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39th European Workshop for Rheumatology Research

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Differential expression of key metabolic genes in antigen-specific B cell subsets in rheumatoid arthritis and systemic lupus erythematosus

Introduction
Antigen-specific B cells (Ag-Bs) differentiate into distinct subsets upon activation: plasmablasts (pBs, CD19^+ IgD^- CD71^+ CD38^- CD20^-) and memory B cells (mBs, CD19^+ IgD^- CD71^- CD38^dim CD20^+). The frequency of antigen-specific plasmablasts and memory B cells has been described to be altered in the blood of Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) patients. Emerging evidence suggests that blood immune cells from RA and SLE patients exhibit different metabolic requirements and profiles when compared to healthy controls (HC). However, the metabolic configurations of these two Ag-Bs subsets in RA and SLE patients are yet to be described.

Objectives
In the present study we aimed to characterize the gene expression of key metabolic enzymes in mBs and pBs from RA and SLE patients.

Methods
Blood samples were obtained from 5 RA, 2 SLE and 3 HC donors. B cells were isolated and sorted into pBs and mBs populations. The gene expression of key metabolic enzymes was assessed by qPCR in both populations. The target genes analyzed were HIF1A, HK2, LDHA, MYC (glycolysis), CPT1A (fatty acid oxidation), SREBF1 (lipid synthesis) and PRKAA1 (mTOR inhibitor).

Results
When comparing to HC there was a higher expression of: HIF1A, LDHA, MYC, PRKAA1 in RA mBs; HIF1A, HK2, LDHA, SREBF1 in RA pBs; HIF1A, HK2, LDHA, CPT1A, SREBF1 in SLE mBs; MYC and PRKAA1 in SLE pBs. When the gene expression was compared between RA and SLE patients, SLE mBs had a higher expression of HK2, CPT1A and SREBF1 while SLE pBs exhibited higher levels of MYC and PRKAA1 expression. Thus, the observed results suggest that RA mBs, RA pBs and SLE mBs exhibit a glycolytic profile when compared to HC. SLE mBs also seem to have higher fatty acid oxidation and lipid synthesis levels when comparing to HC and RA mBs. The higher expression of SREBF1 in RA pBs could also indicate that lipid synthesis is upregulated in this subset. Interestingly, the results in SLE pBs suggest that all the analyzed metabolic pathways are downregulated when comparing to HC and RA pBs. Finally, since PRKAA1 is upregulated in RA mBs and SLE pBs one might hypothesize that inhibition of mTOR is greater in these subsets.

Conclusions
The size of our sample is the principal limitation in this study and therefore we aim to increase it. Nevertheless, these preliminary results seem to indicate that antigen-specific B cells exhibit marked glycolytic profiles in RA and SLE patients. These results are expected in RA given the highly glycolytic requirements of T cells in this disease. Our study demonstrates that B cell metabolism should be characterized in depth in both RA and SLE.

Disclosure of Interest
None declared.
Disclosure of Interest None declared.

P006 INVESTIGATION OF IGA IMMUNE COMPLEX CAPTURE BY FCRL4+ B CELLS IN PERIPHERAL BLOOD AND SYNOVIAL FLUID

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

Introduction Increasing evidence points to a mucosal origin of the autoimmune process in the development of rheumatoid arthritis. A B cell subset identified by surface expression of Fc receptor-like 4 (FcRL4) associates in inflammation in the mucosa associated lymphoid tissue.¹ Our group identified these cells in the synovium of RA patients.² They express high levels of RANKL and participate in the autoimmune response to citrullinated proteins.³ ⁴ Recent in vitro work suggested that FcRL4 may be a low-affinity receptor for aggregated IgA.³ ⁵ FcRL4 +B cells in RA SF express low levels of CD21 and high levels of CD11c, a phenotype they share with Age-Associated B cells (ABC). Very low numbers of ABC are found in blood of healthy individuals but their frequency increases with age however their exact role in RA is not yet understood.⁶

Objectives 1) Investigate the binding activity of IgA immune complexes to FcRL4+ B cells in tonsil, RA synovial fluid, and the peripheral blood of healthy individuals and RA patients. 2) Determine if FcRL4 can specifically capture IgA from the RA SF. 3) Examine the specificity of FcRL4 for secreted and serum IgA and for different IgA iso types.

Methods SF, peripheral blood and tonsil mononuclear cells were labelled for FcRL4, IgA, IgG, and CD19 (PBMCs were also labelled for CD21 and CD11c) and analysed by flow cytometry. Cells pre-treated with a pH3 buffer to remove receptor-bound immunoglobulins were compared with untreated cells to distinguish IgA B cell receptor expression from receptor-bound IgA Immune complexes. An FcRL4 expressing cell line was incubated with RA synovial fluid and purified human IgA to assess FcRL4s specificity.

Results Receptor-bound IgA Immune complexes were observed on ex vivo RA SF and tonsil FcRL4+ B cells but not on FcRL4+ B cells (p=0.001). Intriguingly, this IgA capture was not observed in peripheral blood FcRL4+ ABC from HC or RA patients. Furthermore, the proportion of B cells with IgA BCRs was increased in tonsil and SF FcRL4+ B cells but not in peripheral FcRL4+ B cells, compared to FcRL4- B cells. Using a cell line, we show that FcRL4 specifically binds IgA immune complexes from SF and shows a preference for IgA1 over IgA2 (p=0.02).

Conclusions FcRL4 is a receptor for IgA immune complexes in RA joints. RA SF and tonsil FcRL4+ B cells RA SF differ from peripheral blood FcRL4+ ABC in their in vivo capture of IgA immune complexes and in their frequency of IgA BCRs, suggesting distinct function and origin of these cells.

Disclosure of Interest None declared.

P007 AUTOANTIBODIES’ TITRE CHANGES DURING ANTI-BLYS TREATMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Career situation of first and presenting author Assistant.

Introduction Belimumab, a human monoclonal antibody anti-soluble B lymphocytes stimulator, is the only biological drug approved for Systemic Lupus Erythematosus (SLE). It reduces disease activity and blocks damage progression.¹ ² Anyway, no definitive results have been published regarding its effect on autoantibodies’ titre.

Objectives To analyse the modulation of anti-dsDNA, anti-ENA and anti-phospholipid antibodies’ titre by Belimumab in patients affected by SLE.

Methods 50 SLE¹ patients starting Belimumab were enrolled. Sera were collected at baseline (T0) and every 6 months until 24th month. Clinical features and disease activity index (namely SLEDAI-2K)³ were collected at any timepoints. Anti-dsDNA were analysed by radioimmunological method, anti-cardiolipin (aCL) and anti-beta2 glycoprotein I (anti-B2GPI) were detected by home-made ELISA.⁴ ⁵ Anti-ENA were detected by ELISA; positive sera were further characterized by ELISA to identify anti-Ro, anti-La, anti-ribosomal P protein (ribP), anti-Sm and anti-U1RNP specificities.

Results At T0 anti-dsDNA were positive in 86%, anti-ENA in 50%, a CL in 20% and antibeta2GPI in 28% of cases, respectively. A negative seroconversion was detected in 9/43 anti-dsDNA (21%); in 5/12 anti-beta2GPI IgG (40%) and in 3/10 aCL IgG (30%) sera, mostly at T6. Among anti-ENA specificities 6/10 (60%) anti-ribP and 3/17 (17,6%) anti-Sm positive sera became negative; while anti-Ro and anti-U1RNP antibodies resulted stable during treatment. A significant decrease of anti-dsDNA titre was observed at T6 (p 0,011); T12 (p 0,014); T18 (p 0,0031) and T24 (p 0,013). Anti-beta2GPI IgM isotype showed significant reduction at T6 (p 0,0032), T12 (0,001), T18 (p 0,002) and T24 (p 0,011). ACL antibodies showed significant decrease only for IgG isotype at T18 (p 0,001). Anti-ribP antibodies showed a significant titre decrease at T6 (p 0,015) and T12 (p 0,0078), with complete negative seroconversion at T18. Anti-Sm antibodies significantly dropped down at T6 (p 0,032), than maintained a stable titre during time. A significant correlation between anti-dsDNA titre anti-ribP titre and between SLEDAI ratio (SLEDAI value/SLEDAI T0) and anti-ribP ratio (value/value T0) were found.

Conclusions Belimumab treatment induced a significant reduction of high-affinity anti-dsDNA, anti-Sm and anti-ribP antibodies titre. The decrease of anti-ribP titre correlates with anti-dsDNA titre and disease activity.
Disclosure of Interest None declared.

Conclusions This study shows that in M-CSF-Mφ PADI2 gene expression can be modulated by pro-inflammatory cytokines while PADI4 gene expression cannot be re-induced. Our results reinforce the hypothesis of monocytes and Mφ involvement in generation of the ACPA epitopes in the ST of RA patients.

REFERENCES

Acknowledgements The technical assistance of C. Thomann, G. Offer and I. Belhaouane is gratefully acknowledged.

Disclosure of Interest None declared.

References

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

Introduction Autoantibodies to citrullinated proteins (ACPA) are specifically associated to rheumatoid arthritis (RA) and likely involved in its pathophysiology. ACPA are produced in the inflamed synovial tissue (ST) where peptidylarginine deiminase (PAD) 2 and 4, responsible for fibrin citrullination, generate the ACPA-targeted epitopes. PAD2 and 4 are expressed in the ST and in the subintimal inflammatory infiltrates by CD68+ mononuclear cells. That made macrophages (Mφ) of the ST suspect to synthesize PADs. Moreover, we demonstrated that among various polarised subsets, the M-CSF-Mφ present the highest pro-inflammatory response to ACPA-containing immune complexes. Recently, we confirmed that PAD2 and 4 are expressed in monocytes and showed that PAD2 is expressed at various degrees in monocyte-derived Mφs generated in the presence of IFN-γ, IL-4, IL-10 or M-CSF, while PAD4 is only detected in the IFN-γ Mφs.

Objectives To evaluate expression of PAD2 and 4 in Mφ polarised by M-CSF, by various polarising or pro-inflammatory cytokines, expressed in the ST.

Methods CD14+ monocytes from healthy donors were differentiated in Mφ in the presence of M-CSF and were subsequently exposed for 18 hour either to polarising cytokines or to pro-inflammatory cytokines such as TNF-α, IL-6, IL-8 or IL-17. PADI2 and 4 gene expression was measured by RT-qPCR and expression of the related proteins evaluated by immunoblotting on total cell extracts.

Results PADI2 mRNAs are less detected in M-CSF-Mφ than in the monocytes while PADI4 gene expression is totally suppressed. All the polarised M-CSF-Mφ subsets retain PADI2 gene expression, and interestingly, after IFN-γ stimulation PADI2 mRNAs are detected at a higher relative rate. However IFN-γ, IL-4 or IL-10 does not induce PADI4 gene re-expression. Whereas PAD4 protein remains undetectable, PAD2 is detected in all the polarised M-CSF-Mφ subsets but not more after IFN-γ stimulation.

M-CSF-Mφ activation by TNF-α, IL-8 or IL-17 does not modulate expression of the PADI2 and 4 genes. IL-6 induces a significant decrease of PADI2 gene expression and has no effects on the PADI4 gene. Finally, the protein expression of PAD2 is not modulated in the various activated M-CSF-Mφs, and PAD4 is never induced.

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

Introduction The development of the B cell repertoire is regulated by the process of affinity maturation that occurs within the inner part of the B cell follicles within secondary lymphoid organs (SLOs). In autoimmune process might occur in ectopic lymphoid structures (ELS), aggregates of lymphocytes that form in target organs of disease (i.e. the salivary glands of patients with Sjogren’s Syndrome (SS)). The phenotypical and functional features supporting ELS pathogenic properties have not been identified. Moreover, the functional proof that ELS independently from SLOs contribute to the autoimmune response has not been provided.

Objectives To characterise the transcriptome profile of human ELS isolated from SS salivary glands in comparison with SLO and to dissect, in an animal model of ELS development, the ability of ELS to contribute to the autoreactive response.

Methods Frozen salivary gland biopsies were obtained from SS patients and selected for presence of germinal center +ELS. Samples were stained and microdissected, RNA isolated and transcribed and used for RNAseq using ClonTech SMARTseq v4 kit.

Conclusions Transcript analysis provide evidence that the ectopic ELS are characterised by upregulation of TNF, INFγ, BAFF, APRIL, CXCL12 and CXCL13, FAS and FASL as compared to SLOs. Sequencing unveiled an altered cell-proliferation profile with downregulation of BCL6 and AID, the enzymes responsible for B cell affinity maturation. Selective depletion of FAP +cells induced loss of anatomical segregation of ELS and profoundly compromised their anatomy with significant impact on the local autoimmune response.

Acknowledgements The technical assistance of C. Thomann, G. Offer and I. Belhaouane is gratefully acknowledged.

Disclosure of Interest None declared.
demonstrated that, although characterised by similar anatomical organization, ELS pathogenic cytokine signature is associated with low levels of bcl6 and Aid and aberrant apoptosis, unveiling impaired regulation of the B cell cycle, responsible for the survival of autoreactive, poorly selected B cell clones. Accordingly, selective depletion of ELS in vivo profoundly impact local and systemic autoimmunity.

REFERENCE

Disclosure of Interest None declared.

Characterization of Distinct Dendritic Cell Subsets in the Context of Chronic Inflammation
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Career situation of first and presenting author Assistant.

Introduction Dendritic cells (DCs) are central regulators of the balance between immunity and tolerance, and alteration of the specialized DCs system is a common feature in chronic inflammatory diseases, such as rheumatoid arthritis (RA). In RA, synovial tissues are characterized by aberrant distribution of DCs populations, with altered phenotype. However, an in-depth characterization of the altered phenotype and functions of DCs in RA has been hampered by a lack of specific DCs markers and relevant in vitro culture model.

Objectives To perform an in-depth phenotypic characterization of DCs found in RA joints with an extensive panel of monoclonal antibodies specific of DCs, and to establish a relevant in vitro culture model to generate DCs resembling to those found in joints.

Methods Flow cytometry was used for analysis of cell surface molecules expressed on DCs found in RA synovial fluid (SF). Staining was performed with a panel of 100 mouse monoclonal antibodies specific of DCs, generated by immunization with different subsets of human DCs. Phenotype of DCs from synovial fluid was then compared to the phenotype of monocytes co-cultured in vitro with synoviocytes from RA patients, in presence or not of synovial fluid, or cytokines.

Results In RA SF patients, two main DCs subsets were identified, with distinct surface protein phenotypes. One subset was characterized by markers of plasmacytoid DCs (CD303), whereas the other subset expressed markers of myeloid/conventional DCs (CD11c, DCIR). This last subset of DCs expressed some markers found on inflammatory DCs, the main inducers of Th17 cells in RA joints. Surprisingly, the two subsets of DCs expressed also surface proteins shared by synoviocytes. Culture of human monocytes with synoviocytes and/or combination of pro-inflammatory cytokines (TNF, M-CSF, IL-4) yielded DCs with a phenotype close to the phenotype of conventional DCs found in RA synovial fluid.

Conclusions We uncovered previously unreported phenotypic heterogeneity of DCs in a chronic inflammatory context, and characterized two main subsets of DCs. The next steps will be to understand the consequences of this heterogeneity on the pathogenicity of the disease. This will be facilitated by our relevant in vitro culture model that allows the generation of DCs, closely resembling those found in joints of patients with RA.

Disclosure of Interest None declared.
PATIENTS WITH CANCER AND PREEXISTING AUTOIMMUNE AND INFLAMMATORY DISEASES TREATED BY ANTI-PROGRAMMED DEATH 1 (PD-1) ANTIBODIES AT GUSTAVE ROUSSEY CANCER CENTER

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

Introduction Immune checkpoint inhibitors anti PD-1 are monoclonal antibodies used in cancers.1 PD-1 ligand/PD-1 pathway antagonism between cancer cells and antitumoral cytotoxic CD8+ T cells increase antitumoral immunity but is involved in autoimmune diseases (AID).

Objectives Evaluate tolerance and efficacy of anti PD-1 in AID patients.

Methods Patients had been included in REISAMIC registry (Registry of Severe Adverse Events of Immunomodulating Monoclonal Antibodies in Oncology) between June 1st, 2014, and December 31st, 2017. Patients were treated with anti-PD-1. Exclusion criteria were malignant hematologic disease, second advanced cancer or chronic viral infection. AID subtypes were defined for sensitivity analyses: diseases of clinical interest (DCI), prognostic interest (DPI) and vitiligo. One patient with pre-existing AID were matched on age, sex and cancer type with 3 patients without preexisting AID (controls). Analyses were adjusted for OMS status, corticosteroid, cerebral metastasis, LDH, albuminemia and neutrophil/lymphocyte ratio >3. Endpoints were grade ≥2 irAE free survival and overall survival (OS). Objective response rates (ORR) were described.

Results 641 patients were included in REISAMIC. Among them, 69 patients were excluded. 572 patients had been included: 63 with AID and 509 controls. Among AID patients, we observed 38% DCI, 38% DPI and 24% vitiligo. DCI were Sjögren syndrome (n=4), rheumatoid arthritis (n=4), polymyalgia rheumatica/giant cell arteritis (n=2) and others. DPI were psoriasis (n=11), thyroiditis (n=8) and others. We matched 55 AID patients with 165 controls. Cancer type, TNM or AJCC grade, age and sex were similar between AID patients and controls. Overall survival (HR 0.67, IC95% [0.42–1.08], p=0.098) and irAE free survival (HR 1.27, IC95% [0.84–1.90], p=0.25) were not different between AID patients and controls. After vitiligo exclusion in sensitivity analyses, irAE free survival was shorter for AID patients than controls (HR 1.69, IC95% [1.2–2.87], p=0.05) and more for DCI patients only (HR 2.66, IC95% [1.01–7], p=0.049). ORR were 35.5% and 28.5% for AID patients and controls respectively.

Conclusions Preexisting AID should not be a contraindication for anti PD-1. We suspect that mechanisms involved in AID are important in irAE mechanism and antitumoral response. CD4+ T cells and Th17 cells should be study in these situations.

P014/004 PHENOTYPIC HETEROGENEITY OF REGULATORY T CELLS IN RHEUMATOID ARTHRITIS

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

Introduction Rheumatoid arthritis (RA) is the most frequent chronic inflammatory rheumatism characterized by infiltration of many immune cells into the synovial tissue leading to cartilage and bone degradation over time. The working hypothesis of our team is to use immunomodulatory properties of regulatory T (Treg) cells to restore immune homeostasis in RA. These approaches are based on a better knowledge of the cell populations present in the inflamed tissue and in particular the sub-populations of Treg cells. Heterogeneity of Treg cells is well known however most Treg studies in RA have focused on CD25+FoxP3+ Treg cells in the peripheral blood of patients.

Objectives In this project using innovative approaches such as 18-colors cytometric analyses our project aims to better characterize the Treg subsets to unravel the heterogeneity of the Treg cell subsets in RA.

Methods The phenotypic analyses will be performed using 18-colors conjugated antibodies, analyzed on the LSR II, to allow the simultaneous characterization of CD25+FoxP3+ Tregs, Tr1 cells and CD8+ Tregs within the T lymphocytes. Activation status of the various subsets will be also investigated using various activation markers. The phenotypic characterization will be performed on the synovial fluid (SF) and peripheral blood (PB) from RA and Osteoarthritis patients used as controls.

Results The preliminary results showed that the frequency of CD25+FoxP3+ is higher in the SF compare with PB in both pathologies. Furthermore, the phenotype and activation status of CD25+FoxP3+ Treg cells is different between both fluids with an accumulation of effector activated CD25+FoxP3+ Treg cells in the SF. Interestingly, whereas Tr1 cells are barely detectable in the peripheral blood, the Tr1 subset seems to be enriched in the synovial fluid in both pathologies.

Conclusions This study confirm the presence of activated CD25+FoxP3+ Treg cells in the SF of RA and provide evidence for the presence of other Treg subsets in SF. A better characterization of the suppressive activity of the various Treg subsets need to be investigated and particularly in inflammatory conditions. A better understanding of the suppressive function of the various Treg subsets will thus provide identification of new therapeutic targets in RA patients.

Disclosure of Interest None declared.

REFERENCE

Disclosure of Interest None declared.
Abstracts

P016 VASCULAR INVOLVEMENT IN RHEUMATIC DISEASES – THE ROLE OF IMAGING AND IMMUNOLOGICAL BIOMARKERS

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Career situation of first and presenting author Assistant

Introduction Vascular abnormalities are common complications in rheumatic patients. Their background is multifactorial and still is a subject of the debate.

Objectives This study was designed to evaluate the significance of selected noninvasive imaging indices, immunological and genetic biomarkers in diagnosis of vascular lesions in rheumatic diseases.

Methods The study group included 288 patients with systemic connective tissue diseases (SCTD). The noninvasive evaluation of vascular lesions was made on the basis of carotid intima-media thickness (cIMT), ankle brachial index (ABI), high resistance index (HRI) and ulnar artery intraluminal diameter (UAID) measurements using HDI 3500 (ATL).

We analyzed more than 100 variables: autoantibodies, inflammatory and angiogenic markers, genetic polymorphisms and classical risk factors for atherosclerosis.

Statistical analysis was performed with STATA 11 including: chi2 Yates, chi2Pearson and rank Spearman correlations tests, logistic regression analysis and multivariate stepwise analysis.

Results Macroangiopathy was influenced by selected autoantibodies including antiphospholipid (OR=4.4; 95% CI:1.1–20.7) and anti-endothelial cell (OR=6.6; 95% CI:1.6–28.3) as well as inflammatory biomarkers (OR=3.6; 95% CI:1.1–11.8). The analysis of genetic polymorphisms showed especially an important impact of VEGF 2578 AA genotype on atherosclerosis development (OR=4.8; 95% CI:1.1–21.1). Angiogenic biomarkers were strongly associated with prothrombotic risk (OR=22.8; 95% CI:2.3–230.6). The analysis of relations between imaging indices and vascular manifestations revealed significant association of cIMT with cardiovascular (OR=52.9; 95% CI:7.0–1012.7) and cerebrovascular disease (OR=4.0; 95% CI:1.0–15.3). There was significant reverse correlation between ABI and peripheral vascular disease (R=−0.33; p=0.001). HRI values significantly correlated with thromboembolic disorders (R=−0.29; p=0.03). Finally, UAID was notably related to microangiopathic complications (p<0.05).

Conclusions The protocol for vascular lesions diagnosis in SCTD should be based on the combination of imaging and laboratory biomarkers. Immunological and inflammatory factors are crucial in diagnostics of vascular involvement in rheumatic diseases. IMT and ABI showed a high prognostic value and can be used for the general cardiovascular risk stratification.

Disclosure of Interest None declared.

P018 ANTI-CARbamylated PROTEIN ANTIBODIES AS A CLINical RESPONSE PREDICTOR IN Rheumatoid Arthritis PATients Treated With ABATACEPT

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Career situation of first and presenting author Post-doctoral fellow

Introduction RF and ACPA have been used extensively for the diagnosis of RA, however no clear mechanism of action towards disease pathogenesis and progression has been identified. Importantly, both seropositive and seronegative RA patients experience significant improvement in disease severity following B cell depletion. Therefore, we hypothesized that B cells have a central role in ACPA+ and ACPA− RA irrespective of their capacity to produce auto-antibodies.

Objectives To characterize B and T cell populations, their recruitment to the inflamed joint, B cell cytokine production and CD4+ T cell polarization in RA synovial tissue biopsies and peripheral blood of ACPA+, ACPA− RA and arthralgia subjects.

Methods Synovial tissue biopsies from ACPA+ and ACPA− RA and ACPA+ arthralgia subjects, with paired blood/synovial fluid, were obtained through key-hole arthroscopy and were enzymatically digested. B cell invasion assays and B and CD4+ T cell in vitro stimulation were conducted under hypoxic conditions simulating the unique environment of the inflamed joint. Flow cytometric analysis was performed.

Results Significant accumulation, compared to peripheral blood, of pro-inflammatory B cells and pro-inflammatory cytokine-producing CD4+ T cells in the synovial tissue and fluid of RA patients, irrespective of ACPA status, as well as the synovial tissue of arthralgia subjects. SPICE analysis of peripheral blood B cells, for a panel of chemokine receptors, revealed a disease-specific expression pattern detected in RA and arthralgia subjects. Importantly, the tissue-invading B cells expressed CXCR3, with in vitro blockade of CXCR3 resulting in reduced B cell invasion in response to RA synovial tissue biopsys-conditioned media. Under the unique hypoxic conditions of the inflamed joint, RA patient but not healthy subject-derived B cells produce several pro-inflammatory cytokines including TNF-a and IL-6 and are capable of polarizing CD4+ T cells towards a pro-inflammatory phenotype.

Conclusions Accumulation of pro-inflammatory B cell subpopulations in the synovium of both ACPA+ and ACPA− RA patients underlines a common, antibody-independent, contribution of B cells in synovial inflammation. Arthralgia early in disease, specific chemokine receptor expression and the accumulation of CXCR3+ B cells in the inflamed joint offers an opportunity for therapeutic intervention. Once in the hypoxic environment of the inflamed RA joint, B cells show altered activation, cytokine production and T cell polarization capacity that could prove important for understanding the role of B cells in disease pathogenesis of RA.

Disclosure of Interest None declared.

P017 NON-ANTIBODY MEDIATED PATHOGENIC ROLES FOR SYNOVIAL B CELLS IN ACPA+ AND ACPA− RHEUMATOID ARTHRITIS

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

Introduction The presence of anti-carbamylated protein antibodies (anti-CarbP) has been detected in rheumatoid arthritis...
Anti-CarP autoAbs target proteins that are modified through an irreversible post-translational modification named carbamylation.

Objectives The aim of this work was to assess whether anti-CarP antibodies can be used as a predictive factor of clinical response to abatacept.

Methods Peripheral blood samples of selected patients were collected at the beginning of the therapy with abatacept (T0) and every six months for one year (T6 and T12). A home-made ELISA was applied to determine serum anti-CarP levels. Commercial anti-citrullinated protein antibodies (anti-CCP3) (Inova Diagnostic), Rheumatoid Factor (RF) (Siemens) and high sensitivity C reactive protein (hsCRP) were also tested.

Results Sixty RA patients (49 female (81.2%)), all caucasian, treated with abatacept were enrolled. Fifty-three (88.3%) and fifty-six (93.3%) patients were also treated with corticosteroids and synthetic DMARD respectively. At baseline anti-CarP antibodies were found in 18 (30%) patients; RF and anti-CCP were positive in 35 (58%) and 51 (85%) patients respectively. Comparing anti-CarP+ with anti-CarP- patients at T0, anti-CarP+ group resulted younger (p<0.01) and with a longer disease duration (p<0.05); hsCRP was higher in anti-CarP+ group (p<0.05). Considering the entire cohort, a significant reduction of anti-CarP titre at T6 and T12 of treatment was shown (p<0.0001) while anti-CCP and RF titre did not show any significant change. Thirteen out of 18 patients anti-CarP+ were available for analysis at T6 and in 6 cases turned anti-CarP-. A significant reduction of DAS28-CRP at T6 was found in the subgroup of anti-CarP+ pts in comparison with the negative ones (p=0.03). No significant results were found dividing the cohort using the positivity to anti-CCP and/or RF. Furthermore, stratifying groups of patients for the combination of biomarkers, any groups including anti-CarP resulted in a trend towards a higher DAS28 reduction compared with the combination of anti-CCP+ and RF+ anti-CAP- ones.

Conclusions The precocious onset and a longer disease duration in anti-CarP positive patients might suggest them as a specific risk factors for RA in this subgroup of patients. The link between the anti-CarP positivity at baseline and the higher reduction of disease activity during the first six months of treatment permitted us to hypothesize that anti-CarP antibodies, but not anti-CCP and/or RF, could be a predictive factor of a good clinical response to abatacept.

REFERENCE

Disclosure of Interest None declared.

Abstracts

IS TREATMENT RESPONSE INFLUENCED BY BODY MASS INDEX IN JUVENILE IDIOPATHIC ARTHRITIS?

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

Introduction There is evidence that obesity could be a risk factor for the severity and response to treatment in patients with RA due both to the mechanical effect of overweight and to the potential pro-inflammatory effects of cytokines produced by adipose tissue.

Objectives To evaluate the role of overweight and obesity in a cohort of patients with Juvenile Idiopathic Arthritis (JIA), in terms of incidence, disease activity, outcome and response to treatments.

Methods This single-center retrospective cohort study evaluated 110 children affected by JIA under treatment with anti-rheumatic agents (DMARDs, biologic agents). Changes from baseline in ESR, CRP, number of active joints, and BMI were analyzed under each treatment until last visit. BMI categories of 5–84th (normal weight), 85–94th (overweight), and >95th (obese) percentile were used. Patients with systemic JIA, uveitis, chronic comorbidities, or under other potentially confounding systemic treatments were excluded. Uni- and multivariate analyses were performed.

Results One hundred and ten JIA patients (polyarticular n=50, oligoarticular n=38, psoriatic n=12, enthesitis related arthritis n=8, undifferentiated n=2) were enrolled in the study, 75% girls, 25% boys. The mean age at treatment onset was 6.09 years. Baseline BMI was ≤84 th percentile in 80 patients, 85–94th in 27, and >95 th in 3.

We did not observe a significant association between BMI and ESR, CRP, or number of active joints at baseline, while involvement of the joints of lower limbs was significantly greater (p=0.025) in overweight/obese patients. We observed a trend toward lower remission rates and higher number of relapses, both after DMARDs and biologics, in patients with higher BMI.

Conclusions This study focuses on the relationship between overweight/obesity and JIA. A significant correlation between obesity and a greater involvement of the joints of lower limbs was observed at baseline. Furthermore our data suggest that obesity could negatively influence the course of the disease as well as treatment response.

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Disclosure of Interest None declared.
Introduction Anti-citrullinated protein autoantibodies (ACPA) in RA patients target a wide range of modified proteins and recent findings reveal that monoclonal ACPA are cross-reactive due to recognition of shared citrulline-peptide motifs. Among physiological targets of ACPA, citrullination in neutrophil extracellular trap (NET) products, have been postulated to be important.

Objectives We sought to characterize the anti-nuclear and anti-neutrophil reactivities of different patient-derived monoclonal ACPA.

Methods The study included ten recombinant single B-cell derived RA monoclonal ACPA-IgG with CCP2 reactivity from different cell subsets and compartments (1, 2). They were screened for HEp-2 ANA reactivity, and binding to apoptotic cells or stimulated neutrophils. Binding was compared to CRISPR PAD4 KO cells and neutrophils from PAD2 and PAD4 KO mice. Immunoprecipitation and mass spectrometry were used to identify modified nuclear ACPA targets, and ELISA and Western blot for mAb binding to acetylated epitopes.

Results Four out of ten ACPA clones exhibited strong binding to apoptotic cells, nuclear binding to activated neutrophils, and reactivity to NETs. Three of these were ANA positive. Another NET-reactive ACPA instead displayed a cytoplasmic binding pattern. This cytoplasmic NET-binding was PAD4-dependent, whilst nuclear-mediated NET reactivity was PAD-independent. Using apoptotic cells, acetylated histones were confirmed to be the primary targets of the nuclear reactivity, which could be explained by consensus-motif driven ACPA cross-reactivity. Specifically targeted acetylated histone peptides were identified and the anti-modified protein autoantibody (AMPA) profiles of the ACPA were found to correlate with cell binding.

Conclusions When investigating monoclonal ACPA, our novel data reveal a distinct subset of ACPA with nuclear binding patterns and AMPA activity with acetylated histones (and not citrullinated proteins) in NETs and apoptotic cells.

REFERENCES

Disclosure of Interest None declared.
arthritis (RA). Previous prospective studies have used a clinical definition of arthritis. Thus, we aimed to investigate risk factors of developing arthritis in ACPA-positive subjects with musculoskeletal complaints who did not have any of clinical and ultrasound signs of arthritis.

**Methods** Subjects with positive ACPA-test referred from primary care to rheumatology clinic, lacking arthritis in hands and feet by clinical and ultrasound examination (according to EULAR-OMERACT synovitis definition), were recruited into the Risk-RA research program. Patients included between years 2015–2016 with clinical data up to 2017 were analysed. Blood samples from inclusion were analysed for 13 specific ACPA reactivities using a custom made ImmunoCAP ISAC microarray. Presences of HLA-SE risk gene were analysed using DR low-resolution kit.

**Results** 41% (27 out of 66) of the Risk RA subjects developed arthritis during a median follow up of 8 months. The rest was followed 25 months in median without any signs of arthritis. Subjects developing arthritis tended to have a higher concentration of anti-CCP, more tender joints and rheumatoid factor positivity at inclusion compared to those not developing arthritis. The number of ACPA-reactivities (mean 6 vs 3), the presence of HLA-SE (89% vs 56%) and the occurrence of ultrasound detected tenosynovitis (44% vs 5%) at inclusion were significantly increased in subjects developing arthritis compared to those not developing arthritis.

Univariate cox proportional hazards regression showed a hazard ratio (HR) for arthritis development of 1.1 for every increase in number of ACPA reactivities (95% CI 0.99 to 1.2, p 0.07); HR: 4.4 (95% CI 2.0 to 9.5, p 0.0002) for tenosynovitis and for HR: 4.9 (95% CI 1.5 to 16, p 0.01) for HLA-SE carriers. All subjects with tenosynovitis (n=14) prior to arthritis development were carriers of HLA-SE, except for one subject but similar to the majority this HLA-SE no-carrier also progressed to arthritis.

**Conclusions** Subjects with ACPA-positive musculoskeletal complaints lacking any clinical and ultrasound signs of arthritis are at high risk of developing arthritis, especially carriers of HLA-SE with tenosynovitis. The role of inflammatory spreading from tendons (synovial sheath) to synovial tissue within joints need to be further investigated.

**Disclosure of Interest** None declared.
used the following questionnaires: Female Sexual Function Index (FSFI), Brief Index of Sexual Function for Women (BISF-W), Sexual Quality of Life Questionnaire (SQoL-F), Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire (PISQ-12), Pelvic Floor Distress Inventory Questionnaire (PFQ7), Fatigue Impact Scale (FIS), Beck’s Depression Inventory II (BDI II), Health Assessment Questionnaire (HAQ), Scleroderma Health Assessment Questionnaire (SHAQ) and Human Activity Profile (HAP).

**Results**

Compared to HC, participants with SSc had significantly higher prevalence and greater severity of sexual dysfunction (FSFI, BISF-W: in all subscales as well as total scores), dysfunction of pelvic floor (PISQ-12, PFQ7), and worse sexual quality of life (SQoL-F) (table). Worse scores in SSc patients were associated with higher disease activity [ESSG activity index: SQoL-F (r = −0.364, p = 0.0443)], greater fatigue [FIS correlated negatively with both FSFI, and BISF-W], more severe depression [BDI-II: FSFI (r = −0.553, p = 0.0002), BISF-W (r = −0.514, p = 0.0007)], deteriorated quality of life [SHAQ: FSFI (r = −0.536, p = 0.0003), BISF-W (r = −0.563, p = 0.0001), SQoL-F (r = −0.338, p = 0.0382), PISQ-12 (r = 0.563, p = 0.0051)], and worse ability to perform physical activities [HAP: FSFI (r = 0.407, p = 0.0082), BISF-W (r = 0.409, p = 0.0078)].

**Conclusions**

Women with SSc reported significantly impaired sexual function, sexual quality of life and pelvic floor function than age-matched healthy controls. Worse scores in SSc were associated with disease activity, physical activity, fatigue, depression and quality of life.

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**Disclosure of Interest** None declared.

