receptor the anti-inflammatory response might be impaired and hence enhance cytokine release by the synovium, but this was not observed. The level of sMER and sAXL was either not high enough to suppress the innate immune response or efferocytosis did not play a major role in these RA patients with end stage disease. The putative effect of sMER on the adaptive immune response remains to be investigated and this might contribute to the pathogenesis of RA.

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Disclosure of Interest None declared.

P077

DYSREGULATED MIR-125A PROMOTES JOINT ANGIOGENESIS THROUGH ENHANCED GLYCOLYSIS

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Career situation of first and presenting author Post-doctoral fellow.

Introduction Psoriatic arthritis (PsA) is characterised by an early vascular phase essential for pannus growth, immune responses and disease progression. Recently, numerous studies have highlighted the emerging importance of endothelial cell (EC) metabolism in disease. Herein, we propose microRNA, miR-125, modulates EC bioenergetics and orchestrates joint angiogenesis as characterised by *ex-vivo* associations, *in-vitro* assays and novel CRISPR/cas9 *in-vivo* zebrafish models.

Objectives To elucidate the role of miR-125a and endothelial cell metabolism in dysfunctional angiogenesis in PsA.

Methods MiRNA levels were quantified in synovial tissue and compared to macroscopic synovial vascularity. HUVECs were transfected with anti-miR-125a for 24 hour and tube formation assays, transwell invasion, wound repair and gene expression analysis performed. Real-time analysis of anti-miR-125 treated ECs metabolism was assessed using the XF-24 Extracellular Flux Analyzer (Seahorse Bioscience). Metabolism markers (GAPDH/PKM2/GLUT1/ATP) were assessed *ex vivo* by immunohistochemistry. *In vivo*, miR-125a CRISPR/Cas9-based knock-out zebrafish were generated and vascular development monitored. Finally, miR-125a^{-/-} ECs and zebrafish embryos were treated with an inhibitor of PFKFB3, 3PO.

Results Synovial expression of miR-125 was significantly decreased in PsA versus OA synovial tissue, levels of which were associated with macroscopic and microscopic synovial vascularity. Decreased expression of miR-125a in HMVEC resulted in increased tube formation, invasion and migration properties. Inhibition of miR-125 promoted a metabolic shift towards glycolysis with parallel changes at the gene level. *Ex vivo*, increased vascular expression of glycolytic markers was observed in PsA versus OA synovial tissue. *In vivo*, miR-125a knockout zebrafish displayed increased vascular sprouting similar to the irregular nature of the vasculature within the PsA synovium. Finally, 3PO significantly inhibited anti-miR-125a-induced mechanism whilst normalising the vascular development of miR-125a^{-/-} embryos.

Conclusions Decreased expression of miR-125 in PsA synovium and *in-vivo* models was strongly associated pro-angiogenic mechanisms. Elevated glycolysis following miR-125 inhibition enabled ECs to meet the increased energy demands for new vessel formation. Correcting these miRNA deficiencies and their resulting metabolic shift, either by conventional pharmacological or as novel drug targets, may provide therapeutic benefit, especially in early disease.

Disclosure of Interest None declared.

P078

TRANSFORMING GROWTH FACTOR-BETA DAMPENS PRO-INFLAMMATORY INTERLEUKIN-6 SIGNALING IN CHONDROCYTES BY DECREASING THE INTERLEUKIN-6 RECEPTOR

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Career situation of first and presenting author Young investigator

Introduction The pro-inflammatory cytokine interleukin-6 (IL-6) is present in high levels in arthritic diseases and has been implicated in cartilage degeneration. In contrast, transforming growth factor- β (TGF- β) is a homeostatic regulator of cartilage that decreases the catabolic effects of pro-inflammatory cytokines in chondrocytes. In tissues other than cartilage, multiple mechanisms of cross-talk between TGF- β and IL-6 are reported.

Objectives In this study, we investigated whether TGF- β is able to control IL-6 signaling in chondrocytes.

Methods Human primary chondrocytes and the human G6 chondrocyte cell line were stimulated with rhTGF- β 1 (1.0 ng/ml). We determined mRNA expression levels of IL-6 and IL-6 receptor (IL-6R) using qPCR, measured IL-6 protein release using Luminex technology and IL-6R protein levels by Western Blot. rhIL-1 β was used as a positive control for IL-6 induction. Induction of signal transducer and activator of transcription (STAT)3 phosphorylation by TGF- β was determined using Western Blot in presence of the IL-6R inhibitor Tocilizumab. Induction of suppressor of cytokine signaling (SOCS)3 gene expression was used as a read-out for IL-6/STAT3 mediated transcriptional activity.

Results TGF- β increased both IL-6 mRNA expression and protein release in G6 and primary chondrocytes. Moreover, we observed activation of the main IL-6R downstream signaling protein STAT3 after TGF- β exposure, which could be completely prevented by blockade of the IL-6R, suggesting IL-6 dependency. Surprisingly however, TGF- β did not increase expression of the well known IL-6 target gene SOCS3, despite the increased levels of IL-6 and p-STAT3 we observed in these cells. Moreover, the effect of exogenously added rhIL-6 was markedly inhibited by TGF- β , as 6h pre-treatment prevented rhIL-6-mediated induction of both SOCS3 and p-STAT3. Interestingly, we observed decreased expression of the IL-6R in both primary and G6 chondrocytes after exposure to TGF- β , which might explain the decreased intracellular IL-6 signaling. This effect was not dependent on IL-6 itself, as IL-1 β did not affect IL-6R expression, despite being a strong inducer of IL-6. Finally, we showed that rescuing IL-6R levels using soluble IL-6R dose-dependently restored induction of SOCS3 and p-STAT3 by rhIL-6 in the presence of TGF- β .

