TARGETING T-CELL TRAFFICKING IN A MURINE MODEL OF SJÖGREN’S SYNDROME

Methods Human adipose-derived stem cells (hASCs) were encapsulated via a micromolding method. We first manufactured polydimethylsiloxane chips containing 1600 micromolds with a circular shape, 150 μm in diameter and 100 μm in depth. For cell encapsulation, a solution of 2% alginate (w/v) containing 3 million of hASCs per ml, polymer was deposited onto the chips, loaded in the moulds either by sedimentation or centrifugation and crosslinked using an agarose gel charged with CaCl2. The number of encapsulated cells was evaluated by a CyQUANT assay immediately and 24 hour after encapsulation. The impact of encapsulation on metabolic activity was determined by a presto blue assay 24 hours after encapsulation and 24 hour after their injection through a 26G needle.

Results We successfully obtained cylindrical alginate microparticles presenting a diameter of 103±0.7 μm. Using cell quantification, we determined that the centrifugation method allowed the encapsulation of 3.3±1.5 cells per micromold, whereas the sedimentation method allowed the encapsulation of 7.5±2.3 cells per micromold. We also demonstrated that alginate particles injectable through a 26 G needle had no impact on the viability of encapsulated cells.

Conclusions Our results show that micromolding allows hASCs encapsulation into alginate particles injectable through a 26 G needle without impacting cell viability. Future work will focus on evaluating in vitro long-term encapsulated cell survival and functionality. In case of success, we will then consider intra-articular injection in an animal model of osteoarthritis.

Disclosure of interest None declared

ARGINASE I AND THE METABOLIC CONTROL OF OSTEOCLASTOGENESIS

Methods Submandibular salivary glands of C57BL/6J mice were intra-dually cannulated with luciferase-encoding replication-deficient adenovirus to induce TLS formation as previously described. Mice were administered daily either with PBS or PEPITEM by intraperitoneal injection from day 0, and their salivary glands dissected at day 5 post cannulation. T-cell infiltration into salivary glands was assessed using a combination of flow cytometry, immunofluorescence and qRT-PCR.

Results B cells in sera from cannulated animals express lower levels of both adiponectin receptors 1 and 2 in comparison with non-inflamed control mice. In cannulated animals treated with PEPITEM, histological analysis of salivary glands revealed fewer, as well as less aggregated, infiltrating T cells. Both CD4+ and CD8+ numbers were significantly lower in the salivary glands of PEPITEM-treated animals. Furthermore, administration of PEPITEM also decreased mRNA transcripts for lymphotixin beta, IL-7, lymphoid chemokines (CCL19 and CXCL13) and T cell chemokine receptor CCR7, cytokines and chemokines known to regulate ectopic lymphoepithelial proliferation in pSS. Human samples of pSS are currently being assessed to validate the relevance of this pathway in pSS.

Conclusions These results demonstrate that administration of exogenous PEPITEM can reduce T-cell influx into salivary glands. This may represent a rescue of the homeostatic regulation of leukocyte trafficking, which is disrupted in inflammation. Our work suggests that PEPITEM should be considered to address the therapeutic needs in chronic inflammatory conditions and that the detection of decreased levels of adiponectin receptor could be used as biomarkers in pSS.

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Disclosure of interest None declared
Abstracts

Effect of recArg1 regarding an in vitro model called serum transfer arthritis, where we treated C57BL/6J wildtype mice with the recombinant enzyme. Disease severity was then assessed using clinical scores and paw histology.

Results We observed that ARG1 mRNA expression was downregulated from the progression of a precursor to a mature osteoclast. We incubated day 3 osteoclast precursors with recArg1 and observed that addition of 1000 ng/ml recArg1 abolished osteoclastogenesis. L-Arginine deprivation led to a decrease in the oxygen consumption rate of osteoclast precur- sor cells, assessed 48 hour after RANKL addition. Using serum transfer arthritis, an established murine in vivo model, recArg1 treated mice showed reduced disease severity combined with a significant decrease in the presence of osteoclasts. Treatment efficiency was evaluated using an L-Arginine ELISA, where the amino acid was found to be absent in the serum of treated mice.

Conclusions We propose that the amino acid L-Arginine is critical for the development of osteoclasts from myeloid precursors and hypothesise that its abundance, influenced by recArg1 addition, influences development and severity of osteoclast driven diseases.

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Disclosure of interest None declared

P114 IONISING RADIATION INHIBITS INFLAMMATION IN PATIENTS WITH MUSCULOSKELETAL DISEASES: RADON TREATMENT VS LOW-DOSE RADIATION THERAPY

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Introduction Rheumatoid arthritis (RA) and osteoarthritis (OA) are the most common musculoskeletal diseases (MSD) that affect the joints. Reduced mobility and quality of life are the consequences of the cartilage and bone tissue destruction and the chronic inflammation process, caused by release of bone destruction markers and inflammatory factors including adipokines in the joint. Besides medications, an additional pain relief is achieved by the treatment of patients with low-dose ionising radiation, either as local low-dose radiation therapy (LDRT) or whole-body exposure to radon in radon baths or galleries.

Objectives In the previous work we showed the decrease of serum levels of visfatin and serum carboxy-terminal collagen crosslinks of type-I collagen (CTX-I) in patients treated in radon baths.1 In the present study, we analysed serum samples of patients with MSD, who had been treated locally with photon radiation (LDRT). In addition, we analysed differentiation and activity of osteoclasts that were differentiated in vitro from patient-derived monocytes.

Methods Serum samples were collected from patients before and after treatment. Levels of visfatin and CTX-I were measured by ELISA. Monocytes were isolated from blood samples of patients and cultivated with M-CSF and RANKL on bone slices for 2 weeks. Osteoclasts were defined as TRAP and F-actin positive cells. TRAP activity was measured in the cell supernatants using TRAP Staining Kit.

Results In the serum of patients treated with LDRT, a trend to reduced concentration of CTX-I was observed directly after the therapy. Further, osteoclasts, differentiated in vitro from LDRT patient-derived monocytes, showed reduced TRAP activity.

Conclusions The observations made in this study so far substantiate that the radiation-induced decrease of CTX-I levels could be one main factor that is related to the attenuation of inflammation and to the decrease of disease activity in the patients with MSD. This hypothesis is endorsed by the observed reduced differentiation and activity of in vitro cultured patient-derived osteoclasts.

P115 LOW DOSE RADIATION HAS A POSITIVE IMPACT ON BONE METABOLISM IN AN EXPERIMENTAL MODEL OF INFILAMMATORY ARTHRITIS

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Introduction Rheumatoid arthritis (RA) is, next to inflammation and infiltration of activated immune cells into the synovial joint, characterised by a progressive destruction of cartilage and bone. Although today’s treatment options are very effective for many patients, not all of them respond properly or have to reduce medications due to adverse effects. In these patients it is crucial to slow down bone loss and inflammation in a timely manner to prevent further damage. Here, low-dose radiotherapy (LD-RT) could be an option, as it has been shown to ameliorate inflammation and to reduce pain. Using the human TNFα transgenic (hTNFα tg) mouse model as an experimental model of inflammatory arthritis, we revealed that locally applied LD-RT attenuates inflammation in the joints.

Objectives As little is known about the impact of LD-RT on bone metabolism, we thus focused on the effects of LD-RT on bone homeostasis.

Methods Bone marrow-derived osteoclasts (OC) of hTNFα tg mice were differentiated using M-CSF and RANK-L and...