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**Abstracts**

**P026**

**CONTROL OF A2,6-SIALYATION IN B-CELLS, AND CONSEQUENCES OF REDUCED B-CELL SIALYATION IN PATIENTS WITH RHEUMATOID ARTHRITIS**

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

**Introduction**

Sialic acids are a family of 9-carbon sugars, added to the termini of glycoprotein chains, which are present on the surface of many cells, and secreted proteins.1 Sialic acids on glycan chains of the Fc fragment of IgG molecules can affect how IgG binds Fc receptors. In RA and other autoimmune conditions, disease specific auto-antibodies display decreased Fc sialylation compared to healthy B-cells in response to activation in vitro. This was accompanied by a decrease in expression of both ST6Gal1 and NEU1 mRNA over 48 hours. Surface sialylation was also increased by activated T-cells in co-culture experiments, however sialylation was reduced in B-cell cultures in the presence of TNF.

**References**


**Disclosure of Interest** None declared.

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**P027**

**SRC-LIKE ADAPTOR PROTEIN EXPRESSION IN RHEUMATOID ARTHRITIS**

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

**Introduction**

The Src-like adaptor protein (SLAP) plays a central role in the fine regulation of both B- and T-lymphocyte activation. According to our previous data SLAP is responsible for the proteasomal degradation of the CD3 zeta chain (CD3ζ) of T-lymphocytes.1

**Objectives**

We studied the effect of IL-10, IL-17A and TNF-α treatment on the SLAP expression of CD4+ T- and CD19+ B-lymphocytes.

**Methods**

Peripheral mononuclear cells (PMBC) were isolated from healthy donors and rheumatoid arthritis (RA) patients. CD4+ T-cells or CD19+ B-cells were isolated by negative magnetic separation; stimulated with ConA and goat anti-human IgG + IgM F(ab’)2 fragments respectively. The samples were treated with IL-17A (20 ng/ml and 80 ng/ml, 24 hour), IL-10 (100 U/ml, 48 hour and 72 hour) and TNF-α (20 ng/ml and 60 ng/ml, 24 hour). The SLAP and CD3ζ expression were measured by Western blot.

**Results**

Both the SLAP and the CD3ζ expression of the MTX treated patients’ CD4 cells were higher upon IL-17A and IL-10 treatment, than those of the MTX non-treated RA patients’ or healthy donors’. The TNF-α induced SLAP expression of RA patients’ CD19 B-cells was higher than those of the healthy donors’ B cells (p = 0.05).
Conclusions In addition to proinflammatory and anti-inflammatory cytokines MTX treatment regulates the SLAP and the CD3\(\gamma\) expression of human T-cells.

REFERENCE

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Disclosure of Interest None declared.

PO30 TRANSCRIPTIONAL LANDSCAPES OF MEMORY T CELLS FROM PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS
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Career situation of first and presenting author Post-doctoral fellow.
Introduction Juvenile idiopathic arthritis (JIA) is a chronic inflammatory disease (CID) of unknown origin and is characterized by joint inflammation in children and young adults. Evidence suggests a strong contribution of memory T cells to disease pathogenicity in JIA. While few markers for T cells characterized by joint inflammation in children and young adults.

Objective To characterize the transcriptional profiles of memory T cells that putatively maintain chronic inflammation in JIA patients.

Methods Memory T cells were isolated from the synovial fluid (SF) and the peripheral blood (PB) of oligoarticular JIA patients and purified by fluorescence-activated cell sorting (FACS). Subsequently, single cell sequencing including T cell receptor (TCR) sequencing was performed on 18,000 memory T cells of each JIA patient.

Results Memory T cell populations both from the blood and from the SF are heterogeneous populations according to their transcriptional expression patterns. The SF harbored a larger population of enriched T memory cell clonotypes than the blood. In addition, enriched memory T helper cell clones in the SF showed a transcriptional pattern of activation compared to non-enriched clonotypes. Finally, small subpopulations of enriched memory T helper cell clones in the SF show a transcriptional signature that resembles transcriptomes obtained by bulk sequencing. Thus, a rather small subpopulation of antigen-specific cells might be responsible for the overall transcriptional character of T cells found at the inflamed sites of CIDs.

Conclusions Single cell sequencing combined with TCR sequencing is a powerful tool to identify and characterize subsets of T memory cells in chronic inflammation. The obtained data might be useful to better understand how T cell subsets contribute to disease pathogenicity in CIDs and reveals putative targets that could be therapeutically exploited in order to selectively deplete pathogenic memory T cells.
SIALIC ACIDS INHIBIT NEUTROPHIL EXTRACELLULAR TRAP FORMATION

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

Introduction In rheumatoid arthritis (RA) activated neutrophils produce neutrophil extracellular traps (NETs), which provide a source of autoantigens that drives the autoimmune process.1 To identify novel immune processes that dampened neutrophil activity, we investigated a family of inhibitory glycan-binding receptors (SiglecS) that bind a specific type of glycan; called sialic acids.2 We hypothesize that sialic acid-mediated triggering of siglecS on neutrophils, which express siglec-5, –9 and –14, will reduce their activation. In this study we focused on dampening the activity of neutrophils and thereby NET formation.

Methods Polymorphonuclear cells (PMNs) were isolated from healthy donors. Neutrophil binding of sialic acid-containing glycoconjugates was assessed by flow cytometry. Neutralizing antibodies for siglec-5/14 and –9 were used to block the interaction with the sialic acid glycoconjugates. For functional assays a branched synthetic molecule containing sialic acids (sialic acid dendrimer) was used. PMNs were rested for 1 hour at 37°C followed by stimulation with sialic acids dendrimers for 30 min. Subsequently, IgA coated beads were added for 30 min to activate the neutrophils. NETosis was quantified via Sytoxgreen and visualised via microscopy, and phagocytosis was measured by flow cytometry.

Results Binding of sialic acid glycoconjugates was observed on neutrophils. Neutralizing siglec-5/14 and –9 receptor almost completely abolished sialic acid glycoconjugate binding to neutrophils. Neutrophils activated with IgA beads released NETs, as confirmed via microscopy. Triggering neutrophils with sialic acid dendrimer reduced this process of NETosis. The capacity to engulf IgA beads was not affected by sialic acid dendrimer stimulation.

Conclusions Neutrophils stimulated with sialic acid dendrimers show reduced activation. Patients with RA might benefit from treatment with sialic acid to dampen neutrophil-mediated autoimmune response.

Disclosure of Interest None declared.

REFERENCES

P033/P034

CD38-EXPRESSING MEMORY T CELLS ARE EXPANDED IN PERIPHERAL BLOOD, CONTAINED IN INFLAMED TISSUE AND REPRESENT A POTENTIAL TREATMENT TARGET IN SYSTEMIC LUPUS ERYTHEMATOSUS

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

Introduction The unresponsiveness of long-lived plasma cells (PC) to immunosuppressive and B cell depleting therapies represents a therapeutic challenge in systemic lupus erythematosus (SLE) and other immune-mediated diseases. Novel potential targets such as CD38 have emerged.1

Objectives Here, we aimed to analyze expression levels of CD38 on circulating plasmablast and peripheral blood and tissue residing lymphocyte subsets in SLE to estimate the therapeutic potential of CD38-targeting therapies.

Methods Multicolor flow cytometry was performed to investigate the CD38 expression on peripheral blood mononuclear cells (PBMCs) of SLE patients (n=36), healthy controls (HC, n=20) and multiple myeloma (MM, n=10) patients. In addition, kidney-infiltrating T cells isolated from urine were analyzed in patients with lupus nephritis (LN). To investigate the cytokine secreting potential, cytokines were analyzed intracellularly in CD38-expressing T cells after polyclonal stimulation in vitro with PMA/Ionomycin.

Results Circulating CD19+CD24hiCD27low plasmablasts are more frequent in SLE and MM patients and display higher mean fluorescence intensity for CD38 compared to HC. In SLE, CD38 is significantly higher expressed in both CCR7+ central and CCR7neg effector memory CD4 and CD8 T cells compared to HC. Such cells co-express other markers of T cell activation and recent proliferative history such as HLA-DR and Ki-67, and are preferentially negative for FoxP3 and Helios. CD38 is most exclusively expressed on CXCR3+ memory T cells isolated from urine of patients with LN in contrast to their CXCR3+ counterparts. Upon polyclonal stimulation, cytokine (IFN-g, IL-17, TNFα, IL-2) secreting cells were confined to memory T cells lacking CD38 expression.

Conclusions Expression levels of CD38 are significantly higher in peripheral blood memory B- and T-cell subsets from patients with SLE compared to HC. CD38hi T cells co-express markers of recent activation and proliferative history, are confined to conventional memory T cells and contained in inflamed tissue, suggesting a pathogenic role of a chronically activated memory T cell compartment in SLE. The lack of effector cytokine secretion of such cells is unexpected and merits further investigation. PC depleting therapies targeting CD38 may represent a promising treatment option in SLE given their potential therapeutic effect on activated memory T cells in inflamed tissue.

REFERENCE

Acknowledgments We thank all study participants and blood donors.

Disclosure of Interest None declared.

P034

PRESENCE OF A SPECIFIC DEFECT IN M2 POLARIZATION OF BLOOD MONOCYTES FROM PATIENTS WITH RHEUMATOID ARTHRITIS, ASSOCIATED WITH INCREASED MICRORNA-155

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

Introduction Macrophages contribute in situ to the rheumatoid arthritis (RA) pathogenesis. Two distinct states of

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Disclosure of Interest None declared.
polarization have been recognized: the ‘classically activated’ (M1) and the ‘alternatively activated’ (M2). miRNAs are a recently discovered class of post-transcriptional regulators. Of particular relevance in RA is miR155. Its expression is upregulated in RA synovial monocytes, macrophages and fibroblasts. Its overexpression in macrophages is associated to the production of pro-inflammatory mediators.

Objectives Here, we assessed monocytes capacity of differentiation into M2 and implication of miR155 in RA patients or controls (HD, CTD or SpA).

Methods Purified monocytes were incubated 6 day in the presence human serum AB (SAB) (M2). Expressions of total macrophages markers (CD11b-CD71), M2 markers (CD163, CD206, IL-10 and Arginase) and M1 markers (INOS, IFRS and IL1β) were evaluated by flow cytometry, ELISA or immunofluorescence. The microRNA transfections were performed using AMAXA technology.

Results We observed a significant decrease of macrophages induction only by SAB in RA patients as shown by a decreased expression of CD11b-CD71. We have found a specific decreased level of M2 markers. Moreover, we found ex vivo and in vitro that Adalimumab but not Etanercept or non-anti-TNF drugs were able to partially correct this defect of M2.

Conclusions RA patients have a propensity for preferential maturation towards a pro-inflammatory M1-like phenotype instead of M2, thus contributing to synovial inflammation.

We have hypothesized the involvement of miR155 on this defect. Indeed, miR155 is induced in monocyte or macrophages and drives their inflammatory response by epigenetic regulation that lead to M1 polarization. miR155 is increased in monocytes and M2 RA compared to controls.

Subsequently we transfected monocytes from HD with miR155 mimic and we observed a decrease differentiation in M2. Conversely preliminary experiments on RA monocytes transfection with a miR-155 inhibitor allowed the restoration of M2 polarization.

Conclusions RA patients have a specific impaired maturation of monocytes to M2 as much in phenotype and in function while the differentiation to the M1 phenotype is maintained. The use of monoclonal anti-TNF restores M2 polarization defect while Etanercept or non anti-TNF drugs do not restore M2.

This lack of M2 polarization is associated with miR155 increase in RA patients that leads to IFR5/INOS expression. Preliminary experiments showed that transfection of RA monocytes with an antagoniR155 may correct this lack of M2, justifying the proof of concept trial of monocytes-targeted nanoparticles containing microRNA in CIA mouse models.

Disclosure of Interest None declared.
Methods Phenotypic analysis of peripheral blood B-lymphocytes was made by flow-cytometry in a cohort of SLE patients treated with belimumab. SLE-disease activity was assessed by SLEDAI-2K score. BAFF was tested by ELISA. SPSS was used for statistical analysis.

Results The relative change of BAFF levels at 6 and 12 months from baseline showed linear correlation with the percentage of naïve B-cells (Pearson correlation = 0.645, p = 0.044 and 0.639, p = 0.002, respectively) and of transitional B-cells (Pearson correlation = 0.768, p = 0.009 and 0.623, p = 0.055, respectively). The percentage and absolute number of naïve B-cells showed a progressive decrease during time (ANOVA, p = 0.013 and p = 0.001 respectively). In terms of response prediction, a significant association of SLEDAI percentage improvement at 12 months with the decrease of total number of B-cells within the first 6 months of therapy was observed (Log regression r = 0.707, p = 0.05).

Conclusions BAFF inhibition induces B-cell number modifications in a SLE cohort. The reduction of total number of B-cells within the first six months shows predictive value for SLEDAI response after the first year of therapy.

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Disclosure of Interest None declared.
Circulating follicular helper T cells are increased in systemic sclerosis and promote plasmablast differentiation through the IL-21 pathway which can be inhibited by ruxolitinib

**Results** We observed that cTfh cell numbers are increased in SSc patients compared with HC. Furthermore, the increase in cTfh cells was more potent in patients with severe forms of SSc such as diffuse SSc and in the presence of arterial pulmonary hypertension. cTfh cells from SSc patients present an activated Tfh phenotype, with high expression of BCL-6 and increased capacity to produce IL-21 in comparison to HC. In vitro, cTfh cells from SSc patients had higher capacity to stimulate the differentiation of CD19+CD27+CD38+ B cells and their secretion of IgG and IgM through the IL-21 pathway than cTfh cells from HC. Blocking IL-21 or using the JAK1/2 inhibitor ruxolitinib reduced the Tfh cells’ capacity to stimulate the plasmablasts and Ig production.

**Conclusions** Circulating Tfh cells are increased in SSc and correlate with SSc severity. The IL-21 pathway or JAK1/2 blockade by ruxolitinib could be a promising strategy in the treatment of SSc.

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**Disclosure of Interest** None declared.

**References**


**Disclosure of Interest**

## P040 THE EUROPEAN CONSENSUS FINDING STUDY GROUP ON AUTOANTIBODIES 2017/18 INVESTIGATION.

**CHARACTERISATION OF AUTOANTIBODY CONTENT IN A NEW INTERNATIONAL REFERENCE STANDARD FOR DENSE FINE SPECKLED 70KD (DF570) AUTOANTIBODIES**


**Career situation of first and presenting author:** Instructor.

**Introduction** The European Consensus Finding Study Group on autoantibodies (ECFSG) a.k.a. the EULAR autoantibody study group has been active for 30 years.

**Objectives** To reach consensus about autoantibody measurements in clinical practice, and to evaluate upcoming autoantibody standard reagents concerning autoantibody content.

**Methods** ECFSG focuses on evaluating difficult to interpret serum samples, where differences between assays can be clearly visible. Ten unknown samples are distributed yearly to European laboratories, and analyzed broadly. Results are collected with information about laboratory techniques used, and discussed in relation to clinical information on the donating patients during EWRR. The 2017/2018 investigation contained nine patient samples, and a not yet launched pooled standard.

**Results** Acceptable consensus was reached for the clinical samples. Anti-DF570 pattern was reported from 32/38...
laboratories, whereas 5/38 reported homogenous ANA, one reported unknown pattern. Except for 4 out of 24 laboratories reporting anti-histone and 2 out of 33 laboratories reporting ACPA, both in low levels, no autoantibodies were reported. Consensus was that the sample contained pure anti-DF570.

Conclusions ECFSG helps to keep awareness on differences between autoantibody assays. The anti-DF570 ANA pattern was identified by most laboratories in a reagent that proved to be free of other autoantibodies. The anti-DF570 standard will be available via http://asc.dental.ufl.edu/ReferenceSera.html#text.

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Disclosure of Interest None declared.

Abstracts

P024/005 MOLECULAR MICROMY AND AUTOIMMUNITY: ANTI-P. GINGIVALIS ANTIBODY RESPONSE IN ACPA-POSITIVE RHEUMATOID ARTHRITIS

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

Introduction The presence of anti-citrullinated protein antibodies (ACPAs) is a hallmark of rheumatoid arthritis (RA). ACPAs specifically recognize citrullinated epitopes, a result of a post-translational modification catalyzed by peptidyl arginine deiminas (PAD). Based on the unique feature of the periodontal bacteria Porphyromonas gingivalis (P.gingivalis) to express P. PAD it has been suggested that ACPA-positive RA may be precipitated in the gum mucosa.

Objectives To address this hypothesis our aim was to investigate the antibody response against a citrullinated P.PAD peptide (CPP3) in patients with RA, chronic periodontitis (PD) and in controls. In addition, we generated monoclonal antibodies (mAbs) from gingival tissue B cells of RA patient aiming to investigate whether citrulline-specific B cells may reside in the gingiva.

Methods Gingival tissue-derived single CD19+ B cells from an ACPA-positive RA patient with PD were sorted by flow cytometry. Immunoglobulin variable region genes were sequenced and expressed to generate recombinant mAbs. CPP3-reactivity was analysed by ELISA in serum samples from 66 PD patients, 63 periodontally healthy controls (non-PD), 200 RA patients, and 120 non-RA controls, as well as in 55 mAbs. Differences in antibody levels were examined using Mann-Whitney U test for independent groups.

Results Anti-CPP3 antibody levels were low in non-PD controls, while 65% of PD patients showed elevated levels (p<0.0001). Significantly increased antibody levels were also

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detected in 50% of ACPA-positive RA, 20% of ACPA-negative RA, and in 37% of non-RA controls (p<0.0001). Notably, this antibody response was citrulline-specific, as the antibody response against the arginine-containing control peptide RPP3 was significantly lower in all subsets (p<0.0001). Among 55 mAbs from gingival tissue, 14 (25%) unique clones were CPP3-reactive, of which 4 showed cross-reactivity with RPP3. Interestingly, 4 out of 14 (29%) CPP3-reactive clones also bound citrullinated peptides derived from human a-enolase, filaggrin and histone 4, demonstrating cross-reactivity between a bacterial epitope and human epitopes on a monoclonal level.

Conclusions This study shows that a substantial proportion of systemically healthy individuals possess ACPAs directed against Pg. gingivalis, and these ACPAs also bind epitopes on human proteins. Based on our data, we propose that the ACPA response may be triggered by Pg. gingivalis via an antibody response against CPP3, which cross-reacts with human citrullinated proteins by mechanisms of molecular mimicry.

Disclosure of Interest None declared.

P043 INFECTION WITH CITRULLINATING PORPHYROMONAS GINGIVALIS CAN INDUCE AUTOIMMUNITY TO HUMAN RIBOSOMAL PROTEINS

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Career situation of first and presenting author Young investigator.

Introduction Porphyromonas gingivalis (Pg.) is involved in triggering self-reactive immune responses when citrullinating bacterial or human proteins. However, first evidence to link anti-ribosomal T and B cells responses to rheumatoid arthritis (RA) has been published but the mechanism is still not clear. 1 Infection based autoimmunity induced by citrullination of human proteins with Pg. gingivalis arginine deiminase from RA patient (RA-PPAD) and crossreactivity binding induced by Pg. gingivalis was investigated using affinity purified RA patient antibodies and a monoclonal antibodies to cit-RA-PPAD.

Objectives Antibodies to RA-PPAD isolated from an RA patient sera (RA-PPAD) and crossreactivity binding induced by Pg. gingivalis was investigated using affinity purified RA patient antibodies and a monoclonal antibodies to cit-RA-PPAD.

Methods Screening of RA sera was conducted on 37.830 unique human proteins on protein macroarrays (http://www.engine-gmbh.de) with 30 RA sera. The autoantibody response to 840 different proteins was recorded and bioinformatically evaluated. Protein arrays were citrullinated with PAD2,4,rabbit PAD and RA-PPAD. Sera and affinity purified antibodies were used to detect reactivity to 840 autoantigens.

Results A human protein macroarray consisting of 840 indentified autoantigens from RA patients was modified by human PAD2 and PAD4, rabbit PAD, and RA-PPAD form P g..Using cit specific monoclonal antibodies we identified the ribosomal proteins (RP), RPL18a, RPS27a, modified by PAD2, RPL18a and MRPS11 modifies by Pad4, and RPL7L1 modified by rabbit PAD specifically targeted. In addition 6 RA patient sera and 3 osteoarthritis (OA) control sera were used to identify the citrullinated RA-PPAD specific modified autoantigens not targeted when modified by human PAD2 or PAD4 or rabbit PAD. We identified the RA-PPAD citrullinated ribosomal Proteins RPL3, RPL21, RPS24, RPL9, RPL15, RPS24,RP3a, MRPL28 specifically targeted by RA patients. This identifies ribosomal proteins as major specific RA-PPAD citrullination targets. Moreover, affinity purified antibodies bound to native and citrullinated RA-PPAD from 6 RA patient sera and 3 OA patient sera were tested for crossreactivity on citrullinated human proteinarray. Antibodies to citrullinated ribosomal proteins MRPS11, RPL21, RPS3a, RPL18a, RPS27a, MRPL28 were detected in the RA group but not in the OA control group.

Conclusions Failure of Porphyromonas gingivalis clearance in RA patients leads to infection induced enzymatic mimicry based autoreactivity targeting evolutionary conserved human ribosomal proteins. Autoimmunity to ubiquitous self-antigens may trigger localized tissue damage in RA.

Disclosure of Interest None declared.
EFFECT OF SPECIALIZED 6-MONTH PHYSICAL-NECROSTATIN-1 AMELIORATES NEUTROPHIL ASTHMA

Objectives Our specialized POI intervention led to a significant improvement in the observed parameters that was clinically significant in a substantial proportion of patients, and prevention of the expected worsening of muscle weakness and quality of life.

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Disclosure of Interest None declared.

P046 NECROSTATIN-1 AMELIORATES NEUTROPHIL ASTHMA BY INHIBITING NEUTROPHIL RELEASE NETS

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

Introduction Neutrophilic asthma is Corticosteroid-resistant and increases the burden of global health care. Multiple studies have indicated that there are a large amount of neutrophil extracellular traps (NETs) in the airways of neutrophil asthmatics. Although NETs are able to entrap and kill pathogens, extensive accumulation of NETs which aggravate the condition of asthmatics and promote the progression of the disease.

Methods Mouse models of neutrophil-dominated asthma and phorbolester(PMA) aggregated neutrophil asthma were used in this study to clarify the role of NETs in the pathogenesis of neutrophil asthma. Neutrophil release NETs was detected in bronchoalveolar lavage fluid (BALF)of neutrophil-dominated asthma. Finally a small molecule Necrostatin-1(Nec-1) which has been shown to inhibit neutrophil release NETs was tested for its therapeutic effects against neutrophilic airway inflammation.

Results NETs could induce human epithelium human bronchial epithelial cell death and detachment in vitro study. NETs significantly increased in neutrophil asthma model and PMA aggregated neutrophil asthma. In vivo studies, Nec-1 could relieve airway hyperresponse, also reduced total protein, myeloperoxidase activity and inflammatory cytokines. Histological examination of the lungs also showed that Nec-1 markedly reduced the inflammation. We further explored that Nec-1 could induce human neutrophils and mice BALF neutrophils apoptosis. BALF and lung tissue immunofluorescence showed neutrophils increased expression of cleaved-caspase.

Conclusions NETs could damage airway epithelium and trigger inflammatory responses. Nec-1 inhibit neutrophil release NETs ameliorates neutrophil airway inflammation. May be...
the inherent mechanism of Nec-1 inhibit neutrophil release NETs is related to it specifically promotes neutrophil apoptosis.

REFERENCES

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Disclosure of Interest None declared.

PO47 THE PLASMA CELL BONE MARROWNICHE IN ACPA+ RA PATIENTS CONTAIN CITRULLINE SPECIFIC CELLS
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Career situation of first and presenting author Post-doctoral fellow
Introduction Anti-citrullinated antibodies (ACPA) in RA have been postulated to contribute to disease pathogenesis. This has been supported by studies of monoclonal APCA generated by cloning from single cell sorting of different B cell subsets from peripheral blood and synovial fluid. It has been debated if the bone marrow (BM) compartment of RA patients hosts long lived plasma cells producing ACPA or if ACPA are primarily coming from plasmablasts at the sites of inflammation, e.g. the synovium.
Objectives The main objectives were to study primary lymphoid tissue from RA patients, the BM plasma cell IgG repertoire and further characterize recombinitely expressed antibodies from selected sequences to explore if ACPA producing long lived plasma cells reside in this niche.
Methods For this study we collected bone marrow from proximal or distal femur of RA patients undergoing hip replacement surgery due to secondary osteoarthritis. BM samples were processed from four ACPA-RF+ and one ACPA-RF- RA patients. Mononuclear cells were obtained by Ficoll separation and CD138+ plasma cells were single cell sorted by flow cytometry. Paired heavy and light chains were PCR amplified, sequenced and analyzed by V-Quest and IgBLAST towards the IMGT database to annotate variable gene usage. Selected sequences were cloned and expressed as IgG in Expi293 cells.
Results Overall 465 paired IgG BM plasma cell sequences were obtained, 97 from the ACPA- patient and 368 (range 20–194) from ACPA+ patients. We observed statistically significant changes in heavy chain variable gene usage with lower VH-1 and higher VH-3 frequency in the ACPA+ sequences compared to ACPA- sequences. We also found statistically significant increase in VH N-glycosylation in ACPA+sequences (22.6% ACPA+ vs 12.4% ACPA-; p=0.03). There was however no difference in mutation numbers. From these, 34 clones were selected, based on mutation numbers and presence of Fab N-glycosylation sites, for subsequent mAb-expression. Among the 34 clones we found two CCP2+ and cit-peptide positive clones and one malondialdehyde-acetaldehyde (MAA) adduct reactive clone, originating from different ACPA+ patients.
Conclusions We could identify BM plasma cells producing autoantibodies to malondialdehyde-modified proteins and citrullinated peptides, isolated from RA patients. Hence, we show for the first time, that not just at the site of inflammation but also long-lived plasma cells in bone marrow do produce RA specific autoantibodies.
Acknowledgements Orthopedic staff at Karolinska University Hospital. Danika Shepis, Louise Berg and Christina Gerstner for sorting help.
Disclosure of Interest None declared.

PO48 PREDICTIVE VALUE OF THE DIFFERENT CAPILLAROSCOPIC PATTERNS IN EARLY RAYNAUD PHENOMENON
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Career situation of first and presenting author Assistant.
Introduction The Raynaud phenomenon (RP) affects 3%–5% of the population. Most RPs are primary. The risk of the onset of autoimmune disease associated with RP (ADRF) is around 12%, and the diagnosis is usually made within 2 years of its onset. Capillaroscopy is an innocuous and inexpensive technique that is very useful in the study of RP.
Objectives The primary goal was to evaluate the capillaroscopic findings and theirs different patterns in patients with RF as the only symptom and establish their relationship between the subsequent development of ADRF. We have evaluated the relationship between the presence of the antinuclear antibodies (ANA) and the association with ADRF.
Methods We conducted an observational, descriptive, cross-sectional study of 98 patients with RP as the main manifestation. Periungual capillaroscopy was performed from the 3rd to the 5th finger of both hands. Sociodemographic variables, and results of ANA were described. To assess the validity of capillaroscopy as a diagnostic test, we determined the sensitivity, specificity, and positive and negative predictive value (PPV, NPV).
Results 73.5% were women. The median time of evolution of the RP was 2.5 years. 76% were ANA negative.

The evaluation of the predictive capacity of capillaroscopy for the development of EARP yields the following Results.
- Sensibility: 83,3% (62.6–95.2), specificity: 78% (66.8–86.9), PPV 34,1% (24.5–45.3), PNV 97,1% (93.3–98.8).

The capillaroscopic findings were: 62% no alterations, 21.4% no specific capillaroscopic changes, 14.3% ‘scleroderma’ type capillaroscopic pattern, and 2% characteristic pattern of mixed connective tissue disease (MCTD). None of the patients who did not present alterations developed autoimmun disease. Of the 21 patients who presented nonspecific changes, 5 developed limited systemic sclerosis (SSc), 3 MCTD, and 1 diffuse SSc. Of the 14 patients who presented ‘scleroderma’ type capillaroscopic pattern, 5 develop limited SSc, 3 diffuse SSc, 2 MCTD and 1 did not present autoimmun disease. Of the two patients with characteristic alterations of MCDT, 1 developed this disease. When analyzing the relationship between the presence of ANA and the association
with ADRF we found that it was independent (p: 0.002) of the result of capillaroscopy, being the risk of developing the disease in a patient with positive ANA 8.5 times higher than in an ANA negative patient.

Conclusions Capillaroscopy in patients with RP has a high FNV, which allows us to estimated, with high reliability, the association of this phenomenon with autoimmune disease in patients with normal capillaroscopic patterns.

Disclosure of Interest None declared.

**Abstracts**

**P049** AGE-ASSOCIATED B CELLS IN EARLY DRUG-NAÏVE RHEUMATOID ARTHRITIS PATIENTS

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

Introduction Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterised by joint inflammation and bone destruction. The presence of autoantibodies, years before the clinical onset of disease, and the efficacy of Rituximab, a B-cell depleting therapy, highlight a pathogenic role for B cells. Different groups have recently identified a novel subset of B cells named age-associated B cells (ABCs). Studies in mice autoimmune models and patients suffering from autoimmune diseases described these cells as CD19high CD21- CD11c+. Moreover, a subset of synovial fluid B cells with low levels of CD21, expresses FcRL4 and produces the cytokine RANKL, which stimulates the differentiation and activation of osteoclasts. The ABCs found in peripheral blood could therefore be the precursors of this FcRL4 positive subset found in synovia.

Objectives We aimed to investigate the proportion and phenotype of peripheral blood ABCs in patients suffering from early drug naïve RA.

Methods Newly presenting patients, naïve to immunomodulatory treatment, were recruited from the Newcastle Early Arthritis Clinic, and followed until diagnoses were confirmed. B-cell subsets in peripheral blood were detected and phenotyped using flow cytometry. NanoString nCounter Immunology v2 Panel (NanoString Technologies) was used to detect mRNA from cell lysates of sorted ABCs, naïve, memory and CD5+ B cells from RA patients, age-matched healthy controls and disease controls (Psoriatic Arthritis patients).

Results Our work showed that there are no significant differences in the frequency of ABCs between RA patients, disease controls and age-matched healthy controls. There is a possible trend for increased frequencies of ABCs with age as well as those who have a high disease activity. Our results also show that ABCs resemble a memory B cells phenotype with regard to class-switch immunoglobulins expression, with a significant percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA. Interestingly, the FcRL4+, the proliferating Ki67+ and the T-bet percentage of them being positive for IgG and IgA.

Conclusions These data supports an activated phenotype of the ABCs, which supports the idea that ABCs have a pathogenic role in RA, potentially via autoantibody and T cell stimulatory ability. However, further characterisation of this subset and functional studies are needed.

Disclosure of Interest None declared.

**P050** JAN GÖSTA WALDENSTRÖM AND RHEUMATOLOGY

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

Introduction On 2. September 1943 Jan Waldenström (1906–1996) successfully submitted a paper to Acta Medica Scandinavica describing two patients with a new disease. The discovery was to make him world famous.1 This year marks the 75th anniversary of macroglobulinemia and it coincided with the 10th biennial international workshop of Waldenström’s macroglobulinemia, discussing advances in the genetic basis, pathogenesis and treatments of the disease.2 Jan Waldenström’s 7[MO1] (JW) would have enjoyed this workshop immensely, sharing the information that over 95% of the patients had somatic mutations affecting the MYD88 gene on the 2nd chromosome as well as the impressive advances in treatment. Attending this excellent meeting brought back memories of my time as Waldenström’s PhD student and triggers me to compose this vignette, focusing on connections between the interests of my mentor and rheumatology. For a more comprehensive account of Jan Waldenström’s legacy I recommend Robert Kyle’s superb obituary, published in Blood, a journal JW was attached to from its start.3

After the successful defence of his landmark PhD Thesis on acute intermittent porphyria2 Waldenström’s interest focused on haematology. He worked in Uppsala, a university where the study of proteins had prominence. There The Svedberg had developed the ultracentrifuge and Arne Tiselius the free protein banding technique. Both of these tools contributed to the study of proteins and led to the development of the Svedberg unit (S) used in molecular biology to measure the size of molecules. This year marks the 100th anniversary of the discovery of haemoglobin by Wilhelm von Ebber and the 75th anniversary of its isolation by David E. H. Eveleigh, both of whom were awarded the Nobel Prize in Chemistry in 1929. The Svedberg unit is used to measure the sedimentation coefficient of proteins and other macromolecules.

He noticed the several multiorgan manifestations, the similar-ness to tuberculosis, and considered by many to be a form of tuberculosis. He named the condition uveosclerotic retinochoroiditis. He described 3 cases from this population which were to be the precursors of this FcRL4 positive subset found in synovia. These data supports an activated phenotype of the ABCs, which supports the idea that ABCs have a pathogenic role in RA, potentially via autoantibody and T cell stimulatory ability. However, further characterisation of this subset and functional studies are needed.

Today the diagnosis could have been IgG4 related disease in several of these patients. This early paper also shows a keen interest in inflammatory systemic conditions.

In the early 1940s JW collected serum from some 100 patients with longstanding ESR exceeding 120 mm, and had it analysed by the new technique of free boundary electrophoresis by K.O. Pedersen in the Department of Physical Chemistry. In 1943 he described 3 cases from this population characterised by repeated bouts of decline purpura, leaving spots of brown discoloration, mild anaemia, and on the whole, good general health. Two of the 3 women also had dry eye problems and one had dry mouth and swollen parotis glands. He named the condition ‘purpura
hyperglobulinemica'. Similar patients were soon identified by others and labelled ‘Waldenström’s purpura hyperglobulinemica’, citing his Swedish communication that contained only a brief summary in English. A more comprehensive later report, presented new cases, detailed case histories and a colour illustration of the typical skin changes, figure 1, and discussed the systemic nature of the condition in depth. Other organ manifestations included lymphadenopathy, uveoparotitis, Sjögren’s syndrome and SLE. The serum albumin concentration remained normal in line with the benign nature of the condition.7 The author was surprised that this English paper was hardly ever cited.8

In 1949 Jan Waldenström was appointed as the first professor and chairman of internal medicine at Malmö General Hospital, the new second teaching hospital of the Lund University. In Malmö he continued investigating what he now called gammopathies. The new technique of paper electrophoresis refined by Carl-Bertil Laurell dramatically simplified identification of patients with hypogammaglobinaemia and serum electrophoresis became a routine test. In collaboration with Sten Winblad sera were also routinely examined for presence of antibodies to bacterial antigens by a package called ‘total serology’. Combined, this lead to the distinction between polyclonal reactive and monoclonal malignant conditions, perhaps JW's most important scientific contribution.9

In 1950 JW was invited to speak at the first German congress of Gastroenterology in Germany after the war. There he presented a few cases of a new form of active chronic hepatitis, predominantly in young women with high ESR, very high concentration of gamma-globulin and prominence of plasma cells in the liver. Some but not all developed cirrhosis.10 In 1951 a similar observation was presented as an abstract by Henry Kunkel’s group.11 This condition was also soon observed by other investigators and known under several names. One that has survived is chronic active hepatitis. Sheila Sherlock has summarised the clinical spectrum of the disease based on 115 of her own cases, and emphasised the systemic nature which is characteristic of an autoimmune disorder.12 Ulcerative colitis, skin rashes, glomerulonephritis, pulmonary infiltrates and Hashimoto’s thyroiditis were common. ANAs were found in 40% and rheumatoid factor in 70% of her cases.

