thereby mimicking RA-related conditions. Cytokine/chemokine secretion of IL-6, IL-10, IL-12, IL-8 and CXCL13 was measured using ELISA. Changes of inflammatory pathways including JAK/STAT were evaluated by Western Blot.

Results TLR4-stimulation of M2-macrophages results in suppression of miR-221-3p, while *in vivo* expression in synovial tissue and fluids is significantly increased in RA versus OA or OIA. M2-macrophages with high levels of miR-221-3p lose their anti-inflammatory response and support a M1-like profile. miR-221-3p in combination with miR-155-5p leads to the secretion of M1-specific IL-12 and lowered IL-10. miR-221-3p is affecting the JAK/STAT pathway in M2-macrophages by downregulating JAK3 protein leading to decreased STAT3 activation. Treatment of M2-macrophages using JAK3 inhibitors display the same shift in cytokine/chemokine pattern as increased miR-221-expression.

Conclusions TLR4-activated M2-macrophages exposed to elevated miR-221-3p expression diminished anti-inflammatory response partly by suppressing JAK3/STAT3 pathway. Thus, miR-221-3p is a homeostatic regulator of M2-M1 inflammatory function, which is dysregulated in RA condition.

Acknowledgements Tissue RNA from RA and OA patients was kindly provided by Caroline Ospelt, University of Zurich, Center of Experimental Rheumatology, Switzerland.

Disclosure of Interest None declared.

P071/O13 RNA SEQUENCING DETECTION OF GENE DYSREGULATION IN B CELLS SORTED FROM SALIVARY GLAND TISSUE AND FROM PERIPHERAL BLOOD REVEALS NEW PATHWAYS INVOLVED IN PRIMARY SJÖGREN'S SYNDROME PATHOPHYSIOLOGY

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10.1136/annrheumdis-2018-EWRR2019.60

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

Introduction Primary Sjögren's syndrome (pSS) is a chronic auto-immune disorder characterized by lymphocytic infiltrates and destruction of the salivary glands (SG). Chronic B cell activation, the secretion of autoantibodies and the critical role of BAFF have been demonstrated. However, mechanisms leading to B cells dysregulation remain partially understood.

Objectives To establish transcriptomic maps of the B cells sorted from the SG and from blood in pSS patients and controls using RNASeq.

Methods Patients had pSS according to 2016 EULAR/ACR criteria and controls had sicca symptoms without any antibodies and with normal SG biopsy. B cells were sorted from SG biopsies and from blood using a FACS ARIA. RNASeq profiling was performed using MiSeq. Statistical analysis identified differentially expressed genes between pSS and controls in B cells sorted from SG (9 pSS, 4 controls), from blood (16 pSS, 7 controls); and between B cells sorted from SG and blood in the same patients (4 pSS patients). Functional enrichment analysis used Ingenuity Pathway Analysis.

Results The pSS vs controls comparison in B cells sorted from SG identified up-regulated genes involved in activation of B cells including CD48, CD22 and CD40. TLR10, which is involved in innate immunity was also up-regulated in pSS.

In blood B cells, TLR7 and the downstream signaling molecule IRF7 were up-regulated in pSS. Additionally, IL-6 which is involved in B cells growth was up-regulated. Enrichment analysis highlighted EIF2 signaling pathway, interferon (IFN) signaling pathway and role of JAK in IFN signaling.

The paired comparison between B cells from SG and from blood identified up-regulated genes including CD138, a plasma cell marker, IL-6, TLR5 and IFN induced genes.

The confirmation by qPCR of these results is ongoing.

Conclusions This study allowed to explore the mechanisms that support B cell activation in pSS focusing on tissue resident and circulating cells. Data confirmed B cell activation and differentiation through several markers and highlighted the role of innate immunity and key pathways including IFN and JAK signaling. Precise understanding of these dysregulations should offer development of new targeted therapeutic perspectives.

Disclosure of Interest None declared.

P072 MEFV/MIR-326 AXIS INVOLVEMENT IN HUMAN MACROPHAGE POLARIZATION

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10.1136/annrheumdis-2018-EWRR2019.61

Career situation of first and presenting author Young investigator.

Introduction Familial Mediterranean fever (FMF) is an inherited autoinflammatory disease, characterized by acute self-resolving attacks of fever and serositis, which mainly prevails in populations around the Mediterranean sea. It is caused by mutations in the *MEFV* gene, which encodes the pyrin protein. The alteration of *MEFV* mRNA expression in monocytes is related to both genotype and phenotype of the disease, suggesting that the pathophysiology of FMF can be regulated on a quantitative defect of *MEFV* mRNA.

Objectives Since microRNAs (miRNAs) are implicated in a number of diseases including FMF, the present study aimed at identifying miRNA regulators of *MEFV* expression involved in monocyte inflammatory response.

Methods MiRWalk2.0 database was used to identify putative miRNA target sequences within the 3'-UTR mRNA of *MEFV.* Human primary $CD14^+$ monocytes were sorted from peripheral blood of healthy donors using magnetic microbeads and differentiated into M1 or M2 macrophages following IFNγ/LPS or IL4/IL13 stimulation, respectively. Using RT-qPCR, M1/M2 polarization was validated by measuring the expression of prototypic M1 and M2 markers: the chemokine *CXCL10* and the macrophage mannose receptor 1 (*MRC1* also known as CD206), respectively, as well as the *MEFV* mRNA. We used loss-of-function method to evaluate the effect of candidate miRNA on $CD14^+$ monocytes, i.e. its role on macrophages classical versus alternative polarization. IL-10 expression was quantified using ELISA.

Results *In silico* analyses revealed that miR-326 targets putatively the 3'UTR mRNA of *MEFV.* miRNAs and mRNAs

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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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10.1136/annrheumdis-2018-EWRR2019.62

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, ClC-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 *Tyrobp*, 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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10.1136/annrheumdis-2018-EWRR2019.63

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)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNFα) after 16 hour of stimulation. In contrast, at day 4, the cells showed a decreased expression of the osteoclast-promoting factor TNFα. 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 TNFα, 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

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