (ACKR2$^{+/+}$) and ACKR2$^{-/-}$ mice. Bone marrow-derived macrophages (BMDM), when polarized to M1 and M2 phenotypes, exhibited lower response when exposed to LPS and silica *in vitro,* as attested by reduction in the levels of TNF-alpha release. Also, macrophages recovered from the peritoneal cavity of ACKR2$^{-/-}$ mice were lessen sitiveto LPS and silica stimulation than those from ACKR2$^{+/+}$ animals. Additionally, by means of intravital microscopy of cremaster venules we noted that, under normal condition, peripheral leukocytes from ACKR2$^{-/-}$ mice exhibited less rolling and adhesion to endothelium as compared to those from ACKR2$^{+/+}$ ones**. Conclusion**: In conclusion, our findings show that macrophages from ACKR2 knockout mice were less responsive in *in vivo* and *in vitro* systems, suggesting that they might contribute to the refractoriness of the knockout mice to silica-induced lung fibrosis. More experiments are under way to identify the mechanism involved in this phenomenon.

Financial support: FIOCRUZ, CNPq, FAPERJand CAPES.

04.055 Study of the leishmanicidal activity of *Bauhinia forficata*, *Punica granatum*, *Eugenia uniflora*, **and** *Persea americana*. Nunes ICM, Ferreira SCA, Albuquerque LWN, Alves AA, Moreira MSA, Santos MS, Leite AB, Silva AE, Queiroz AC UFAL

Introduction: Leishmaniasisis a global term for cutaneous and visceral diseases caused by the vector-borne parasites of the genus *Leishmania* (MCGWIRE; SATOSKAR, 2014). It is considered an endemic parasitosis in 100 countries and remains one of the most neglected diseases worldwide, affecting predominantly the poorest, especially developing countries (WHO, 2019).At present, the available treatments are limited and with high toxicity. Thus, this work proposes to evaluate the leishmanicidal activity of plants *Bauhinia forficata*, *Punica granatum*, *Eugenia uniflora*, and *Persea americana*, which are in the National List of Medicinal Plants of Interest to Health Unic System of Brazil (RENISUS)against *Leishmania chagasi* and *Leishmania amazonensis*. **Methods**: For the experiment, the cytotoxicity of the plants was investigated in J774.A1 cell line (macrophages) by the colorimetric test of MTT. Macrophages were infected with the protozoan and subsequently treated with aqueous extractive solution of the plant species or standard drugs such as pentamidine, meglumine antimoniate and miltefosine. The treatment with the standard drugs and medicinal plants was carried out for 48 hours. Moreover, the nitric oxide (NO) production was evaluated in culture supernatant of cells infected with *L. chagasi* and *L. amazonensis* was evaluated. **Results**: The aqueousextractivesolutionsoftheplantsdidnotdemonstratecytotoxiceffectstothe host cell up to the highest tested concentration (100 µg/mL). Also, the plants did not present deleterious effect against amastigotes of the *L. amazonenses* or *L. chagasi*. *P. granatum* and *E. uniflora* demonstrated a significant effect on the increase of NO production in *L. chagasi*-infected macrophages when compared to control. Likewise, *P. granatum* induced the increase of NO production in macrophages infected with *L. amazonensis* when compared to the medium group**. Conclusion**: At the concentrations tested, these plants did not present leishmanicidal activity, however they demonstrated a significant effect on the increase of NO, an important microbicidal agent in host defense. Therefore, new studies with these plants at different concentrations are necessary to ensure a better understanding of the pharmacological action of these plants. **Reference**: MCGWIRE, B.S.; SATOSKAR, A.R. Leishmaniasis: clinical syndromes and treatment. Q.J.M., v. 107, p. 7-14, 2014. OMS. Diponível em: <http://www.who.int/leishmaniasis/en/>. Acesso em: 09 de junho de 2019. **Funding**: CNPq , UFAL, INCT-INOFAR (573.564/2008-6), FAPEAL (PRONEM 20110722-006-0018-0010), Decit-SCTIE-MS/FAPEAL/SESAU-AL (PPSUS 60030 000820/2016). Keywords: Leishmanicidalactivity; Medicinal plants; *Leishmania.*

04.056 Repeated exposures to polyinosinic-polycytidylic acid, **a Toll-like receptor 3 agonist**, **causes glucocorticoid-insensitive airway hyper-reactivity and inflammation in A/J mice**. Procópio CAM, Santana ACC, Sa YAPJ, Nascimento ALD, Gomes HS, Coutinho DS, Ferreira TPT, Martins PMRS, Martins MA Fiocruz