In Malmö JW soon emerged as a charismatic leader, equally popular among patients, medical students, staff, and highly respected by Malmö’s ambitious administrators. Within few years the department, although frugally staffed, became the leading academic internal medicine unit in the country. JW was a firm believer in the blessing of un-fragmented internal medicine, although expecting members of the staff to select an area of special expertise within it. Rheumatology was only established as a speciality in Sweden in 1969 and the first generation of rheumatologists were specialists in internal medicine. But in Malmö autoimmune disorders like SLE were specialité de la maison and Talbott and Ferrandis’ ‘Collagen Diseases’ was obligatory reading.13 The book from 1956 still rests on my shelf.

International visitors were frequent guests and fellows came to work with the famous professor. Patients with rare or unclear disease were referred to him from all over Sweden. One example which directly affected me as the most junior house officer was two cases with frequent infections, antibody deficiency disease, labelled as adult ‘acquired’ hypogammaglobinaemia. The professor had interviewed the women who came from different hospitals in the country and not simultaneously. In spending good time talking to them he happened to find out that both had roots in a village 100 miles to the north of Malmö. I was given the task to find out if they had common ancestors. After some months of searching in old church registers this in fact turned out to be the case, hinting at a possible genetic etiology. When I showed the pedigree starting in the mid-eighteenth century to the professor, JW was of course pleased, and when I presented the brief report with his name after mine he said ‘Fine, send it to The Lancet’. Unfortunately he erased his own name from the manuscript although it clearly was his idea based on his ground work. The paper was accepted without changes.14 The story was later supported by a following larger report.15 The disease now is named common variable immunodeficiency and genomic technology including next-generation sequencing reveals its complex genetic basis and explains links to autoimmunity.16 The Lancet paper was my first publication as internist and it opened the way to USA. Just coming from JW's department in Malmö usually resulted in red carpet reception.

Several of JW’s international contacts and visitors were prominent in rheumatology. Henry Kunkel, Morris Ziff, Eric Bywaters, Barbra Ansell, Norman Talal, Eng Tan, Bob Winchester and Ralph C Williams are some names that come to mind (figure 2). He never passed New York without visiting Henry Kunkel at the Rockefeller Institute where he enjoyed making ward rounds. A paper titled ‘Forty years with the gammaglobulins’17 gives further personal proof of JW's close ties with rheumatology, which certainly facilitated my path into the specialty. My own visits with Henry Kunkel’s small group usually included a seminar where the presenter was allowed to use the blackboard but not to show slides. The group then had lunch and after lunch went to the library and browsed through the new journals of the day. Electronic journals had not been born.

REFERENCES

Abstracts
Charaterization of the Anti-Centromere Antibody Response in Systemic Sclerosis Patients Suggests a Broad and Active B Cell Response

Introduction Systemic Sclerosis (SSc) is a rare, heterogeneous autoimmune disease characterized by microvascular damage, organ fibrosis and immune dysfunction. Autoantibodies are detected in >95% of patients, the most prevalent being anti-centromere (ACA) and anti-topoisomerase (ATA) antibodies. Although used for diagnosis, little is known about the underlying auto-reactive B cell responses. In particular, the ACA B cell response has been poorly studied.

Objective Characterization of the ACA B cell response in SSc patients.

Methods ACA IgG, IgA and IgM levels were measured in serum samples of 167 ACA IgG+ SSc patients. Patients were divided in a SSc (fulfilling ACR 2013 criteria, n=132) and a very early SSc group (fulfilling VEDOSS criteria, n=35). Additionally, PBMCs from ACA IgG+ SSc patients (and ATA IgG + SSc and healthy donors (HD) as control) were cultured either in the presence of CD40L expressing fibroblasts, IL-21 and BAFF or without stimulation. Levels of ACA IgG, IgA and IgM (and total Ig) were measured after one week of culture using ELISA.

Results ACA IgG+ SSc patients displayed a broad isotype usage with 75% being ACA IgA+ and 68% being ACA IgM+ in serum. Patients within the SSc group showed higher ACA IgG levels and a higher percentage of ACA IgM positivity compared to the very early SSc group. ACA IgA and IgM could be measured in ACA SSc PBMC culture medium following stimulation, but not in ATA SSc and HD, indicating the presence of circulating ACA B cells of all three isotypes. In cultures that yielded sufficient Ig production, ACA IgG was detectable in 7/9 ACA SSc patients, ACA IgA in 3/7 and ACA IgM in 2/7. Furthermore, ACA IgG production was also detected in the absence of stimulation in 5/9 patients, suggesting the presence of ACA-producing plasmablasts in the circulation. No spontaneous production of anti-Tetanus Toxoid antibodies, a control recall response, was observed.

Conclusions ACA+ SSc patients display a broad range of isotype usage in their ACA response, reflected both by ACA serum levels and presence of ACA IgG-, IgA- and ACA IgM-producing B cells in the peripheral blood. Additionally, ACA IgG production by unstimulated PBMCs points towards continuous differentiation of memory cells into antibody secreting cells. These data, together with differential isotype profiles between very early SSc and SSc patients, provide insight into the ACA B cell response and its potential involvement in disease-relevant pathogenetic processes.

Disclosure of Interest None declared.

Auto-Antibodies against Post Translational Modified Proteins in Osteoarthritis: Pilot Data Comparing Synovial Fluid and Sera

Introduction Osteoarthritis (OA) is a highly prevalent disease and a leading cause of disability worldwide. OA is age-related and as such has a potential devastating impact on our ageing populations. The pathogenesis of OA remains poorly understood. Inflammatory responses are prevalent in driving processes associated with aging, and increased levels of IgG autoantibodies (auto-Abs) have been associated with age. In OA, auto-Abs are also frequently observed, notably towards post translational modified (PTM) proteins. Such PTM are often the result of inflammation-driven mechanisms such as oxidative stress, carbamylation and citrullination in OA joints.

Objective To establish a profile of auto-Abs associated with PTM in OA and controls.

Methods Serum was collected from healthy controls (HC) and OA patients as well as rheumatoid arthritis (RA); knee synovial fluid (SF) samples were collected from OA/RA patients. All participants gave informed consent. The levels of antibodies against citrullinated protein antigens (ACPA) was measured (Phadia CCP-test), antibodies against carbamylated proteins (anti-CarP) were measured in collaboration with Leiden using an in-house ELISA, antibodies against glycated-collagen proteins (anti-ROS-CII) were analysed using an in house ELISA.

Results ACPA positivity was present in 6% (n=24/392) of OA patients’ serum, which was 3 times more frequent than in HC (~2%), but was present in 48% (n=22/46) of OA SF. RA frequencies were 73% (n=41/56) in SF and 65% (n=55/85) in serum. Anti-CarP auto-antibodies were detected in 11% (n=12/111) of OA sera and in 37% (n=127/340) of RA sera (SF results are not yet available). Anti-collagen auto-Abs in serum were observed at similar frequencies: 18% of OA (n=6/34) and 18% (n=14/78) of RA patients, but only 6.7% (n=3/45) of RA fluids were positive. Collagen modified by reactive oxygen species enables detection of auto-Ab (anti-ROS-CII); these were observed in 35% (n=12/34) of OA and 32% (n=25/78) of RA sera; this differed from SF with 20% positive (n=9/45) in OA and 60% (n=34/56) in RA.

Conclusions In OA patients, autoimmune responses against native proteins (especially collagens and proteoglycans) have been reported since the 1980’s, notably in relation with severity. However, our data demonstrate that auto-Abs may also result from the accumulation of PTMs induced by chemical reactions in the inflamed joint. These auto-Abs against PTM-antigens may also be contributing to disease severity, as these are notably frequently detected in SF even if a bit less frequently than in RA in our pilot cohorts.

Disclosure of Interest None declared.
LncRNA HOTAIR PROMOTES PROLIFERATION AND INVASION OF FIBROBLAST-LIKE SYNOVIOCYTES AS MICRORNA SPONGING IN RA PATIENTS

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

Introduction Long non-coding RNAs (lncRNAs) have drawn increasing attention because of the pivotal roles which they play in various types of autoimmune diseases, including rheumatoid arthritis (RA). LncRNA HOTAIR is a crucial lncRNA function as an oncogene in multiple cancers. Fibroblast-like synoviocytes (FLSs), a prominent component of hyperplastic synovial pannus tissue, are critical to synovial aggression and joint destruction in RA. However, the functions of lncRNA and the molecular mechanisms remain to be further elucidated in FLSs of RA patients.

Objectives Our present study aimed to investigate the expression and roles of lncRNA HOTAIR in RA-FLSs and explore its possible mechanism.

Methods FLSs were cultured from synovial tissues of joint. LncRNA and microRNA expression profiles in FLSs were screened by microarray, and then we validated the results by Real-time Quantitative polymerase chain reaction (qRT-PCR). Small interfering RNA (siRNA) was then used to knock down the expression of HOTAIR in order to determine its role in RA FLSs. Cell viability was evaluated using the CCK-8 assay and flow cytometry. Cell invasion was analyzed by transwell chamber methodology. Bioinformatics analysis was performed to predict the possible competitive endogenous RNA (ceRNA) mechanisms via miRanda, PITA, RNAhybrid, as well as KEGG and Gene Ontology (GO) analysis.

Results Both microarray analysis and qRT-PCR showed the expressions of lncRNA HOTAIR were up-regulated in RA FLSs compared with healthy controls (HCs). Transfection of HOTAIR-siRNA significantly decreased the expression of lncRNA HOTAIR in RA FLSs. HOTAIR knockdown largely inhibited cell proliferation and invasion of RA FLSs. Furthermore, the bioinformatics analysis predicted that some of microRNAs and mRNAs may be the downstream molecules of lncRNA HOTAIR. Considering the microRNA expression profiles detected by microarrays and the results from qRT-PCR, we designated miR-138 and miR-17~5p as potential ceRNAs which lncRNA HOTAIR could directly bind to. In addition, the expressions of miR-138 and miR-17~5p were markedly downregulated in RA FLSs, whereas the knockdown of lncRNA HOTAIR upregulated the expressions compared with the negative control group (NC-siRNA).

Conclusions Our study illuminated that elevated lncRNA HOTAIR expression promoted the proliferation and invasion of RA FLSs. Meanwhile, it may function as a novel microRNAs sponging agent and regulate RA FLSs pathological behaviors via miR-138 or miR-17~5p associated ceRNA network. In summary, the regulation of lncRNA HOTAIR may be a promising therapeutic strategy for RA in the future.

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Disclosure of Interest None declared

IRF1 IS CRITICAL FOR THE TNF-DRIVEN INTERFERON RESPONSE IN RHEUMATOID FIBROBLAST-LIKE SYNOVIOCYTES

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Career situation of first and presenting author Young investigator.

Introduction Rheumatoid arthritis (RA) is an autoimmune disease, which is characterized by persistent, synovial inflammation. Major drivers of synovial inflammation are cytokines and chemokines. Among them TNF activates fibroblast-like synoviocytes (FLS), which leads to the production of inflammatory mediators. The pathways and transcription factors that determine the inflammatory response in FLS are largely unexplored. Here, we investigated the potential contribution of the transcription-factor IRF1 to the inflammatory gene expression in FLS.

Methods Expression of IRF1 in synovial tissues was assessed by immunohistochemistry (IHC). RA-FLSs were isolated according to established protocols and cultured using 2-D or 3-D culture techniques. IRF1 expression in response to TNF was determined by western blots, qPCR or IHC. FLSs were also stimulated with TNF in the presence or absence of IRF1 siRNA pools. Global changes of mRNA expression were assessed by RNA-sequencing. Janus kinase activity was blocked by baricitinib or tofacitinib.

Results Our data reveal that TNF regulates the expression of IRF1 in human FLS as well as in the huTNFtg mouse model of arthritis. Transcriptomic analyses of IRF-1-deficient TNF-stimulated FLS define the interferon (IFN) pathway as a major target of IRF-1. Further experiments show that TNF-induced IRF-1 expression promotes the expression of IFN-β, which leads to an activation of the JAK-STAT pathway. Blockade of the JAK-STAT pathway, by the Janus kinase inhibitors baricitinib or tofacitinib, reduces the expression of IFN-regulated genes (IRGs), such as CXCL9, CXCL10, CXCL11 and TNFSF13B, in TNF-activated FLS.

Conclusions Our data reveal that IRF1 is crucial for the IFN-response of FLS and support the idea that IRF1 plays a critical role for the development of synovial inflammation.

Disclosure of Interest None declared.
**Abstracts**

**P055** CXCL13 AS BIOMARKER FOR HISTOLOGICAL INVOLVEMENT IN SJOGREN’S SYNDROME

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Introduction Sjögren’s syndrome (SS) is an autoimmune condition characterised by systemic B cell activation, autoantibody production and ectopic germinal centers (GC) formation within salivary gland (SG). The extent of SG infiltrate has been proposed as biomarker of disease severity. Plasma levels of CXCL13 correlate with GC activity in animal models and disease severity in SS, suggesting its potential use as a surrogate serum marker to monitor local B cell activation.

Objectives To evaluate the potential role of CXCL13 as biomarker of SG pathology in two independent SS cohorts.

Methods 109 patients with SS were recruited at Sapienza University of Rome (Italy) (n=60), or at Queen Elizabeth Hospital in Birmingham and Barts Health NHS Trust in London (n=49). Both sera and matched paraffin-embedded minor SG biopsy were available. Sica (n=57) and healthy subjects (HS) (n=19) sera were used as control. CXCL13 gene expression was also assessed in 25 frozen SGs.

Results CXCL13 serum levels were higher in SS patients [90.3 (84.2) pg/ml], compared to both sica [61.9 (38.6) pg/ml, p=0.0005] and HS [36.5 (40.18) pg/ml, p<0.0001]. No differences in average CXCL13 levels were detected between the two SS cohorts [83 (95.2) pg/ml and 103 (64.9) pg/ml, respectively]. In both Italian and British cohorts, serum levels of CXCL13 correlated with the percentage of SG infiltration (p=0.0008 and p=0.0004, respectively), FS (p=0.0010 and p=0.0103, respectively) and mean foci area (p=0.0167 and p=0.0054, respectively); higher serum levels were observed in patients with segregated foci (p=0.0553 and p=0.0019, respectively) and GCs (p=0.0147 and p=0.0044, respectively).

Higher CXCL13 serum levels were also associated with anti-Ro/SSA antibodies (p=0.0007, Italian cohort and p=0.0294, British cohort) as well as rheumatoid factor (RF) (p=0.0037, Italian Cohort and p=0.0033, British Cohort). Tissue expression of CXCL13 was moderately correlate with the mean foci area and percentage of infiltration. Higher expression of CXCL13 transcripts was observed in SGs with segregated foci (p=0.0057) and GCs (p=0.0162). Serum levels of CXCL13 did not correlate with local mRNA levels of the same cytokine.

Conclusions Our data foster the use of CXCL13 to monitor the extent of local pathology in SS and its validation in longitudinal clinical studies. Both serum and tissue expression of CXCL13 correlate with SS histological severity, suggesting a major role of this chemokine in SG lymphocytes recruitment and organization.

Disclosure of Interest None declared.

**P056** A NOVEL PGC-1β/NFATC-1 PATHWAY IN MONOCYTES FACILITATES OSTEOCLASTOGENESIS IN RHEUMATOID ARTHRITIS

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Career situation of first and presenting author Resident.

Introduction The underlying mechanism of excessive osteoclastogenesis causing bone erosion in rheumatoid arthritis (RA) remains elusive. PGC-1β is implicated in transcriptional regulation of osteoclastogenesis but its role in RA pathogenesis is unknown.

Objectives To investigate whether PGC-1β regulated osteoclastogenesis in RA.

Methods PGC-1β expression in peripheral CD14+ monocytes from RA patients were detected by immunofluorescence, flow cytometry and western blot. Peripheral CD14+ monocytes from RA patients or healthy controls were transfected with lentivirus for PGC-1β gene silencing or over-expression and cultured with M-CSF and RANKL. Mature osteoclasts and their bone resorption activity were determined by TRAP, F-actin and toluidine blue staining. DC-STAMP and bone degrading enzymes as well as signaling molecules were detected by western blot.

Results

1. Increased nuclear accumulation of PGC-1β was observed in peripheral CD14+ monocytes from RA patients and their relative PGC-1β protein expression was higher than that in healthy controls. Cultured with RANKL and M-CSF, the cell counts of mature osteoclasts on day 14 and 21 and the pit area of bone resorption lacunae on day 21 were significantly higher in RA patients than those in healthy controls.

2. PGC-1β knockdown in monocytes suppressed the expression of cathepsin K, TRAP and MMP-9 as well as osteoclast differentiation and bone resorption activity, while PGC-1β over-expression markedly promoted these indicators and osteoclastogenesis. Further, over-expressed PGC-1β increased the nuclear expression of NFATc-1, VIVIT, inhibitor of NFATc-1 activation, limited the effect of over-expressed PGC-1β on promoting the expression of cathepsin K, TRAP and MMP-9 in peripheral CD14+ monocytes from healthy controls.

3. ChIP-QPCR analysis confirmed the immunoprecipitation of PGC-1β and the NFATc-1 promoter which indicated that PGC-1β binds to the NFATc-1 promoter region. Dual-luciferase reporter gene assay showed that over-expressed PGC-1β on the peripheral CD14+ monocytes from healthy controls increased the transcriptional activity of NFATc-1 in a dose-dependent manner.

Conclusions Our data revealed a novel PGC-1β/NFATc-1 pathway contributing to excessive osteoclastogenesis in RA, which implied a potential therapeutic target of PGC-1β for osteoclast inhibition in RA.

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Disclosure of Interest None declared.
ARTEMETHER SUPPRESSES MIGRATION AND INVASION OF FIBROBLAST-LIKE SYNOVIOCYTES THROUGH INHIBITION OF THE RHO SIGNALLING PATHWAY IN RHEUMATOID ARTHRITIS

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

Introduction: Experiments on collagen-induced arthritis rats showed that artemether, a new antimalarial drug derived from artemisinin, can reduce inflammatory cell infiltration, tissue edema, and bone erosion in the paws, which implied its potential efficacy in rheumatoid arthritis (RA). Fibroblast-like synoviocytes (FLS) in RA manifest tumor-like properties including increased adherence and invasiveness of adjacent cartilage and bone, resulting in joint destruction. However, effects of artemether on the aggressive properties of RA-FLS have not yet been elucidated.

Objectives: The aim of this study was to investigate the role and underlying mechanism of artemether on the migration and invasion of RA-FLS.

Methods: Synovial tissues were obtained by closed needle biopsy from 6 active RA patients and FLS were isolated and cultured in vitro. RA-FLS were treated with artemether at various concentrations, while methotrexate (MTX) and hydroxychloroquine (HCQ) were employed as controls. Cell viability, proliferation, cell-cycle, apoptosis, migration, invasion, and pseudopodium formation of RA-FLS were assessed by CCK-8 assays, EdU stained, Annexin V-FITC/PI stained, transwell assays, or F-actin staining respectively. The protein expression of RhoA, Rac1, and CDC42 were measured by Western blot.

Results:
1. The IC50 value of artemether, MTX and HCQ on RA-FLS were 988 μM, 181 nM and 5433 μM, respectively.

2. The transwell assays of migration and invasion showed that artemether (20 μM) significantly inhibited migration and invasion of RA-FLS and reduced lamellipodia and filopodia formations. Under the same IC50 concentration, artemether (20 μM) showed similar inhibitory effects as MTX (10 nM), while HCQ (20 μM) showed no effect on migration and invasion of RA-FLS.

3. Rho family proteins play important roles in modulating cell migration and invasion. Western blot analysis showed that artemether (20 μM) and MTX (10 nM) significantly reduced the expression of RhoA, Rac1, and CDC42 in RA-FLS, which indicated that artemether could inhibit the Rho signaling pathway.

Conclusions: Artemether can suppress the migration and invasion capability of RA-FLS through inhibiting the Rho signaling pathway.

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Disclosure of Interest: None declared.

CHEMOKINE CCL18 ENRICHED IN SYNOVIAL FLUID IS INVOLVED IN JOINT DESTRUCTION THROUGH PROMOTING MIGRATION OF FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

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Career situation of first and presenting author: Young investigator.

Introduction: CC chemokine ligand 18 (CCL18) which is either constitutively expressed or induced in monocytes/macrophages and dendritic cells has been reported to be highly expressed in peripheral blood and synovial fluid of rheumatoid arthritis (RA) patients compared with healthy controls, indicating the possible role of CCL18 in the development and pathogenesis of RA.

Objectives: To explore the association of serum and synovial fluid CCL18 with clinical and radiographic outcome in RA and its potential effect on RA fibroblast-like synoviocytes (FLS).

Methods: Consecutive patients with active RA (DAS28-CRP >2.6) were recruited. Synovial fluid was collected from inflamed joints if available. Demographic and clinical data were collected according to the 2017 EULAR recommendation. Serum and synovial fluid CCL18 was detected by ELISA. RA-FLS was cultured in vitro with RA synovial fluid and neutralizing antibody to CCL18. Migration/invasion ability was analyzed by Transwell assay.

Results:
1. Among 83 RA patients, age (median and IQR, similarly hereinafter) was 50 (41–58) years old and 63 patients (78%) were female, with median disease duration 36 (12–102) months, median DAS28-CRP 5.0 (4.6–6.1).

2. Serum CCL18 was 107 (80–126) ng/mL, which was significantly higher than healthy controls [n=25, 51 (29–70) ng/mL, p<0.001]. Serum CCL18 correlated slightly but significantly with CRP (r=0.385, p<0.001), ESR (r=0.239, p=0.03), PrGA (r=0.249, p=0.03), DAS28-CRP (r=0.368, p=0.001), DAS28-ESR (r=0.336, p=0.003), SDAI (r=0.360, p=0.001), CDAI (r=0.328, p=0.004) and HAQ (r=0.325, p=0.004).

3. Synovial fluid CCL18 of 31 patients was 719 (415–1271) ng/mL, which was significantly higher than corresponding serum level (Paired test, p<0.001). Among them, 13 patients who were treated according to treat to target strategy received X-ray assessment of hand/wrist both at baseline and month 12. Six patients who had one-year radiographic progression (a change of the Sharp/van der Heijde modified sharp score ≥0.5 units) showed higher synovial fluid CCL18 than other 7 patients without radiographic progression [1481 (1244–2034) ng/mL vs. 458 (405–681) ng/mL, p=0.004].

4. When incubated with RA synovial fluid, the migration ability of RA-FLS was significantly increased; but this effect was inhibited by neutralizing antibody to CCL18.

Conclusions: CCL18 elevates especially in synovial fluid of RA patients, which may correlate with one-year radiographic progression through promoting migration of RA-FLS.

Disclosure of Interest: None declared.
DISSECTING THE ROLE OF GREMLIN 1 IN SYSTEMIC SCLEROSIS

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

Introduction Systemic Sclerosis (SSc) is a multi-system autoimmune disease, its exact aetiology remains unknown. Despite its heterogeneity, SSc is characterised by three common hallmarks: vascular disruption, production of auto-antibodies and aberrant accumulation of extra cellular matrix (ECM) proteins, leading to fibrosis. Unfortunately, the disease has no cure and few effective therapies.1 The aberrant regulation of developmental pathways, including that of bone morphogenetic proteins (BMP) and Gremlin 1, could yield alternative therapeutic targets.

Gremlin 1 is a known BMP antagonist. However, research has found it is implicated in several pathologies including fibrotic diseases such as idiopathic pulmonary fibrosis.2 A paper published in 2014 showed that recombinant Gremlin 1 is pro-fibrotic in normal human dermal fibroblasts (NHDFF).3

Objectives The aim is to investigate the role Gremlin 1 plays in the development of fibrosis in patients with SSc. The key steps to achieving this will involve firstly confirming overexpression of Gremlin 1 leads to an increase in key ECM proteins.

Methods Once confirmed chemical, RNA and clinical antibody interference work will be carried out. Several key molecular techniques will be utilised in the project including transfection, western blotting and reverse transcription polymerase chain reaction.

Results Electroporation was used to transfect NHDFF with a Gremlin 1 overexpression vector. Gremlin 1 overexpression showed an increase in Collagen 1 and a statistically significant increase in the myofibroblast marker Alpha Smooth Muscle Actin, compared to control. RNAi work is currently being carried out to assess novel downstream targets. Data suggests cross-talk between the transforming growth factor beta pathway, interaction of Gremlin 1 directly with the vascular endothelial growth factor receptor or a feedback loop involving sonic hedgehog could be involved in the pathway.

Conclusions Due to the rarity, lack of effective therapies and incomplete knowledge of its aetiology research into SSc is required in order to enhance understanding and therapeutic options.

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Disclosure of Interest None declared.
INHIBITION OF ARGINASE-1 EXPRESSION BY THE TRANSCR  

A27

Introduction Activins and inhibins belong to the transforming growth factor β family. Activins are disulphide-linked homodimers consisting of two inhibin β chains (βA, βB) that are expressed in many cell types. However, activin A (βA βA) is the only activin that is expressed in bone and cartilage. Moreover, activin A has been demonstrated not only to stimulate receptor activator of NF-κB ligand (RANKL)-induced osteoclast (OC) differentiation but also to inhibit osteoblast differentiation.

Objectives Here we investigate the impact of activin A on joint destruction in rheumatoid arthritis.

Methods Synovial tissue samples from rheumatoid arthritis (RA) and osteoarthritis (OA) patients were analysed by immunohistochemical staining. For in vitro experiments, synovial fibroblasts (FLS) were isolated from hind paws of WT mice. Effects of cytokines on the secretion of activin A by mouse FLS were evaluated by ELISA. Bone marrow-derived macrophages (BMM) were isolated from female and male mice and differentiated into osteoclasts in the presence of macrophage colony-stimulating factor (M-CSF) and RANKL with or without activin A. OC differentiation was characterised by TRAP staining. Resorption activity was determined by quantification of osteoclast-mediated pit formation on a calcium phosphate-coated plate. Furthermore, osteoclast-specific gene expression as well as the activation of SMAD2 in BMMs, OCS and FLS were analysed by immunoblotting. The interaction of phospho-SMAD2 with NFATc1 was evaluated by co-immunoprecipitation using Dynabeads.

Results We demonstrate that activin A is highly abundant in the synovium of RA but not of OA patients. In vitro, activin A secretion by FLS was strongly enhanced by pro-inflammatory cytokines. Furthermore, activin A strongly enhanced the RANKL-mediated differentiation of BMMs into mature OCs, reflected by a significantly increased OC number and OC size. Moreover, concomitant administration of activin A led to a significant increase of the total resorption area as well as resorption area per pit, indicating an increased activity of individual OCs. Furthermore, activin A enhanced the RANKL-induced expression OC differentiation markers, but alone was not able to induce OC differentiation. Analyses of signaling pathways revealed that activin A induce the activation of SMAD2 in BMMs and OCs. Finally, upon co-stimulation with RANKL, activin A resulted in an increased interaction between activated SMAD2 and NFATc1.

Conclusions The data strongly suggest that increased expression of activin A in the arthritic joint is associated with enhanced osteoclast formation, promoting joint destruction in rheumatoid arthritis.

Disclosure of Interest None declared.

REGULATION OF JOINT DESTRUCTION BY ACTIVIN A IN RHEUMATOID ARTHRITIS

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Introduction Chronic inflammatory joint disorders are associated with bone destruction by osteoclasts (OC), which derive from myeloid precursors. Recent findings reveal that OC are

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Abstracts

P063/011 INHIBITION OF ARGINASE-1 EXPRESSION BY THE TRANSCRITION FACTOR FRA-1 IN MACROPHAGES EXACERBATES RHEUMATOID ARTHRITIS INFLAMMATION

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Conclusion Our data show for the first time that Fra-1 is a pivot between pro- and anti-inflammatory macrophage. By inhibiting Arg1 activity, Fra-1 exacerbates RA inflammation and joint destruction.

Disclosure of Interest None declared.

P065 REGULATION OF JOINT DESTRUCTION BY ACTIVIN A IN RHEUMATOID ARTHRITIS

Institute of Musculoskeletal Medicine, Münster, Germany

Introduction Activins and inhibins belong to the transforming growth factor β family. Activins are disulphide-linked homodimers consisting of two inhibin β chains (βA, βB) that are expressed in many cell types. However, activin A (βA βA) is the only activin that is expressed in bone and cartilage. Moreover, activin A has been demonstrated not only to stimulate receptor activator of NF-κB ligand (RANKL)-induced osteoclast (OC) differentiation but also to inhibit osteoblast differentiation.

Objectives Here we investigate the impact of activin A on joint destruction in rheumatoid arthritis.

Methods Synovial tissue samples from rheumatoid arthritis (RA) and osteoarthritis (OA) patients were analysed by immunohistochemical staining. For in vitro experiments, synovial fibroblasts (FLS) were isolated from hind paws of WT mice. Effects of cytokines on the secretion of activin A by mouse FLS were evaluated by ELISA. Bone marrow-derived macrophages (BMM) were isolated from female and male mice and differentiated into osteoclasts in the presence of macrophage colony-stimulating factor (M-CSF) and RANKL with or without activin A. OC differentiation was characterised by TRAP staining. Resorption activity was determined by quantification of osteoclast-mediated pit formation on a calcium phosphate-coated plate. Furthermore, osteoclast-specific gene expression as well as the activation of SMAD2 in BMMs, OCS and FLS were analysed by immunoblotting. The interaction of phospho-SMAD2 with NFATc1 was evaluated by co-immunoprecipitation using Dynabeads.

Results We demonstrate that activin A is highly abundant in the synovium of RA but not of OA patients. In vitro, activin A secretion by FLS was strongly enhanced by pro-inflammatory cytokines. Furthermore, activin A strongly enhanced the RANKL-mediated differentiation of BMMs into mature OCs, reflected by a significantly increased OC number and OC size. Moreover, concomitant administration of activin A led to a significant increase of the total resorption area as well as resorption area per pit, indicating an increased activity of individual OCs. Furthermore, activin A enhanced the RANKL-induced expression OC differentiation markers, but alone was not able to induce OC differentiation. Analyses of signaling pathways revealed that activin A induce the activation of SMAD2 in BMMs and OCs. Finally, upon co-stimulation with RANKL, activin A resulted in an increased interaction between activated SMAD2 and NFATc1.

Conclusions The data strongly suggest that increased expression of activin A in the arthritic joint is associated with enhanced osteoclast formation, promoting joint destruction in rheumatoid arthritis.

Disclosure of Interest None declared.

P066 MIR-342–3P PROMOTES CELL SURVIVAL AND MOTILITY OF OSTEOCLAST PRECURSORS

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Career situation of first and presenting author Young investigator.

Introduction Chronic inflammatory joint disorders are associated with bone destruction by osteoclasts (OC), which derive from myeloid precursors. Recent findings reveal that OC are

Career situation of first and presenting author Student for a master or a PhD.
not only professional bone-resorbing cells but also directly involved in controlling inflammatory responses. Depending on the pathophysiological context, OC can polarize the immune response towards tolerance or inflammation. Aiming at identifying key regulators of inflammatory OC functions, we have defined a miRNA-based signature, which includes miR-342-3p. MiRNAs are key regulators of gene expression that control cellular processes, including osteoclastogenesis, and few miRNAs have been described in the differentiation of myeloid precursors into mature OC.

**Objectives** To study the role of miR-342-3p in inflammatory OC.

**Methods** OC were derived from the murine monocyte RAW264.7 cells. The expression levels of miR-342-3p and OC-specific genes were monitored by qRT-PCR. RAW264.7 cells were transfected with either miR-342-3p mimics, neutralizing molecules or control miRNAs. Cell survival and motility were assessed at 48 hours after RANKL incubation using quantification of the caspase3-7 activity, ATP production and BrDU incorporation. Motility of OC precursors was monitored using time-laps during the course of OC differentiation. The K/BxN serum-transfer arthritis (STA) model was performed in 8 weeks old C57BL/6 males and bone marrow was flushed. Primary OC were generated from either total bone marrow or sorted CD11b+ and CD11c+ cell subsets of healthy and arthritic mice.

**Results** The expression of miR-342-3p was transiently up-regulated in the early phase of OC generation and was down-regulated after 24–48 hours in OC precursors. While pre-miR-342-3p promoted the motility of RAW264.7 cells, anti-miR-342-3p inhibited all motility parameters recorded (p<0.0001, ANOVA test). Anti-miR-342-3p reduced the proliferation (p<0.01) and cell survival of OC precursors through a pro-apoptotic effect, as assessed by increased caspase3-7 activity (p<0.01). Overall, miR-342-3p neutralization in OC precursors reduced OC numbers (p<0.001) compared to the pre-miR-342-3p condition. In primary cells, miR-342-3p was up-regulated in bone marrow-derived mature OC from arthritic mice compared to healthy controls (p=0.03; STA n=5/group).

**Conclusions** Our data suggest that miR-342-3p promotes the early phase of osteoclastogenesis by enhancing the cell survival and motility of OC precursors. The up-regulation of miR-342-3p in OC isolated from arthritic mice may reflect the increased osteoclastogenic potential of inflammatory precursors in arthritis.

**Disclosure of Interest** None declared.

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**PO67**

**TOFACITINIB IMPAIRS MONOCYTE-DERIVED DENDRITIC CELL DIFFERENTIATION IN RHEUMATOID ARTHRITIS AND PSORIATIC ARTHRITIS**

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

**Introduction** Tofacitinib is an oral Janus kinase inhibitor, recently approved for the treatment of rheumatoid arthritis (RA) and psoriatic arthritis (PsA). Although its mechanism of action has been explored in circulating cells, including neutrophils and lymphocytes, its effect on dendritic cells development and function remains still to be elucidated. Monocyte-derived dendritic cells (Mo-DC) are a subset of inflammatory DC derived from circulating monocytes with a key role in inflammation and infection.

**Objectives** The aim of this project is to evaluate the effect of Tofacitinib on inflammatory Mo-DC differentiation from RA and PsA patients, an important step in innate immunity.

**Methods** Monocytes were isolated from blood of healthy donor (HC), RA and PsA patients by magnetic separation and differentiated in the presence of GM-CSF/IL-4 cocktail for 7 days. To evaluate the effect of Tofacitinib on Mo-DC differentiation, monocytes were pre-treated with 1 μM Tofacitinib (or DMSO as control). CD209 (immature DC marker) was evaluated by flow cytometry in the CD11c+ population. Non-specific macrophage-activating agents (using Lucifer Yellow) and receptor-mediated endocytosis (using DQ™ Ovalbumin) were investigated by flow cytometry. The effect of Tofacitinib on NADPH oxidases (NOX) 5 and 2 expression, known players in Mo-DC differentiation, was evaluated by Western blot analysis. Finally, the frequency of CD209+ cells and their chemokine receptor expression (CXCRCXCR3/5 and CCR6/7) were evaluated by flow cytometry in peripheral blood (PBMC), synovial fluid (SFMC) mononuclear cells and synovial tissue cell suspensions from RA and PsA patients.

**Results** Pre-treatment of Mo-DC with Tofacitinib inhibited Mo-DC differentiation in RA and PsA patients, as evident by reduced CD209 marker expression. The decreased ability of monocytes to differentiate into DC in the presence of Tofacitinib was translated into a functional impairment of endocytic ability, in particular in PsA patients, as observed by the decreased uptake of both DQ™ Ovalbumin and Lucifer Yellow. In addition, Tofacitinib decreased NOX5 and increased NOX2 protein expression in Mo-DC of PsA and RA Mo-DC, altering the NOX2/NOX5 balance. Finally, we identified CD209+ cells in the periphery of RA and PsA patients, which were enriched in SFMC and synovial tissue cell suspension, and presented with an increased expression of CCR7 and CXCR3.

**Conclusions** Together, these observations suggest a novel mechanism of action of Tofacitinib in RA and PsA, by inhibiting Mo-DC development, which may alter migration of DC to the joint and subsequent activation of the immune response.