Conclusions Our study reveals a novel, protective effect of TGF- β that could help preserve cartilage homeostasis under inflammatory conditions by dampening IL-6 signaling via IL-6R down regulation. In arthritic diseases, the presence of soluble IL-6R might bypass this protective TGF- β effect and contribute to cartilage damage.

Disclosure of Interest None declared

P079

IS IGM RHEUMATOID FACTOR PRESENT ON CIRCULATING EXTRACELLULAR VESICLES OF RHEUMATOID ARTHRITIS PATIENTS A POTENTIAL BIOMARKER FOR DISEASE SEVERITY?

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Introduction Rheumatoid factor (RF) are autoantibodies and measured in serum of rheumatoid arthritis patients (RA) to diagnose the disease. High serum RF levels are also predictors of more severe disease forms although the functional role of RF is still unclear.¹ Most research on RA has been focused on cytokines as main effectors in the disease process but cell-cell communication involves a much broader scope of responses such as via the release of extracellular vesicles (EVs).² EVs are small particles of around 100 nm and released by cells. They mediate intercellular communication by exchange their content (RNA, miRNA and proteins).

Objectives As EVs are communicators that carry a multitude of signals and can be derived from all cells including B-cells we set out to determine whether RF is detectable on circulating EVs in RA patients and if this relates to parameters of disease activity.

Methods EVs were isolated from platelet-free plasma of 41 RA patients by size exclusion chromatography. We quantified the particle and protein concentration, using NanoSight particle tracking analysis and micro-BCA. Tender (TJC) and swollen joints (SJC) were assessed by physicians and the global patient visual analogue score (VAS) was determined by the patient. IgM-RF, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were determined by standard laboratory blood tests in our hospital. Disease activity score (DAS28) was calculated.

Results In plasma of 28 out of 41 RA patients RF was detectable, and in 13 out of these 28 RA +RF was also detected on their isolated pEVs (RF⁺pEVs). No statistical differences were found on particle size, protein content per particle and amount of particles of pEVs between seronegative (RA-) and seropositive (RA+) patients (116 nm, 39fg, 5.4×10^{10} and 114 nm, 58fg, 1.8×10^{10} , respectively). Comparing disease parameters no differences were found between RA+ and RA- patients, except for increased ESR levels in RA+ patients. However, RA+ patients having circulating RF⁺pEVs showed significant higher CRP and ESR levels and VAS and DAS28 scores as compared to RA+ patients without circulating RF⁺pEVs while no differences were found on TJC and SJC.

Conclusions This study shows for the first time the presence of RF on pEVs in a subset of RA+ patients and this could be a plasma marker to distinguish RA patients with a more

severe disease. We found that RF+ EVs is not a reflection of the number of affected joints but might be more indicative for the underlying pathophysiology of RA.

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Disclosure of Interest None declared.

P080

THE EFFECT OF ACTIVIN A AND FOLLISTATIN ON THE INTERACTION OF ENDOTHELIAL CELLS AND RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Introduction The autoregulatory cycle of activin A and its antagonist follistatin is well known in the hypothalamic-pituitary-gonadal axis, but activins also play an important role in autoimmune diseases, such as rheumatoid arthritis (RA). Due to inflammation, activin A is released, then inducing its antagonist follistatin. This negative feedback mechanism has been primarily described in hepatocytes, but is inactive in synovial fibroblasts from patients with rheumatoid arthritis (RASf). Synovial fibroblasts are mediators of cartilage destruction in RA. In inflamed synovium, there is an interaction of RASf with endothelial cells. Due to tissue hyperplasia and inflammation, neoangiogenesis, mediated in part by local fibroblasts, is increased in RA synovium. The effect of activin and follistatin on the interaction of RASf and endothelial cells is still unknown.

Objectives This study focuses on the effect of the activin A and follistatin on the interaction of RASf and endothelial cells.

Methods RASf were isolated from synovial tissue of patients with RA undergoing joint replacement surgery. Endothelial cells (HUVEC) were commercially obtained. RASf and HUVEC were stimulated in mono-, or coculture with activin A (15 ng/ml), follistatin (500 ng/ml) or IL-1 β (1 ng/ml). After 18 hour the supernatants were collected and the concentrations of activin A, VEGF and IL-6 were measured by ELISA.

Results IL-1 β induced activin A release in RASf and HUVECs alone as well as in direct coculture. The stimulation with follistatin and IL-1 β at the same time reduced the activin A concentration in HUVECs as well as in cocultures in comparison to stimulation with IL-1 β alone ($p < 0.05$; $n = 4$). In monocultures of RASf this reduction could not be detected.

With respect to HUVECs, a significant reduction ($p < 0.05$) of the proinflammatory cytokine IL-6 after stimulation with activin A and IL-1 β in comparison to the stimulation with IL-1 β alone could be observed.

The release of VEGF, which plays an important role in inflammation-induced neoangiogenesis, was induced in RASf with IL-1 β (89%), activin A (55%), activin A and IL-1 β (148%), follistatin and IL-1 β (84%). In coculture with HUVECs, the induction was less pronounced as in