Background: Cytastins are cysteine protease-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 Th17 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 one 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 (1.13±0.13, vehicle: 1.35±0.14) (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 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.

References:

THU0074 ANTI-ARTHRITIC EFFECT OF RECOMBINANT CYSTATIN 3 FROM FASCIOLA HEPATICA IN COLLAGEN-INDUCED ARTHRITIS

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

Objectives: We explored the effect of exosomes derived 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 Treg/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.

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 T cells cis-simers and Th17 cells decreased. miR-21 was highly expressed in RA patients. The balance of Tregs/Th17 decreased when miR-21 was overexpressed. The expression of Tregs/Th17 decreased when miR-21 was knocked down and overexpressed. In addition, STAT3 in CD4+ T cells were overexpressed and co-culture with DPSCs-exo, which inhibited Th17.

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

References:

Disclosure of Interests: None declared.

THU0075 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 (AAIA). Previously we have demonstrated that SHH signaling promoted the tumor subset and a decrease in Treg cells resulting imbalance of Treg/Th17 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 administrated 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.