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**PO68**

**RHEUMATOID ARTHRITIS PERIPHERAL CD14+ MONOCYTES ARE HYPER-INFLAMMATORY, HYPER-GLYCOLYTIC AND RETAIN A MEMORY BIAS TOWARD M1 MACROPHAGES**

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

**Introduction** Myeloid cells with a monocyte/macrophage phenotype are present in large numbers in the rheumatoid arthritis (RA) joint, significantly contributing to disease.
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 viability 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++. mRNA levels of differentiating NHK cells were determined by RT-qPCR.

Results Constitutive IL-38 overexpression reduced the proliferation of NHK cells and led 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.
RNA sequencing detection of gene dysregulation in B cells sorted from salivary gland tissue from primary Sjögren’s syndrome pathophysiology

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Abstracts

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.

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

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 oxLDL levels on the differentiation and activation of osteoclasts remains elusive.

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+/Ly6Chigh and CD11b−/Ly6C+ osteoclast precursors from day 3 and mature osteoclasts from day 6 were determined with staining for tartrate-resistant acid phosphatase (TRAP) and resorption capacity using hydroxyapatite-like-coated plates. RNA expression was analyzed with Luminex analysis. Secretion of pro-/anti-inflammatory mediators was analyzed with FACS. Underlying epigenetic programming was studied using chromatin immunoprecipitation. Secretion of pro-/anti-inflammatory mediators was analyzed with Luminex analysis. Secretion of pro-/anti-inflammatory mediators was analyzed with Luminex analysis.

Results Whereas basal levels of bone resorption were comparable between WT and Apoe−/− mice, induction of AIA resulted in a strong decrease in the numbers 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, CAII, 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.

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

Introduction The alarmin S100A9 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 S100A9 has been 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 TNFα-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 B cell depletion while long-lived plasma cells are not targeted. Thus, there is a need for novel therapeutics targeting the B-cell line in AAV. NF-kB 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-kB 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-kB signalling was targeted in these cultures by small molecule inhibitors of NF-kB inducing kinase (NIK, non-canonical NF-kB signalling) and Inhibitor of κB kinase β (IKKβ, canonical NF-kB signalling). Downstream NF-kB signalling and nuclear NF-kB 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 IKKβ effectively inhibited downstream non-canonical or canonical NF-kB signalling, respectively. In a B cell stimulation assay, NIK and IKKβ 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 IKKβ 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-kB signalling in AAV B cells results in the modulation of various B cell responses. Ongoing studies will indicate whether targeting of NF-κB 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 synovial 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, TNFα, IL-1b 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 TNFα (Pearson r=0.5387) or IL-1β (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
Dysregulated miR-125a promotes joint angiogenesis through enhanced glycolysis

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Conclusion: Decreased expression of miR-125 in PsA synovium and in-vitro models was strongly associated with anti-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.
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

References

Disclosure of Interest None declared.

Abstracts

P079 IS IgM RHEUMATOID FACTOR PRESENT ON CIRCULATING EXTRACELLULAR VESICLES OF RHEUMATOID ARTHRITIS PATIENTS A POTENTIAL BIOMARKER FOR DISEASE SEVERITY?

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

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. 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). EVs are small particles of around 100 nm and released by cells. They mediate intercellular communication by exchanging 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.

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-1β (1 ng/ml). After 18 hour the supernatants were collected and the concentration in HUVECs as well as in cocultures in comparison to the stimulation with IL-1β alone could be observed.

Results IL-1β induced activin A release in RASF and HUVECs alone as well as in direct coculture. The stimulation with follistatin and IL-1β at the same time reduced the activin A concentration in HUVECs as well as in cocultures in comparison to stimulation with IL-1β 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-1β in comparison to the stimulation with IL-1β alone could be observed.

The release of VEGF, which plays an important role in inflammation-induced neoangiogenesis, was induced in RASF with IL-1β (89%), activin A (55%), activin A and IL-1β (148%), follistatin and IL-1β (84%). In coculture with HUVECs, the induction was less pronounced as in
monoculture (IL-1β (73%), activin A (22%), activin A and IL-1β (101%), follistatin and IL-1β (67%)).

**Conclusions** The autoregulatory cycle of activin A and follistatin is active in HUVECs, but not in RASF. In direct coculture of HUVECs and RASF, the effects of HUVECs appear to outweigh resulting in a significant reduction of IL-1β in the presence of follistatin.

**Disclosure of Interest** None declared.

**Abstracts**

**P081/O20 LASP1 REGULATES CELL-TO-CELL CONTACT FORMATIONS OF FIBROBLAST-LIKE SYNOVIOCYTES IN RA**

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

**Introduction** In rheumatoid arthritis (RA), fibroblast-like synoviocytes (FLS) undergo a stable transformation resulting in an aggressive, tumour-like phenotype that mediates cartilage damage by increased levels of MMPs and adhesion molecules such as β1 integrins. In this context, the tumour-associated protein Lasp1 is of interest because it modulates actin organization and focal adhesion turnover.

**Objectives** The effects of Lasp1 deficiency on RA-FLS cell-to-cell contact formations, the disease course and joint destruction have been investigated in this study.

**Methods** Lasp1 expression was analysed in RA synovial tissue and in murine models of arthritis (hTNFtg mice and G6PI mouse model). Hind paws were analysed using WB analyses and immunofluorescence stainings, primary FLS were isolated and cultivated, respectively. Furthermore, Lasp1-/- mice were interbred with hTNFtg mice and offsprings were analysed for the progression of joint destruction by clinical evaluation and histopathology. Migration characteristics of FLS derived from wild type (wt), Lasp1-/-, hTNFtg and Lasp1-/-hTNFtg mice were analysed by live cell imaging. Additionally, we used an in vitro 3D organ culture system for functional analyses.

**Results** Upregulated Lasp1 levels in RA synovial tissue and FLS were observed. In line with the human data, increased levels of Lasp1 were found in murine FLS derived from wild type (wt), Lasp1-/-, hTNFtg and Lasp1-/-hTNFtg. Furthermore, Lasp1 deletion in the hTNFtg background resulted in an organised cellular lining layer comparable with wt FLS matrices.

Conclusions Lasp1 represents an interesting target involved in RA-caused joint destruction, because its loss resulted in significantly reduced cartilage destruction in vivo and RA-FLS interactions and migration rates in vitro.

**Disclosure of Interest** None declared.

**P082/O24 SCLEROSTIN DEFICIENCY AFFECTS RANKL-MEDIATED OSTEOCLAST DIFFERENTIATION**

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

**Introduction** Sclerostin is a Wnt inhibitor and has anti-anabolic effects on bone formation by negatively regulating osteoblast differentiation. Sclerostin loss-of-function leads to a higher bone mass and bone strength. Therefore, inhibition of sclerostin is currently considered as a promising treatment for osteoporosis. Surprisingly, in a TNFα-dependent arthritis mouse model (hTNFtg) the genetic deficiency of sclerostin caused a deterioration of disease severity. hTNFtg mice lacking sclerostin displayed enhanced and bone erosion associated with an elevated number of osteoclasts within the joint.

**Objectives** In order to understand the underlying mechanisms, we aimed to investigate the direct and indirect impact of sclerostin on osteoclast differentiation and bone erosion in arthritis.

**Methods** Sclerostin knockout (sost-) mice were crossbred with hTNFtg mice to obtain sost-/hTNFtg mice, from which synovial fibroblasts (SF) were isolated. Cocultures of synovial fibroblasts and green fluorescent protein (GFP+) bone marrow-derived macrophages (BMM) were performed and osteoclastogenesis was analysed. Receptor activator of NF-κB ligand (RANKL) and macrophage colony stimulating factor (MCSF) expression was measured by ELISA and IL-1α expression by Western Blot. Viability of osteoclast precursors was measured by MTT Assay.

**Results** In cocultures of SF and GFP+ BMM, osteoclast formation was enhanced by sost-/hTNFtg SF compared to hTNFtg SF. Expression of RANKL and MCSF, two crucial factors for osteoclast differentiation, was not different between the genotypes. Interestingly, stimulation of wildtype BMM with conditioned media (CM) from hTNFtg or sost-/hTNFtg synovial fibroblasts showed no TRAP+ cells at all. However, CM supplemented with RANKL lead to an elevated number of osteoclasts using sost-/hTNFtg CM compared to hTNFtg CM, pointing to a secreted factor promoting osteoclast development. In this regard, the osteogenic factor IL-1α, was higher expressed in sost-/hTNFtg SF than in hTNFtg SF. Moreover, the treatment of cocultures with recombinant sclerostin lead to a decreased number of osteoclasts in both genotypes. Accordingly, sclerostin inhibited osteoclastogenesis in monocytes when administered in the pre-differentiation phase, whereas no effect was observed in the differentiation phase, indicating an inhibitory effect of sclerostin mainly on osteoclast precursors.

**Conclusions** Sclerostin deficiency in hTNFtg SF promotes RANKL-mediated osteoclast differentiation, which is likely dependent on the inhibitory effect of sclerostin itself and/or on the promoting effect of higher levels of IL-1α.

**Disclosure of Interest** None declared.
Abstracts

P083/023 NOVEL SUBCLASS OF NON-CLASSICAL MONOCYTES ARE CRITICAL FOR INFLAMMATION

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

Introduction Monocytes in mice are distinguishable by expression of Ly6c. Ly6c\textsuperscript{hi} (classical) monocytes are associated with pro-inflammatory responses, while Ly6c\textsuperscript{lo} (non-classical) are involved in patrolling endothelial membranes. We have previously shown that depletion of monocytes prevents serum transfer induced arthritis (STIA) in mice, and that Ly6c\textsuperscript{lo} monocytes are the critical population.

Objectives We aim to contrast Ly6c\textsuperscript{lo} monocytes from the circulation, lining vessels, and tissue to determine their involvement in inflammation.

Methods Female 8–10 week old NR4A1\textsuperscript{−/−}, CX3CR1\textsuperscript{EGRc. zsGFP} and C57Bl/6 mice were used in all studies. CX3CR1\textsuperscript{EGRc. zsGFP} were utilized for cell tracking studies and joint shielded bone marrow chimeras via administration of tamoxifen (tam). Intravascular monocytes were identified by I.V. anti-CD45 labeling before perfusion. STIA was induced via I.V. KBxN sera. Cell populations were quantified by flow cytometry and FACS sorted for RNA-seq. Monocytes were identified CD45\textsuperscript{+} CD11b\textsuperscript{+} Ly6G\textsuperscript{−} TIM4\textsuperscript{−} CD64 Ly6c\textsuperscript{lo} and subdivided into intravascular (labeled, CD45\textsuperscript{+}) and extravascular (labeled CD45\textsuperscript{−}) and extravascular (no label).

Results NR4A1\textsuperscript{−/−} mice retain only 5% of circulating Ly6c\textsuperscript{lo} monocytes but all joint Ly6c\textsuperscript{lo} cells. STIA was comparable in NR4A1\textsuperscript{−/−} and C57Bl/6 mice suggesting circulating Ly6c\textsuperscript{lo} are redundant. Transcriptional profiling of Ly6c\textsuperscript{lo} cells identified distinct pathways enriched in upregulated genes between Ly6c\textsuperscript{lo} from joint and blood. In the joint we identified three populations of Ly6c\textsuperscript{lo} monocytes: extravascular unlabeled cells, labeled trans-vascular cells, and labeled intravascular cells adherent to endothelium. Mice given tam D8 of gestation had GFP\textsuperscript{+} microglia only, whereas D15 tam induced GFP\textsuperscript{+} synovial macrophages and unlabeled Ly6c\textsuperscript{lo} monocytes. Both labeled Ly6c\textsuperscript{lo} populations were GFP\textsuperscript{−}, indicating unlabeled and unlabeled Ly6c\textsuperscript{lo} arise from different progenitors. This was confirmed by bone marrow chimera studies showing labeled Ly6c\textsuperscript{lo} cells are replenished from blood monocytes. Clodronate loaded liposomes depleted labeled CD43\textsuperscript{+} cells but did not affect CD43\textsuperscript{−} cells or unlabeled cells. With our previous finding that clo-lip prevents STIA, these suggest adherent CD43\textsuperscript{−} Ly6c\textsuperscript{lo} cells are essential. This is supported by the finding that labeled Ly6c\textsuperscript{lo} monocytes expand rapidly during the first 1 hour of STIA. Adherent CD43\textsuperscript{−} cells expand especially rapidly, increasing in population size by 30x.

Conclusions We have identified and described two previously uncharacterized populations of Ly6c\textsuperscript{lo} cells in the joint- intra-vascular adherent and trans-vascular which have distinct origins and phenotype from both extravascular and circulating Ly6c\textsuperscript{lo}. The findings presented here strongly suggest adherent Ly6c\textsuperscript{lo} monocytes are a key effector cell in inflammatory arthritis.

Disclosure of Interest None declared.

P084 SALIVARY GLAND EPITHELIAL CELLS FROM SJÖGREN’S PATIENTS INCREASE B LYMPHOCYTES SURVIVAL AND ACTIVATION

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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 autoimmune disorder characterized by lymphocytic infiltrates and destruction of the salivary glands (SG). Several lines of evidence support the hypothesis that SG epithelial cells (SGECs) are not only the target of autoimmunity in pSS but may also play a role for its initiation and maintenance.

Objectives To study the survival and the activation of B lymphocytes cocultured with SGECs from pSS patients compared to controls.

Methods Primary cultures of SGECs were established from minor SG biopsies. Patients had pSS according to 2016 EULAR/ACR criteria and had sicca symptoms without any antibodies and with normal SG biopsies. The coculture involved B lymphocytes isolated by CD19 magnetic bead positive selection from healthy donors’ blood (purity >80%). Several conditions of stimulation were tested: IFNa 2400 U/mL, IFNg 5 ng/mL, Poly(IC) 10 µg/mL or 30 µg/mL. After 5 days, the viability, the activation (CD38) and the differentiation (CD27) of B lymphocytes were assessed by flow cytometry. Mann-Whitney (unpaired data) and Wilcoxon (paired data) were used for statistical analysis.

Results A significant increase of B lymphocytes survival was observed when cocultured with SGECs compared to B lymphocytes cultured alone, in all conditions of stimulation (p<0.05). The survival of B lymphocytes (percentage of alive cocultured B lymphocytes- percentage of alive cultured alone B lymphocytes) was increased when the cocultures were performed with SGECs from pSS patients (n=5) compared to SGECs from controls (n=5), in all conditions of stimulation (p<0.05), except IFNg. Moreover, there was a trend for an increase of B lymphocytes activation, assessed by higher percentages of CD38\textsuperscript{+} B lymphocytes when the cocultures were performed with SGECs from pSS patients compared to SGECs from controls. This difference was statistically significant (p<0.05) in the condition stimulated with TLR3 agonist (Poly(IC) 10 µg/mL). The percentage of CD27\textsuperscript{+} B lymphocytes was not affected by the cocultures and no difference between pSS and controls SGECs was observed.

Conclusions This coculture model showed a differential effect of SGECs from pSS compared to controls on B lymphocytes survival. Interestingly, there was also a trend for a higher activation level of B lymphocytes when cocultured with SGECs from pSS compared to controls. These results suggest that SGECs could play a major role in pSS pathophysiology through B lymphocytes support and activation.

Disclosure of Interest None declared.

P085 ACTIVATED MEMORY T CELLS PRODUCE LIGANDS THAT CAUSE NF-KB-DEPENDENT INFLAMMATORY ACTIVATION OF THE ENDOTHELIUM

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Career situation of first and presenting author Master or a PhD.

Introduction Inflammation of the endothelium is associated with the activation of T cells. The mechanisms underlying the activation of the endothelium are still unclear. We have previously shown that CD4\textsuperscript{+} T cells from pSS patients are the critical cells for the activation of the endothelium in vitro and in vivo.

Methods We investigated whether CD4\textsuperscript{+} T cells from pSS patients produce ligands that activate the endothelium.

Results CD4\textsuperscript{+} T cells from pSS patients produce ligands that activate the endothelium.

Conclusions CD4\textsuperscript{+} T cells from pSS patients produce ligands that activate the endothelium.

Disclosure of Interest None declared.
Career situation of first and presenting author: Student for a master or a PhD.

Introduction: Endothelial cells (EC) are important contributors to inflammation via expression of inflammatory mediators, including chemokines and adhesion molecules. Production of these inflammatory mediators can be induced via canonical and NF-κB-inducing kinase (NIK)-dependent noncanonical NF-κB signalling. The ligands activating these pathways are well studied, but less is known about the cells producing ligands that can activate NF-κB signalling in EC.

Objectives: To study the effects of soluble factors produced by activated memory T (T_m) cells on NF-κB dependent inflammatory activation of EC.

Methods: CD4+CD45RO+ Memory T cells were isolated from healthy PBMC using MACS sorting and cultured in medium containing anti-CD3 and anti-CD28 for 72 hours, after which supernatant was harvested. Human umbilical cord EC (HUVEC) were stimulated with 50% T_m supernatant (Tm sup). After 72 hours of stimulation, HUVEC protein and RNA was harvested and downstream expression of inflammatory mediators was analysed using qPCR and Western Blot. Culture supernatant was analysed by ELISA to detect presence of inflammatory properties of IL-22.

Results: Stimulation with Tm sup led to activation of both canonical NF-κB signalling, indicated by increased levels of phosphorylated (p)-IκBα, and noncanonical NF-κB signalling, indicated by increased p100 to p52 processing. HUVEC stimulated with Tm sup had increased mRNA levels of all tested inflammatory mediators compared to non-treated cells. Gene expression of chemokines (CXCL1, CXCL5, IL6, IL8 and GM-CSF) after Tm sup stimulation was significantly reduced after treatment with iIKKβ (iIKKb) and to a lesser, but still significant, extent after treatment with iNIK. Interestingly, treatment with iIKKβ also led to a reduction in mRNA levels of the adhesion molecules VCAM-1 and ICAM-1, while this effect was minimal after iNIK treatment. In addition, treatment with either iIKKβ or iNIK led to a significant reduction in CXCL5 in the culture supernatant of HUVEC stimulated with Tm sup.

Conclusions: This study provides new insights into the cellular interactions leading to production of inflammatory mediators by EC. Our findings demonstrate that activated T_m cells factors produce factors that can cause NF-κB-dependent inflammatory activation of EC. Targeting canonical NF-κB signaling and, although to a lesser extent, NIK-dependent NF-κB signaling reduces inflammatory activation of the endothelium.

Disclosure of Interest: None declared.
Abstracts

by exposure to pathogens or vaccines, which evolved as a protective mechanism against infections. TI is characterized by rewiring of functional, epigenetic and metabolic programs of innate immune cells such as monocytes and macrophages, which sustain enhanced production of pro-inflammatory cytokines. Since aberrant activation of TI is implicated in inflammatory diseases, tight regulatory mechanisms are likely in place, but the mechanisms responsible for this modulation remain elusive.

Objectives Scope of this study was to evaluated the role of IL-37, an anti-inflammatory cytokine that curbs inflammation as well as modulates metabolic pathways, as an endogenous regulator of trained immunity.

Methods The effects of recombinant IL-37 were evaluated in a mouse model of TI induced by the administration of beta-glucan in vivo (survival to a lethal inoculum of infectious agents, production of inflammatory cytokines, recruitment of inflammatory cells at the sites of infection). Subsequently, the effects of IL-37 were evaluated ex vivo on splenic and bone marrow monocytes (production of inflammatory cytokines, metabolomic analysis of the activation status of the main pathways of cellular energy metabolism). Finally, we evaluated the association between IL-37 gene polymorphisms and the induction of TI in monocytes of healthy donors with in vitro functional studies.

Results The exogenous administration of IL-37 abrogates the pro-inflammatory effects of TI, significantly reducing the production of pro-inflammatory cytokines and the survival of experimental animals subjected to a disseminated infection model. The inhibitory effects of IL-37 on TI are also associated with reduced recruitment of neutrophils at sites of inflammation. IL-37 and TI programs have differential and opposite effects on the modulation of cellular energy metabolism of monocytes. In humans, polymorphisms in the IL-37 gene are associated with reduced activation of TI programs and reduced production of inflammatory cytokines by healthy donor monocytes.

Conclusions In conclusion, IL-37 emerges as an endogenous regulator of TI, which makes this cytokine a potential therapeutic target in immune-mediated pathologies.

Disclosure of Interest None declared.

Characterization of novel humanized IL-17 mouse preclinical platforms for the efficacy evaluation of anti-human IL17 therapeutics

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

Introduction As the IL17/IL23 axis has proven pathogenic for many inflammatory conditions like rheumatoid arthritis, psoriasis, IBD, multiple sclerosis (MS) and others, IL17 inhibition is an attractive target for the treatment of these diseases. Indeed, biologics targeting human IL17 (hIL17) have recently proven a successful treatment for psoriasis with similar therapeutics being currently under development for this and other diseases.

Objectives To describe novel preclinical platforms based on a hIL17A transgenic mouse model for the efficacy evaluation of human therapeutics, targeting psoriasis and MS.

Methods Human IL17A transgenic mice were generated using as transgene a 170 kb genomic DNA fragment comprising hIL17A intron exon sequences flanked by extended 5’ and 3’ regulatory regions. These mice were further crossed with IL17AKO1 resulting in a mouse line (TghIL17) that exclusively expresses hIL17A. We standardized IMQ-induced psoriasis and MOG-induced Experimental Autoimmune Encephalomyelitis (EAE) induction protocols in these mice and characterized their response to anti hIL17 treatment. Disease severity was evaluated using established clinical and histopathological scoring scales.

Results TghIL17 mice have no overt pathology, and express hIL17A upon induction. IMQ-induced psoriasis induction in TghIL17 mice resulted in the development of skin pathology characterized clinically by skin erythema, thickening and scaling and histopathologically by acanthosis, hyperkeratosis and lymphocytic infiltration. Treatment with secukinumab resulted in alleviation of both clinical and histopathological psoriasis hallmarks to levels comparable to those observed in the IL17KO mice. MOG-induced EAE in TghIL17 mice resulted in clinical symptoms comparable to the ones observed in WT mice that involved, at the peak of the disease, paraplegia with forelimb weakness or paralysis. Treatment of EAE-affected TghIL17 mice with secukinumab ameliorated the pathological findings to a level similar to the one observed in IL17KO mice.

Conclusions TghIL17 mice upon induction develop pathologies similar to WT animals demonstrating that human IL17A efficiently replaces its mouse counterpart. Using these mice with standardized IMQ-induced psoriasis and MOG-induced EAE protocols we established and validateed with commercially approved anti-hIL17 biologicals, preclinical platforms that allow the efficient and reproducible evaluation of anti-hIL17 therapeutics.

REFERENCE

Disclosure of Interest None declared.

Mucosal-associated invariant T (MAIT)-cell-derived IL-17A and IL-17F production is IL-23-independent and biased towards IL-17F

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Career situation of first and presenting author Young investigator.

Introduction The requirement for IL-23 in driving IL-17A and IL-17F production in humans is incompletely understood. Preclinical data support IL-17F, together with IL-17A, as a key driver of chronic tissue inflammation. MAIT cells, an innate T-cell population, uniformly express RORγt but only a minority have been shown to produce IL-17A. Disregulation in frequency and function of MAIT cells has been associated with IL-17A-mediated inflammatory diseases, including PsA and AxSpA and IL-17F production in MAIT cells remains largely unexplored.

Disclosure of Interest None declared.
Objectives To explore the importance of IL-23 signalling in MAIT-cell-derived IL-17A and IL-17F production, examine the presence of MAIT cells in psoriatic lesional skin and assess the contribution of MAIT-cell-derived IL-17A and IL-17F using in vitro models of skin inflammation.

Methods IL-17A and IL-17F production by MAIT cells was assessed by flow cytometry, ELISA, qPCR and CyTOF upon activation by anti-CD3/CD28 or fixed E. coli via MR1-presented riboflavin metabolites, ±recombinant cytokines or an IL-23-neutralising antibody. RNAseq was utilised to observe MAIT cells in psoriatic lesional skin. MAIT cell supernatant, generated by FACS sorting, was cultured with normal human dermal fibroblasts (NHDFs) in the presence of IL-17 isoform-specific antibodies, including bimekizumab, a monoclonal antibody that potently suppresses IL-17A and IL-17F.

Results Optimal MAIT cell IL-17A and IL-17F production occurred upon T-cell receptor triggering with IL-12 and IL-18, independently of IL-23. IL-17F expression was greater at both gene and protein levels than IL-17A. The kinetics and threshold of activation for IL-17A and IL-17F suggest tighter regulation compared with other inflammatory cytokines, including IFNγ and TNF. Optimal MAIT cell IL-17A and IL-17F production requires monocytes, which contribute to IL-12 production upon IL-18 stimulation. MAIT cells were abundant in psoriatic lesional skin. NHDFs cultured with supernatant generated from activated MAIT cells produced inflammatory mediators IL-6, IL-8 and CCL2, which were reduced upon inhibition of either IL-17A or IL-17F, with optimal suppression achieved following dual neutralisation with bimekizumab.

Conclusions IL-17A and IL-17F can be produced from MAIT cells independently of IL-23, and contribute to inflammatory responses in NHDFs. These results support dual neutralisation of IL-17A and IL-17F as a complete and targeted approach to suppress IL-17-driven inflammatory responses.

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Disclosure of Interest S. Cole Employee of: UCB Pharma, A. Maroof Employee of: UCB Pharma (also has a patent pending).

PO90 PRO-INFLAMMATORY CYTOKINES AND CELL INTERACTIONS BETWEEN PBMC AND SYNOVIOCYTES INDUCE RETRACTION AND FORMATION OF PSEUDOPODIA

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

Introduction Rheumatoid synovitis is infiltrated by immune cells, which interact with synoviocytes inducing abnormal proliferation and massive production of pro-inflammatory cytokines.

Objectives The aim was to evaluate the effect of inflammatory environment and cell interactions on morphological parameters of synoviocytes alone or in co-culture with peripheral blood mononuclear cells (PBMC).

Methods Synoviocytes from different donors: healthy (HE) or rheumatoid arthritis (RA) were exposed or not to inflammatory conditions (IL-17 alone, TNF alone and their combination) during 48 hours and observed with different microscopes (optical, digital holographic and holographic-tomographic). Quantification of morphological parameters included cell confluence, area, motility speed and number of pseudopodia/cell. Co-cultures between normal PBMC and synoviocytes with or without phytohemagglutinin (PHA) or cytokines (IL-17/TNF) were used to mimic the in vivo situation.

Results Inflammatory cytokines induced a change in synoviocyte morphology, inducing a retracted cell with a higher number of pseudopodia. Several parameters decreased in inflammatory conditions: cell confluence (Ctrl:31.7%±2.5%, IL-17:21.8%±2.0%, TNF:19.2%±1.5%, IL-17/TNF:19.8%±1.6%, p<0.01), area (Ctrl:4491±254 μm², IL-17:3537±265 μm², TNF:2862±217 μm², IL-17/TNF:2583±211 μm², p<0.01) and motility speed (Ctrl:446±7 μm/h, IL-17:177±2 μm/h, TNF:161±2 μm/h, IL-17/TNF:159±1 μm/h, p<0.01). The cell membrane exhibited a much larger number of pseudopodia in inflammatory conditions (ctrl: only 18% of cells had more than 4 pseudopodia vs. IL-17/TNF: 82%, p<0.01). The same impact on cell morphology was observed in co-culture of synoviocytes and PBMC, affecting both cell types: synoviocytes were retracted (HE: Ctrl:3092±274 μm², cocult:3037±168 μm², cocult +PHA:364±184 μm², cocult +IL-17/TNF: 2949±154 μm², p<0.01) and inversely PBMC proliferated in inflammatory and PHA conditions (HE: Ctrl:47.9±1.4% cocult:50.7±4.8%, co-cult +PHA:54.7±6.0%, co-cult +IL-17/TNF: 59.0±5.4%, p<0.01), indicating that cell activation induced a morphological change of cells. With RA but not normal synoviocytes, co-culture was not sufficient to activate both PBMC and synoviocytes. The morphological effect came only from the inflammatory environment and not from cell interactions as if it did not exist and did not occur (RA: Ctrl:4491±254 μm², cocult:4573±275 μm² (ns), cocult +PHA:3220±184 μm², co-cult +IL-17/TNF: 2313±122 μm², p<0.01).

Conclusions The inflammatory environment or cell interactions induced massive changes in synoviocytes, with cell retraction and increase of pseudopodia number, leading to better interactions with other cells. Except in the case of RA, the inflammatory environment was absolutely required for such changes.

Disclosure of Interest None declared.

PO91 EFFECTS OF BIOLOGICS ON IL-17A AND TNF INDUCED CYTOKINE SECRETION ON SYNOVIAL FIBROBLASTS FROM RHEUMATOID AND PSORIATIC ARTHRITIS PATIENTS

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Career situation of first and presenting author Post-doctoral fellow.
Introduction Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are both rheumatic autoimmune diseases, which share a number of similarities but also display differences. For example, the anti-IL-17A biologic secukinumab turned out to be more effective in PsA than in RA. The anti-TNF biologic adalimumab on the other hand is equally indicated for both diseases. Synovial fibroblasts (SF) are one of the key effector cell types in the pathophysiology of RA and PsA.

Objectives We therefore investigated whether the effect of the two cytokines IL-17A and TNF-α as well as the effect of their corresponding biologics differs between RASF and PsASF, thus contributing to the difference seen in the therapeutic response. The effect of the IL-17A homolog IL-17F was also analyzed.

Methods SF were isolated from patients with PsA or RA, each undergoing surgery. SF from RA and PsA patients were stimulated with recombinant IL-17A, IL-17F and TNF-α alone or with respective combinations. Dose-response curve analysis was performed with IL-17A. The biologics secukinumab and adalimumab were used to block the effects on the SF. As a measure of the proinflammatory response, secretion of the cytokine IL-6 was quantified using an immunoassay.

Results RASF as well as PsASF responded to IL-17A (IL-17A: 13.7-fold vs 6.9-fold; n=3), while IL-17F alone caused no induction of IL-6 secretion in either SF type. However, when used in combination with TNF-α, both IL-17 isofoms, IL-17A and IL-17F, increased IL-6 secretion due to a strong synergistic effect with TNF-α. Surprisingly, these effects were notably stronger for RASF than for PsASF (IL-17A: 544-fold vs 127-fold, IL-17F: 54-fold vs 27-fold; n=3). However, adalimumab and secukinumab were similarly effective in abolishing the synergistic effect of IL-17A and TNF-α in RASF as well as PsASF. Conclusions SF appear not to contribute to the differences in the therapeutic effectiveness of the anti-IL17A biologic secukinumab as the response to IL-17A alone and IL-17A together with TNF-α is not stronger for PsASF than for RASF. Furthermore, secukinumab was similarly effective for both SF types. The data also suggest that in a proinflammatory milieu with increased TNF levels IL-17A as well as IL-17F play a role in the SF-mediated pathophysiology of PsA and, therefore, approaches targeting TNF are effective in both diseases.

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P092/O15 SYNOVIAL DERIVED TH17-LYMPHOCYTES FROM PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS INDUCE CARTILAGE DEGRADATION BY SYNOVIAL FIBROBLASTS MEDIATED BY MMP9 IN AN EXPERIMENTAL ANIMAL MODEL

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

Introduction The synovial fluid of Juvenile idiopathic Arthritis (JIA) is rich of Th17 and of Th-17-derived CD4+ CD161+ cells, also called non-classic-Th1. How such subpopulations drive the JIA joint damage is still a subject of great interest especially in light of the possible use of biological drugs able to selectively inhibit the activity of specific cytokines.

Objectives To clarify the role of Th17 and non-classical Th1 lymphocytes in the pathogenesis of joint cartilage destruction by synovial fibroblasts (SFs).

Methods The role of different subsets of CD4+T cells was observed in the activation of SFs in terms of cartilage degradation by normal and JIA SFs and induction of proteases both in vitro and in vivo, using a SCID Mouse model through the ‘inverse wrap’ implantation technique.

Results JIA SFs produce large amounts of MMP9 and efficiently degrade fragments of human cartilage wrapped in a collagen matrix containing the fibroblasts themselves and grafted under-skin on SCID mice (the ‘inverse wrap model’). Similar effects were observed with SFs of healthy subjects incubated with conditioned media of Th17 and of non-classical Th1. We shown that Th17 induce MMP9 in SFs, while non-classic Th1 act mainly by inducing urokinase-plasminogen-activator over-activity.

Conclusions IL-17 triggers the pathogenic chain leading to joint damage in patients with JIA.

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Disclosure of Interest None declared.
recombinant IL-36 using isolated keratinocytes and bone marrow differentiated dendritic cells. We then assessed the sensitivity of IL-36R<sup>AK</sup> mice to IMQ-induced psoriasis, and compared it to mice presenting a complete IL-36R deficiency (IL-36R<sup>−/−</sup>) and to their respective littermate controls (IL-36R<sup>+/+</sup> and IL-36R<sup>fl/fl</sup>). The severity of skin inflammation was assessed by ear thickness measured with a caliper. H and E staining was performed on treated and control ears. Total RNA was extracted from ears and mRNA levels of various cytokines were assessed by RT-qPCR.

**Results** IL-36R<sup>−/−</sup> mice were strongly resistant to the induction of IMQ-induced psoriasis as assessed by ear thickness. IL-36R<sup>AK</sup> mice showed a similar macroscopic protection as IL-36R<sup>−/−</sup> mice, demonstrating that IL-36 signaling in keratinocytes is critical in this model of psoriasis. Several pro-inflammatory genes upregulated by IMQ in IL-36R<sup>+/+</sup> and IL-36R<sup>fl/fl</sup> mice were not stimulated in neither IL-36R<sup>−/−</sup> nor in IL-36R<sup>AK</sup> mice. These genes included notably IL-17A or IL-22, both known to be crucial in psoriasis. Histological findings in IMQ-induced psoriasis include keratinocyte altered differentiation and hyperproliferation as well as inflammatory cell infiltration. Surprisingly and in contrast to IL-36R<sup>−/−</sup> mice, epidermis thickness was not reduced in IL-36R<sup>AK</sup> compared to IL-36R<sup>fl/fl</sup> control mice. This finding suggests that IL-36 signaling in keratinocytes does not induce keratinocyte hyper-proliferation but rather controls the development of downstream inflammatory responses in IMQ-treated ears.

**Conclusions** IL-36R signaling in keratinocytes is mandatory for the development of IMQ-induced psoriasis in mice. FACS studies are ongoing to characterize the inflammatory cell infiltrates in the different mouse lines.

**Disclosure of Interest** None declared.

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**P094 EFFECTIVENESS AND SECURITY OF SECUKINUMAB IN PATIENTS WITH PSORIATIC ARTHRITIS IN REAL CLINICAL PRACTICE**

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

**Introduction** Recently new therapeutic targets have been approved for the treatment of psoriatic arthritis (PsA). We have limited experience in real clinical practice.

**Objectives** To assess efficacy, safety and tolerability of secukinumab in patients with active PsA.

**Methods** Descriptive, retrospective observational study on the efficacy and safety of secukinumab in patients who met CASPAR classification criteria for PsA in follow-up in the Virgen de Valme hospital area. We evaluate, measures of disease activity by DAPSA in peripheral forms and ASDAS in axial forms.

**Statistical analysis** The quantitative variables are expressed with means and standard deviations or medians and quartiles if the distributions are asymmetric, and the qualitative variables with percentages.

**Results** 12 patients were reviewed. 58.3% were male. The average age of these patients is 47.67 years. The mean time of evolution in years of the disease was 8.33. 75% of the sample was not a smoker, and 83.3% of patients didn’t consume alcohol excessively. 25% were hypertensive. 8.3% had an associated dyslipidemia, and 16.7% had a symptomatic hyperuricemia.

