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THU0074

ANTI-ARTHRITIC EFFECT OF RECOMBINANT CYSTATIN 3 FROM *FASCIOLA HEPATICA* IN COLLAGEN-INDUCED ARTHRITIS

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Background: Cystatins are cysteine proteases-inhibitors secreted by *Fasciola hepatica* in order to modulate the host immune response to promote survival of the parasite. These molecules are able to inhibit different mammal cathepsins, to regulate the immune balance via Th2 and T regulatory responses, to downregulate antigen presentation and the release of pro-inflammatory cytokines (1,2) – mechanisms that are important in the development and maintenance of several immunopathology, as rheumatoid arthritis (RA) (3).

Objectives: To evaluate the therapeutic effect of recombinants cystatin 1 and cystatin 3 from *Fasciola hepatica* in a mice model of collagen-induced arthritis (CIA).

Methods: Twenty-seven DBA/1J mice were induced with CIA by an injection of collagen type-II and Freund's adjuvant at days 0 and 18. Animals were randomly divided into three groups: vehicle (n=9, treated with phosphate-buffered saline), cystatin 1 (n=9, treated with 100 µg/dose of recombinant cystatin 1) and cystatin 3 (n=9, treated with 100 µg/dose of recombinant cystatin 3). Treatment started after day 18 by intraperitoneal injection once a day until the end of the experiment, at day 45 after CIA induction. Clinical arthritis score, nociception, paw edema, body and spleen weight were evaluated. Lymphocytes were isolated from lymph nodes and CD4+CD25+Foxp3+ T regulatory subset was assessed by flow cytometry. Data are expressed as mean ± SEM and were evaluated by one-way or two-way ANOVA followed by Bonferroni post-test.

Results: Treatment with cystatin 1 did not alter any of the analyzed parameters. On the other hand, cystatin 3 was able to reduce clinical arthritis score from day 38 with 32% of reduction at day 45 (9.22±1.22) compared to vehicle (13.56±0.73) (p<0.05). In addition, treatment with cystatin 3 diminished nociception (cystatin 3: 4.0±0.36g, vehicle: 2.7±0.32g) (p<0.05) and paw edema (cystatin 3: 0.051±0.012ml, vehicle: 0.093±0.007ml) (p<0.05). Moreover, the treatment did not alter body weight (cystatin 3: 21.67±0.31g, vehicle: 21.05±0.38g) and spleen weight (cystatin 3: 7.04±0.31, vehicle: 7.16±0.38), as well as the T regulatory population (cystatin 3: 63.38±3.66%; vehicle: 58.31±6.77%).

Conclusion: Treatment with cystatin 3 improved collagen-induced arthritis by attenuating the disease score, nociception and paw edema. Moreover, the treatment did not induce body weight loss or spleen weight alteration. These results suggest that recombinant cystatin 3 from *Fasciola hepatica* has the potential as a treatment for inflammatory and autoimmune diseases such as RA.

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THU0075

MIR-21 IN EXOSOMES DRIVEN FROM DENTAL PULP STEM CELLS AMELIORATE THE TREGS/TH17 IMMUNE RESPONSE VIA TARGETING STAT3 IN COLLAGEN-INDUCED ARTHRITIS MICE

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Background: Rheumatoid arthritis (RA) is a common and systemic autoimmune disease¹. It is vital for deciphering pathogenesis to define the precise CD4⁺Tcell subsets that are expanded or dysregulated in RA patients². An increase in the Th17 cell subset and a decrease in Tregs resulting imbalance of Tregs/Th17 may be responsible for the occurrence and development of RA³. Dental pulp stem cells (DPSCs) appear be a new therapeutic tool for immunological diseases^{4,5}. In RA, the mechanism of the effect of miR-21 driven from DPSCs exosomes (DPSCs-exo) on the balance of Tregs/Th17 is still unclear.

Objectives: We explored the effect of exosomes driven from DPSCs on RA and investigated the correlation between DPSCs-exo and Tregs/Th17 balance.

Methods: Exosomes were isolated through differential centrifugation. Collagen-induced arthritis (CIA) mice were built to detect histological change and Tregs/Th17 ratio. CD4⁺T cells were isolated from PBMCs. Exosomes and CD4⁺T cells were co-cultured to study the regulation of Tregs/Th17 balance. The relationship between miR-21 and Tregs/Th17 balance was studied by flow cytometry, qRT-PCR and Western blotting etc.

Results: After Dpscs-exo treatment, the clinical scores and paw swelling of CIA mice decreased. At the same time, the expression of Treg cells increased and the expression of Th17 cells decreased in the spleen, changing the ratio of Tregs/Th17. Exosomes and CD4⁺T cells were co-culture, showing that Treg cells increased and Th17 cells decreased. MiR-21 is highly expressed in RA patients. MiR-21 in exosomes were Knockout and overexpressed. The balance of Tregs/Th17 decreased when miR-21 were knockout and increased when miR-21 were overexpressed. In addition, STAT3 in CD4⁺T cells were overexpressed and co-culture with DPSCs-exo, which inhibited Tregs/Th17.

Conclusion: We elucidated the mechanism of the regulation of Tregs/Th17 balance by DPSCs-exo, that is, miR-21 in exosomes driven from dental pulp stem cells ameliorate the Tregs/Th17 immune response via targeting STAT3 in collagen-induced arthritis mice.

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THU0076

SONIC HEDGEHOG PROMOTES SYNOVIAL HYPERPLASIA AND BONE DAMAGE THROUGH P38 MAPK SIGNALING IN EXPERIMENTAL ARTHRITIS

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Background: Abnormal activation of sonic hedgehog (SHH) signaling has been found in synovium from patients with rheumatoid arthritis (RA). Inhibition of SHH signaling is reported to attenuate inflammation and cartilage damage in adjuvant-induced arthritis (AIA). Previously we have demonstrated that SHH signaling promoted the tumor-like behavior of fibroblast-like synoviocytes (FLSs) through p38 MAPK *in vitro*.

Objectives: In the current study, we aim to further explore the role of SHH-p38 MAPK signaling in regulating synovial hyperplasia and bone erosion in experimental arthritis.

Methods: Collagen-induced arthritis (CIA) mouse model was induced and the mice were injected with adenovirus associated virus (AAV) overexpressing SHH or treated with small molecule inhibitors GDC-0449. SB203580 was administered for the inhibition of p38 MAPK. The severity of paw inflammation was graded and serum levels of TNFα, IL-6 were detected. The histological features of arthritis were evaluated by H&E staining. The bone erosion was identified by micro-CT assessment and the number and function of osteoclasts were determined.