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-1 β - 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-1 β and S100A8/A9 levels are highest) strongly attracted particularly PMNs, which clustered around ASCs in the synovium 6 hour after injection. IL-1 β -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-1 β -stimulated ASCs (IL-1 β -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-1 β -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-1 β -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-1 β -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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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 S1P₁ 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 S1P₁ receptor in a murine model of SS.

Methods Cenerimod, an orally active, selective S1P₁ 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 (LT α , LT β , TNF α , 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 S1P₁ 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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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

immune-regulating response when exposed to an inflammatory environment through the secretion of soluble mediators and cell-cell contact mechanisms. Several molecules are implicated in ADSC-mediated immunomodulation such as PGE₂, IDO, IL-6, TGF- β and TSG6. However, while ADSC have shown remarkable results *in vitro* and in animal models, their efficacy in clinical applications has yet to be fully elucidated.

Objectives Considering the diversity of the local inflammatory microenvironment in the joints of RA patients and to further explore the behaviour of ADSC within their microenvironment, we aimed to study the effect of synovial fluid (SF) collected from patients with RA on ADSC proliferation and immune-modulating properties.

Methods ADSC isolated from 6 healthy subjects were cultured for 24 hours in the presence or absence of RASF (diluted to 5%) obtained from 10 different RA patients or a control SF obtained from a patient undergoing non-inflammatory rheumatism related surgery. ADSC proliferation was assessed by an MTT colorimetric assay. After SF treatment, ADSC were harvested for RNA extraction and subsequent RT-qPCR to quantify the gene expression of COX2, IL6, IDO, TSG6, TGF- β . To evaluate the inflammatory status of SF, pro-inflammatory cytokines present in SF were first identified using an antibody-based membrane array and then quantified by ELISA or CBA.

Results RASF differentially affected the proliferation of ADSC with 2 out of 10 inducing ADSC proliferation whilst the remaining inhibited proliferation. Gene expression of COX2, IDO, IL-6, TSG6 and TGF- β was simultaneously induced in only 4 of the 10 RASF included in this study. We thus investigated if these four RASF had any particular inflammatory signature that could explain their effect on ADSC. Higher concentrations of one or more of the following markers IL-6, TNF α , MCP1, ICAM1, IL1 α were detected in these SF compared to others, which correlates with a more potent pro-inflammatory environment. Conversely, RASF that had no effect on ADSC gene expression presented a less inflammatory profile. Recombinant cytokines and neutralisation assays are underway to determine which specific molecules mediated the SF effect on ADSC.

Conclusions Proper licensing of ADSC to an anti-inflammatory phenotype is usually done *in vitro* by stimulation with high concentrations of pro-inflammatory cytokines which do not necessarily reflect the concentrations present in the synovial fluid. This study sheds further light on how ADSC respond to local inflammatory stimuli in order to better direct their immunomodulatory ability and efficacy in clinical therapy.

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TREATMENT WITH RITUXIMAB TO DEMAND IN RHEUMATOID ARTHRITIS. EFFECTIVENESS ANALYSIS

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Introduction Rituximab (RTX) is a chimeric anti-CD20 monoclonal antibody that has been shown to be effective in the treatment of rheumatoid arthritis (RA). At present we have different treatment regimens in terms of the time of

administration, every 6 months or on demand and with respect to the dose used: cycles of 1000 mg x 2 or 500 mg x 2.

Objectives Our objective was to analyse the effectiveness on-demand treatment regimen of RTX in RA

Methods Retrospective observational study on RA with RTX treatment between January 2006 and February 2015 was carried out. Administration was performed if DAS28 >3.2. The median time of retreatment and the number of patients portrayed at 8, 12, 16, 20 and 24 months were calculated

Results 57 patients were included, mean age 55 years, 70% women; 93% FR+, 31.6% ACPA + and 63.2% with erosive disease. The median time of evolution of RA at the start of RTX 8.1 years. All patients had high activity at the start of RTX [median DAS28=5.6; HAQ=1.75]. Prior to RTX, all patients were treated with another biological (24.5% with two or more). 56% of patients were receiving DMARDs.

The median time of retreat was 7 months.

The number of patients portrayed was: at 8 months 55.8%, at 12 months 69.4%, at 16 months 80%, at 20 months 81.3% and at 24 months at 88.6%. Those not evaluated in each quarter are those who are discontinued RTX before two years of follow-up (four due to inefficacy and one due to adverse reaction) or, at the time of completing the study, have not completed two years of treatment. The effectiveness considered as the median number of cycles received at two years was 2.

Conclusions Although the median time to retreatment was seven months, there are some patients who remain unplastered for about two years. 80% of the patients had been portrayed at 16 months, half with 1 cycle of RTX and the other half with 2. The pattern on demand even allowed to decrease the doses of concomitant DMARDs. A subsequent individualised analysis of patients will allow us to evaluate the possible characteristics that can affect the effectiveness of the pattern on demand

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THE IFN TYPE I GENE EXPRESSION IN A CARDIOVASCULAR DISEASE CONTINUUM OF RHEUMATOID ARTHRITIS

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Introduction Rheumatoid Arthritis (RA) patients have a prominent increase in cardiovascular (CV) comorbidity not fully explained by traditional risk factors.¹ Interferons type 1 (IFN-1) have been associated with premature CV disease (CVD) in SLE² and are implicated in several aspects of atherosclerosis and acute coronary syndromes.³ In RA a subpopulation of patients also display an IFN-1 signature in the blood,⁴ which is associated with response to biologics. The IFN-1 signature association with CVD in RA remains unclear.

Objectives To analyse expression of interferon type 1 response genes (ISGs) along a CVD continuum in patients with RA using a well phenotyped cohort of RA patients whereby