Oligoarthritis form was the most frequent patterns of presentation with 46%, followed by polyarthritis with 27%. All patients had previously failed at least one DMARD, the most frequent being Methotrexate (41.7%). Up to 75% of the patients were naïve to biological therapy. In patients refractory to biological therapy, the most commonly used drug in first choice was etanercept.

In peripheral presentation 80% of the patients had a moderate DAPSA at the beginning of the treatment and of these 62% passed to a low activity, while 38% they remained with moderate activity.

In patients with high activity, up to 70% went to a low activity DAPSA, and in 30% it decreased to moderate activity.

We have survival with the drug that has been 12.67 months with a DS of 7.07 months.

Treatment has been suspended in 3 patients, in one of them due to severe skin and joint breakout, in another due to nausea and headaches, and the last due to ineffectiveness.

Finally, regarding the safety data we have not had any severe adverse effects. 8.3% had mild adverse effects, the most frequent being infectious symptoms of the upper respiratory tract.

8.3% had a primary failure (no response at any time) compared to 25% that has presented a secondary failure after a good initial response to treatment.

**Conclusions** Secukinumab provided sustained improvements in signs and symptoms in patients of active and was well tolerated, with a safety profile consistent with that reported previously.

**REFERENCE**


**Disclosure of Interest** None declared.

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**P095 INFLAMMATORY BOWEL DISEASE, DURING ANTI IL 17 TREATMENT**

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

**Introduction** IL 17-A blocking with Secukinumab (SEK) has proved efficacy in the treatment of psoriasis and psoriatic arthritis (PsOA). Inflammatory bowel disease (IBD) is more common among patients with psoriasis and is considered as a part of the spectrum of spondyloarthritis. IL-17 is a cytokine required for the normal homoeostasis of the bowel and its inhibition could initiate a subclinical disease.

**Objectives** We have observed 2 patients with IBD during therapy anti-IL17 for PsOA.

**Results** Case 1 A 20-year-old woman with a diagnosis of plaque psoriasis since 12 years; she has been treated with topical treatment, phototherapy and Methotrexate. In November 2017 She presented skin worsening, (PASI 20, DLQI 20 and 25) – (Suppl 1):A1–A83

A41
weeks. After the 2nd dose she began with abdominal pain and increased depositions. Acute phase reactants were increased major PCR 194 mg/dl ESR 87 mg/dl. In colonoscopy, biopsies objective and inflammatory lesions with extensive areas of mucosal ulceration compatible with Crohn’s disease were observed.

Case 2 A 59-year-old man with a diagnosis of ankylosing spondylitis of 30 years of evolution without a clinical response to NSAIDs and sulphasalazine, was treated with SEK 150 mg. After the second dose during induction, He presented fever 38.9°C, abdominal pain, bloody diarrhea, and nocturnal tenesmo. A colonoscopy showed typical ulcerative colitis lesions with severe activity.

Conclusions SEK is a very potent treatment for psoriasis and psoriatic arthritis, with positive results n Spondylitis, but not effective for IBD. II-17 is a cytokine with a role in the normal intestinal homeostasis.

Psoriasis and Spondyloarthritis patients have an association with IBD and uveitis.

We present 2 patients who were diagnosed of IBD shortly after start SEK, anti-IL17. We think they could have a previous undiagnosed IBD; SEK could have trigger a flare but We can’t rule out a role of IL-17 inhibition. During clinical trials for SEK, only 0.7 cases per 100 patients-year of IBD were reported. Caution must be taken before start SEK for patients to detect possible symptoms of IBD.

REFERENCE


Disclosure of Interest None declared.

P096 THE ROLE OF THE IL-23/TH17 AXIS AS MODULATOR OF B CELL-MEDIATED (AUTO)IMMUNE RESPONSES

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

Introduction Rheumatoid arthritis (RA) is characterized by chronic synovitis and joint destruction. Autoantibodies and autoreactive B cells are a hallmark of RA. Furthermore, Th17 cells have been demonstrated to be crucial for disease development. However, checkpoints and mechanisms regulating the onset of rheumatoid arthritis (RA) remain largely unclear.

Objectives Recently, we have demonstrated, that Th17 cells suppress the enzyme ST6 α-galactoside b-2,6-sialyltransferase in developing plasma cells. Thereby, Th17 cells are able to increase the inflammatory activity of autoantibodies in an IL-23 dependent manner by regulating the degree of Fc-glycosylation. However, the molecular mechanisms that mediate this IL-23/Th17-mediated proinflammatory reprogramming of B cells and the relevance for arthritis remains to be determined in detail.

Methods K/BxN mice were treated twice a week with neutralizing antibodies against IL-17A and IL-22 from week 1 until week 9. Both, clinical, histological and immunological parameters of arthritis were assessed. Serum was collected weekly and autoantibody titers were determined using ELISA. Collected Sera were used for K/BxN serum transfer to evaluated IgG activity. Additionally, the expression levels of the corresponding interleukin receptors (IL17RA and IL22Ra1) were analyzed during collagen-induced arthritis.

Results Here we show, that in contrast the upregulation of IL-22Ra1 on plasmablasts in the spleen, lymph node and bone-marrow during collagen-induced arthritis, its blockade during K/BxN arthritis did not prevent the onset of arthritis. Also, the IgG from K/BxN mice remained its inflammatory activity. We also show, that while IL17RA is basically absent on B cells during arthritis, the neutralization of IL-17A during K/BxN arthritis led to a strongly delayed development of autoimmune arthritis. Furthermore, depletion of IL17A led to a decreased titer of autoantibodies during early phases of K/BxN arthritis. However, in the K/BxN serum transfer mice which received serum that was produced in the absence of IL-17 were not protected against inflammatory arthritis.

Conclusions Our data indicate that IL-22 is not directly involved in the regulation of IgG activity during K/BxN arthritis. Furthermore, our data indicate that IL-17A is indeed a crucial cytokine during inflammatory processes inside the joint and may be involved in the germinal center reaction or plasma cell survival. However, neither IL-17 nor IL-22 is involved in the IL-23/Th17-mediated proinflammatory reprogramming of B cells to produce highly pathogenic autoantibodies. Therefore, other cytokines produced by Th17 cells and/or mechanisms depending on cell-cell contact proteins might orchestrate the Th17/B-cell crosstalk leading to the downregulation of S6Gal1 in developing plasma cells.

Disclosure of Interest None declared.

P097 INTRACELLULAR INTERLEUKIN-1 RECEPTOR ANTAGONIST RELEASED UPON CELL DEATH ACTS AS AN ALARMIN INHIBITOR IN ALDARA CREAM-INDUCED PSORIASIS-LIKE SKIN INFLAMMATION

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

Introduction The inflammatory effects of interleukin (IL)-1 are tightly controlled by IL-1 receptor antagonist (IL-1Ra), which blocks the binding of both IL-1α and IL-1β to IL-1R1. Four IL-1Ra isoforms are produced from the same gene by the use of different first exons, alternative mRNA splicing and translation initiation sites. One IL-1Ra isoform is secreted (sIL-1Ra), whereas the three others are intracellular (icIL-1Ra1, 2, 3) due to the absence of a signal peptide. In contrast to the well-characterized function of the secreted isoform, the biological role of the intracellular isoforms remains largely unclear. The icIL-1Ra1 isoform is constitutively expressed and represents the major isoform in keratinocytes.
**Objectives** To investigate the role of icIL-1Ra1 in a mouse model of psoriasis-like skin inflammation induced by Aldara cream, an FDA-approved drug with the active ingredient imiquimod, a toll-like receptor 7 agonist.

**Methods** We generated a mouse line specifically lacking icIL-1Ra1, in which expression of the other three IL-1Ra isoforms was not affected. Psoriasis-like skin inflammation was induced on mouse ears by the topical application of Aldara cream. Skin inflammation was assessed using a caliper to measure ear thickness. Ear mRNA levels of cytokines were measured by real-time PCR. An intracellular or extracellular role of icIL-1Ra1 was investigated *ex vivo* on ear biopsies and *in vitro* in primary keratinocytes by real-time PCR or ELISA. Injection of neutralizing anti-IL-1α antibodies into Aldara-treated icIL-1Ra1 deficient mice significantly reduced the severity of skin inflammation.

**Results** Naïve icIL-1Ra1 deficient mice exhibited a normal phenotype. However icIL-1Ra1 deficiency resulted in an enhanced severity of Aldara cream-induced skin inflammation as demonstrated by increased ear thickness. In addition, mRNA levels of several pro-inflammatory cytokines were significantly increased in icIL-1Ra1 deficient mice as compared to wild-type (WT) littermates. By immunofluorescence IL-1Ra was detected only in keratinocytes of naïve and Aldara-treated WT mice, but totally absent in icIL-1Ra1 deficient mice. Using ear biopsies and keratinocytes from WT mice, we observed that Aldara cream led to the release of both icIL-1Ra1 and the alarmin IL-1α, accompanied by caspase 1/11-independent cell lysis. Injection of neutralizing anti-IL-1α antibodies into Aldara-treated icIL-1Ra1 deficient mice significantly reduced the severity of skin inflammation.

**Conclusions** These data suggest that icIL-1Ra1 is stocked in keratinocytes in order to immediately counteract the inflammatory effects of IL-1α that is released upon cell lysis, thus acting as an intrinsic alarmin inhibitor.

**Disclosure of Interest** None declared.
order to evaluate the diagnostic accuracy of miR-146a in PB and SF for distinguishing RA patients from healthy controls (HCs). Concentrations of IL-17A in matched serum and SF samples were determined by Human IL-17A ELISA kit (Gene probe, Diaclone). The results were compared to HCs as well as within the RA group.

Results miR-146a was overexpressed in 70.83% of RA SF (p=0.007) when compared to HCs SF. The ROC curve analysis showed diagnostic accuracy for miR-146a in SF with AUC=0.769 (95 CI: 0.600–0.938) with 75.0% sensitivity and 72.3% specificity (p=0.006). SF levels of miR-146a were overexpressed in 52.17% of the RA patients compared to its systemic levels. Levels of IL-17A were higher in RA SF compared to serum (8.645 pg/ml versus 0.315 pg/ml, p=0.012).

Conclusions The difference between the systemic and local levels of miR-146a and IL-17A in RA patients indicates that the local inflammatory process leads to their deregulation with a possible role of both molecules in the disease pathogenesis. The higher local levels of miR-146a and IL-17A suggest the possibility relationship between miR-146a expression and the production of IL-17 from IL-17 expressing cells.

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Disclosure of Interest
None declared.

P101

SYSTEMIC AND LOCAL IL-17A AND MIR-155 LEVELS IN RHEUMATOID ARTHRITIS PATIENTS

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10.1136/annrheumdis-2018-EWRR2019.89

Career situation of first and presenting author Assistant. Introduction Interleukin 17 (IL-17) is a proinflammatory cytokine, which overproduction promotes the autoimmune reaction in rheumatoid arthritis (RA). Posttranscriptional regulation of IL-17 by specific microRNAs (miRNAs) is of great interest in the recent years. Studies have shown that miR-155 enhances Treg and Th17 cells differentiation and IL-17A production by directly targeting the suppressor of cytokine signaling (SOCS) 1.

Objectives To examine a possible deregulation of the expression levels of miR-155 and levels of IL-17A in peripheral blood (PB) and synovial fluid (SF) of RA patients.

Methods Expression levels of miR-155 were determined in matched PB and SF samples of RA patients by relative quantitation method 2-ΔΔCt. As reference control for normalization RNU6B gene was used. Receiver operating characteristic (ROC) curve analysis using RQ values was constructed in order to evaluate the diagnostic accuracy of miR-155 in PB and SF for distinguishing RA patients from healthy controls (HCs). Concentrations of IL-17A in matched serum and SF samples were determined by Human IL-17A ELISA kit (Gene probe, Diaclone). The results were compared to HCs as well as within the RA group.

Results miR-155 was overexpressed in 79.17% of RA SF (p=1.63 × 10^{-4}) when compared to HCs SF. The ROC curve analysis showed diagnostic accuracy for miR-155 in SF with AUC=0.858 (95 CI: 0.757–0.959) with 81.3% sensitivity and 81.8% specificity (p=2.3 × 10^{-8}). SF levels of miR-155 were overexpressed in 76.09% of the RA patients compared to its systemic levels. Levels of IL-17A were higher in RA SF compared to serum (8.645 pg/ml versus 0.315 pg/ml, p=0.012).

Conclusions The difference between the systemic and local levels of miR-155 and IL-17A in RA patients shows that the inflammatory process leads to their deregulation with a possible role of both molecules in the disease pathogenesis. The higher local levels of miR-155 and IL-17A confirm the data about the possible role of miR-155 in regulating the production of IL-17.

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Disclosure of Interest
None declared.

P102

HIGH DIMENSIONAL PROFILING OF REGULATORY T CELLS IN SPONDYLOARTHRITIS REVEALS CELLULAR HETEROGENEITY


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

Introduction The Spondyloarthritides (SpA) are immune-mediated conditions characterised by spinal and joint inflammation. Numerous studies implicate Th17 lymphocytes in pathogenicity but few reports exist on the role played by regulatory T cells (Treg) in the disease.

Objectives 1. To describe Treg frequency and phenotype in SpA patients compared to controls.

2. To identify differential expression of trafficking molecules and activation markers within Tregs at the inflammatory site.

Methods A total of 61 patients with SpA (38 with AS, 23 with PsA) and 16 age-matched healthy controls were recruited. Peripheral blood (PB) and paired synovial fluid (SF) mononuclear cells (n=8) were stained for with 3 multicolor flow cytometry panels, including a total of 35 surface and
in intracellular protein markers. Manual gating was done in parallel with unsupervised data analysis using FlowSOM and SPADE.

**Results**

No difference in PB Treg frequency was observed between SpA and healthy controls. In the SF Tregs were enriched relative to the blood memory (CD45RA-) compartment (mean: 11.7 vs 4.7%; p=0.01) with a higher expression of Foxp3 (p=0.04). The distribution of trafficking markers between patients and controls showed considerable heterogeneity, as visualised on SPADE analysis, but little difference in their relative frequency.

Intracellular cytokine staining demonstrated that Tregs from AS and PsA patients have higher potential to secrete IL-17A compared to controls (IL-17A+Tregs mean: 2.8 vs 1.2%; p=0.02). Production of this cytokine overlapped with expression of CD161 and CCR6, both markers associated with Th17. Interestingly, this subset, despite expressing normal levels of Foxp3, expressed lower levels of TIGIT (p=0.04) and Helios (p<0.001) when compared to conventional Tregs. **Conclusions**

High-dimensional immunoprofiling in SpA patients shows normal frequency of Tregs in the PB, but increased Tregs with activated phenotype in the inflammatory site. The presence of phenotypical markers commonly associated with Th17 in Tregs of non thymic origin are intriguing findings and require further evaluation.

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None.

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**Disclosure of Interest**

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**Disclosure of Interest**

None.

**Career situation of first and presenting author**

Student for a master or a PhD.

**Introduction**

Interleukin-17A (IL-17A) contributes to the pathogenesis of psoriatic arthritis (PsA) as evidenced by the success of biologics targeting IL-17 pathway in PsA patients. Both CD4+ and CD8+ T cells are reportedly producers of IL-17A in PsA with IL-17A+ CD8+ T cells specifically enriched in PsA synovial fluid (SF). However, most current findings regarding IL-17A-producing cells in PsA patients are coming from intracellular staining using flow cytometry.

**Objectives**

To confirm and compare ex vivo production of IL-17A by CD4+ and CD8+ T cells from PsA synovial fluid using flow cytometry, enzyme-linked immunosorbent assay (ELISA) and RT-qPCR.

**Methods**

Fresh SF of established PsA patients were collected and part of the cells were directly stained intracellularly for IL-17A and IFNγ after 4 hours’ (hrs) phorbol myristate acetate and ionomycin (PMA and iono) stimulation. Blood samples of early PsA patients and age/sex-matched healthy volunteers were included as controls for PsA SF. In addition, the rest of SF cells underwent density gradient separation and mononuclear cells (SFMCs) were stored till use. CD4+ and CD8+ T cells were sorted from SFMCs, and ex vivo cultured with soluble anti-CD3/anti-CD28 (aCD3 and 28), PMA and iono or without stimulation for 4 or 72 hours. Furthermore, SF CD4+ and CD8+ T cells were co-cultured with either allogeneic PsA fibroblast-like synoviocytes (FLS) or autologous PsA CD14+ monocytes with aCD3 and 28 activation for 72 hours. Culture supernatants were tested for ELISA and cells were analyzed with intracellular staining or RT-qPCR.

**Results**

Accumulation of IL-17A+ CD8+ T cells was significantly higher in PsA SF compared to in blood of either PsA patients or healthy volunteers. Although both CD4+ and CD8+ T cells from SFMCs are IL-17A+ with flow cytometry, only CD4+ T cells produce measurable amounts of IL-17A in culture supernatants after 72 hours of aCD3 and 28 activation. Similar results were found after 3 days’ co-culture of aCD3 and CD28 stimulated CD4+ or CD8+ T cells with PsA FLS. Also, when either co-cultured with autologous CD14+ monocytes, only CD4+ T cells showed mRNA IL-17A expression and IL-17A production. In contrast, if PMA and iono were used to stimulate CD4+ or CD8+ T cells, both produce IL-17A in supernatants after 4 or 72 hours.

**Conclusions**

Although both CD4+ and CD8+ T cells show IL-17A positivity in PsA SF with flow cytometry, the use of strong stimuli such as PMA and iono during intracellular staining may account for the IL-17A positivity in CD8+ T cells. The contribution of CD8+ T cells to IL-17A production in local PsA joints might be limited as normal TCR activation, mimicked by aCD3 and 28, didn’t induce IL-17A release in CD8+ T cells in contrast to CD4+ T cells.

**Disclosure of Interest**

None declared.

**P104**

**ANAEROPLASMA, A POTENTIAL ANTI-INFLAMMATORY PROBIOTIC FOR THE TREATMENT OF CHRONIC INTESTINAL INFLAMMATION**

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**Career situation of first and presenting author**

Post-doctoral fellow.

**Introduction**

The human intestine is colonized with billions of microorganisms, which form the gut microbiota, consisting of up to 1000 different bacterial species. Recent studies have implicated the intestinal microbiota in the pathogenesis of chronic inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, and Sjögren’s syndrome. Yet, we still lack the knowledge which bacteria of the gut microbiota induce, promote or inhibit chronic inflammatory inflammation.

**Objectives**

The aim of our work is to identify members of the intestinal microbiota with pro- or anti-inflammatory properties...
for targeted and therapeutic manipulation of the microbiota in chronic inflammatory diseases.

**Methods** We have developed high-resolution microbiota flow cytometry which allows us to analyze the microbiota on a single cell level. This provides a non-invasive, fast and efficient diagnostic tool to visualize dramatic changes of microbiota composition in inflammatory diseases, fast and efficiently, and isolate distinct bacteria for functional and molecular analyses.

**Results** We have identified bacteria belonging to the genus Anaeroplasma, which enhances the levels of mucosal IgA. Adoptive transfer of Anaeroplasma increases the numbers of IgA+ germinal center B cells in the Peyer’s patches and of IgA-secreting plasma cells in the lamina propria of the small intestine leading to significantly enhanced mucosal IgA levels. Anaeroplasma controls IgA expression presumably its ability to induce expression of the regulatory cytokine TGF-β in T cells, as we show here.

**Conclusions** The anti-inflammatory properties of Anaeroplasma to induce the anti-inflammatory cytokine TGF-β, thereby also strengthening the intestinal barrier by enhancing mucosal IgA, qualify Anaeroplasma as potent probiotic for the prevention and treatment of chronic inflammation.

**Disclosure of Interest** None declared.

### P105 IDENTIFICATION OF RARE CODING VARIANTS IN IL-1-RELATED PATHWAYS IN PATIENTS WITH ADULT-ONSET STILL’S DISEASE

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**Career situation of first and presenting author** Assistant.

**Introduction** Adult-onset Still’s disease (AOSD) is a rare auto-inflammatory disease characterized by fever, arthritis, and multi-organ involvement. Inflammation in AOSD is mediated by interleukin (IL)-1β, as confirmed by the clinical efficacy of selective blockers. The genetic predisposition to this IL-1-driven inflammation remains nevertheless elusive. Previous studies failed to identify associations between polymorphisms in the IL-1 genes and AOSD, thus pointing at more complex genetic mechanisms. These cannot be investigated with traditional techniques for genetic partitioning, such as GWAS, which only assess common variants and polymorphisms. Studies focusing on highly penetrant rare variants or different types of mutations (i.e. small copy-number variations; insertions/deletions) are warranted.

**Objectives** We hypothesized that genetically determined changes in IL-1-related pathways resulting in excessive IL-1β activity lead to the development of autoinflammation in AOSD. Scope of this study was to unravel the combined mutational variation of a network of IL-1-related receptors, pathways, counter-regulators, and cellular processes possibly involved in the pathogenesis of IL-1-mediated inflammation.

**Methods** We collected clinical and genetic data from a large cohort of 76 AOSD patients and developed an innovative platform based on molecular inversion probes technology, which enables highly multiplexed targeted-resequencing of the coding sequence of 48 genes related to the IL-1-pathway, and allows studying rare and common variants in one assay. We have also screened 500 healthy controls, and 1000s of samples with other disorders using the same assay.

**Results** We identified rare and unique (i.e. private variants) in the IL-1 pathway in several individuals with AOSD. Whether any of these are involved in a strong predisposition to AOSD is currently followed-up. Rare genetic variants have been identified in six IL-1-pathway ‘clusters’:

1. Inflammasomes;
2. IL-1 pathway;
3. IL-1 family;
4. IL-18 pathway;
5. Autophagy;
6. ROS production.

**Conclusions** Unraveling the genetic bases of inflammation in AOSD deepens our understanding of the human innate immune system. This study platform may now serve for the genetic analysis of other IL-1-mediated conditions (i.e. gout and other autoinflammatory diseases), whose genetic predisposition remains elusive. Equally important, the identification of pathways amenable to targeting with small molecules or biologics may translate into remarkable clinical implications.

**Disclosure of Interest** None declared.

### P106/Q25 DNA METHYLATION IN LYMPHOCYTE SUBSETS AS A MEDIATOR OF GENETIC RISK IN EARLY RHEUMATOID ARTHRITIS

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

**Introduction** Genome-wide association studies (GWAS) have identified over 100 RA-associated risk loci, whose enrichment for lymphocyte-specific enhancer elements is consistent with a regulatory function of many causal variants in these cells. Epigenetic modifications have also been strongly implicated in RA pathogenesis.

**Objectives** To investigate the role of DNA methylation as a mediator of RA genetic risk.

**Methods** CD4+ T lymphocyte-specific DNA and RNA were extracted from freshly isolated blood of 43 RA and 60 disease control patients, along with equivalent material from B-lymphocytes of 46 RA and 73 controls. Comparator groups were drug-naïve and matched for age, sex, and acute phase response. Genotyping, gene expression and methylation
polymorphisms in SLC2A9 and SLC22A12 genes are related to hyperuricemia, gout and also to hypouricemia

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

Introduction Serum uric acid concentration is significantly influenced by urate transporters, such as ABCG2 (encoded by ABCG2 gene), GLUT9 (SLC2A9 gene) and URAT1 (SLC22A12 gene). The main function of ABCG2 is uric acid secretion, whereas GLUT9 and URAT1 also ensure reabsorption. Pathogenic allelic variants in SLC2A9 and SLC22A12 are not only associated with hyperuricemia and gout, but they also lead to rare hereditary renal hypouricemia (type 1 – OMIM #220150 or type 2 – OMIM # 612076).

Objectives Previously, we analyzed ABCG2 gene and detected non-synonymous variants that lead to hyperuricemia and early onset of the gout.1 The aim of this study was to find a possible correlation between variants in SLC2A9 and SLC22A12 and hypouricemia, hyperuricemia and gout.2

Methods We recruited a cohort of 232 individuals with primary gout and hyperuricemia. We examined coding regions of SLC2A9 (13 exons) and SLC22A12 (10 exons) by Sanger sequencing. We also analyzed SLC2A9 and SLC22A12 in five patients with suspect hypouricemia.

Results In the context of 232 individuals, we detected five synonymous variants, 18 intron variants and seven misense variants in SLC22A12: A17T, G25R, T275M, D281H, V282I, R294H, and P350L. In SLC22A12 gene, we found six synonymous variants and seven intron variants.

We detected several pathogenic variants in patients with suspect hypouricemia. Intronic variant c.1419+1G>A in SLC2A9 most likely affects the splicing. In SLC22A12, we found rare pathogenic variants T467M and L415_G417del. These variants have according to our previous study high frequency in the Czech and Slovak Roma population.3

Conclusions The uric acid level is determined by a complex network of urate transporters that can not only lead to hyperuricemia, but in rare cases also to hypouricemia.

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Disclosure of Interest None declared.
Expression levels of miR-21 and miR-29 in the epigenetic changes by inhibition of DOT1L affect A48ARD.

Results We report that hypoxia exacerbates CCL20 and IL-1β release in response to LPS and increases glycolytic intermediates at the expense of carnitine. Modulation of carnitine identified a novel role for FAO in the production of CCL20 in response to LPS. Transcriptomics of RA blood monocytes and RA-SF macrophages revealed that fatty acid metabolism was altered and CCL20 was increased when monocytes enter the RA milieu. In vitro analysis of monocytes showed that RA-SF increases carnitine abundance and CCL20 production in hypoxia, which was exacerbated by exogenous carnitine.

Conclusions This work has revealed a novel inflammatory mechanism in RA which links FAO to CCL20 production in human monocytes. This may contribute to RA disease pathogenesis by promoting the recruitment of Th17 cells and osteoclastogenesis.

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Disclosure of Interest None declared.

EPIDEMIC CHANGES BY INHIBITION OF DOT1L AFFECT WNT SIGNALING, PROLIFERATION AND CELL CYCLE IN DERMAL FIBROBLASTS, WITH NO OVERALL EFFECT ON COLLAGEN DEPOSITION IN MODELS OF FIBROSIS

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

Introduction The role of epigenetic factors in the pathophysiology of fibrosis, a hallmark of Systemic Sclerosis, is increasingly explored. DOT1L, the unique H3K79-methyltransferase, methylates histone 3 at the Lysine residue at position 79, thereby regulating gene expression programs. In cartilage and bone, DOT1L has cell-type specific effects on Wnt signaling, a pathway suggested to play an important role in fibrosis.

Objectives To study the role of DOT1L in fibrosis.

Methods Primary human dermal fibroblasts were treated with DOT1L-inhibitor EPZ-5676 or vehicle and stimulated with TGF-β. Expression of smooth muscle alpha 2 actin (ACTA2) and Wnt target genes was measured by RT-qPCR. Western Blot was done for dimethylated H3K79 and β-catenin. Picrosirius Red staining measured collagen deposition. 5-Bromo-2’-deoxy-uridine (BrdU) labeling for proliferation and flow cytometry with Propidium Iodide for cell cycle analysis was done. ColIa1;Cre-ERT2;DOT1L−/−mice, injected under the curve (AUC) for miR-21 was 0.634 (95% CI=0.479–0.790), p=0.147 with 64.7% sensitivity and 64.3% specificity. AUC for miR-29 was 0.605 (95% CI=0.420–0.790), with 64.3% sensitivity and 52.9% specificity but without statistical significance (p=0.257). The multimarker analysis of the ROC curves for both miRNAs showed AUC=0.714 (95% CI=0.569–0.860), p=0.021 with 79.4% sensitivity and 42.9% specificity. Levels of miR-29 correlated with the levels of miR-21 in the serum (with Spearman correlation coefficient 0.517, p=0.0017) and with the presence of anti-Scl70 antibodies in the serum (with Spearman correlation coefficient 0.438, p=0.010).

Conclusions Our data showed a deregulation of miR-21 and miR-29 in the serum of patients with SSc which could suggest their potential role in the disease pathogenesis. Further analysis with higher number of patients is needed to confirm if these miRNAs could be used in the clinical practice as diagnostic biomarkers as well as biomarkers for both disease activity and progression.

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Disclosure of Interest None declared.
with tamoxifen to induce a fibroblast-specific DOT1L knockout, were injected subcutaneously with bleomycin or vehicle. Injected skin was analyzed by OH-prolin assay and by histology.

Results The DOT1L-inhibitor EPZ-5676 reduced H3K79 dimethylation in all samples. In breast dermal fibroblasts, the induction of ACTA2 with TGF-β was reduced with DOT1L inhibition, while in abdominal dermal fibroblasts this induction was more pronounced. After 48 hours of TGF-β, collagen deposition was higher in DOT1L-inhibited fibroblasts. After 72 hours of TGF-β however, this deposition was comparable with controls. DOT1L inhibition induced canonical Wnt signaling in fibroblasts, with a small increase in active β-catenin and expression of Lymphoid enhancer-binding factor 1 (LEF1). With DOT1L inhibition, more proliferation of fibroblasts, but also proportionally more cells in the G1/G0 phase were observed. In cultured dermal fibroblasts, but also proportionally more cells in the G0/G1 phase and less cells in S and M/G2 phase were seen.

Wnt signaling in fibroblasts, with a small increase in active β-catenin and expression of Lymphoid enhancer-binding factor 1 (LEF1). With DOT1L inhibition, more proliferation of fibroblasts, but also proportionally more cells in the G1/G0 phase and less cells in S and M/G2 phase were seen. In vivo, subcutaneous bleomycin increased murine dermal thickness and skin collagen content. No difference was observed between wild type and mice with a fibroblast-specific deletion of DOT1L.

Conclusions In an in vitro model of fibrosis, ACTA2 induction in DOT1L-inhibited human dermal fibroblasts was dependent on the fibroblast origin. DOT1L inhibition resulted in an earlier deposition of collagen, without differences in deposition at the end points. Inhibition of DOT1L-induced canonical Wnt signaling and proliferation but also led to a higher proportion of cells in the G0/G1 phase. In an in vivo murine model of skin fibrosis, no difference in bleomycin-induced skin thickness and collagen content was found when the DOT1L gene was deleted in fibroblasts.

Disclosure of Interest None declared.

P111 TENOCYTES EXTRACTED FROM ROTATOR CUFF TENDONS INDUCE TNAP-DEPENDENT MINERAL DEPOSITION AND EXPRESS GENES RELATED TO A HYPTERTROPHIC CHONDROCYTE DIFFERENTIATION

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Career situation of first and presenting author Resident.

Introduction Calcific tendinopathy represents 10% to 42% of chronic painful shoulders. These calcium deposits are composed of carbonated apatite. Although the disease is 10% frequent, its origin stays still largely unknown. Our previous results showed that calcific deposits are surrounded by chondrocyte-like cells expressing TNAP (Tissue Nonspecific Alkaline Phosphatase) and ENPP1 (Ectonucleotidepyrophosphatase/phosphodiesterase 1), two key enzymes involved in the mineralization process.

Objectives To study the ability of cells extracted from rotator cuff tendons to produce apatite crystals and to analyze the phenotype of these mineralizing cells.

Methods Tenocytes were extracted from rotator cuff tendons removed during shoulder total replacement. To evaluate their ability to mineralize, they were cultured in an osteogenic medium (OM) for 21 days. Mineral deposition then was assessed by staining with Alizarin red. Tenocytes total RNA was extracted and analyzed by RT-qPCRs. TNAP enzymatic activity was also assessed in the cells. A TNAP inhibitor was used to delineate its implication in the mineralization process.

Results Tendon samples were obtained from 5 patients (age 69.6±3.13 years). Cells extracted from these tendons expressed collagen I, collagen III, Scleraxis and Mkx (Mohawk homeobox), as expected for tenocytes. However, Tenomodulin was very weakly expressed and lost after passage 1. These cells were able to mineralize in the OM although no mineralization was observed in the control medium. qPCR analyses showed a significant increase of TNAP and ENPP1 expression by cells cultured in OM (p<0.05). Osteoblast markers (Runx2, osteocalcin, osteopontin, BSP) were not increased by the OM. COMP (Cartilage Oligomeric Matrix Protein), a chondrocyte marker was significantly increased, as well as MMP13 (Matrix Metallopeptidase 13) and Collagen X suggesting a hypertrophic differentiation. In parallel, in the OM, TNAP enzymatic activity was significantly higher at 14 and 21 days compared to the control medium. An inhibitor of TNAP completely prevented mineral deposition in OM and reduced expression of the hypertrophic chondrocyte markers MMP13 and Collagen X.

Conclusions Tenocyte-like cells extracted from tendons of the rotator cuff are able to induce mineralization in an osteogenic medium. The cells express genes associated with a hypertrophic chondrocyte phenotype (TNAP, COL10 and MMP13) and TNAP seems to have a crucial role in the induced mineralization. These results suggest that tenocytes could differentiate into hypertrophic chondrocyte which induce TNAP-dependent apatite deposition in calcific tendonitis.

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Disclosure of Interest None declared.

P112 STUDYING CELLULAR SENESCENCE IN HUMAN LYMPH NODE STROMAL CELLS DURING THE EARLIEST PHASES OF RHEUMATOID ARTHRITIS

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

Introduction Cellular senescence is a state of proliferation arrest of cells. The persistence and accumulation of senescent cells has been implicated in the pathogenesis of age-related diseases. Ageing is an important risk factor of rheumatoid arthritis (RA), a prototypic autoimmune disease in which loss of immune tolerance and systemic autoimmunity precedes clinical onset of disease. Through their intimate contact with lymphocytes, lymph node stromal cells (LNSCs) are important regulators of peripheral tolerance. Therefore, malfunctioning senescent LNSCs may potentially lead to defective peripheral
tolerance and the development of systemic autoimmune disease.

Objectives To determine the extent of cellular senescence of LNSCs during early phases of systemic autoimmunity.

Methods We included individuals with arthralgia without any evidence of arthritis who were positive for IgM rheumatoid factor (IgM-RF) and/or anti-citrullinated protein antibodies (ACPA; RA-risk group), early arthritis patients (ACR/EULAR 2010 criteria; disease duration <1 year) and seronegative healthy controls. All study subjects underwent ultrasound-guided inguinal lymph node biopsy. LNSCs were cultured from freshly collected lymph node needle biopsies and passages 0–9 were used for experiments. Flow cytometry, qPCR and microscopy were used to measure cell size, granularity, senescence-associated gene expression levels, telomere attrition and senescence-associated β-galactosidase (SA-β-gal) activity.

Results Preliminary flow cytometry data shows that the cell size of LNSCs from RA patients (n=11) and RA-risk individuals (n=7) is increased compared with healthy LNSCs (n=7), while granularity was specifically increased in LNSCs from RA patients (n=9). Initial SA-β-gal stainings indicate higher activity in LNSCs from RA-risk (n=3) and RA patients (n=4) compared with healthy controls (n=2), however this data needs to be carefully interpreted and more donors should be analysed. Expression levels of senescence-associated genes significantly increased over culture passages and significantly higher p21 and p53 levels were observed in passage 9 compared with healthy controls. All study subjects underwent ultrasound-guided inguinal lymph node biopsy. LNSCs were cultured from freshly collected lymph node needle biopsies obtained from 24 patients with RA, 23 individuals positive for autoantibodies but without clinical apparent disease (RA-risk group) and 14 seronegative healthy controls. Expression of PADI enzymes, citrullinated proteins, DEAF1, AIRE and PTAs was investigated in mRNA and protein level. Expression of immunomodulatory molecules in LNSCs was assessed after stimulation with IFN-γ (n=15).

Results Citrullinated proteins, targeted by ACPA, were found in human LN tissue as well as in cultured LNSCs. In addition, we observed the expression of transcription factors AIRE and DEAF1 as well as disease-related PTAs in LNSCs with some PTAs showing a distinct expression pattern in autoimmune LNSCs compared to healthy controls. TGF-β was constitutively expressed by LNSCs while CD86 or IL-10 were not detected. Upon IFN-γ stimulation LNSCs upregulated MHC class II, co-stimulatory molecules CD40 and CD80 as well as T cell negative regulators CD274, NOS2 and IDO. Overall, no clear differences between donor groups were observed for these markers with exception of a slightly lower induction of CD40 and NOS2 in RA LNSCs.

