PRÊMIO JOSÉ RIBEIRO DO VALLE 2009

O prêmio José Ribeiro do Valle, oferecido a cada ano pela SBFTE, visa identificar a cada ano os melhores trabalhos científicos desenvolvidos por jovens investigadores na área da Farmacologia. Entre os trabalhos inscritos para esta décima-segunda edição do prêmio, foram selecionados cinco finalistas, que fizeram apresentações de seus respectivos trabalhos perante comissão julgadora, em sessão pública durante o 41º Congresso Brasileiro de Farmacologia e Terapêutica Experimental, em Ribeirão Preto, SP. O resultado foi o seguinte:

Primeiro lugar

Silvio Manfredo Vieira

04.095 The intracellular pattern recognition receptor NOD2 is crucial for development of arthritis. Vieira SM[1], Pinto LG[1], Lemos HP[1], Cunha TM[1], Lima JB[2], Talbot J[1], Almeida SCL[3], Verri WA, Jr[1], Louzada Jr P[3], Zamboni DS[2], Ferreira SH[1], Cunha FQ[1] [1]FMRP-USP - Farmacologia, [2]FMRP-USP - Biologia Celular, Molecular e Bioagentes Patogênicos, [3]HC-FMRP-USP - Clínica Médica

Introduction: Nucleotide-binding oligomerization domains containing 1 and 2 (NOD1 and NOD2) are intracellular sensor proteins belonging to the pattern recognition receptors family, which play an important role in the immune response. The NOD-like receptors can sense pathogens, products of damaged cells or endogenous metabolites and could potentially be involved in the initiation, amplification and progression of the inflammatory response in rheumatic diseases. **Methods:** Synovial and peripheral blood mononuclear cells (SPBMCs) from RA and osteoarthritis (OA) patients were taken for real-time RT-PCR or stimulated with MDP (a Nod2 agonist) for ELISA, Caspase-1 activation and western blot assays. Adult C57BL/6 (WT), *Nod1*$^{-/-}$, *Nod2*$^{-/-}$, *Caspase-1*$^{-/-}$ and *RIPK2*$^{-/-}$ mice weighing 18-23 g were used. Firstly, for the development of experimental arthritis model, mice were immunized with methylated bovine serum albumin (mBSA) and complete Freud´s adjuvant through subcutaneous (s.c.) injection. Twenty-one days after the initial injection, arthritis was induced in the immunized mice by intra-articular (i.a.) injection of mBSA dissolved in PBS. Synovial membranes of arthritic and non-arthritic WT and knockout mice were taken to real-time RT-PCR. Migration assays, mechanical hypernociception and histological analysis were used to evaluate neutrophil (NØ) recruitment to knee joints, decrease of nociceptive withdrawal threshold and cartilage loss, respectively. Proteoglycan content of cartilage was measured by dimethylmethylene blue assay of papain digests. ELISA assays were used to evaluate cytokines production in joint of arthritic and non-arthritic WT and knockout mice. This study was approved by Ethics Committee of FMRP/USP (nº. 127/2008). **Results:** Real-time RT-PCR analysis shows that NOD2, RIPK2 and IL-1β mRNA expression is augmented in SPBMCs from RA patients when compared with cells from OA patients. MDP induced *in vitro* high levels of Caspase-1 activation as wells as increase in IL-23, IL-1β, TNF-α and CXCL8 release by SPBMCs of RA patients in comparison with OA patients' cells. mBSA-induced arthritis, NØ migration, mechanical hypernociception and cartilage loss were completely abrogated in *Nod2*$^{-/-}$ and markedly reduced in *RIPK2*$^{-/-}$ and *Caspase-1*$^{-/-}$ mice in comparison with WT or *Nod1*$^{-/-}$ mice. mBSA challenge into joints increased Nod2 mRNA expression in synovial membrane of WT arthritic mice in comparison with non-arthritic mice. Joint synovial membrane mRNA expression of IL-6, IL-23 and IL-1β and the levels of IL-17, IL-23, IL-1β, TNF-α and CXCL1 were significantly reduced in *Nod2*$^{-/-}$ mice in comparison with WT or *Nod1*$^{-/-}$ mice. The levels of IL-17, IL-1β and CXCL1 were also significantly reduced in Caspase-1$^{-/-}$ mice after mBSA-challenge. **Discussion:** These results strongly suggest that NOD2 signaling, but not NOD1, plays a fundamental role in the pathogenesis of RA**.** Furthermore, RIPK2/Caspase-1 pathway seems to be the downstream signaling of NOD2 in joint inflammation and might favor Th17 immune response. Together, it is reasonable to propose NOD2 signaling as a novel target to development of new drug to control RA. **Financial support:** FAPEAM, FAPESP and FAEPA.

