quantification in polarized macrophages showed that miR-326 is mainly expressed by the M2-type macrophages, and *MEFV* by the M1-type macrophages. Loss-of-function studies showed that neutralization of miR-326 in M2 macrophages induced the expression of *MEFV* and *CXCL10* while reducing *MRC1* expression level. Furthermore, enforced expression of miR-326 in M1 macrophages significantly repressed *MEFV* expression and induced the production of IL-10.

Conclusions A miR-326/*MEFV* axis seems to be implicated in macrophage polarization and might explain the observed monocyte versatility in FMF.

Disclosure of Interest None declared.

P073

HIGH LDL LEVELS LESSEN BONE DESTRUCTION DURING ANTIGEN-INDUCED ARTHRITIS BY INHIBITING OSTEOCLAST FORMATION AND FUNCTION

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

Introduction Rheumatoid arthritis (RA) is a chronic inflammatory disease, characterized by severe joint inflammation and bone destruction as the result of increased numbers and activity of osteoclasts. In RA, joint destruction is associated with high levels of low-density lipoprotein (LDL), which in inflammatory environments is oxidized into oxLDL. However, the effects of high (ox)LDL levels on the differentiation and activation of osteoclasts remains elusive.

Objectives Here, we investigated the effects of high LDL and oxLDL levels on osteoclast differentiation.

Methods Antigen-induced arthritis (AIA) was induced in *Apoe*^{-/-} mice that spontaneously develop high LDL levels. Bone erosion was assessed with histology and numbers of osteoclasts were determined with staining for tartrate-resistant acid phosphatase (TRAP). Numbers of CD11b⁺/Ly6C^{high} and CD11b⁺/Ly6C^{high} osteoclast precursors were determined by flow cytometry. *In vitro* osteoclast differentiation from bone marrow cells was induced with macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B (RANK) for 7 days. Gene expression was determined with qPCR.

Results Whereas basal levels of bone resorption were comparable between WT and *Apoe*^{-/-} mice, induction of AIA resulted in significantly lower bone resorption in *Apoe*^{-/-} mice as compared to WT mice, which was associated with lower number of TRAP⁺ osteoclasts along the bone surface. However, the absence of *Apoe* did not result in altered numbers of osteoclast precursors in the bone marrow of naïve mice, whereas even increased numbers were observed in *Apoe*^{-/-} mice during AIA. Moreover, *in vitro* osteoclastogenesis showed comparable numbers and mRNA expression of osteoclast markers, such as c-Fms, RANK, NFATc1, DC-STAMP, TRAP, CTR, CIC-7, CA-II, Cat K and MMP-9. Addition of oxLDL, but not LDL, to pre-osteoclasts from day 3 and mature osteoclasts from day 6 of osteoclastogenesis strongly reduced the number of TRAP⁺ osteoclasts and their resorptive capacity. This was accompanied by a decreased expression of various osteoclast markers.

Interestingly, oxLDL decreased the expression of osteoclast-associated receptor (*Oscar*) and the DNAX adaptor protein-12 encoding gene *Tyrbp*, which regulate the immunoreceptor tyrosine-based activation motif (ITAM) mediated co-stimulation signaling pathway that is strongly involved in osteoclastogenesis.

Conclusions *Apoe*^{-/-} mice have decreased bone resorption during experimental RA, probably via oxLDL-mediated interference in the co-stimulatory pathway during osteoclastogenesis.

Disclosure of Interest None declared.

P074

THE ALARMIN S100A9 HAMPERS OSTEOCLAST DIFFERENTIATION FROM CIRCULATING PRECURSORS BY REDUCING THE EXPRESSION OF RANK

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

Introduction The alarmin S100A8/A9 is produced in high levels in inflamed synovium during arthritic diseases and has been implicated in sterile inflammation-induced bone resorption. We have previously shown that this alarmin increases the bone-resorptive capacity of mature osteoclasts. However, the effects on osteoclast differentiation remains elusive.

Objectives Here, we investigated the effects of S100A9 on osteoclast differentiation from CD14⁺ circulating precursors.

Methods CD14⁺ monocytes were isolated from buffy coats of healthy donors and differentiated towards osteoclasts with macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B (RANK) ligand in the presence or absence of S100A9. Differentiation state of osteoclasts was determined by tartrate-resistant acid phosphatase (TRAP) staining and resorption capacity using hydroxyapatite-like-coated plates. RNA expression was analyzed with RNA sequencing and qPCR. RANK expression was assessed using FACS. Underlying epigenetic programming was studied using chromatin immunoprecipitation. Secretion of pro-/anti-inflammatory mediators was analyzed with Luminex analysis.

Results S100A9 stimulation during monocyte-to-osteoclast differentiation resulted in a strong decrease in the numbers of multinucleated osteoclasts, underlined by a decreased resorptive capacity. The thus differentiated cells showed a high production of pro-inflammatory factors, such as interleukin (IL)-1 β , IL-6, IL-8, and tumor necrosis factor- α (TNF α) after 16 hour of stimulation. In contrast, at day 4, the cells showed a decreased expression of the osteoclast-promoting factor TNF α . Interestingly, S100A9 stimulation during the first 16 hour of culture was sufficient to reduce osteoclastogenesis. We observed that within this time frame, S100A9 inhibited the M-CSF-mediated induction of RANK, which associated with changes in various histone marks at the epigenetic level. This S100A9-induced reduction in RANK could be partially reversed by blocking TNF α , but not interleukin-1 (IL-1).

Conclusions Whereas S100A8/A9 was previously shown to stimulate the resorptive capacity of mature osteoclasts, we here show that early S100A9 stimulation impedes monocyte-to-osteoclast differentiation via reduction of RANK expression

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