Conclusions We present for the first time that human LNSCs express several PTAs and the transcription factors AIRE and DEAF1, driving PTA expression. Additionally, human LNSCs express molecules involved in citrullination, antigen presentation and immunomodulation. Moreover, antigens targeted by ACPAs are present in LN tissue and LNSCs. These data suggest that LNSCs have the machinery to regulate peripheral tolerance.

Disclosure of Interest None declared.

P113/Q17 HUMAN LYMPH NODE STROMAL CELLS EXPRESS SELF-ANTIGENS TARGETED BY ANTI-CITRULLINATED PROTEIN ANTIBODIES: ROLE FOR TOLERANCE INDUCTION IN RHEUMATOID ARTHRITIS

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

Introduction In rheumatoid arthritis (RA) the cause for loss of tolerance and anti-citrullinated protein antibody (ACPA) production remains unidentified. Mouse studies showed that peripheral tolerance can be maintained through presentation of peripheral tissue antigens (PTAs) by lymph node stromal cells (LNSCs). We hypothesize that deregulation of peripheral tolerance mechanisms mediated by LNSCs might underlie pathogenesis of RA. Here we investigated the expression of PTAs, citrullinated proteins and immunomodulatory molecules by human LNSCs during health and autoimmunity.

Methods LN tissue sections and LNSCs were prepared from freshly collected lymph node needle biopsies obtained from 24 patients with RA, 23 individuals positive for autoantibodies but without clinical apparent disease (RA-risk group) and 14 seronegative healthy controls. Expression of PADI enzymes, citrullinated proteins, DEAF1, AIRE and PTAs was investigated at mRNA and protein level. Expression of immunomodulatory molecules in LNSCs was assessed after stimulation with IFN-γ (n=15).

Results Citrullinated proteins, targeted by ACPA, were found in human LN tissue as well as in cultured LNSCs. In addition, we observed the expression of transcription factors AIRE and DEAF1 as well as disease-related PTAs in LNSCs with some PTAs showing a distinct expression pattern in autoimmune LNSCs compared to healthy controls. TGF-β was constitutively expressed by LNSCs while CD86 or IL-10 were not detected. Upon IFN-γ stimulation LNSCs upregulated MHC class II, co-stimulatory molecules CD40 and CD80 as well as T cell negative regulators CD274, NOS2 and IDO. Overall, no clear differences between donor groups were observed for these markers with exception of a slightly lower induction of CD40 and NOS2 in RA LNSCs.

Conclusions We present for the first time that human LNSCs express several PTAs and the transcription factors AIRE and DEAF1, driving PTA expression. Additionally, human LNSCs express molecules involved in citrullination, antigen presentation and immunomodulation. Moreover, antigens targeted by ACPAs are present in LN tissue and LNSCs. These data suggest that LNSCs have the machinery to regulate peripheral tolerance.

Disclosure of Interest None declared.

P114 TISSUE REGENERATION AND BONE REPAIR UPON TNF BLOCKADE IN EXPERIMENTAL ARTHRITIS

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

Introduction Partial bone repair upon treatment with biological DMARDs has been reported in patients with Rheumatoid Arthritis (RA). Previous in vivo PET/CT studies using the human tumor necrosis factor transgenic mouse model (hTNFtg) have been demonstrated the reversibility of pre-existing inflammatory, erosive arthritis with complete remission of synovial inflammation and repair of bone erosions upon TNF blockade.

Objectives To investigate spontaneous repair and regenerative processes of structural joint damage in ankle and knee joint, menisci and patella tendon upon TNF blockade in hTNFtg mice.

Methods Two cohorts of arthritic hTNFtg mice were treated with anti-TNF ab (infliximab, 10 mg/kg, 3x per week, i.p.,
4 weeks): (I) 8 week old mice with established inflammatory, erosive arthritis and (II) 12 week old hTNFtg mice with severe inflammatory, erosive arthritis (n=8–10 animals). Clinical signs of arthritis were weekly assessed. Knee and ankle joints were used for μCT (Scanco, μCT35) and subsequent histology. Toluidine blue staining indicated proteoglycan contents, tartrate-resistant acid phosphatase staining identified osteoclasts. Immunohistochemical stainings were performed for collagen type II (col II) and osterix. Expression of chondrogenic markers was analysed from RNA paw extracts.

**Results** TNF blockade significantly improved clinical signs in both treatment cohorts. Compared to eroded bone surfaces at week 8 (before treatment), TNF blockade led to improved bone architecture with regular, smooth bone surfaces in CT scans from knee and ankle. Histologically, treatment allowed complete resolution of synovitis, osteoclast activity and subchondral bone erosions. Despite sites of intact bone tissue, regenerative processes of cortical and subchondral bone were found. Erosions of bone and calcified cartilage were filled with col II positive cartilaginous or fibro-cartilaginous tissue. Hyperproliferative chondrocytes and endochondral ossifications were found in knees. Consistently, chondrogenic markers like Sox9 transcription factor and col II were markedly increased, accompanied by decreased expression of cartilage degrading enzymes (MMP3, MMP13) upon treatment. Of note, menisci and patella tendon showed massive hyperproliferative chondrocytes, col II and osteorix staining.

Anti-TNF treatment at later stages with severe bone destruction (at week 12) could not completely resolve synovitis and restore structural damage. Pannus tissue transformed into chondrocyte rich, fibrocartilaginous tissue. Callus and ossifications were formed in knees.

**Conclusions** Therapeutic TNF blockade of inflammation-mediated joint damage enables the initiation of directed but also undirected regenerative processes. Remission of synovitis seems to be essential to promote bone and calcified cartilage repair.

**Disclosure of Interest** None declared.

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**P115/019 IMMUNOMODULATORY ACTIVITY OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS OF ANKYLOSING SPONDYLITIS PATIENTS**

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

**Introduction** Ankylosing spondylitis (AS) is a chronic autoimmune disease characterized by inflammation and pathological new bone formation at axial joints with resulting spinal segments fusion. The immune abnormalities observed in AS patients concern mainly T lymphocytes and manifest by increased numbers of circulating Th17 cells and defects of regulatory T (Treg) cells. Adipose mesenchymal stromal/stem cells (ASCs) exert immunosuppressive effects on different immune cells, including T lymphocytes, and may be promising option for successful therapy of AS.

**Objectives** In this study we have focused on immunoregulatory activity of ASCs from AS patients (AS/ASCs) toward healthy donor peripheral blood mononuclear cells (PBMCs) and T lymphocytes with special regard to Th17 and Treg cell subsets.

**Methods** AS/ASCs of 15 AS patients and commercially available ASC lines from healthy donors (hASC) were used. ASCs were co-cultured with either anti-CD3/CD28-stimulated CD4+ T lymphocytes or mitogen-stimulated PBMCs of allogenic healthy volunteers. Expression of CD25 and FoxP3 transcription factor in T cells and these cell proliferation were evaluated by flow cytometry. Concentrations of cytokines associated with Th1 (IFNγ), Th17 (IL-17AF) or Treg (IL-10, TGFβ) cells as well as of factors related to immunomodulatory function of ASCs (PGE2, kynurenines) were measured by ELISAs in co-culture supernatants.

**Results** In the co-cultures of ASCs with PBMCs both hASCs and AS/ASCs exerted similar inhibitory effect on the proliferation response of T CD4+ and CD8+ cells. Similar results were obtained in co-cultures of ASCs with purified T CD4+ cells. In the co-cultures of hASCs and AS/ASCs with PBMCs as well as with purified T CD4+ cells the significant increases of kynurenines and PGE2 production were observed. In co-cultures of ASCs and PBMCs there was a marked decrease of IFNg and significant increase of IL-17AF. Concomitantly, Treg proportion in these co-cultures was elevated. In contrary, in the co-cultures of activated T CD4+ cells with ASCs there was some increase of IFNg and IL-17AF whereas Treg proportion was downregulated.

**Conclusions** AS/ASCs and hASCs exert comparable effects on T cells. Since different effects were observed in the co-cultures of ASCs with PBMCs than with CD4+ cells, it seems that ASCs activity toward Th17 and Treg cells is dependent on the presence of accessory cells.

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**Disclosure of Interest** None declared.

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**P116 BASIC CHARACTERISTICS OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS OF ANKYLOSING SPONDYLITIS PATIENTS**

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

**Introduction** Application of mesenchymal stem/stromal cells (MSCs), endowed with immunosuppressive and regenerative properties, may be promising option for successful therapy of ankylosing spondylitis (AS) patients, characterised by inflammation and pathological bone remodelling. Adipose tissue is an
Visfatin down-regulates growth promoting lncRNA H19 during osteogenic differentiation of mesenchymal stromal cells

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P117

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

Introduction Osteoarthritis (OA) and osteoporosis are destructive bone diseases causing chronic pain and leading to disability. Mesenchymal stromal cells (MSC) are essential to bone health and tissue repair. Adipokines such as visfatin alter the osteogenic potential of MSCs and contribute to the loss of bone homeostasis. Long non-coding RNA H19 is one of the first IncRNAs discovered and relevant for distinct processes, e.g., during embryonic growth and tumor formation. IncRNAs interact directly with DNA, RNA as well as proteins, modulating transcription, protein expression and protein function. H19 upregulation was shown in osteogenic differentiation of MSCs. H19 increases the osteogenic potential via TGFβ1 and Wnt/b-catenin pathways.

Objectives To investigate the influence of visfatin on H19 expression during osteogenesis.

Methods Human MSCs from healthy donors (hMSC) and primary human MSCs from osteoarthritis patients (phMSC) after knee replacement surgery were treated with differentiation medium to induce osteogenic differentiation (OD). Matrix mineralization (MM) was quantified after 21 days by Alizarin Red. H19 expression by real-time PCR and IL-6 production by ELISA were measured.

Results lncRNA H19 was up-regulated during OD. Although the H19 upregulation was not altered by co-stimulation with resistin, leptin or TNFα, visfatin co-stimulation during OD down-regulated H19 expression up to 10-fold as compared to unstimulated MSCs. The effect was significant in phMSCs at two of three measured time points (day 7 p=0.03; day 14 p=0.002, n=3) and in hMSCs at day 14 (p=0.0003, n=4). Visfatin co-stimulation of MSCs in OD increased MM, as well as IL-6 levels. However, TNF did not alter H19 expression or increase MM.

Conclusions Visfatin co-stimulation during osteogenesis down-regulated lncRNA H19 expression, indicating a loss of the growth-promoting effects of lncRNA H19 in affected areas of destructive bone disease. This regulatory effect was specific to visfatin and did not occur upon co-stimulation with other adipokines or inflammatory stimuli such as TNF supporting a TNF-independent effect of visfatin.

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Disclosure of Interest None declared.

Podoplanin regulates the migration of mesenchymal stromal cells and their interaction with platelets

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P118

Career situation of first and presenting author Young investigator.

Introduction Within the rheumatoid joint, mesenchymal stromal cells (MSC) up-regulate podoplanin with unknown consequences for disease pathogenesis. The function of podoplanin has been linked to enhanced migratory potential and interactions with platelets. However, it is unclear how these two cell types interact with one another, given that MSC and platelets are usually located in different anatomical compartments (tissue vs blood respectively) separated by the blood vascular endothelial cells (EC).

Objectives Here, we examined the functional consequences of podoplanin expression on the migratory potential of MSC and their interactions with circulating platelets.
Methods Human MSC were isolated from healthy controls. Comparisons were made between podoplanin positive and negative MSC. MSC migration across 8 um pore filters following treatment with anti-siRNA podoplanin or Rho GTPases inhibitors was assessed. MSC-platelet interactions were assessed by culturing MSC on the basal surface of 3 um pore filters and perfusing fluorescently labelled platelets in whole blood over the apical surface. In some cases, the apical surface of the filter was pre-coated with EC, forming an EC-MSC co-culture, prior to platelet perfusion.

Results Expression of podoplanin significantly enhanced the migration of MSC compared to MSC lacking podoplanin. Rac-1 inhibition altered the membrane localisation of podoplanin and in turn significantly reduced MSC migration. Blocking Rac-1 activity had no effect on the migration of MSC lacking podoplanin, indicating it was responsible for regulation of migration through podoplanin. When podoplanin-expressing MSC were seeded on the basal surface of a porous filter, they were able to capture platelets perfused over the uncoated apical surface and induce platelet aggregation. Similar microthrombi were observed when EC were co-cultured on the apical surface. Confocal imaging shows podoplanin-expressing MSC extending processes into the EC layer, which could interact with circulating platelets. In both models, platelet aggregation induced by podoplanin-expressing MSC was inhibited by recombinant soluble CLEC-2.

Conclusions Podoplanin enhances the migratory capacity of tissue-resident MSC enabling them to move more rapidly within the rheumatoid joint. Moreover, podoplanin allows MSC to interact with both circulating and tissue platelets to elicit either protective or pathogenic responses.

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Disclosure of Interest None declared.

P120

IL-23 RECEPTOR SIGNALING IS IMPORTANT DURING PHYSIOLOGICAL BONE REMODELING AND RADIAL BONE GROWTH THROUGH REGULATION OF OSTEOSTERT DIFFERENTIATION

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

Introduction In mice, systemic exposure of IL-23 induces chronic arthritis, increased osteoclast differentiation and systemic bone loss. However, the role of IL-23R signaling during physiological bone remodeling is not fully elucidated.

Objectives To examine the role of IL-23R signaling during physiological bone remodeling.

Methods Femurs of naïve 7-, 12- and 26-week-old IL-23R<sup>GFP</sup>/GFP (IL-23R<sup>−/−</sup>) and IL-23R<sup>−/+</sup> (WT) littermate mice were used for micro-CT analysis of the bone and a three-point bending test for bone strength. Bone marrow (BM) cells were either cultured towards osteoclasts with M-CSF and RANKL or were cultured towards osteoblasts with β-glycerophosphate and vitamin C. Osteoclast differentiation and activity were assessed.
using tartrate-resistant acid phosphatase (TRAP) staining and bone resorption assay, respectively. Osteoblast differentiation was assessed by alkaline phosphatase staining and activity was determined by calcium measurement in the supernatant.

**Results** Trabecular bone volume, thickness and number as well as cortical volume and thickness, and femur length were significantly lower in 12-week-old IL-23R<sup>-/-</sup> mice compared to WT. In addition, three-point bending data revealed reduced maximum force in IL-23R<sup>-/-</sup> femurs. Surprisingly, bone volume was similar between both groups at the age of 26 weeks. However, similar to 12-week-old mice, endocortical volume and femur perimeter were significantly lower in IL-23R<sup>-/-</sup> mice compared to WT. To further study the temporal differences in bone phenotype, we studied osteoclasts and osteoblasts from 7- and 12-week-old mice *in vitro*. Osteoclast differentiation and function were similar at both ages between WT and IL-23R<sup>-/-</sup> mice. Interestingly, BM cells of 7-week-old IL-23R<sup>-/-</sup> mice had reduced capacity to differentiate to osteoclasts, compared to WT. In contrast, these cells showed higher differentiation and significantly higher calcium uptake than WT mice at 12 weeks.

**Conclusions** IL-23R<sup>-/-</sup> mice have temporally dynamic changes in bone metabolism, which is possibly related to alterations in osteoblasts, however, the exact mechanism still needs to be elucidated.

**Disclosure of Interest** None declared.

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**P121/O21 MIR-146A AN IMPORTANT KEY PLAYER IN BONE METABOLISM**

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

**Introduction** Micro RNAs (miRNAs) play a crucial role in the regulation of bone metabolism. MiR-146a, an important anti-inflammatory miRNA, was found to negatively impact osteogenesis and bone regeneration *in vitro*, by controlling differentiation of mesenchymal stem cells. But to date the role of miR-146a in bone remodelling, its influence on bone stability and development of osteoporosis is not known.

**Objectives** Our aim is to analyse the function of miR-146a in bone metabolism.

**Methods** Systemic bone, tibiae and femur, of wt and miR-146a deficient animals was assessed histologically and via μCT analysis, over a period of 3 to 18 months of age. Serum cytokine levels were analysed by ELisa. mRNA expression levels in bone were analysed by qPCR. To induce osteoporosis, ovariectomy (OVX) induced bone loss was performed.

**Results** When we analysed bone volume of long bones histologically as well as with μCT analysis we detected significantly increased trabecular bone mass in miR-146a deficient compared to wt animals, starting at an age of 6 months. In addition cortical thickness of systemic bones from miR-146a knock out animals was significantly increased compared to control mice. Analysis of serum in aged miR-146a deficient animals displayed elevated activity of bone resorbing osteoclasts as amounts of CTX I in miR-146a<sup>-/-</sup> mice were significantly increased compared to wt animals. Q-PCR analysis of important osteoclast as well as osteoblast marker genes in bones *ex vivo* displayed elevated expression of signature molecules of both cell types in aged miR-146a deficient mice, suggesting a regulatory role of miR-146a in both osteoclasts as well as osteoblasts. When we induced osteoporosis using the OVX disease model, histological analysis of long bones showed significant trabecular bone loss in ovariectomized wt mice. In contrast, we detected no trabecular bone loss in ovariectomized miR-146a knock out animals, suggesting that loss of miR-146a deficiency protects bone loss induced by estrogen deficiency.

**Conclusions** MiR-146a seems to control bone turnover and miR-146a deficient mice accrue bone over time. Moreover this miRNA has a negative influence on bone loss occurring during oestrogen loss induced osteoporosis. Therefore miR-146a could be possibly used as a therapeutic target in the treatment of osteoporosis.

**Disclosure of Interest** None declared.

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**P122 IMPORTANT ROLE OF DENDRITIC CELLS IN INFLAMMATORY ARTHRITIS**

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

**Introduction** Important role of dendritic cells in inflammatory arthritis.

**Objectives** Investigation of the role of CD11c<sup>+</sup> cells in joint inflammation and destruction.

**Methods** We analyzed histological sections of K/BxN serum transfer arthritis as well as hTNFtg arthritis for the presence of CD11c<sup>+</sup> cells by immunohistochemistry. We used CD11c-diphteria toxin receptor (DTR) transgenic mice. K/BxN serum transfer arthritis was induced, and mice were given either DT or PBS or in wt and BARF3 deficient mice. In addition CD11c DTR mice were crossed into hTNFtg animals and also received either DT or PBS. The severity of arthritis was determined clinically and histologically.

**Results** Both CD8<sup>+</sup>CD11c<sup>+</sup> and CD11b<sup>+</sup>CD11c<sup>+</sup>, can be found in synovial tissue in TNF driven arthritis. Upon depletion of CD11c<sup>+</sup> cells clinical signs of K/BxN serum transfer arthritis were significantly reduced. Histological analysis found reduced synovial inflammation after the depletion of CD11c<sup>+</sup> cells in K/BxN arthritis. In addition, local bone destruction and the number of osteoclasts was also significantly reduced. In addition to K/BxN arthritis, we found that also in TNF-driven arthritis depletion of CD11c<sup>+</sup> cells led to a striking reduction of synovial inflammation and a complete depletion of osteoclasts.

**Conclusions** These data show that in addition to initiating an adaptive immune response, CD11c<sup>+</sup> dendritic cells, are also involved in innate effector mechanisms of inflammatory arthritis.
EXTRACELLULAR MATRIX ATTENUATED MATRIX-DEGRADING EFFECTS OF VISFATIN DURING ADIPOGENIC MSC DIFFERENTIATION

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OSTEOCYTE-DERIVED PODOPLANIN IS AN IMPORTANT REGULATOR OF BONE REMODELLING IN THE KBXN SERUM TRANSFER MODEL OF RHEUMATOID ARTHRITIS

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

Introduction
Osteoporosis (OP) is the most common age-related disorder characterized by bone loss and correlates with increased bone marrow adiposity due to a shift of osteogenic towards adipogenic differentiation of bone marrow mesenchymal stem cells (MSC). Therefore, bone marrow adipocytes are in direct contact with the altered bone matrix in OP. Adipose tissue is metabolically active. Therefore, adipocyte-derived factors such as adipokines might influence MSC differentiation.

Objectives
We analyzed the presence of adipokines (visfatin, resistin and leptin) in bone tissue and their effects on MSC differentiation under standard culture vs. spongiosa.

Methods
RNA and MSC were isolated from spongiosa of femoral heads of osteoarthritis patients after hip replacement surgery, or after osteoropic femoral neck fracture. Adipogenic MSC differentiation was performed with/without adipokines and visfatin inhibitor Apo866 as well as SB203580 p38-MAPK inhibitor. For the transfer and differentiation of MSC on cancellous bone, bone fragments were purified and sterilized. Gene expression was evaluated by Realtime PCR. Protein production was analyzed by ELISA.

Results
Visfatin and leptin levels were increased in OP bone vs. non-ostearthrotic OA bone, however, resistin was reduced. Visfatin induced the secretion of proinflammatory factors during adipogenesis in standard cell culture as well as on cancellous bone. Visfatin-induced cytokine release was markedly reduced during differentiation on spongiosa (e.g. 14d IL6, x-fold: standard culture 151±1100, spong. 40±30, n=7). Significantly elevated MMP13 mRNA as well as protein expression induced by visfatin could be observed during adipogenesis in standard cell culture as well as on spongiosa, however visfatin-mediated MMP13 expression was reduced on cancellous bone (e.g. 21d, x-fold: standard culture 81±89, spong. 13±21, n=7). The visfatin inhibitor Apo866 inhibited the visfatin-induced cytokine release. However, the MMP13 expression was not influenced during adipogenesis in culture (n=4). In contrast to Apo866, the p38-MAPK inhibitor did not reduce cytokine release during adipogenesis.

Conclusions
Visfatin and leptin levels were elevated in osteoporotic bone. Therefore, visfatin-mediated increase of MMPs and proinflammatory cytokines during adipogenic differentiation might influence bone turnover at the adipose tissue/bone interface. Our results support the idea that the extracellular matrix attenuates visfatin-mediated detrimental effects during adipogenesis. The observed visfatin-mediated effects most likely depend on different signaling pathways.

Disclosure of Interest
None declared.
IL-36 AXIS IS AN EMERGING THERAPEUTIC TARGET IN PSORIATIC ARTHRITIS

MTOR BLOCKADE BY RAPAMYCIN DECREASES ARTHRITIS AND SPONDYLITIS DEVELOPMENT AND SEVERITY IN HLA-B27 TRANSGENIC RATS

Disclosure of Interest None declared.

P125 IL-36 AXIS IS AN EMERGING THERAPEUTIC TARGET IN PSORIATIC ARTHRITIS SYNOVIAL TISSUE

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

Introduction The IL-36 family of cytokines includes three agonists (IL-36α, β and γ) and two established or hypothetical antagonists (respectively IL-36Ra and IL-38). IL-36 agonists are pro-inflammatory cytokines highly expressed in skin,cleaved and activated by neutrophil proteases and involved in the pathogenesis of psoriasis. They have only limited effect in driving synovial inflammation in rheumatoid arthritis (RA) but little is known about the expression and biologic functions of the IL-36 axis in synovial tissue of psoriatic arthritis (PsA).

Objectives In this study, we aimed to investigate the expression pattern and role of the IL-36 cytokines in early treatment-naïve PsA synovium in comparison with RA.

Methods Synovial tissue samples were collected from patients with early (disease duration <12 months) RA and PsA DMARDs (Disease Modifying Anti-Rheumatic Drugs) and treatment-naïve. All patients underwent an ultrasound-guided synovial biopsy before starting the treatment and after six months of treatment with conventional DMARDs. The expression of IL-36 family members was investigated in synovial tissue at gene level by RNA-sequencing (87 RA, 15 PsA), at protein level by immunostaining (20 RA, 26 PsA) and in plasma by ELISA (22 RA, 38 PsA). RA and PsA-fibroblasts-like-synoviocytes (FLS) were treated in vitro to assess their response to IL-36 stimulation.

Results Gene and protein expression of IL-36 agonists was comparable between RA and PsA synovial tissue; conversely, the antagonists were significantly lower in PsA compared to RA. Accordingly, the agonists/antagonists ratio was considerably higher in PsA synovium, also characterized by a strong neutrophil-signature, suggesting an activation of the IL-36 pro-inflammatory pathway. Among the immune cells infiltrating the PsA synovium, macrophages (CD68+) and plasma cells (CD138+) were the primary IL36α-expressing cells. At baseline, the synovial expression of IL-36α was significantly higher in PsA patients who did not respond to DMARDs treatment at 12 months; this differential synovial expression of IL-36α between responders and non-responders was also maintained at six months. In keeping with this observation, we showed that treatment with DMARDs did not reduce the expression of IL-36 in PsA cells in vitro. Finally, we observed that PsA-FLS produced significantly higher levels of IL-8 upon stimulation with IL-36α in comparison with cells isolated from RA patients.

Conclusions The impaired balance between IL-36 agonists and antagonists observed in PsA synovial tissue might contribute to the persistent inflammation characterising the diseased tissue. The exogenous replacement of the IL-36 antagonists may be a novel promising therapeutic target for PsA patients.

Disclosure of Interest None declared.

References
reduced bone, cartilage destruction (p<0.001) and less new bone formation (p<0.001) in peripheral joints. Comparably, therapeutic treatment with rapamycin decreased spondylitis severity (1 versus 2.8; p<0.05) and arthritis severity (4 versus 8.8; p<0.05) compared to controls. In the rapamycin treatment group, IL-17A (p<0.05) and IL-17F (p<0.05) mRNA were decreased in the MCP joints.

**Conclusions** mTOR blockade decreases IL-17A and TNF production *in vitro* in Spa PBMCs and reduces osteoblastic differentiation of human Spa FLS. In the *M. tub* transgenic rat model of SpA, mTOR blockade reduces arthritis and spondylitis development and severity by targeting inflammation and bone remodeling.

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spondyloarthritis, a prototype chronic inflammatory disease. Furthermore, it attenuated inflammation and improved disease activity, function, and quality of life.

REFERENCE

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Disclosure of Interest
None declared.

P130 PEFICITINIB TARGETS SYNOVIAL FIBROBLASTS IN RHEUMATOID ARTHRITIS

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Introduction
Tofacitinib and baricitinib are the first approved Janus kinase inhibitors (JAKi) in Europe for treatment of rheumatoid arthritis (RA). Other JAKi differing in the inhibition profile of the four isoforms JAK1, JAK2, JAK3 and TYK2 are currently examined in clinical trials. Especially filgotinib, inhibiting mainly JAK1/2, and the panJAKi peficitinib are well tolerated up to doses causing Cmax values higher than 1 μM. However, less is known about the effect of JAKi in RA on synovial fibroblasts (RASF) activated by IL-1β. RASF contribute to the growing pannus and develop an aggressive cartilage-destructive phenotype.

Objectives
The aim of the study was to examine the effect of JAKi on activated RASF and further characterize the most effective inhibitor.

Methods
Isolated human RASF were pretreated with JAKi for 2 hour and then stimulated with IL-1β and JAKi with/out soluble IL-6 receptor (sIL-6R). IL-6 and MMP-3 were measured in supernatants by ELISA. Stimulation with oncostatin M served as control. The proliferation of RASF was detected using a BrdU-incorporation assay. The effect of peficitinib on RASF migration was investigated by cell culture inserts using a FCS gradient. For adhesion assays, RASF were treated with JAKi, detached and seeded in culture plates. Then, plates were extensively shaken and adherent RASF quantified by counting crystal violet stained cells. Cell viability, cytotoxicity and apoptosis were measured using commercially available assays.

Results
Addition of the sIL-6R caused an additional release of IL-6 in RASF treated with IL-1β alone. This release was fully blocked by tofacitinib at 0.5 and 1 μM (both p<0.01). However, tofacitinib was not able to reduce the IL-6 release of IL-1β activated RASF without sIL-6R. In contrast, peficitinib attenuated the IL-6 levels by 62% at 5 μM (p<0.001, n=7) and by 24% at 1 μM. Filgotinib resulted in a reduction of 30% at 3 μM only (p<0.05). The MMP-3 release was reduced by peficitinib (88%, p<0.001) at 5 μM. Peficitinib was the only JAKi able to reduce the proliferation of activated RASF by 23% at 1 μM (p<0.05). Furthermore, peficitinib at 1 and 5 μM decreased the migration of RASF by 38% and 92% (p<0.001, n=4). Peficitinib up to 5 μM did not act cytotoxic on RASF even after 48 hour.

Conclusions
JAKi suppress the inflammatory response induced by transsignaling of IL-6 in RASF. Peficitinib appears to be superior to other JAKi in suppressing the IL-1β induced response and the proliferation of activated RASF. Thus, patients suffering specifically from severe synovial proliferation could benefit from treatment with peficitinib.

Disclosure of Interest
None declared.

P131 TARGETING ACTIVATED SYNOVIAL FIBROBLASTS USING PHOTODYNAMIC THERAPY IN HUMAN RHEUMATOID ARTHRITIS SYNOVIAL TISSUE

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Introduction
Activated synovial fibroblasts (ASF) play an important role in the pathogenesis of rheumatoid arthritis (RA). They contribute to the pro-inflammatory environment in the joint and degradation of cartilage and bone. Depleting ASF could ameliorate both these hallmarks of RA. ASF are characterized by the expression of fibroblast activation protein (FAP).

Objectives
Here, we investigated the potential of FAP-targeted photodynamic therapy (tPDT) using the photosensitizer IRDye700DX conjugated to the anti-FAP antibody 28H1 (28H1–700DX) to selectively kill these cells.

Methods
To demonstrate the proof-of-concept of FAP-based tPDT in ex vivo human tissue, RA synovial tissue obtained during joint replacement surgery was trypsin digested and fibroblasts were cultured for at least 5 passages. Cells were incubated with or without 1 ug/well 28H1–700DX for 4 hour, washed with PBS and either exposed to 690 nm light or not exposed. A luminescent cell viability assay (CellTiter-Glo) was used to measure cell viability. In parallel, 6 mm biopsies of synovial tissue were taken and used for FAP-based tPDT (n=8 patients). They were subjected to tPDT as described above and formalin fixed after 1 hour. To measure cell death, 5 μm paraffin slices were stained for FAR, gamma-H2AX and caspase-3 expression for activated fibroblasts, DNA double-strand breaks and early apoptosis, respectively.

Results
FAP-tPDT performed on the cultured fibroblasts showed a light dose dependent increase in cell death when incubated with 28H1–700DX for 4 hour. Cell viability was not affected when cells were incubated with 28H1–700DX without illumination (101.37%±3.9% remaining compared to 100%;±5.07% in the normalized control). Radiant exposure of 17.6 J/cm2 did not significantly decrease cell viability (6.4%±9.8% decrease, ns). Radiant exposures of 52.8, 105.6 and 158.4 J/cm2 significantly decreased cell viability (38.6%±6.9%, 67.5%±10.9% and 80.6%±5.8% respectively, p<0.001 for all). After FAP-tPDT, the human synovial biopsies showed a significantly increased staining of the caspase-3 marker, but not of gamma-H2AX (Friedman’s ANOVA, p=0.007 and p=0.810 respectively). Pairwise comparison
showed that caspase-3 scores were significantly higher in biopsies treated with tPDT compared to those incubated with buffer (p=0.009).

Conclusions FAP-tPDT induces cell death of FAP-positive activated fibroblasts in synovial tissue from RA patients. This is a first indication that FAP-targeted PDT can be a feasible new treatment strategy in RA.

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P132 EFFECTS OF RESVERATROL AND A NOVEL RESVERATROL-SALICYLATE HYBRID MOLECULE ON ACTIVATION OF HUMAN CD4+ T-CELLS

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OBJECTIVE

To compare the effects of resveratrol and a novel resveratrol-salicylate hybrid molecule C-10 (Aldawsari et al, 2016) on the activation of human CD4+ T-cells.

METHODS

CD4+ T-cells were isolated from healthy donors and pre-incubated with different concentrations of resveratrol or C-10 before being stimulated with anti-CD3/anti-CD28 antibodies. After 24 hours, the up-regulation of the early activation markers CD25, CD69, CD71 and CD98 was analyzed and phosphorylation of signal transduction molecules were determined by western blot and/or flow cytometry.

RESULTS

Inhibition of IL-2, IFN-γ and particularly TNF-α release was significantly more effective when the cells were treated with C-10 as compared to resveratrol. Moreover, the proliferation rate was significantly more decreased in the presence of C-10. The expression of CD25, CD69, CD71 and CD98 was reduced to a similar degree by both compounds. Furthermore, phosphorylation of Akt and STAT-5 was substantially attenuated by C-10 and to a lesser degree also by resveratrol. All T cell subsets investigated (Th1, Th2, Th17) were affected at a similar degree but the most pronounced effect was seen in naïve T cells.

Conclusions Our data demonstrate that C-10 suppressed cytokine secretion and proliferation more effectively than resveratrol. Both compounds influence the phosphorylation of important signalling molecules. The effect exerted on STAT-5 activation may be the key mechanism for inhibition of T cell activation. Thus, the resveratrol-salicylate hybrid molecule C-10 might be considered a candidate drug for treatment of RA and other T-cell driven autoimmune diseases.

REFERENCE


Disclosure of Interest None declared.

P133 CLINICAL AND IMMUNOLOGICAL EFFECTS OF TOFACITINIB THERAPY IN RHEUMATOID ARTHRITIS

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

Introduction During the treatment of rheumatoid arthritis (RA) we intend to achieve remission or low disease activity. In this regard, the most effective way is targeted therapy. Oral JAK inhibitor, tofacitinib appeared as a new therapeutic option, beside biological therapies, which has already proven its safety and effectiveness in RA.

Objectives The aim of this study was to assess the clinical and immunological effects of one-year tofacitinib therapy in patients with RA.

Methods Altogether 30 RA patients with active disease were recruited and treated with tofacitinib in this 12 months follow-up study. Mean duration of rheumatoid arthritis were 7.7 ±5.0 years. Half of the patients haven’t received biological treatment prior tofacitinib therapy, other half of the patients switched to tofacitinib therapy after completing washout. 15 patients received 2 × 5 mg and 15 patients received 2 × 10 mg tofacitinib daily for 12 months. Assessments were performed at baseline, month 6 and 12. Levels of CRP and IgM rheumatoid factor (RF) antibodies were measured by quantitative nephelometry and levels of anti-CCP assessed by ELISA. Lymphocyte subsets (CD3+, CD4+, CD8+ T cells, CD19+B cells and CD56+/CD3- NK cells) were assessed by cytokerometry. In addition, disease activity (DAS28), age and disease duration were also measured.

Results Tofacitinib therapy was clinically effective, significant improvements in physical function were observed in 26 patients. 4 patients (two from both arms) quit the study due ineffectiveness. There were significant decrease in levels of DAS28 (p<0.001), CRP (p<0.001) and HAQ value (p<0.05) in 12 months. There were no changes in levels of RF and anti-CCP. Numbers and ratio of CD3+ and CD4+ T cells were significantly decreased (p<0.05), however significant increase was seen in the numbers and ratio of CD19+B cells after 12 months (p<0.05). There were no significant changes in numbers and ratio of NK cells. There was significant correlation between absolute number of CD8+ T cells and disease
duration at baseline (R=0.466, p=0.009). There were no other significant correlations.

Conclusions One year tofacitinib therapy effectively reduced disease activity and systemic inflammation. The therapy also improved functional capacity. JAK inhibition significantly decreased the number and ratio of CD3+ and CD4+ T cells and increased number and ratio of CD19+ B cells in 12 months. Tofacitinib is a safe and effective treatment for rheumatoid arthritis. It makes an effect, partly, via modulating lymphocyte subsets.

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Disclosure of Interest None declared.