Introduction: Asthma and COPD exacerbation by viral infection usually denotes worsening of symptoms and lung inflammation, in association with loss of sensitivity to glucocorticoid therapy. Since prior investigations demonstrate that the polyinosinic-polycytidylic acid (Poly(I:C))can mimic several pivotal aspects of lung inflammation caused by viral infection, we have done investigations into the efficacy of glucocorticoid treatment on the lung inflammatory response induced by Poly(I:C) in mice. **Methods**: A/J mice were challenged with Poly(I:C) (intranasal, 50µg/50µL) for 3 consecutive days and treated with dexamethasone (3 mg/kg, oral) or vehicle 1 h before provocation. Whole body noninvasive barometric pletysmography, leukocyte accumulation in bronchoalveolar lavage (BAL) fluid, myeloperoxidase levels and cytokine generation were evaluated 24 h after the last challenge. The Committee on Use of Laboratory Animals of the Oswaldo Cruz Institute (license L-030/2015; Rio de Janeiro, Brazil) approved all protocols and experimental procedures involving animals. **Results**: We found that three repeated daily exposures of Poly(I:C) intranasally in A/J mice promoted significant airway inflammatory response, accompanied by airway hyper-reactivity following exposure to methacholine aerosol 24 h after the last provocation. In the BAL effluent the mononuclear cell counts increased from 0.72± 0.09 to 1.21± 0.11 ($x10^5$) (Mean ± SEM, n=6), whereas neutrophil levels increased from 0.08± 0.02 to 1.58± 0.1 ($x10^5$) (Mean ± SEM, n=6), under conditions where eosinophils were not detected. The levels of myeloperoxidase (0,11 ± 0,01 to 0,4 ± 0,05 (OD)(Mean ± SEM, n=6) as well as of the chemokines KC (10,44 ± 0,6 to 25,04 ± 2,88(pg/mg of tissue)(Mean ± SEM, n=6), MIP-1α(2,81 ± 0,2 to 5,340 ± 0,5(pg/mg of tissue)(Mean ± SEM, n=6) and MCP-1 (22,27 ± 4,4 to 43,79 ± 5,1(pg/mg of tissue)(Mean ± SEM, n=6) in lung tissue were also significantly increased. All these changes, including Poly(I:C)-induced airway hyper-reactivity, were shown to be insensitive to dexamethasone (3 mg/kg), except the elevations in MIP-1αand MCP-1 lung tissue levels which appeared inhibited in 97% and 98 %, respectively. **Conclusion**: We conclude that repeated exposure of Poly(I:C)causes glucocorticoid-insensitive airway inflammation and hyper-reactivity *in vivo*, suggesting that this short-term murine model of Poly(I:C)-induced steroid-resistance can be useful as an in vivo system to assess molecular mechanisms and drug candidates related to glucocorticoid resistance and viral exacerbation of chronic inflammatory lung diseases such as asthma and COPD. **Financial Support**: CNPq, FAPERJ and CAPES.

04.057 Characterization and membrane expression of Siglec receptors on human monocytes. Silva PCS, Formiga RO, Amaral FC, Spiller F UFSC

Introduction: Sialic acid-binding immunoglobulin-like lectins (Siglecs) are receptors expressed mainly in immune cells. Siglec receptors have strong immunomodulatory properties through sialic acid binding and their ability to recruit phosphatases to dampen cellular signaling (VARKI, A. Trends Mol Med, v. 14, p. 351, 2008). Previous studies have demonstrated an increased expression of Siglec-3 in peripheral blood monocytes from pigs after *H. parasuis*-induced sepsis (ÁLVAREZ, E. Comp Immunol Microbiol Infect Dis, v. 64, p. 31, 2019). This work aimed to characterize levels of Siglec receptor on human monocytes in whole blood stimulated or unstimulated with LPS. **Methods:** Blood samples were collected from healthy donors (from 18 to 60 years old) using a vacuum tube containing K_3EDTA (Labor Import, Brazil). Total leukocytes were obtained after red blood cells lysis and incubated separately with anti-Siglec-3; -5; -7; -8; -9; -10 and anti-CD66b (granulocyte marker). Median of fluorescence intensity (MFI) and frequency of Siglec expression on mononuclear CD66b-negative cells were evaluated for each Siglec on non-stimulated cells. Furthermore, LPS-induced cell activation (Sigma Aldrich, St. Louis, Missouri, USA) was assessed using different concentrations (0.01; 0.1 and 1.0 µg/mL) and several time points (0.5 h, 1.5 h, 3 h and 6 h) at 37°C/5% CO2, to evaluate Siglecs expression on these cells. Analysis of results was obtained by FACSVerse™ BD flow cytometer (BD Biosciences, San Jose, CA, USA) using FlowJo X software (Tree Star Inc, Ashland, OR, USA). **Results**: Characterization of membrane Siglec expression on non-stimulated monocytes showed positive cells for Siglec-3, -5, -7 and -9 and negative for Siglec-8 and -10, presenting higher expression for Siglec-3 (MFI= 953) and -9 (MFI= 2406). Expression of Siglec-3 increase in a dose-dependent manner after LPS stimulation showing an MFI of 2628, 2793, 3323 for 0.01, 0.1 and 1 µg/mL, respectively when compared to unstimulated cells (1456). Expression of Siglec-9 also increase after LPS stimulation with a MFI of 1732, 1514, and 1113 for 0.01, 0.1, and 1 µg/mL, respectively, when compared to control group (unstimulated= 980). Time-response assay for Siglec-9 showed the higher expression of this receptor 6 h after LPS stimulation (Siglec-9 – Control = 2801; LPS 6 h = 6495). **Conclusion**: These results showed an upregulation of Siglec-3 and -9 and suggests a correlation between cell activation and Siglec expression on monocytes, which may be important for further investigations of immune modulatory properties of those receptors. **Acknowledgments**: CNPq/CAPES CAAE: 82815718.2.0000.0121

