Objectives This study aimed to assess whether peripheral monocytes in RA are pre-programmed to become M1 pro-inflammatory macrophages.

Methods Blood was collected from healthy donors, at-risk individuals (Those with arthralgia, ACPA+/RF+, normal CRP and no evidence of synovitis) and established RA patients. CD14+ monocytes were isolated from peripheral blood mononuclear cells using a CD14 magnetic bead separation kit. Cells were stimulated with LPS (100 ng/ml) for 3–24 hours and to assess the effects of STAT3 inhibition, cells were pre-treated with STAT3i (10 μ M) for 30mins. A Human Cytokine and Chemokine PCR array was carried out and those genes most differentially expression were further validated in a larger cohort of patients using RT-PCR. The metabolic profile of cells was analysed using Seahorse XFE Technology, which concomitantly analysis glycolysis and mitochondrial respiration in real-time. Gene and protein expression of key inflammatory and glycolytic markers was also carried out by RT-PCR, western blotting and ELISA.

Results CD14+ RA monocytes are hyper-inflammatory upon stimulation, with significantly higher expression of IL-1 β , TNF α , IL-6, IL-27, CXCL10 and CXCL11 compared to healthy controls, which is indicative of a M1-like pro-inflammatory phenotype. These hyper-inflammatory cells are highly glycolytic, with increased expression of HIF1 α , HK and PFKFB3, key glycolytic enzymes. Both baseline glycolysis and baseline oxidative phosphorylation are increased in RA CD14+ monocytes, paralleled by increased ATP synthesis and maximal respiratory capacity, suggesting a hyper-energetic phenotype. This hyper-inflammatory, hyper-glycolytic phenotype is mediated by STAT3, as selective STAT3 inhibition can significantly decrease M1-like cytokines and PFKFB3 and HK2 expression. In addition, STAT3 inhibition significantly decreases both oxidative phosphorylation and glycolysis pathways. Finally, this pro-inflammatory phenotype is evident in CD14+ monocytes from arthralgia ACPA+/RF+ people at risk of developing disease, demonstrating that these processes may precede clinical manifestations in RA.

Conclusions This study demonstrates the unique inflammatory and metabolic phenotype of RA monocytes, suggesting that peripheral CD14+ monocytes may be pre-programmed to become M1-like pro-inflammatory macrophages. In addition, the observation of this phenotype in at-risk individuals indicates that these features may precede clinical manifestations of RA and therefore could be useful as a biomarker for early diagnosis.

Disclosure of Interest None declared.

P069

CONSTITUTIVE OVEREXPRESSION OF INTERLEUKIN 38 HAS A NEGATIVE IMPACT ON HUMAN NHK KERATINOCYTE FITNESS

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

Introduction Interleukin (IL)-38 is a member of the IL-1 cytokine family. Based on its sequence homology with the IL-1 and IL-36 receptor antagonists, IL-38 was proposed to act as an anti-inflammatory molecule. However, few data are

available to date about its biological role and mechanism of action. Highest constitutive IL-38 expression is detected in the skin, where the cytokine is produced mainly by differentiated keratinocytes.

Objectives The aim of this study is to investigate the function of IL-38 in human keratinocytes, using the immortalized normal human keratinocyte (NHK) cell line stably transfected with vectors allowing for constitutive or inducible IL-38 overexpression.

Methods NHK cell proliferation and mortality were assessed respectively by cell counting and by measuring LDH activity in the supernatant of high density cell culture. Differentiation of NHK cells was initiated by switching to a culture medium containing high Ca⁺⁺ and serum. mRNA levels of differentiating NHK cells were determined by RTqPCR.

Results Constitutive IL-38 overexpression reduced the proliferation of NHK cells and lead to increased mortality. Differentiating NHK cells constitutively overexpressing IL-38 had increased mRNA levels for the early differentiation marker keratin 10 as compared to empty vector transfected control cells. On the contrary, expression of the late differentiation marker involucrin was reduced in NHK cells constitutively overexpressing IL-38. To further explore potential effects of IL-38 on keratinocyte differentiation, while circumventing the impact of its constitutive overexpression on cell fitness, we set up a doxycyclin-inducible Tet-On system to overexpress IL-38 in NHK cells.

Conclusions We are currently optimizing 2D and 3D culture conditions for *in vitro* differentiation of NHK cells to mimic more closely the situation in human skin. We will then study the impact of IL-38 on NHK cell differentiation using cells transfected with the inducible IL-38 expression system. In conclusion, our results suggest that constitutive overexpression of IL-38 decreases proliferation and viability of NHK cells. We will thus use an inducible expression system to further investigate the effects of IL-38 on NHK cells.

Disclosure of Interest None declared.

P070/O09

MIR-221-3P IS UPREGULATED IN RHEUMATOID ARTHRITIS AND AFFECTS JAK3/STAT3 SIGNALING IN M2-MACROPHAGES

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

Introduction Recurrent activation of synovial macrophages is considered as intrinsic factor that precedes rheumatoid arthritis (RA)-related autonomous cytokine cascades. microRNAs (miRs) and Toll-like receptors (TLRs) play a pivotal role in regulating the inflammatory response in innate immune cells and are at dysregulated levels in RA patients.

Objectives Here we explored TLR-regulated miRs that tune the inflammatory response of M2-type macrophages.

Methods Based on a miR-screen in TLR-stimulated human M2-macrophages we selected miR-221-3p to analyze its expression profile in clinical samples from RA, other inflammatory arthritis (OIA), osteoarthritis (OA) and healthy donors (HD) by qPCR. We challenged M1- and M2-macrophages with elevated miR-221-3p and/or miR-155-5p expression

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.

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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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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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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 IFN γ /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