P134/O32

SELECTIVE EXPANSION OF A THYMIC-DERIVED AND FUNCTIONALLY COMPETENT REGULATORY T CELL POPULATION BY LOW-DOSE IL-2 THERAPY IN PATIENTS WITH REFRACTORY SLE

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Career situation of first and presenting author: Instructor.

Objectives An acquired deficiency of interleukin-2 (IL-2) and related defects in regulatory T cell (Treg) homeostasis play a crucial role in the pathogenesis of SLE. Here, we report the responses of Treg and other lymphocyte subsets to low-dose IL-2 therapy observed during an open-label, uncontrolled, dose adaption, phase 1/2a single-center clinical trial in patients with active and refractory SLE.

Methods 12 patients with active and refractory SLE (SLEDAI >6) were treated at our site with a low-dose IL-2 regimen consisting of four separate treatment cycles each with daily subcutaneous injections of recombinant human IL-2 (aldesleukin) at single daily doses of either 0.75, 1.5 or 3.0 million IU for five consecutive days. Cells from peripheral blood were analyzed by flow cytometry before and one day after each treatment cycle.

Results All patients showed highly significant cycle- and dose-dependent increases in the proportions and absolute numbers of CD3+CD4+FoxP3+CD127lo Treg and of CD25hi expressing cells among Treg. By contrast, we observed no relevant changes in the absolute numbers of CD3+CD4+FoxP3 conventional T cells (Tcon), of CD3+CD8+ T cells, of CD3+CD56+NNK T cells and of CD3-CD56+NNK cells. The IL-2 expanded Treg population displayed a preserved suppressive capacity and expressed high levels of the Treg-associated molecules Helios, CD39 and CD137. In addition, we noted robust and dose-dependent increases in the proportions of Ki67+ cells among Tcon and also among other lymphocyte subsets were observed at the end of each treatment cycle, the calculated ratio between Ki67+ Treg and Ki67+ Tcon continuously increased and was significantly higher at the end of the treatment phase, suggesting a preferential targeting of the Treg population.

Conclusions Low-dose IL-2 therapy promotes the selective expansion of a functionally competent and thymic-derived Treg population in patients with refractory SLE. This study also provides novel insights into the pharmacodynamics and the broad biologic effects of low-dose IL-2 therapy.

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pathway, played an important role in the development of RA. LY294002 could delay the onset and reduce the severity of arthritis in CIA mice through the promotion of neutrophils apoptosis. It might open a new door to the future clinical treatment of RA.

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Disclosure of Interest None declared.

P138010 INVESTIGATING GENE EXPRESSION PATTERNS AND FUNCTION OF TOLERODGENIC DENDRITIC CELLS
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Career situation of first and presenting author Post-doctoral fellow.

Introduction Dendritic cells (DCs) are professional antigen-presenting cells and play a major role in immune system responses and function. They express a repertoire of toll-like receptors (TLRs) that recognise pathogens. Tolerogenic dendritic cell (tolDC) therapy is a promising cellular therapy that aims to restore tolerance in autoimmune disease. Exposure to dexamethasone leads to the expression of TLR2; however, expression of this marker is not homogeneous. Some tolDCs have low TLR2 expression while some others have high TLR2 expression. Despite these differences in TLR2 expression, we hypothesised that tolDCs are homogenous in terms of their gene expression and function.

Objectives The study aimed to investigate the gene expression and function of tolDCs to determine if expression patterns and function seen in low TLR2 expressing tolDCs are similar to those of high TLR2 expressing tolDCs.

Methods Peripheral blood CD14+ monocytes, obtained from healthy individuals (n=8) were differentiated for 7 days into tolDCs using IL-4, granulocyte-macrophage colony-stimulating factor (GM-CSF), dexamethasone and Vitamin D3. As a control, mature DCs were differentiated using IL-4, GM-CSF and IFN-γ. TolDCs were stained for TLR2 and fluorescence activated cell sorted (FACS) as the 20% lowest TLR2 expressing tolDCs and the 20% highest TLR2 expressing tolDCs. TolDC phenotype was explored using flow cytometry, gene expression of pro-inflammatory, anti-inflammatory and migratory genes by quantitative PCR and CD40-Ligand re-stimulation and mixed lymphocyte reaction assay to investigate function.

Results Phenotypic marker expression was different between tolDCs and mature DCs, with tolDCs expressing low levels of CD83 and CD86 and high levels of HLA-DR, latency-associated peptide (LAP) and TLR2. In general, TLR2Low and TLR2High tolDCs showed similar phenotypic properties, gene expression patterns and tolerogenic functions. TLR2High tolDCs had increased LAP and HLA-DR expression. TLR2Low tolDCs had higher gene expression for CCR7 and TNF-α. Cytokine (IL-6 and IL-10) production for both TLR2Low and TLR2High tolDCs was not significantly different upon re-stimulation with CD40-ligand and both populations had similar immunosuppressive capacity for CD4+ T cells compared to mature DCs.

Conclusions Both populations of tolDCs displayed similar gene expression profiles and phenotypic properties for the majority of characteristics investigated. The minor differences observed may be attributable to stochastic differences in dendritic cell exposure to, or uptake of dexamethasone. Despite these differences, all tolDCs function similarly, which is the most important factor when considering tolDCs as a therapy.

Disclosure of Interest None declared.

P140 IDENTIFICATION OF AFFIMERS THAT BIND TO THE IL-7R AND INHIBITS THE IL-7 SIGNALLING CASCADE
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Career situation of first and presenting author Student for a master or a PhD.

Introduction IL-7R is a heterodimer constituted by the IL-7R alpha (α) chain (CD127) and the common gamma (γ) chain (CD132). IL-7 binding to IL-7R expressed on CD4+ T cells induces a survival signal. The IL-7/IL-7R signalling axis has been validated as a therapeutic target for treatment of both T-cell driven autoimmune diseases (AIDs) and T Acute Lymphoblastic Leukaemia (T-ALL). Affimers are small and stable artificial proteins which bind with nanomolar affinities to human proteins and can block protein-protein interactions. They are becoming widespread owing to their stability, ease of production and versatility.

Objectives Identify Affimers that recognise the IL-7Rα and inhibits the IL-7 signalling cascade. This may result in an attractive approach for the treatment of both T-cell driven autoimmune diseases and T-ALL.

Methods The type-II Affimer library (1010) was interrogated by Phage display using fully glycosylated human IL-7Rα ectodomain (ECD). PhageELISA and DNA sequencing were used to either obtain or elucidate the unique binders (Affimers), respectively. Affimers were produced as His-Tagged proteins (~13 kDa) in E. coli and purified using IMAC. Affimer binding to IL-7Rα was confirmed by pull-down assays (soluble) and Flow cytometry (membrane). An IL-7 reporter assay using HEK-IL7R (HEK293 cells stably transfected with the IL-7R) was developed and the biological effect of the Affimers was elucidated.

Results We have screened an Affimer library using human ECD-IL7Rα and after three consecutive panning rounds, 20 Affimers were raised as shown by PhageELISA and DNA sequencing. From these, 17 were able to pull-down the soluble ECD-IL7Rα and 7 stained specifically HEK-IL7R cells (by flow cytometry using anti-His Tag Abs). Finally, we have identified 3 Affimers (1, 42 and 96) that showed inhibition of the IL-7 signalling cascade on HEK-IL7R cells.

Conclusions Our work demonstrates the possibility of screening an Affimer library for a cytokine-receptor target, and selecting specific binders, some of which showed the desired antagonist activity of the cytokine signalling cascade. IL-7 itself is a validated target, so this work offers an alternative to antibody-mediated protein interference. With further biological
validation including animal models, it may even offer a novel therapeutic tool for AIDs and T-ALL.

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P141 DIFFERENTIAL DNA METHYLATION IN PERIPHERAL NAÏVE CD4+ T-CELLS IN EARLY RHEUMATOID ARTHRITIS PATIENTS

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

Introduction Alterations in DNA methylation patterns (epimutations) have been related to several diseases, including Rheumatoid Arthritis (RA). We hypothesise that such epimutations occur early in the RA disease process. T-cells are important cells in early pathogenesis, therefore we choose to analyse patterns of differential methylation (DM) of the DNA of CD4+ T-cells.

Objectives To identify changes in DNA methylation pattern of naïve CD4+ T cells in early, drug naïve RA patients, towards understanding early events in disease pathogenesis.

Methods DNA methylation of 480,000 CpGs (Illumina methylation genome-wide array) were analysed in cell sorted, naïve CD4+ T cell from 6 healthy control (HC) and 10 RA patients. To priorities DM gene, we designed a scoring system.1 We examined potential interactions between DM genes using the STRING-database of protein-protein network analysis. Flow cytometry was used to characterise subpopulations of naïve CD4+ T cells in early RA for 4 DM genes, coding for cell surface molecules.

Results We observed 648 DM-genes in naïve T-cells in early RA (354 hypo and 294 hyper-methylated). STRING analysis of these 648 genes/proteins revealed central of JAK1/STATs signalling, associated with an IL6/IL6R/STAT3 signalling node, which is generally already elevated in RA.

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

Introduction The use of methotrexate (MTX) for the treatment of rheumatoid arthritis (RA) is limited by serious adverse effects. Some effects, such as stomatitis and gastric ulcer, can be alleviated by folate supplement. Others are folate-independent, e.g. fibrosis of liver and lung. Moreover, MTX further elevates the micronuclei count, which is generally already increased in RA.

In RA, inflamed tissue is characterized by up to 100-fold increased concentrations of reactive oxygen species (ROS), including hydrogen peroxide) compared to healthy tissue.

A novel MTX prodrug, ROS101 in development for the treatment of RA, releases MTX at exposure to ROS. This restricts MTX exposure to target tissues with increased ROS levels, e.g. the synovial membrane in RA.

Objectives To investigate the effect and toxicity of a targeted MTX prodrug in a rat CIA model of rheumatoid arthritis.

Methods ROS101 was tested in a collagen induced arthritis (CIA) model in dark agouti (DA) rats (10 per group) against vehicle and 0.3 mg MTX i.p./day at equimolar doses. Arthritis was induced by s.c. injection of an emulsion of collagen type II in complete Freund’s adjuvant. Treatment was initiated at a mean arthritis severity score of 2/60. Blinded disease evaluation took place 3 times/week from day 10. Bone marrow cells for micronucleus test were obtained from the femur, pictures captured with LARS.4.8 software and analyzed blinded. Individual rat data were included until termination.

Results Mean and maximum arthritis severity score was reduced in rats in the ROS101 group compared to vehicle (mean score 6.3±3.1 vs. 14.1±3.3, mean±SEM); (max score 12.6±5.3 vs. 32.3±5.3, mean±SEM, p<0.05). All rats in the ROS101 group completed the study until day 32. Reduced arthritis severity in the MTX group after day 22 was accompanied by overt toxicity, causing all rats to be terminated from day 24 to 29. There was no significant difference in body weight between rats in the ROS101 and vehicle groups, whereas body weight in the MTX group decreased significantly from day 22 due to toxicity, including GI effects. Bone marrow micronucleus count was reduced in the ROS101...
EFFECT OF LOW-DOSE IONIZING RADIATION ON THE INFLAMMATORY PHENOTYPE OF ADIPOCYTES AND DIFFERENTIATION OF OSTEOCLASTS (IN VITRO)

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

Introduction Adipose tissue is a complex endocrine organ that produces a variety of immune and inflammatory mediators. Adipocytes, the dominant cell type of adipose tissue, are known to support inflammatory processes in musculoskeletal diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA) by release of different cytokines and adipokines. During this process, osteoclastogenesis is also enhanced and results in an imbalance of bone metabolism. Low-dose radiation therapy (LD-RT) is known to attenuate inflammation and to increase the mobility of patients suffering from RA or OA.1

Objectives In our previous work, we observed a decrease of visfatin levels in serum of patients treated with low-dose ionizing radiation during exposure to the alpha-emitter radon.2 In the same study, a decrease of markers for bone resorption after radon exposure was detected. Based on this, we next compared the response of human adipocytes derived from subcutaneous and infrapatellar adipose tissue to ionizing radiation with respect to release of adipokines and other inflammatory factors (IL-6, IL-8). In parallel, we analyzed the effect of ionizing radiation on differentiation capacity of osteoclast (OC) precursors into mature, bone resorbing OC.

Methods Human subcutaneous preadipocytes and human infrapatellar preadipocytes were irradiated with different doses of ionizing radiation, and release of inflammatory factors was measured in the cell culture supernatants using ELISA. OC precursors were isolated from human donor blood, differentiated according to standard protocols and analyzed by fluorescent stainings for cell nuclei, tartrate-resistant acidic phosphatase (TRAP) and actin filaments.

Results The results revealed that the release of adipokines and inflammatory cytokines (IL-6, IL-8) was not significantly affected by ionizing radiation. Further, it was found that differentiation of OC precursor cells into mature OC is reduced after irradiation.

Conclusions The observations made in this study suggest that adipocytes are probably not the main source of modified adipokine levels in the arthritic joint. However, an observed tendency of adipocytes to increase fat accumulation after irradiation suggests radiation-induced changes in functionality of human adipocytes which could have an indirect impact on the radiation response of the tissue. OC respond to radiation by reduced differentiation and structural changes, but the impact on functionality needs to be further tested.

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HCQ ALLEVIATES 5-FU-INDUCED INTESTINAL INFLAMMATION THROUGH INHIBITING TLR9-DEPENDENT DNA SENSING PATHWAY

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Career situation of first and presenting author Young investigator.

Introduction Evidences revealed that chemotherapies could trigger DNA release, then induce inflammation of intestinal tissues which damp the effect of anti-cancer treatment.1 2 DNA released induces the translocation of TLR9 to endolysosomes and subsequent nuclear factor-kB (NF-kB) activation, which leads to interleukin-1β (IL-1β) secretion and inflammation.3 Targeting TLR9-dependent DNA sensing pathway may be a valuable therapy for chemotherapy induced intestinal mucositis.

Objectives This study aims to investigate whether hydroxychloroquine (HCQ), suppresses 5-FU-induced intestinal mucositis through inhibiting TLR9-dependent DNA sensing pathway.

Methods The effect of HCQ on 5-FU-induced intestinal mucositis were examined in vivo and in vitro. We established 5-FU-induced intestinal mucositis model and assessed body weight, diarrhea score and histopathologic changes following HCQ treatment in vivo, then TLR9 and NF-kB expression of small intestine and IL-1β secretion of serum were analyzed. Bone marrow-derived macrophages (BMDMs) were cultured, transfected with calf-thymus DNA(CT-DNA) and treated with HCQ for 6 hour in vitro. TLR9 and NF-kB expression and IL-1β secretion in BMDMs were then investigated.

Results HCQ treatment markedly attenuated body weight loss, severity of diarrhea, intestine shortening, and destruction of small intestinal in histopathology of 5-FU- treated mice in vivo. Also HCQ treatment inhibited TLR9 and NF-kB expression in small intestine of 5-FU- treated mice and pre-inflamatory IL-1β secretion in serum of 5-FU- treated mice. Meanwhile administration of HCQ reduced the number of macrophages in small intestine of 5-FU-treated mice. Then BMDMs were cultured, transfected with CT-DNA and treated with HCQ in vitro. We found HCQ efficiently inhibited TLR9 expression, translocation of NF-kB to nucleus, and IL-1β secretion in supernatant of CT-DNA stimulated BMDMs.

Conclusions These results provide a new insight into the mechanism of chemotheraphy induced intestinal mucositis and...
Antibodies and Activity of Disease


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Disclosure of Interest None declared.

**REFERENCES**


**Citation**

Young investigator.

**Introduction**

Currently there is very little information on optimal levels of golimumab in patients with polyarthritis.

**Objectives**

The main objective was to analyze the minimal serum concentration of golimumab (GOL), anti-golimumab antibodies (AcGOL) and its relation to the activity of the disease measured by DAS 28 index, in rheumatoid arthritis (RA) and psoriatic arthritis (PA).

**Methods**

We conducted a retrospective observational study with RA or PA patients on golimumab treatment between 2011 and 2016.

We analyzed the age, sex, diagnosis, dose, dose interval, duration of treatment, concomitant disease modifying drugs (DMARDs), minimal concentration of GOL (GOL Cmin), AcGOL, and DAS28 levels. Sampling was performed on the day corresponding to the dose of golimumab, prior to administration, in order to obtain the minimum levels of the drug. The analytical technique used for the determination was the Promonitor® sandwich ELISA.

**Results**

10 RA, 5 AP patients were selected. Median age 53 years. 67% women. All with golimumab 50 mg with an administration interval of 28 to 41 days. Duration of treatment 16.73 months (median). 80% underwent combination therapy with DMARDs.

Only one patient (RA) presented levels of AcGOL, with undetectable Cmin, DAS28 of 4.05.

Golimumab levels in patients without AcGOL and DAS28 <3.2: 1 Cmin <0.25 mcg/mL, 5 Cmin 0.26–0.5 mcg/mL, 1 Cmin 0.5–1.4 mcg/mL and 1 2mcg/mL Cmin. In patients with DAS28 >3.2 were: 2 Cmin <0.25 mcg/mL, 2 Cmin 0.26–0.5 mcg/mL, 2 Cmin 0.5–1.4 mcg/mL and 1 Cmin of 2.2 mcg/mL.

**Conclusions**

Very variable GOL Cmin values (<0.25–2.2 mcg/mL) were obtained. 80% with DAS28 <3.2 had a GOL Cmin 0.26–0.5 mcg/mL. Because of the limited information currently available on the optimal range of golimumab levels, further studies are needed to evaluate the relationship between serum drug levels and patient disease activity data.

Disclosure of Interest None declared.
**P148**

**JAK-INHIBITION BY BARICITINIB AND TOFACITINIB AMELIORATES PATHOLOGICAL BONE LOSS**


Career situation of first and presenting author

**Student** for a master or a PhD.

**Introduction** Many cytokines relevant to rheumatoid arthritis (RA) rely on the janus kinase – signal transducer and activator of transcription (JAK-STAT) signaling pathway. The JAK-inhibitors tofacitinib and baricitinib, targeting JAK3/JAK1 and JAK1/JAK2 respectively, have been approved for treatment of RA.

While currently available therapies reduce inflammation, erosive damage in involved joints is still irreversible. However, preliminary data suggests an influence of JAK inhibition on local bone healing.

**Objectives** To study the role of JAK-inhibition in osteoblast and osteoclast-mediated bone homeostasis and its capacity to alleviate structural bone damage in vivo.

**Methods** For steady state analysis C57BL/6 (WT) mice received tofacitinib QD by oral gavage for 6 weeks. WT mice of the ovariectomy-induced osteoporosis model (OVX) obtained tofacitinib BID by oral gavage for 6 weeks. For the serum-induced arthritis model (SIA) WT mice received tofacitinib or baricitinib BID by oral gavage for 14 days. Readout covered serum cytokine levels (ELISA), µCT and mRNA analysis of bone (qPCR) and clinical scoring. Murine osteoclasts (OC) were assessed for differentiation (TRAP staining) and resorption (von Kossa staining). Murine mesenchymal stem cell (MSC)-derived and primary osteoblasts (OB) were analyzed for differentiation (qPCR) and function (Alizarin red staining).

**Results** In unchallenged WT mice, treatment with tofacitinib increased trabecular bone density in tibia and reduced RANKL expression during JAK-inhibition. Moreover, Dkk1 expression at day 7. Accordingly, primary OBs showed increased trabecular induction and led to decreased IGF1 and increased bone formation in vivo, in both steady-state and pathological conditions, presumably as a result of increased mineralization capacity by osteoblasts.

**Conclusions** Our findings indicate that JAK-inhibition by tofacitinib and baricitinib increases bone formation in vivo, in both steady-state and pathological conditions, presumably as a result of increased mineralization capacity by osteoblasts.

**REFERENCE**


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**P149/006**

**HIGHER EXPRESSION OF SIGLEC-7/9 ON GRANULOCYTES AND MONOCYTES HAS A PROTECTIVE ROLE IN RHEUMATOID ARTHRITIS**

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Career situation of first and presenting author

**Student** for a master or a PhD.

**Introduction** Prominent features of rheumatoid arthritis (RA) are pain, joint swelling and erosion induced by massive infiltration of immune cells into the inflamed tissue. Among these immune cells, granulocytes and monocytes play a crucial role. Both express sialic acid-binding immunoglobulin-like lectins (Siglecs) —7 and 9 (in mice Siglec-E), trans-membrane proteins generally transmitting inhibitory signals through immunoreceptor tyrosine-based inhibitory motifs in their cytoplasmic tails. So far, there has been only limited research on the effects of Siglecs in RA.

**Objectives** To evaluate the potential role of Siglec 7- and 9 during disease progression in RA by comparing the expression on blood leukocytes with disease activity in RA patients. Additionally we monitored Siglec-E knockout mice in an experimental arthritis model.

**Methods** Blood samples from 45 healthy donors (HD) and 37 RA patients were analyzed by flow cytometry for Siglec-7 and 9 expressions on NK cells (CD14+/CD56+/CD3-), granulocytes (CD16+/CD19-) and monocytes (CD14+/CD16-). Expression was correlated with autoantibody positivity and disease severity.

Siglec-E knockout mice and wildtype controls were used in a serum transfer induced arthritis model and disease manifestation was monitored by measurement of paw swelling. Additionally we performed functional ex vivo assays to study Siglec impact on granulocytes and monocytes.

**Results** There was a diminished Siglec-7/9 expression on NK cells from RA patients, especially in highly CCP positive patients (>500 U/ml), while no differences were observed in neutrophils and monocytes compared to healthy controls. Interestingly we observed a correlation between a higher DAS28 score and lower Siglec-9 expression levels on granulocytes in seropositive patients.

Siglec-E knockout mice displayed higher disease scores and paw swelling in serum transfer induced arthritis, especially at the peak and the resolution phase. Functional studies unraveled that granulocytes are in a more active state if Siglec-7 and 9 are missing.

**Conclusions** Together our data support that Siglec-7/9 expression negatively correlates with pathogenesis and disease manifestation in RA patients. We showed in vivo and ex vivo, that Siglecs possess an important role concerning granulocyte and monocyte function and are capable of dampening immune response. Due to their inhibitory potential, Siglecs should be considered as potential therapeutic starting point in future studies.
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Disclosure of Interest None declared.

IMPACT OF RADON SPA ON PAIN AND THE IMMUNE SYSTEM OF PATIENTS WITH MUSCULOSKELETAL DISORDERS

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

Introduction The pain reducing effects of certain natural springs containing the radioactive noble gas radon have been described for centuries. Today, one expects that small cumulative dose of radiation of about 0.3 mSv originating from radon progeny impacts on the immune system and bone metabolism of patients suffering from chronic painful degenerative and/or inflammatory diseases. However, osteoimmunological analyses of patients during radon spa were lacking.

Objectives We initiated the observational and explorative RAD-ON01 study to analyse for the first time the impact of radon spa on the immune status in the whole blood of 100 patients.

Methods Whole blood of the patients was drawn before and at weeks 6, 12 and 30 after therapy. Deep immunophenotyping was performed by multicolour flow cytometry and cytokine analyses by ELISA.

Results The RAD-ON01 study confirmed a long-lasting pain reduction. While the major immune cells were only marginally affected, in particular regulatory T cells and dendritic cells were temporarily increased and activation markers on immune cells were decreased. Further, a decrease of serum markers related to bone erosion was observed. The cytokine analyses showed that temporarily increased TGFβ following radon spa correlates with reduced pain perception of the patients. To exclude placebo effects, in November 2018 the RAD-ON02 study (EUDRACT: 2016-002085-31) started. With this prospective, temporarily placebo-controlled and double-blinded trial the evidence level of radon spa application and knowledge on osteoimmunological modes of action of radon should be improved.

Conclusions We conclude that patients with musculoskeletal disorders do benefit from radon spa and that osteoimmunological mechanisms are modulated by exposure of the patients to very low doses of radiation. Future randomized studies should improve the evidence level for radon spa as therapeutic option for chronic painful degenerative and/or inflammatory diseases.

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Disclosure of Interest None declared.

NOCICEPTIVE PAIN IN ACUTE EXPERIMENTAL SYNOVITIS IS PARTLY MEDIATED BY THE ALARMIN S100A9

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

Introduction Inflammatory mediators like S100A8 and -A9 released by the synovium have been implicated in the regulation of pain. They may regulate pain either via direct stimulation of nerve endings in the synovium or via stimulation at the site of the dorsal root ganglia (DRG), enabling an increased phagocyte infiltration and causing sensitization.

Objectives To elucidate the role of S100A9 in the pain response after induction of an acute synovitis using streptococcal cell walls (SCW) as a trigger.

Methods Acute synovitis was induced by a single i.a. injection of SCW in the knee joint of C57Bl6 (WT) mice and S100A9+/- mice, control mice received a saline injection. Serum S100A8/A9 levels were investigated by ELISA. Joint swelling and cell influx was assessed by 99mTc accumulation and histology. Pain response were investigated using an Incapacitance Tester (weight bearing), Catwalk (gait analysis) and von Frey’s filaments (mechanical allodynia). Gene expression of inflammatory mediators and neuron activation markers in DRG were determined by q-PCR. Monocyte influx and protein expression was monitored by immunohistochemistry (IHC).

Results A single i.a. injection of SCW resulted in increased synovial and serum levels of S100A8 and S100A9 at day 1, which returned to basal levels at day 7. Joint swelling and cell influx were similar in WT and S100A9+/- mice at day 1 day excluding a role for S100A8/9 in synovitis. WT mice showed a marked and significant decrease in percentage of weight bearing on the SCW injected hindpaw (28%) compared to saline injection (47%, p<0.001) at day 1, whereas S100A9+/- mice did not. In addition, gait showed increased ‘limping’ in the WT mice, whereas the S100A9+/- mice did not. Both mouse strains showed a similar reduction of paw withdrawal threshold, excluding a role for S100A8/9 in alldynia. DRG showed no increased phagocyte infiltration after SCW injection and no change in gene expression of MCP-1, KC, IL-1β or TNF was measured. In addition, F4/80 staining was unchanged in both WT and S100A9+/- mice. However, neuron activation markers NAV1.7, ATF3 and GAP43 were significantly increased at 1 day after SCW injection in WT mice, compared to saline injected mice (p=0.022, 0.004 and 0.030, respectively) and not in S100A9+/- mice, which is in line in with the reduced pain response observed earlier in S100A9+/- mice. The difference in NAV1.7 expression in the DRG was further confirmed at protein level with IHC.

Conclusions These findings show that S100A9 is an important mediator of inflammatory nociceptive pain response, and not of peripheral sensitization. During the acute phase of inflammation S100A8/A9 is likely involved via direct activation of nerve endings in the synovium and not via macrophage infiltration in the DRG.

Disclosure of Interest None declared.
P152 INTERFERON-I ACTIVITY IN EARLY AND ESTABLISHED RHEUMATOID ARTHRITIS (RA) WITH AND WITHOUT CARDIOVASCULAR ABNORMALITIES IN CARDIAC MAGNETIC RESONANCE (CMR)

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Disclosure of Interest

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

Introduction RA is a heterogeneous disease and there is a substantial evidence to indicate the contribution of type I interferon (IFN-I) in 20%-40% of RA patients with possibly more local IFNβ role; compared to systemic IFN-α action in SLE. Patients with RA have an increased risk of cardiovascular disease (CVD) equivalent to type 2 diabetes, predominantly driven by excess atherosclerosis (ATS). Pre-clinical and human data suggest IFN-I plays a key role in the development of ATS. IFN-I has been shown to underlie cardiovascular (CV) abnormalities in SLE.

Objectives IFN-I is important at the initiation of the pathological processes in early RA and increased IFN-I activity is associated with CVD in RA.

Methods The Viral group recently published a continuous 2-score IFN system, IFN scores A and B (as opposed to an often used categorical classification of IFN high/low). We applied this scoring system in a cohort of early (ERA n=75) and established RA (EstRA n=101) as well as in HC n=71. Next RA patients were stratified for CVD using multi-parametric cardiac MRI (CMR) evaluation that included aortic distensibility (vascular stiffness), LV mass/BSA (LV geometry) and Myocardial T1 (indicating myocardial fibrosis) as follows: (i) ‘ERA-no CVD’ n=37 (no abnormalities on CMR), (ii) ‘ERA-sub CVD’ n=37 (at least one of the three parameters abnormal), (iii) ‘EstRA-no CVD’, n=14 (iv) ‘EstRA-sub CVD’, n=54 (v) ‘RA-CVD’, n=32 (defined as per ‘Major Adverse Cardiovascular Event’ (MACE)).

Results We confirmed a higher IFN score B than A in RA patients, similar to the observation made in the original paper that developed the scoring system. Significantly higher IFN score B was observed in ERA than in EstRA and HCs. There was no association between IFN scores and markers of inflammation. Increase in expression of IFN score B was observed across a CVD continuum (i.e. from RA-no CVD to RA-sub CVD to RA-CVD) in EstRA but not in ERA.

Conclusions IFN-I may play a particularly important pathological role at time of development of disease (ERA). CVD stratification suggests that genes included in IFN Scores A and B may be implicated in the progression along a CVD continuum; and appearing to associate with a pro-atherogenic role. This observation only in EstRA may reflect CVD burden over time. If confirmed, these data imply multiple organ specificities for the IFN scores. Further work is planned to interrogate IFN status in RA-CVD, towards improved risk stratification and tailored management of CVD co-morbidity.

REFERENCE


Disclosure of Interest None declared.

P154 LOCAL LOW DOSE RADIATION INDUCES SYSTEMIC IMMUNE ALTERATIONS IN TWO EXPERIMENTAL MODELS OF IMMUNOLOGIC ARTHRITIS

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Disclosure of Interest None declared.

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

Introduction Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory autoimmune disease that mainly affects the joints with its hallmarks being synovial inflammation followed by cartilage and bone destruction. There are a plethora of treatment options available, however, not all patients respond properly. In these patients it is crucial to slow down bone loss and inflammation in a timely manner to prevent further damage. Here, a therapy with low-dose ionizing radiation, the so called low-dose radiotherapy (LD-RT), could be an additional option. Detailed knowledge on the underlying mechanisms of reduced bone destruction and immune-mediated pain levels in patients following LD-RT is still scarce. We already showed that LD-RT locally slows down disease progression in human TNFα transgenic (hTNFα tg) animals by mainly having an impact on bone metabolism.

Objectives We now aim to take a closer look on systemic immune-mediated effects of LD-RT in both, hTNFα tg mice, as a cytokine mediated model, and in the KRN serum transfer model, as an example for arthritogenic antibody dependence.

Methods hTNFα tg or serum-injected C57Bl/6 mice were locally irradiated with a single dose per fraction of 0.5 Gy. After an observation period of 7 days (serum-injected mice) or 30 days (hTNFα-tg mice), blood, bone marrow as well as hind paws and synovial fluid was taken and analyzed using multicolor flow cytometry or histomorphometry.

Results Treatment of hind paws of serum-injected C57Bl/6 mice with 1 × 0.5 Gy resulted in a systemic immune modulation. In the peripheral blood, an increase of eosinophils and a decrease of B cells and NK cells were observed. In the bone marrow, the prominent alteration was a shift from CD8+ to CD4+ T cells in both, the irradiated and non-irradiated leg. Furthermore, dendritic cells were decreased. In hTNFα tg animals, inflammation was modulated in a systemic manner after local LD-RT, while bone protection was a local effect.

Conclusions We conclude that LD-RT is not only a valuable tool for locally reducing inflammation and bone loss in the affected joints, but also has beneficial systemic immune-mediated effects. The observed immune modulations in the peripheral blood were similar to those observed for patients treated with LD-RT within the observational IMMO-LDRT01 study (NCT02633079). In the future, placebo-controlled studies with high patient numbers are desirable to clinically prove LD-RT as therapy for RA.

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Disclosure of Interest None declared.
IMMO-LDRT01 TRIAL: IMMUNOMODULATORY EFFECTS OF LOW DOSE RADIATION THERAPY OF CHRONIC DEGENERATIVE AND INFLAMMATORY DISEASES

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

Introduction The treatment of chronic inflammatory and/or degenerative diseases by low dose radiation therapy (LDRT) is a successful alternative treatment option, especially for patients who were refractory for treatment with drugs. Generally, LDRT aims to reduce pain of patients and to increase their mobility. Although LDRT has successfully been applied since the late 19th century, the underlying immunological mechanisms are only fragmentarily analyzed.

Objectives Therefore we have aimed to analyze the impact of locally delivered LDRT on systemic immune changes.

Methods For this the IMMO-LDRT01 study (NCT02653079) was initiated in 2016. The study will include 130 patients suffering from chronic inflammatory and/or degenerative diseases, such as arthritis, arthrosis or benign calcaneodynia. The patients are treated with six local irradiations (single dose per fraction of 0.5Gy) in three weeks. If necessary, the serial irradiation can be repeated after 8–12 weeks, in order to achieve a further reduction of the pain. Blood samples are taken before and after each serial irradiation, as well as during follow-up appointments of the patients. For detailed immunophenotyping of whole blood, we established a multicolor flow cytometry assay, which allows the monitoring of seven main immune cell types, 26 immune cell subtypes, and the activation status of the immune cells.

Results To date 80 patients have been included in the study. First evaluations showed a significant reduction of B cells and cytotoxic T cells in the peripheral blood of patients. In particular, LDRT impacted on activation markers of immune cells. One has to stress that the immunological effects of LDRT were dependent on the particular type of disease of the patients. Currently, the data of the immunophenotyping are correlated to pain perception and quality of life of the patients.

Conclusions These analyses will be helpful to optimize LDRT. A better patient stratification based on pain- and immune-related biomarkers is envisaged for the future, alongside with initiation of deliberated randomized clinical trials.

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Disclosure of Interest None declared.

SYSTEMIC INFLAMMATION ORIGINATING FROM THE SKIN OR THE INTESTINE COMBINED WITH BIOMECHANICAL STRESS HAS DIFFERENT EFFECTS ON THE JOINTS IN AN IN VIVO MOUSE MODEL

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

Introduction Psoriasis (PsO) and inflammatory bowel disease (IBD) share a wide range of comorbidities, including psoriatic arthritis (PsA). Entheses, the attachment sites of tendons and ligaments into the bones, spread the mechanical forces generated by movement onto the bone and are considered as a primary disease localization in PsA. Increasing evidence supports the hypothesis that biomechanical stress, together with inflammatory triggers from distant sites, such as the skin or the intestine, can contribute to the onset of PsA by inducing microdamage in the enthese.

Objectives In this study, we aimed to investigate the role of biomechanical stress together with cutaneous or intestinal inflammation in initiating joint disease.

Methods Eight-week-old male C57/B16 mice were treated with imiquimod cream (IMQ) on a shaved area of the back skin or with dextran sodium sulphate (DSS) dissolved in the drinking water to induce PsO-like skin or IBD-like gut inflammation. Control mice were left untreated. Afterwards, half of the mice were subject to a forced treadmill running protocol to increase biomechanical stress. Control mice with or without IMQ or DSS treatment did not run. Severity of cutaneous or intestinal inflammation was assessed clinically and by histology; knees and paws were analyzed by microCT, histology and immunohistochemistry.

Results Local induction of cutaneous or intestinal inflammation led to a systemic response, as detected by splenomegaly, trabecular subchondral bone loss and bone marrow hypercellularity.

On a background of PsO- but not of IBD-like inflammation, discrete signs of synovitis determined by the presence of CD45+ cells and overexpression of IL-17 were detected, with no significant impact of the running protocol. The CD45+ cells in the IMQ non-running condition were identified as F4/80+ cells and overexpression of IL-17 were detected, with no significant impact of the running protocol. The CD45+ cells in the IMQ non-running condition were identified as F4/80+ cells, in contrast to the running condition where the CD45+ cells could not be further identified, showing negative results for F4/80, CD3, MPO and MCSF-R stainings.