04.058 Effect of the Biochanin A on inflammatory in obese ovariectomized mice. Araújo LFLMF[1], Almeida RG[1], Araújo JMD[1], Santos WM[1], Camargo EA, Neres WS[1], Félix FB[2] [1]UFS, [2]UFMG

Introduction: Adipose tissue is regulated by profile of cytokines and leukocytes that define its metabolic state. In the obesity, the adipocytes hypertrophy promotes tissue hypoxia, release of Th1 cytokines profile by M1 macrophages and neutrophils, that accumulate around the necrotic adipocytes, characterizing the regions known as crown-like structures (CLS) (LUMENG, C.N. Mol Aspects Med, v.34, p.12, 2013). In contrast, in normal adipose tissue, the Th2 cytokines profile released by M2 macrophages, eosinophils and lymphocytes improves insulin sensitivity (WU, D. Science, v.332, p.243, 2011). It is known that estrogen inhibits adipogenesis and improving insulin sensitivity (YONEZAWA, R. Am J Physiol Endocrinol Metab, v. 303, p.445, 2012). However, the hormone replacement therapy demonstrates benefit and harmful effects. In view of this, the phytoestrogen Biochanin A (BCA) has been few explored in obesity. Nevertheless, its anti-inflammatory action has been demonstrated in other models (CHOI, E.M. J Environ Sci Health A Tox Hazard Subst Environ Eng, v.20, p.1, 2019; XUE, Z. J Agric Food Chem, v.65, p. 3842, 2017). Thus, the purpose of the study was to investigate the BCA effects on inflammatory parameters in adipose tissue of ovariectomized obese mice. **Methods:** Ovariectomy (OVX) surgery was performed in mice C57BL/6. After 15 days the animals received during 13 weeks, standard diet (OVX DP) or high fat diet (OVX DH) or DH and BCA (OVX DH BCA, 2mg / kg, ip, daily / last 4 weeks). Finally, the perigonadal adipose tissue, gastrocnemius muscle, uterus, liver and blood were collected. The biochemical parameters of triglycerides, glucose, total cholesterol, HDL-c and LDL-c were evaluated in blood. In adipose tissue was investigated hypertrophy and hyperplasia and eosinophils amount, by the eosinophil peroxidase activity assay (EPO). The oxidative stress was performed in liver by assay of sulfhydryl and TBARS (Thiobarbituric acid reactive substances). To evaluate change in muscle mass and uterine edema, the gastrocnemius muscle and uterus were weighed, respectively. All experimental procedures were approved by the UFS Animal Research Ethics Committee under number 57/2016. **Results**: The results showed that BCA reduced adiposity index and adipocytes hypertrophy in 30%, and promoted hyperplasia (increase in 45% of the adipocyte number), in relation to OVX DH. In addition, there was increase in EPO activity (56%) and concentration of antioxidant group sulfhydryl (40%), in adipose tissue and liver, respectively. BCA treatment did not alter muscle mass, uterus weight and biochemical parameters. **Conclusion**: The dates demonstrate that BCA has anti-inflammatory effect on adipose tissue, reducing adiposity and increasing the eosinophils amount in the tissue. Additionally, the treatment has an antioxidant action on the liver of these animals. These results are important to support future studies that evaluate anti-inflammatory mechanism of BCA in ovariectomized obese mice. **Acknowledgments**: We also thank Prof[a]. Rosilene Calazans for assisting technically in histological procedures. **Financial Support**: CNPq