cells were pretreated with different concentrations of JAKi or vehicle control and then stimulated with IL1-β (10 or 20 ng/ml) or Oncostatin M (OSM, 100 ng/ml). After the indicated time (17–24 hour), the supernatants were collected and the concentrations of IL-6 were measured by ELISA. An assay combining the measurement of cell viability, cytotoxicity and apoptosis was performed to exclude effects of JAKi caused by cell toxicity.

Results In a pretest-setting, RA-FLS were pretreated with JAKi for 2 hour with concentrations of 1 μM and 10 μM and then stimulated with IL1-β (20 ng/ml) for 18 hour. At the concentration of 10 μM, Peficitinib, Filgotinib and Upadacitinib reduced the IL-6 release by RA-FLS, whereas Tofacitinib and Baricitinib did not change the IL-6-levels. Tofacitinib and Baricitinib at 1 and 5 μM only reduced the cytokine release if the IL-6 pathway was activated selectively with OSM (n=3). In further analyses, RA-FLS were again pretreated with Filgotinib and Peficitinib for 2 hour with clinically relevant concentrations (range between 0.01 μM and 5 μM) and then stimulated with IL1-β (10 ng/ml) for 17 hour. At the concentration of 5 μM, Peficitinib caused a reduction of IL-6 levels of 66% compared to control with IL1-β ($p<0.01$, n=5). However, a reduction of only 24% ($p=0.12$, n=5) could be observed at 1 μM. Filgotinib did not decrease IL-6 levels. Peficitinib did not change the viability, cytotoxicity or apoptosis (n=2), confirming that the observed effects were JAKi dependent.

Conclusions Peficitinib reduced the inflammatory response of RA-FLS after activation with IL1-β and appeared to be superior to Tofacitinib and Baricitinib in targeting RA-FLS. The lack of effect of Tofacitinib and Baricitinib on RA-FLS activated by IL1-β could explain the treatment failure in some patients.

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P118 TRANSFORMING GROWTH FACTOR BETA INDUCED (TGFBI,) A NEW PLAYER IN THE THERAPEUTIC EFFECT OF MESENCHYMAL STEM CELLS

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Introduction Osteoarthritis (OA) is the most common form of chronic joint diseases. In recent years, stem cell-based therapies have been investigated as an alternative approach to treat OA. Mesenchymal Stem Cells (MSCs) have demonstrated therapeutic efficacy in the context of pre-clinical studies. More than the capacity of these cells to differentiate into chondrocytes, their effect is primarily associated with a paracrine function.

Objectives Because the Transforming Growth Factor β (TGFβ) pathway plays a critical role in joint homeostasis, we investigated whether the therapeutic effect of MSCs could be mediated by members of the TGFβ family. Using a secretome analysis, we identified Transforming Growth Factor β Induced (TGFβI), a potential candidate for a chondroprotective role of MSCs.

Methods Murine chondrocytes were isolated from 3 days old C57BL/6 mice and cultured for 5 days. OA-like chondrocytes were obtained by incubation with IL1β for 24 hour. Bone marrow-derived MSCs were used to produce a conditioned medium for 24 hour following or not a pre-activation step with TGFβ3 for 24 hour. For co-culture experiments, MSCs were seeded on transwells and pre-activated or not with TGFβ3 for 24 hour. Supernatants or MSC-containing transwells were added to OA-like chondrocytes (ratio 1 MSC:3 chondrocytes) for 24 hour before cell recovery for RT-qPCR analysis. For silencing experiments, siRNA directed against TGFβI were transfected in MSCs and chondrocytes with oligofectamine and lipofectamine, respectively. The Collagenase-Induced OsteoArthritis murine model (CIOA) was induced by collagenase injection in knee joints of C57BL/6 mice at day 0 and 2. MSCs were transfected twice with siRNA before being injected in knee joints at day 7 (250,000 cells/5 μL). At day 42, paws fixed in 4% formaldehyde and scanned by microCT and confocal laser scanning microscopy before being processed for histology.

Results Addition of conditioned medium from MSCs or MSCs in transwells on OA-like murine chondrocytes allows to reinduce expression of the chondrocyte markers, aggrecan and type IIB collagen. Addition of transwells containing pre-activated MSCs on OA-like chondrocytes not only increased the expression of aggrecan and type IIB collagen but decreased the expression of catabolic markers, MMP13 and ADAMTS5. This effect was associated with an increased expression of TGFβI in both chondrocytes and MSCs. We therefore investigated the consequence of TGFβI silencing in either chondrocytes or MSCs. Interestingly, silencing of TGFβI in MSCs resulted in a significant reduction of their therapeutic effect. In the CIOA model, down-regulation of TGFβI in MSCs resulted in lower therapeutic effect on OA features as visualised by a reduced protective effect on cartilage and sub-chondral bone histomorphometric parameters.

Conclusions Altogether, our results indicated that TGFβI secreted by MSCs can regulate cartilage homeostasis by regulating the expression of anabolic and catabolic mediators and that TGFβI participates to the protective effect of MSCs in OA.

Disclosure of interest None declared

P119 LOCAL ADMINISTERED ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS REDUCE EXPERIMENTAL OA-PATHOLOGY VIA INTERLEUKIN-1B-MEDIATED IMMUNOMODULATION OF PRO-INFLAMMATORY PMNS

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Introduction Adipose-derived mesenchymal stromal cells (ASCs) exhibit anti-inflammatory characteristics and reduce development of joint pathology after injection into murine experimental inflammatory osteoarthritis (CiOA) joints.[1,2] This protection is only achieved when ASCs are applied in early CiOA. This early, but not the late phase of CiOA, is characterised by strongly elevated levels of S100A8/A9 and interleukin-1 beta (IL-1β),[3] suggesting that the inflammatory environment mediates the protective effect of ASCs.