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Segundo lugar

Priscilla Christina Olsen

01.017 JMF2-1, a non-anesthetic analog of lidocaine, inhibits T cell proliferation and survival by increasing intracellular cAMP levels. Olsen PC[1], Coelho LP[1], Jurgilas PB[1], Costa JCS[2], Viola JP[3], Cordeiro RSB[1], Silva PMR[1], Martins MA[1] [1]FIOCRUZ - Inflammation, [2]Farmanguinhos-FIOCRUZ, [3]INCa - Cellular Biology

Introduction: Our previous findings showed that inhalation of JMF2-1, an analog of lidocaine with reduced anesthetic activity, prevents cardinal features of asthma. We found that JMF2-1 reduces airway hyperresponsiveness, T_H2 cytokine generation and lung eosinophilic inflammatory infiltrate. These effects were likely due to an inhibition of T cell function and survival. In the current study, we elucidated the molecular mechanisms of these effects. **Methods:** Lymphocytes obtained from lymph nodes of DO11.10 or BALB/c mice were stimulated with OVA allergen (0,5 mg/mL) or anti-CD3 mAb (5 μg/mL) for 72 or 48 hours, respectively, in the presence or absence of JMF2-1 (300 μM). Apoptosis and proliferation were confirmed by staining DNA with propidium iodide and by analyzing Sub-G_0 and S+G_2 population through flow cytometry. A pan-caspase inhibitor and a specific inhibitor of caspase-8, ZVAD and ZIETD (50 μM) respectively, were used as a pre-treatment to confirm the apoptosis pathway. ELISA technique was used to quantify cytokine production in supernatants of DO11.10 stimulated cells pre-treated with caspase inhibitor and treated consecutively with JMF2-1. Western blotting with GATA-3 monoclonal antibody was performed to determine expression of this protein in stimulated lymphocytes treated with JMF2-1. Radioimmunoassay was used to quantify cAMP in lymphocytes 20 minutes after JMF2-1 treatment. (Protocol number of Animal Ethics Committee approval 00085-02). **Results:** We found that JMF2-1 increased the apoptosis rate of T cells activated either by OVA allergen (59.62 %) or anti-CD3 mAb (44,75%) without modifying the survival rate of unstimulated lymphocytes *in vitro*. Apoptosis induced by JMF2-1 was partially reversed by both caspase inhibitors (ZVAD prevented 46,53% and ZIETD 19,65% of cell death induced by JMF2-1). Caspase blockade prevented cell death caused by JMF2-1 but it did not affect the inhibition of cytokine production. Furthermore, JMF2-1 increased cAMP intracellular concentrations and inhibited GATA-3 expression in these cells. **Discussion:** These results show that JMF2-1 acts by increasing cAMP concentrations in lymphocytes leading to two separate mechanisms: (i) inhibition of proliferation and cytokine production, by inactivating GATA-3, as well as (ii) stimulating T cell apoptosis, via activation of caspase-8 pathway. Altogether, these findings help to explain the anti-inflammatory effects of JMF2-1 observed in *in vivo* systems of experimental asthma. **Financial Support:** CNPq, CAPES, FAPERJ, PDTIS.

Menção Honrosa

Cibelle Ramos Fiuza

05.017 Endothelins as pronociceptive mediators of the rat trigeminal system: role of ET_A and ET_B receptors. Fiuza CR, Chichorro JG, Bressan E, Claudino RF, Leite DFP, Rae GA UFSC Pharmacology

Eduardo Ekundi Valentim

04.012 Hydrogen sulfide reduces carrageenan-dependent joint inflammation through a caspase-1 deficiency and increased levels of IL-10 dependent mechanism. Ekundi-Valentim E, Teixeira SA, Barreto MAA, Moreira, DF, Belizário JE, Muscará MN, Costa SKP USP-ICB - Farmacologia

Remo Castro Russo

08.002 PI3Kγ plays a critical role in bleomycin-induced pulmonary injury and fibrosis. Russo RC[1], Garcia CC[1], Guabiraba R[1], Barcelos LS[2], Sousa LP[1], Roffe E[1], Souza ALS[1], Cassali GD[3], Pinho V[4], Mirolo M[5], Doni A[5], Locati M[5], Teixeira MM[1] [1]ICB-UFMG - Bioquímica e Imunologia, [2]ICB-UFMG - Biofísica, [3]ICB-UFMG - Patologia Geral, [4]ICB-UFMG - Morfologia, [5]Instituto Clinico Humanitas - Translational Medicine

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