that is partially TNF α -mediated. This suggests that the timing of exposure to S100A8/A9 is an important determinant for monocyte-to-osteoclast differentiation.

Disclosure of Interest None declared.

P075

TARGETING NF-KB SIGNALLING IN B CELLS: A POTENTIAL NEW TREATMENT MODALITY FOR ANCA-ASSOCIATED VASCULITIS

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

Introduction The pivotal role of B cells in the pathogenesis of autoimmune diseases such as ANCA-associated vasculitis (AAV) is well-established and further substantiated by beneficial therapeutic effects of rituximab (anti-CD20 B cell targeted therapy). However, this results in prolonged B cell depletion while long-lived plasma cells are not targeted. Thus, there is a need for novel therapeutics targeting the B-cell lineage in AAV. NF- κ B signalling pathways that act downstream of various B cell surface receptors, including the B cell receptor, CD40, BAFFR and TLRs, are crucially involved in B cell responses and may be suitable as novel targets.

Objectives To identify whether inhibition of NF- κ B signalling by novel pharmacological inhibitors is effective in targeting B cell responses in general and more specifically blocks (auto) antibody production and plasmablast differentiation in B cells from AAV patients.

Methods PBMC and sorted B cells from AAV patients and healthy donors were cultured with T cell-dependent (anti-IgM + anti CD40 + IL-21) and T cell-independent (CpG + IL-2) stimuli. NF- κ B signalling was targeted in these cultures by small molecule inhibitors of NF- κ B inducing kinase (NIK, non-canonical NF- κ B signalling) and Inhibitor of κ B kinase β (IKK β , canonical NF- κ B signalling). Downstream NF- κ B signalling and nuclear NF- κ B translocation was determined by Western blot and confocal imaging. Effects on B cell proliferation and differentiation were determined by CFSE dilution assays and flow cytometric analysis of B cell markers. (Auto)antibody production was measured by ELISA.

Results In B cells of AAV patients and healthy donors, targeting of NIK and IKK β effectively inhibited downstream non-canonical or canonical NF- κ B signalling, respectively. In a B cell stimulation assay, NIK and IKK β inhibition significantly reduced T cell-dependent (anti-IgM + anti-CD40 + IL-21) and T cell-independent (CpG + IL-2) B cell proliferation. In addition, B cell differentiation towards plasmablasts (CD27++/CD38+) and functional antibody production was attenuated by both NIK and IKK β inhibitors. Interestingly, the effects of NIK inhibition appeared to be B cell-specific as T cell proliferation was largely unaffected.

Conclusions These data demonstrate that inhibition of NF- κ B signalling in AAV B cells results in the modulation of various

B cell responses. Ongoing studies will indicate whether targeting of NF- κ B signalling in B cells may be an effective novel treatment modality for AAV.

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P076

SYNOVIAL TISSUE FROM RHEUMATOID ARTHRITIS PATIENTS SHED THEIR ANTI-INFLAMMATORY – AND EFFEROCYTOSIS RECEPTOR MER

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Career situation of first and presenting author Student for a master or a PhD.

Introduction We recently showed that the tyrosine-protein kinase MER, a member of the TAM (TYRO, AXL, MER) receptor family plays a protective role in mouse models of rheumatoid arthritis (RA).^{1 2} In both humans and mice, MER can be proteolytically cleaved and this soluble MER (sMER) acts as a decoy receptor for the TAM receptor ligands Growth Arrest-Specific 6 (GAS6) and Protein S. A recent study showed that sMER correlates with disease activity and bone destruction in RA patients.³ In this study, we measured whether sMER was also increased in the circulation of arthritic mice and in synovial fluid and conditioned medium of synovium explants from RA patients.

Methods KRN serum transfer arthritis was induced and mice were sacrificed at day 7 of full blown arthritis. Ankle joints were immunostained for MER (n=15) and sMER levels were measured in serum at day 0 and 7 (n=13). Human synovial explants of RA (n=15) and osteoarthritis (OA)(n=17) patients were immunostained for MER. Synovial explants were cultured 24 hours and released sMER and GAS6 was measured by ELISA. The level of sMER, sAXL, GAS6, IL-6, TNF α , IL-1 β was measured in synovial fluid of RA (n=21) and OA patients (n=11) by ELISA or multiplex array.

Results Serum sMER levels were increased in arthritic mice whereas the amount of MER⁺ synovial cells was unaltered. In human RA synovium, numerous MER⁺ macrophages were present in the lining and sublining. We found significantly enhanced sMER and GAS6 in the synovium culture media (p=0.0045 and 0.0177) and synovial fluid (p=0.0001 and 0.0189) of RA patients as compared to OA patients. Soluble AXL levels in synovial fluid of RA and OA patients was high but did not differ (p=0.5256). In synovial fluid of RA patients, sMER and GAS6 did not correlate with TNF α (Pearson r=0.5587) or IL-1 β (Pearson r=-0.1667), and negatively correlated with IL-6 (Pearson r=-0.9608).

Conclusions Systemic sMER levels are enhanced in experimental arthritis, in line with the observation of Xu et al in RA patients. Synovial shedding of MER was higher in RA- than in OA patients, but this was not reflected by differences in MER positive synovial cells. As sMER acts as a decoy

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 .