Furthermore, forced exercise and PsO-like inflammation both induced overexpression of IL-17 at the enthese and acted synergistically when combined.

Conclusions Local induction of PsO- or IBD-like inflammation triggers a systemic response with inflammation-associated bone loss and discrete signs of joint disease. PsO-like inflammation in combination with biomechanical stress increased the degree of synovitis and enthesis, showing that systemic inflammation combined with biomechanical stress may contribute to disease manifestations in PsA.

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RADIORESISTANT AND RADIONSENSITIVE CELLS CONTRIBUTE TO IL-18BP PRODUCTION IN A MODEL OF MACROPHAGE ACTIVATION SYNDROME

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Career situation of first and presenting author Student for a master or a PhD.
**Introduction** Interleukin (IL)-18 is a pro-inflammatory cytokine, the activity of which is regulated by its natural inhibitor IL-18 binding protein (IL-18BP). If the balance between IL-18 and IL-18BP is disregulated, abnormal levels of free bioactive IL-18 (sIL-18) are detected, such as in the sera of patients with macrophage activation syndrome (MAS). We showed that endogenous IL-18BP exerts a protective role in a murine model of MAS induced by repeated injections of the TLR9 agonist CpG. IL-18BP production is strongly increased in liver, lung, and spleen in this model, but its cellular origin is unknown.

**Objectives** To study the relative contribution of radioresistant and radiosensitive cells to IL-18BP production using bone marrow (BM) transfer experiments.

**Methods** Following whole-body irradiation, WT or Il18bp-/ recipient mice were reconstituted with WT or Il18bp+/ BM to create 4 groups of mice: WT recipient mice transferred with WT BM (WT/WT), WT/Il18bp-/-, Il18bp-/WT, and Il18bp-/-/ Il18bp-/- BM chimeric mice were challenged with CpG injections on days 0, 2 and 4 and sacrificed on day 7. We assessed body weight during the course of the experiment, blood cell counts before and after CpG injections and spleen weight after sacrifice. Liver, lung and spleen mRNA levels of Il18, Il18bp, Ifng, and IFN-γ signature genes Cxcl9 and Citi were determined by RT-qPCR. Circulating levels of IL-18BP, fIL-18, and CXCL9 were measured by ELISA.

**Results** The severity of CpG-induced MAS, as assessed by body weight changes, spleen weight and blood cell counts, was increased in Il18bp+/Il18bp-/- mice, but not significantly different between the three other groups. As reflected by a higher disease severity in Il18bp+/-Il18bp-/- mice, an enhanced IFN-γ signature and elevated levels of circulating sIL-18 were observed in these mice. In the other groups, IL-18BP levels were still sufficient to inhibit IL-18 activity. Indeed, circulating IL-18BP levels dropped drastically only in the Il18bp+/Il18bp-/- group. Despite this, Il18bp mRNA levels, as assessed by RT-qPCR, varied in the different organs, consistent with the relative contribution of radioresistant versus radiosensitive cells. Indeed, following CpG stimulation radioresistant cells were the main contributors in liver (65%) and lung (90%), whereas radiosensitive cells were the primary source of IL-18BP in spleen (80%).

**Conclusions** Our results demonstrate that IL-18BP is produced by both radioresistant and radiosensitive cells and is present at high levels in the circulation to prevent the deleterious systemic effects of IL-18 in MAS.

**Disclosure of Interest** None declared.

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**Introduction** Tumour necrosis factor (TNF) is important in immune-mediated inflammatory diseases such as spondyloarthritis (SpA). Transmembrane (tm)TNF-transgenic (tg) mice that overexpress tmTNF develop SpA symptoms, including inflammation, ectopic lymphoid structures (ELS) in bone marrow (BM), bone destruction and bone formation. SpA patients have extensive angiogenesis in inflammatory and bone forming regions.

**Objectives** To investigate whether there is a link between pathological angiogenesis and ELS in tmTNF tg mice in the BM.

**Methods** Ankles, femora, tibiae, vertebrae and spleens from 6 and 12 weeks and 8 months old tmTNF tg mice and wild-type (WT) littermates (n=5 per age per group) were dissected and analyzed with confocal microscopy and analyzed 12 week old tmTNF tg and WT mice (n=4) with flow cytometry. To study the importance of TNF-R signaling, tmTNF tg mice lacking TNF-R (tmTNF tggTNF-R/-) or TNF-RII (tmTNF tggTNF-R-II-/-) (n=4 per group) were used.

**Results** Immunofluorescent evaluation demonstrated that BM of tmTNF tg mice contained significantly more and extensive ELS. These ELS are limited to BM of the vertebrae and ankles, and are in close proximity of MECAT9+ high endothelial venules (HEVs). ELS predominantly consisted of B220+ B cells, of which most are IgD- naive B cells. Preliminary flow cytometric analysis revealed a trend towards an increase in IgD-CD95- germinal center B cells and CCRX5+PD-1+FoxP3+CTLA4+ T follicular helper cells and CCRX5+PD-1+FoxP3+CTLA4+ in the vertebrae of tmTNF tg mice compared to WT littermates. Meanwhile, B cell lineages in the BM of tmTNF tg hind limbs were not altered. Furthermore, preliminary data indicates that BM and spleen from tmTNF tg mice contain more IgD+ plasma cells compared to WT littermates. tmTNF tggTNF-R/- mice did not display lymphoid aggregates or HEVs in the BM, while tmTNF tggTNF-R-II-/- mice did, although to a lesser extent than tmTNF tg mice.

**Conclusions** tmTNF overexpression in mice results in extensive ELS associated with HEVs in the BM, which is likely to be mediated through TNF-R signaling. HEV formation may lead to persistence of inflammation in the BM which contributes to pathology.

**REFERENCE**

**Disclosure of Interest** None declared.
Introduction Gout flares are characteristically mediated by the pro-inflammatory cytokine interleukin (IL)-1β. Uptake of monosodium urate (MSU) crystals by macrophages activates the nucleotide-binding domain and leucine-rich repeat containing family, pyrin domain containing 3 (NLRP3) inflammasome, which converts intracellular pro-interleukin-1β (pro-IL-1β) to mature bioactive IL-1β by proteolytic cleavage. Deposition of MSU crystals alone is not sufficient to trigger a gout flare. In the presence of concomitant pro-inflammatory stimuli, i.e. Toll-Like Receptor (TLR) agonists, transcription of il-1β gene is induced and pro-IL-1β is rapidly converted into its active form. IL-1β binds to its receptor (IL-1R1) and induces a cascade of secondary inflammatory mediators including prostaglandins, cytokines and chemokines. Recently, OLT1177™, a β-sulfonil nitrile compound, safe in humans, was shown to inhibit the NLRP3 inflammasome, reverse the metabolic cost of inflammation and inhibit joint inflammation in murine models of acute arthritis.

Objectives To explore the mechanism by which oral OLT1177™ inhibits joint inflammation in humans with gout flares.

Methods 29 patients with a gout flare were treated within 4 days after the start of the symptoms with three different doses of OLT1177™ for 7 days (EudraCT: 2016-000943-14). Blood was drawn at baseline, days 3, 7 and 14 (7 days after finishing treatment). Haematology, hsCRP and SAA were measured as markers for systemic inflammation. Plasma was collected for assessment of circulating cytokines. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured under unstimulated or stimulated conditions with a TLR ligand (Pam3Cys and LPS) in combination with MSU crystals after which intra- and extracellular cytokine production was assessed.

Results Plasma IL-1β are increased in samples from intercritical gout patients and individuals with gout flares when compared to healthy controls (healthy controls hsCRP and SAA and acute phase proteins of individuals treated with OLT1177™ during a gout flare show a dose-dependent reduction during course of treatment. Circulating IL-1β and IL-6, but not TNFsα, was reduced and stimulated cytokine production of PBMCs declined during treatment. In unstimulated PBMCs on day 3, ratio of intracellular pro-IL-1β and IL-1β revealed inhibition of the NLRP3 inflammasome by oral OLT1177™. Moreover, in vitro and ex vivo data show OLT1177™ spontaneously increases the level of IL-1 receptor antagonist (IL-1Ra).

Conclusions Oral OLT1177™, safe in humans, inhibits inflammation and increases systemic IL-1Ra concentrations in patients with an acute gout flare by inhibiting the NLRP3 inflammasome product, IL-1β.

REFERENCES

Acknowledgements We wish to acknowledge the gout patients who participated as well as Daniëlle Poeth and Dorine Baselmans for their commitment to the conduct of this study.

Potential investigators conflict of interest D5 is Founder and CEO of Olatec, CD is CSO and SAB member of Olatec, LJ is SAB member of Olatec.

Disclosure of Interest None declared.

P160 THE FIRST PHASE 2A PROOF-OF-CONCEPT STUDY OF A SELECTIVE NLRP3 INFLAMMASOME INHIBITOR, DAPANSUTRILE™ (OLT1177™), IN ACUTE GOUT

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Career situation of first and presenting author Young investigator.

Introduction Acute gout is a severe debilitating type of arthritis that is treated in the acute phase with potent anti-inflammatory drugs. To date, prednisolone, colchicine and/or non-steroidal anti-inflammatory drugs are the standard of care despite serious side effects of chronic use, especially in the older population most often presenting with an acute gout attack. In addition, interleukin-1 (IL-1) biologics (e.g., canakinumab/rilonacept/analina) have proven efficacy in RCTs; however, these biologics have not been broadly adopted due to barriers such as the requirement of parenteral administration, cost and risk of infection. There is an unmet need for a safe, oral, cost effective IL-1 inhibitor targeting the NLRP3 inflammasome pathway.

Dapansutrile™ (OLT1177™) has been shown to inhibit IL-1β and IL-18 release in human macrophages and to prevent NLRP3 inflammasome activation, with no effect on the priming phase of the NLRP3 inflammasome formation or on TNF-alpha. Dapansutrile™ has no effect on the AIM2 or NLRC4 inflammasomes. In addition to acute gout, dapansutrile™ is under clinical development in heart failure and it has demonstrated positive results in numerous preclinical models. Dapansutrile™ Phase 1 dose escalation clinical trial demonstrated safety at doses up to 1000 mg/day for 8 days.

Objectives The Phase 2a study is a dose ranging, proof-of-concept trial to demonstrate the clinical effectiveness, pharmacodynamics (e.g., cytokine levels and other relevant biomarkers), safety/tolerability and pharmacokinetics of dapansutrile™ in four cohorts at doses of 100 mg QD, 300 mg QD, 500 mg BID or 500 mg QID.

Methods An adaptive dose design was used with planned enrollment of 8 patients per cohort to assess the efficacy of dapansutrile™ in treating the clinical signs and symptoms of acute gout over an 8 day treatment period. Clinical effect was targeted to be greater than 50% pain reduction from baseline at approximately 72 hours after the first dose. Cohorts 1, 2 and 3 were administered dapansutrile™ doses of 500 mg BID, 500 mg QID and 300 mg dapansutrile™ (200 mg at 08.00 hour and 100 mg at 20.00 hour), respectively. Cohort 4 is currently enrolling subjects given 100 mg dapansutrile™ QD. VAS pain, general disability and walking disability scores were measured by daily diary and blood sampling was conducted on study days 0 (baseline), 3, 7 and 14 to assess PK and PD (including plasma cytokine levels, hsCRP, inflammasome activity, etc). Safety was measured over the duration of
the study with clinic visits on study days 0 (baseline), 3, 7, 14 and a follow-up telephonic visit on day 35.

**Results** A significant clinical and inflammatory cytokine response at Day 3 was seen in all dose groups and will be elaborated upon once the final datasets become available. There were no metabolic, physiological or hematological changes and all doses were well tolerated.

**Conclusions** On the basis of both the clinical response and the biomarkers, dapsansutri™ is a safe and effective anti-inflammatory oral NLRP3 inhibitor in the treatment of acute gout with a broad therapeutic range and promise for further clinical development in this indication.

**Acknowledgements** We wish to acknowledge the gout patients willing to participate as well as Daniëlle Poeth and Dorine Baselmans for their commitment to the conduct of this study.

**Potential investigators conflicts of interest** DS is founder and CEO of Olatec, CS is CMO of Olatec, RB is COO of Olatec, CD is CSO and SAB member of Olatec, LJ is SAB member of Olatec.

**Disclosure of Interest** None declared.

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**P161 THE RELATION BETWEEN THE INFLAMMATORY STATUS OF HUMAN END STAGE OA SYNOVIM AND LEVELS OF LOW DENSITY LIPOPROTEIN**

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

**Introduction** High systemic levels of low density lipoprotein (LDL) is a shared metabolic risk factor for osteoarthritis (OA) and atherosclerosis, which may point to common biochemical pathways. One of the key events in atherosclerosis is formation and uptake of oxidized LDL (oxLDL) as a consequence of macrophage-mediated inflammation. In mice, high LDL levels enhanced synovial activation during development of collagenase-induced OA, and injection of oxidized LDL in naïve macrophage-depleted joints led to infiltration of myeloid progenitor cells. However, a role for (ox)LDL in human OA has not yet been shown.

**Objectives** In the present study, we investigated whether the inflammatory status of OA synovium is associated to systemic and local levels of (ox)LDL.

**Methods** Blood was collected from patients planned to undergo total knee replacement and LDL serum levels were determined (n=16). After surgery, synovial explants from the same patients (2–8 per patient) were collected and incubated at 37°C for 24 hours in presence of LDL (50 µg ApoB/mL), oxLDL (50 µg ApoB/mL) or control medium. Levels of IL-1β, TNF-α, IL-10 and MCP-1 secreted in the conditioned medium were measured using Lumixen, and S100A8/A9 using ELISA. Immunohistochemical staining of ApoB was performed to assess presence and uptake of (ox)LDL in the synovium. Hematoxylin and eosin-stained sections were used to score cell infiltration and synovial hyperplasia.

**Results** Around 50% of the patients in our cohort showed synovial activation exemplified by high secretion levels of IL-1β, TNF-α and IL-10. Cellular infiltration and synovial hyperplasia was observed in the majority of synovial tissue samples, their grades did however not correlate with cytokine secretion. Secretion levels of IL-1β positively correlated with serum levels of LDL and total cholesterol and did not correlate with BMI, suggesting a link that runs via systemic metabolic processes rather than increased weight-mediated joint loading. Staining of LDL associated protein ApoB in the synovia showed distinct speck-like staining within macrophages and endothelial cells especially, suggesting oxLDL accumulation in these cells. Unexpectedly, presence of high concentrations of LDL or oxLDL during incubation of synovial explants did not significantly affect secretion of IL-1β, TNF-α, IL-10 and MCP-1.

**Conclusions** IL-1β secretion by end stage OA synovium is associated to systemic levels of LDL. Intervention with high concentrations of LDL or oxLDL for 24 hours after synovium extraction did however not alter cytokine secretion, suggesting that LDL and synovitis are linked through more long-term local interactions, or alternatively via systemic inflammatory factors.

**Disclosure of Interest** None declared.

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**P162 EXTRACELLULAR VESICLES FROM PATIENTS WITH ANKYLOSING SPONDYLITIS CONVEY PROTEINS RELATED TO IMMUNE RESPONSE: NEW SIGNALING PATHWAYS?**

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**Career situation of first and presenting author** Young Investigator.

**Introduction** Ankylosing spondylitis (AS) is a chronic inflammatory disease, whose pathogenesis is still unclear. Many studies show the proteins in extracellular vesicle (EVs) would change regularly in many diseases. However, none of study has focused on the proteins profiles of serum-derived EVs in AS patients.

**Objectives** The study aim to detect the proteins profiles of serum-derived EVs in AS patients by Label-free-LC-MS/MS technology.

**Methods** EVs were isolated by ExoQuick-TC™ kit. The protein contents were evaluated by Label-free-LC-MS/MS technology. Venn diagram, volcano plot, hierarchical cluster, Gene Ontologies (GO), Encyclopedia of Genes and Genomes (KEGG) pathway and Protein-Protein Interaction (PPI) network analysis were constructed. Enzyme-linked immunosorbent assay (ELISA) was used to confirm the expression of the differently-expressed proteins.

**Results** Label-free-LC-MS/MS results indicated a total of 677 proteins were detected, of which 65 were unique to AS group, 545 were identified in both AS and control group, and 67 were unique to control group. A heat map of the hierarchical clustering revealed a clearly distinct expression of proteins in AS group, compared with control group. With the cut off criteria of |log2 (fold-change)| ≥ 1, p < 0.01 and unique peptides ≥ 2, 31 up-regulated proteins and 42 down-regulated proteins were found in the AS group. Among them, Serum amyloid A-1 (SAA1) protein and Complement C4-B were highly expressed in AS group. PPI network analysis yielded a highly clustered network (clustering coefficient: 0.584), with prothrombin (F2) and c-reactive protein (CRP) as the central/hub proteins. GO analysis of the biological process indicated that the differentially expressed proteins were significantly enriched.
in the ‘Complement activation’. GO analysis of the cellular components demonstrated that most proteins were annotated as exosome, vesicle and micro-particle. KEGG pathway analysis revealed proteins were significantly enriched in ‘Complement and coagulation cascades’, ‘Staphylococcus aureus infection’, ‘Systemic lupus erythematosus’ and ‘PI3K-Akt signaling pathway’. ELISA demonstrated that both EVs-derived SAA1 and serum SAA1 were more abundant in AS group, showed a consistent with the Label-free-LC-MS/MS data.

Conclusions Differentially expressed proteins were found in serum-derived EVs of AS patients, and the functional analysis indicated that they may potentially involved in immune response.

REFERENCE

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Disclosure of Interest None declared.
Molecular and cellular biomarkers that impact of disease activity, duration and inflammatory status, skeletal muscle involvement by manual muscle testing (MMT-8, iDXA Lunar, and by bioelectrical impedance: BIA2000-M), and physical activity (by Human Activity Profile questionnaire, HAP). Routine biochemistry analysis was performed after 8 hours of fasting. Disease activity was evaluated by MITAX and MYOACT activity score, muscle involvement by manual muscle test (MMT-8) and functional index 2 (FI2).

Results Compared to HC, patients with IIM had significantly increased body fat% (BF%) but significantly decreased lean body mass (LBM), bone mineral density (BMD) and increased extracellular mass/body cell mass (ECM/BCM) ratio, which reflects worse muscle predispositions for physical activity and deteriorated nutritional status. Disease duration negatively correlated with BMD and LBM (assessed by BIA). Disease activity assessed by MITAX and MYOACT positively correlated with LBM (assessed by both BIA and DXA), as well as with basal metabolic rate (BMR), and fat free mass (FFM). CRP was positively associated with BF% (iDXA and BIA). Higher BF% (iDXA) was associated with worse physical endurance (FI2) and worse ability to perform physical activity (HAP). MMT-8 score negatively correlated with ECM/BCM ratio.

Conclusions In this study, we propose for the first time a SF-based miRNA signature as well as 5 myeloid cell subsets that discriminate between oJIA and SA and might be used as potential diagnosis markers in juvenile arthritis. Moreover, these markers give an insight into the mechanisms that are differentially altered between inflammatory and infectious arthritis.

Disclosure of Interest None declared.

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P166 IMPACT OF DISEASE ACTIVITY, DURATION AND MUSCLE INVOLVEMENT ON CHANGES OF BODY COMPOSITION IN MYOSITIS PATIENTS

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

Introduction Skeletal muscle, pulmonary and articular involvement in idiopathic inflammatory myopathies (IIM) limit the mobility/self-sufficiency of patients, and can have a negative impact on body composition.

Objectives To assess body composition and physical activity of IIM patients and healthy controls (HC).

Methods 54 patients with IIM (45 females; mean age 57.7; disease duration 5.8 years; PM, 22/DM, 25/IMNM, 7) and 54 age-/sex-matched HC (45 females, mean age 57.7) without rheumatic/tumor diseases were included. PM/DM patients fulfilled Bohan/Peter criteria for PM/DM. We assessed anthropometric parameters and body composition (by densitometry: iDXA Lunar, and by bioelectric impedance: BIA2000-M), and physical activity (by Human Activity Profile questionnaire, HAP). Routine biochemistry analysis was performed after 8 hours of fasting. Disease activity was evaluated by MITAX and MYOACT activity score, muscle involvement by manual muscle test (MMT-8) and functional index 2 (FI2).

Results Compared to HC, patients with IIM had significantly increased body fat% (BF%) but significantly decreased lean body mass (LBM), bone mineral density (BMD) and increased extracellular mass/body cell mass (ECM/BCM) ratio, which reflects worse muscle predispositions for physical activity and deteriorated nutritional status. Disease duration negatively correlated with BMD and LBM (assessed by BIA). Disease activity assessed by MITAX and MYOACT positively correlated with LBM (assessed by both BIA and DXA), as well as with basal metabolic rate (BMR), and fat free mass (FFM). CRP was positively associated with BF% (iDXA and BIA). Higher BF% (iDXA) was associated with worse physical endurance (FI2) and worse ability to perform physical activity (HAP). MMT-8 score negatively correlated with ECM/BCM ratio.

Conclusions We found significant negative changes in body composition of our IIM patients compared to healthy age-/sex-matched individuals, associated with their disease activity and duration, inflammatory status, skeletal muscle involvement, and physical activity. These data could reflect their impaired nutritional status and predispositions for physical exercise, aerobic fitness and performance.
Introduction Fibrosis of the skin and visceral organs, especially digestive tract, and musculoskeletal involvement in systemic sclerosis (SSc) can have a negative impact on body composition and physical activity.

Objectives The aim was to assess body composition and physical activity of SSc patients and healthy controls (HC) and the association with selected inflammatory cytokines in SSc.

Methods 59 patients with SSc (50 females; mean age 52.5; disease duration 6.7 years; kSSc:34/dcSSc:25) and 59 age-/sex-matched HC (50 females, mean age 52.5) without rheumatic or tumour diseases were included. SSc patients fulfilled ACR/EULAR 2013 criteria. We assessed body composition (densitometry: iDXA Lunar, bioelectric impedance: BIA-2000-M), physical activity (Human Activity Profile, HAP questionnaire), disease activity (ESSG activity index) and serum levels of 27 cytokines (commercial multiplex ELISA kit, Bio-Rad Laboratories). Data are presented as mean ±SD.

Results Compared to HC, patients with SSc had significantly lower body mass index (BMI), body fat% (BF%) and visceral fat weight (VF), and also significantly decreased lean body mass (LBM), and bone mineral density (BMD). Compared to HC, patients with SSc had increased extracellular mass/body cell mass (ECM/BCM) ratio, reflecting deteriorated nutritional status and worse muscle predispositions for physical activity. Increased ECM/BCM in SSc positively correlated with disease activity (ESSG), skin score (mRSS) and inflammation (CRP, ESR), and was associated with worse quality of life (HAQ, SHAQ), fatigue (FSS), and decreased physical activity (HAP). ESSG negatively correlated with BF%. HAP positively correlated with BMD. Increased serum levels of several inflammatory cytokines were associated with alterations of body composition.

Conclusions Compared to healthy age-/sex-matched individuals we found significant negative changes in body composition of our SSc patients, which are associated with the disease activity and physical activity, and could reflect their nutritional status, and gastrointestinal and musculoskeletal involvement. Serum levels of certain inflammatory cytokines were associated with alterations of body composition in SSc patients.
Disclosure of Interest None declared.

P169 EFFECT OF MACROPHAGE MIGRATION INHIBITORY FACTOR ON HUMAN MACROPHAGES FROM ARTHRITIS PATIENTS

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

Introduction Macrophage migration inhibitory factor (MIF) is a key regulator of pro-inflammatory cytokines and has been implicated in angiogenesis and pathogenesis of several diseases such as rheumatoid arthritis (RA). Macrophages are considered to be one of the key players in the hyperplastic synovial tissue that invades and degrades adjacent cartilage and bone in patients with inflammatory arthritis.

Objectives In this study a comparative analysis was performed to examine the expression of MIF, and the effect it has on the macrophage polarisation and on the angiogenic and inflammatory mechanisms of macrophages in patients with RA, Psoriatic Arthritis (PsA), Osteoarthritis (OA) and in Arthralgia patients.

Methods PBMCs (Peripheral Blood Mononuclear Cells) were isolated from healthy donors, and patients with OA, RA, PsA and Arthralgia. Primary macrophages (Mfs) were subsequently differentiated and polarised from circulating CD14+ monocytes into M1 and M2 phenotypes. The levels of MIF expression in PBMC, Mf and Synovial tissue was evaluated by real-time PCR (RT-PCR) and Immunohistochemistry (IHC). The effect of MIF on polarisation of Mfs was investigated by examining M1 and M2 Mf specific markers by RT-PCR and Flow Cytometry. Polarised Mf supernatants were harvested and assayed for soluble MIF by ELISA. The effect of MIF on angiogenic and inflammatory markers (MCP-1, IL-6, IL-8, Ang-2, VEGF, Hif1α, PDGF-B, bFGF, RANTES and ICAM-1) of polarised Mfs was investigated by Real-PCR, Western blot and ELISA.

Results MIF expression was significantly increased in RA tissue compared to OA and PsA. In contrast MIF expression in RA PBMCs was significantly decreased when compared to HC, Arthralgia, and PsA. RA tissue biopsies demonstrated significantly higher MIF expression when compared to PsA, OA, and Arthralgia. In polarised macrophages MIF expression was found to be increased in RA and PsA compared to healthy controls. Addition of rhMIF activated pro-inflammatory and angiogenic responses in unpolarised and polarised HC Mfs with increases in gene expression levels of IL-1β, IL-6, MCP-1, ICAM-1, Hey-1, VEGF and Hif1α. Soluble IL-6 expression was also elevated in M0 macrophages.

Conclusions MIF may have a key role in promoting pathogenesis of RA and has a good potential as a therapeutic for RA.

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Abstracts

P171 HSP90 IN AXIAL SPONDYLOARTHRITIS, PSORIATIC ARTHRITIS AND RHEUMATOID ARTHRITIS
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Disclosure of Interest None.

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References

None.

Career situation of first and presenting author Young investigator.

Introduction Hsp90 is a highly conserved molecular chaperone that regulates activation of innate immunity, antigen presentation, and the induction of proinflammatory cytokines and chemokines. These properties predispose Hsp90 to its potential role in the pathogenesis of autoimmune inflammatory rheumatic diseases.

Objectives The aim of this study was to assess plasma Hsp90 in patients with axial spondyloarthritis (axSpA), psoriatic arthritis (PsA), rheumatoid arthritis (RA) and healthy controls (HC) and to determine its potential association with disease activity and clinical features.

Methods A total of 136 patients, that were divided into groups according to the type of a rheumatic disease (SpA, RA, PsA), and age-/sex- matched healthy individuals were included. Plasma Hsp90 levels were measured by ELISA (eBioscience, Vienna, Austria). Data are presented as median (IQR).

Results Plasma Hsp90 levels were significantly increased in axSpA and in RA patients compared to HC. The increased plasma levels of Hsp90 in PsA compared to HC did not reach statistical significance. Hsp90 concentrations were higher in RA patients with an altered serum lipid profile: low-density lipoprotein (LDL: r=0.352, p=0.048), high-density lipoprotein (HDL: r=−0.349, p=0.046) and atherogenic index (calculated as log (triglycerides/HDL)) (AI: r=0.454, p=0.009). Furthermore, Hsp90 levels in r-axSpA patients positively correlated with the MRI presence of active inflammatory lesions in sacroiliac joints (SPARCC MRI score for SI joints: r=0.594, p=0.020). Increased Hsp90 levels in PsA patients were associated with the count of joint deformities (r=0.526, p=0.025). No further statistically significant associations were found between Hsp90 plasma levels and RA-, axSpA- or PsA-specific clinical features.

Conclusions We demonstrated elevated plasma concentrations of Hsp90 in RA and in axSpA patients compared to healthy controls. Hsp90 could be associated with early alterations of serum lipids and development of atherosclerosis in RA. Furthermore, in r-axSpA, Hsp90 may represent an independent marker of SI joint inflammation, whereas in PsA, plasma Hsp90 correlates with joint deformities. These data suggest that Hsp90 could become a potential biomarker of structural changes in SpA.

REFERENCE

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P172 NLRP3 INFLAMMASOME-MEDIATED PYROPTOSIS AGGRAVATES THE AIRWAY INFLAMMATION IN TOLUENE DIISOCYANATE-INDUCED ASTHMATIC MICE
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Disclosure of Interest None.

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Career situation of first and presenting author Young investigator.

Introduction NLRP3 inflammasome-mediated pyroptosis is a highly inflammatory event. This cell death process is characterized by cell explosion and IL-1β release.1 Our previous data have revealed that NLRP3 inflammasome-mediated pyroptosis plays an essential role in the pathogenesis of collagen-induced arthritis mice and lupus-like mice. While blockade of NLRP3 inflammasome-mediated pyroptosis alleviates the inflammation and organ damage in these autoimmune mice models.

Objectives Interesting, Toluene diisocyanate(TDI), the most common organic compound causing occupational asthma, has been proven to drive bronchial epithelium damage and IL-1β production in patients with TDI-induced asthma. Therefore, in this study, we investigated whether NLRP3 inflammasome-mediated pyroptosis was involved in bronchial epithelial injury and airway inflammation in TDI-induced asthma.

Methods In vivo, 16HBEs (a human bronchial epithelial cell line) were stimulated with TDI. Then the cell death form was identified. In vivo, TDI-induced asthmatic mice were established after sensitization and challenge with TDI. After treatment with the NLRP3 inflammasome specific inhibitor, the airway hyperresponsiveness (AHR) and airway inflammation in asthmatic mice were assessed.

Results Here we found that TDI induced 16HBEs pyroptosis in a time and dose-dependent manner, as evidenced by the increased ratio of caspase-1 "PI" cells and the enhanced levels of LDL and IL-1β. Importantly, the NLRP3 inflammasome specific inhibitor significantly blocked TDI-induced pyroptosis. In TDI-induced asthmatic mice, NLRP3 inflammasome inhibitor attenuated AHR and airway inflammatory infiltration by inhibiting the activation of caspase-1 and the cleavage of pyroptotic executioner GSDMD in the lungs. Moreover, NLRP3 inflammasome inhibitor decreased the levels of IL-1β in the plasma and bronchoalveolar lavage fluid (BALF), accompanied by lower level of IgE in the plasma and Th2-related cytokines in the BALF.

Conclusions Our data demonstrated for the first time that bronchial epithelial pyroptosis was critical for TDI-induced asthma pathogenesis via the activation of NLRP3 inflammasome and the cleavage of GSDMD. Inhibition of NLRP3 inflammasome-mediated pyroptosis may be a valuable therapeutic strategy for TDI-induced asthma.

REFERENCE

Jian Zhuang and Yi He contributed equally as co-first authors.
Haoyan Cui and Erwei Sun are corresponding authors.
Disclosure of Interest None declared.

**P173** COMPLEX ASSESSMENT OF BONE MINERAL DENSITY, FRACTURE RISK, VITAMIN D STATUS AND BONE METABOLISM IN HUNGARIAN SYSTEMIC SCLEROSIS PATIENTS

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Career situation of first and presenting author Instructor. Osteoporosis has been associated with systemic sclerosis (SSc).

Objectives We wished to determine bone alterations in SSc patients by conventional densitometry (DXA), peripheral quantitative computed tomography (pQCT) and bone biomarkers.

Methods We included 44 SSc patients and 33 age-matched healthy controls. Lumbar spine and femoral neck bone mineral density (BMD) was assessed by DXA. Volumetric BMD was measured by pQCT at the radius. FRAX, 25-hydroxyvitamin-D3 (25-OH-D3), parathyroid hormone, osteocalcin, C-terminal collagen telopeptide and procollagen type I amino-terminal propeptide were also assessed.

Results SSc patients had lower L2–4 BMD (0.880±0.108 vs. 0.996±0.181 g/cm2; p=0.019) and femoral neck (FN) BMD (0.786±0.134 vs. 0.910±0.090 g/cm2; p=0.007) by DXA. In SSc vs controls, pQCT indicated lower mean cortical (328.03±103.32 vs. 487.06±42.45 mg/cm2; p<0.001) and trabecular density (150.93±61.91 vs. 184.76±33.03 mg/cm2; p=0.037). Vitamin D3 deficiency was more common in SSc vs controls (60.0% vs 39.3%; p=0.003). L2–4 (p=0.002) and FN BMD (p=0.015) positively correlated with BMI. pQCT assessments confirmed an inverse correlation between pulmonary manifestation and total (p=0.024), trabecular (p=0.035) and cortical density (p=0.015). Anti-ScI70 positivity inversely correlated with pQCT total density (p=0.015) and the presence of digital ulcers with cortical density (p=0.001). We also found that vertebral and FN BMD as determined by DXA significantly correlated with pQCT total, trabecular and cortical density (p<0.05).

Conclusions The results of our study suggest that bone loss in SSc patients may be associated with lower BMI, anti ScI70 positivity, and the presence of pulmonary manifestations and digital ulcers. Both DXA and pQCT are appropriate tools to evaluate the bone alterations in SSc patients.

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Disclosure of Interest None declared.
COMPARATIVE METABOLOMIC AND LIPIDOMIC ANALYSIS OF SERUM SAMPLES FROM PATIENTS WITH SERONEGATIVE RHEUMATOID ARTHRITIS AND PSORIATIC ARTHRITIS

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Introduction Rheumatoid arthritis (RA) is a chronic autoimmunem disease causing polyarthritis and joint destruction and affecting 1% of the population. Psoriatic arthritis (PsA) is a chronic inflammatory arthropathy which affects 20%–30% of patients with psoriasis. The diagnosis of RA is based on the clinical symptoms and the serological titer of rheumatoid factor (RF) and the anti-citrullinated peptide antibodies (anti-CCP). Although most RA patients are seropositive, in about 15%–20% of the cases the level of RF and anti-CCP remains within the normal range.

Objectives Since the symptoms between RA and PsA can be very similar, the differential diagnosis between seronegative RA (negRA) and PsA is often difficult. Early differential diagnosis is important for the choice of the best treatment strategy. By assessing the serum metabolomic and lipidomic profiles of PsA and negRA patients, we aimed at identifying potential biomarkers to distinguish these pathologies.

Methods Serum from 31 negRA and 77 PsA patients were collected for metabolic profiling using 1H NMR spectroscopy. For all samples, regular 1H acquisition with presaturation and Carr–Purcell–Meiboom–Gill (CPMG) spectra were acquired using a 600 MHz Bruker NMR spectrometer. Spectra were processed with TopSpin using 0.3 Hz of line broadening and manual phasing. For metabolomics and lipidomics integration, spectra were subjected to bucketing by AMIX from 0.75–8.5 ppm (excluding the solvent region) with the spectral area normalised to the sum of all points. For statistical analysis Metaboanalyst software was used, where after interquantile range filtering and auto-scaling, partial least squares discriminant analysis (PLS-DA) was performed. Che- nomx software was applied for the identification of metabolites.

Results In the untargeted metabolomic analysis using the CPMG pulse sequence, PLS-DA was able to separate the negRA from PSA patients. Based on our PLS-DA results Methionine, Glutamine, 4-Hydroxyphenyllactate and O-Acetyl-carnitine were identified in spectral regions separating the two groups. Regular 1H spectra were used for the untargeted lipidomic analysis. Due to the broad characteristic of the lipid signals seven groups of lipids (L1-L7) were assessed and designated by moieties. PLS-DA was performed, and the results suggest that the lipid profiles from those groups could differentiate between negRA and PsA.

Conclusions Our findings show that there are differences between the lipidomic and metabolomic profiling of negRA and PsA patients, and targeted analysis based on our present data is planned in order to identify more distinguishing metabolites and lipids.

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Disclosure of Interest None declared.
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