Objectives To examine the mechanism behind ASC-mediated amelioration of CiOA pathology.
Methods CiOA was induced by intra-articular collagenase injection. Knee joint sections were stained with haematoxylin/eosin, the PMN-specific antibody NIMP-R14, or CD271 to locate ASCs. Gene expression and protein release of chemokines by IL-1β- or S100A8/A9-stimulated ASCs were assessed with qPCR and Luminex. Migration of MACS-isolated PMNs towards ASC-conditioned medium through Transwell membranes was examined using flow cytometry. ASC-PMN co-cultures were analysed with microscopy and Luminex. Phagocytic capacity of PMNs was measured with fluorescent labelled zymosan particles.
Results Intra-articular ASC injection on day 7 of CiOA (when IL-1β and S100A8/A9 levels are highest) strongly attracted particularly PMNs, which clustered around ASCs in the synovium 6 hour after injection. IL-1β-stimulation of ASCs *in vitro* strongly increased protein expression of PMN-attracting chemokines KC, CXCL5, and CXCL7, whereas S100A8/A9 did not. Migration of PMNs towards conditioned medium of IL-1β-stimulated ASCs (IL-1β-CM) was significantly enhanced (two-fold increase) when compared to CM of non-stimulated ASCs (NS-CM). After 6 hour co-culturing ASCs with PMNs, both the number of ASCs clustering with PMNs and clustered PMNs per ASC were significantly increased after IL-1β-stimulation. Interestingly, association of PMNs with ASCs significantly diminished the release of KC protein by ASCs (69% lower after 24 hour), and also strongly reduced the release of S100A8/A9 protein by the PMNs. Finally, phagocytosis of zymosan by PMNs was strongly enhanced after priming with IL-1β-CM.
Conclusions Local application of ASCs in inflamed CiOA joints results in attraction and clustering of PMNs with ASCs in the synovium, which is likely mediated by IL-1β-induced up-regulation of chemokine release by ASCs. This results in lowered levels of pro-inflammatory S100A8/A9 and enhanced phagocytic capacity of PMNs, enabling the clearance of debris to cease synovitis and promote tissue repair.

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P120 EFFECT OF CENERIMOD, A SPHINGOSINE-1-PHOSPHATE RECEPTOR 1 (S1P1) MODULATOR, ON THE FORMATION OF TERTIARY LYMPHOID STRUCTURES IN A MOUSE MODEL OF SJÖGREN'S SYNDROME

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Introduction Tertiary lymphoid structures (TLS) often develop in target tissues of autoimmune diseases (AID) such as systemic lupus erythematosus (SLE), multiple sclerosis (MS), rheumatoid arthritis, and Sjögren's syndrome (SS). These structures consist of aggregates of B and T cells with varying degree of organisation and are proposed to promote the generation of autoreactive effector cells and autoantibody production. Modulation of the $S1P_1$ receptor inhibits egress of pathogenic lymphocytes from lymphoid organs and reduces their availability in circulation. This has proven to be an effective target for the treatment of AID including MS and is currently being considered for early phase clinical trials in SLE and SS.
Objectives To investigate the functional targeting of the $S1P_1$ receptor in a murine model of SS.
Methods Cenerimod, an orally active, selective $S1P_1$ receptor modulator, was administered either preventively (early in inflammation) or therapeutically (established inflammation) in an inducible model of SS to evaluate the efficacy of cenerimod *in vivo*. Histology, flow cytometry and qPCR were used to analyse the tissue samples.
Results Cenerimod induced disaggregation of the lymphocytic structures and resolution of salivary gland (SG) inflammation with a concomitant decrease in focus score, lymphoid structure size and T/B-cell follicular organisation. Mice treated with cenerimod displayed significantly decreased T (naïve, central memory and effector) and B (including CD138 +plasma cells) lymphocyte infiltration in cannulated SG, relative to vehicle treated mice. Interestingly, the lymphocytes from cenerimod treated mice exhibited significantly reduced proliferation as well as reduction in pro-inflammatory cytokine RNAs such as IL-17, IL-21, and IL-6. Furthermore, the gene expression profile associated with TLS formation (LTα, LTβ, TNFα, CXCL13, CCL19) was less pronounced in cenerimod treated samples. The cervical lymph nodes draining the salivary glands showed a slight reduction in T lymphocytes, but no significant defects were observed in structure, organisation and production of lymphoid chemokine/cytokines, suggesting that homeostatic regulation of tertiary and physiological lymphoid organs differentially relies on lymphocyte/stromal cell cross-talk during inflammation.
Conclusions Together, these data demonstrate that the $S1P_1$ receptor modulator cenerimod regulates TLS in mice and might therefore be a potential treatment option in AID with TLS formation such as SS and SLE. The data also unveil differential requirements for the establishment and maintenance of secondary versus tertiary lymphoid structures.
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P121 RHEUMATOID SYNOVIAL FLUIDS DIFFERENTIALLY AFFECT ADSC PROLIFERATION AND IMMUNOMODULATORY POTENTIAL

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Introduction Adipose-derived mesenchymal stem cells (ADSC) are currently considered as a potential innovative therapy for rheumatoid arthritis (RA) due to their immune-modulating properties. It has been well established that ADSC exert an