OR=2.812; 1.2 vs. 1.1; OR=2.252). Genotype and allele frequencies of rs3212227 polymorphism of the IL12B were similar in cases and controls. The mean ($\pm$ SEM) levels of IL-12p40 and IL-23 in AS patients (176.5 $\pm$ 20.7 pg/ml; 26.98 $\pm$ 3.4 pg/ml, respectively) was significantly higher than in controls (88.8 $\pm$ 9.6 pg/ml; 12.1 $\pm$ 1.5; respectively) ($p < 0.001$). Stratification based on IL12Bpro revealed significantly higher serum levels of IL-12p40 in AS patients with IL12Bpro 1.1 and 1.2 genotypes than controls. Highest IL-23 serum levels were associated with IL12Bpro 2.2 genotype. With regard of polymorphic variability of IL12B 3' UTR, AC genotype in AS has been linked to a higher IL-12p40 production and lower one of IL-23.

Conclusions: Allelic variants in the IL12B genes have the potential to alter the expression of both IL-12p40 and IL-23 cytokines in specific manner for AS patients. Carriage of the IL12Bpro variant 1-allele and of the 3'-UTR A-allele in the genotype regardless of homo- or heterozygosity could enhance systemic IL-12p40 and IL-23 levels.

Disclosure of Interest: None declared

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SAT0021

INNATE LYMPHOID CELLS ARE NOT A MAIN SOURCE OF IL-17A IN THE INFLAMED SPONDYLOARTHRITIS JOINT

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Background: Clinical trials of the anti-IL-17A antibody secukinumab demonstrated the crucial role of IL-17A cytokine in the pathogenesis of spondyloarthritis (SpA), however, its cellular source in this condition remains controversial. Group 3 innate lymphoid cells (ILC3s) have been recently identified in a number of different tissues as potent producers of proinflammatory cytokines, including IL-17A and IL-22.

Objectives: In this study we set out to characterise the presence and composition of ILCs and investigate whether these cells are the important source of IL-17A in the synovial tissue of patients with SpA.

Methods: Matched synovial tissue (ST), synovial fluid (SF) and peripheral blood (PB) were obtained from SpA and rheumatoid arthritis (RA) patients with actively inflamed knee joints. ILCs subsets were characterised by flow cytometry. Gene expression analysis at the single-cell level was performed directly *ex vivo* and after stimulation with PMA ionomycin. IL-17A ELISPOT assay was used to detect IL-17A-secreting cells.

Results: Analysis revealed that ILCs, and particularly NKp44-positive ILC3s, are expanded in the inflamed arthritic joint. Single cell expression analysis revealed that ST ILCs are clearly distinguishable from ST T cells and from their PB counterparts. We detected expression of Th17 signature transcripts *RORC*, *AHR* and *IL-23R* in the notable fraction of ST ILC3s. Furthermore these cells were capable to induce IL-22, but not IL-17A expression in response to *in vitro* re-stimulation

Conclusions: We demonstrate in this study that ILC3s are absolutely and relatively enriched in the synovial joint of patients with SpA, however these cells are not a significant source of IL-17A cytokine in this pathology.

Disclosure of Interest: None declared

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SAT0022

MIR-15A/16 SUPPRESS INFLAMMATORY RESPONSE AND CELL INVASION AND MIGRATION IN FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS VIA TARGETING SOX5

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Background: Aberrant expressions of miRNAs play a critical role in the inflammatory and immune response in rheumatoid arthritis (RA). The microRNA cluster which encodes for miR-15a and miR-16, located at 13q14.3. Both these microRNAs have been implicated in the pathogenesis of many diseases, but little is known of their role in RA.

Objectives: In this study, we aim to investigate the underlying molecular mechanisms of MiR-15a/16 in RA, which will provide new insight into understanding the pathogenesis of RA and identifying novel therapeutics targets for this disease.

Methods: The expression of MiR-15a/16 in the peripheral blood mononuclear cell (PBMC) and synovium from RA, osteoarthritis (OA) and healthy controls (HC) was determined by RT-qPCR and Western blotting. Mimics of MiR-15a/16 were

transfected into the human rheumatoid fibroblast-like synoviocytes (FLS) MH7A cell line. The effect of MiR-15a/16 on proinflammatory cytokines expression, migration and invasion of FLS was detected by RT-qPCR, transwell and F-actin staining. The potentially target gene of miR-15a/16 was predicted by bioinformatics analysis. The 3'UTR of Sox5 containing wild-type or mutated miR-15a/16 binding sites was cloned to the downstream of a luciferase vector and transfected into MH7A, respectively.

Results: There is no significant difference of MiR-15a/16 expression in PBMC in RA and HC at baseline. However, the MiR-15a/16 expression is lower in PBMC from non-responders (defined DAS28 changes < 1.2 after 3 months DMARDs therapy) than those in responders after 3 months DMARDs therapy. The levels of MiR-15a/16 expression were significant decreased in the synovium from RA patients, as compared with OA patients. Transfection of MH7A with MiR-15a and 16 mimic suppressed IL-1b, TNFa, IL-17 expression, decreased cell migration and invasion and affected the cytoskeletal reorganisation in RA-FLS. We co-transfected MH7A with luciferase-reporter constructs with either wild-type or mutated miR-132 or miR-15a/16 mimics. Luciferase assay showed that either miR-15a or 16 reduced the luciferase intensity of Sox5 3'UTR. However, mutated miR-15a/16 alleviated the inhibitory effect of miR-15a/16 on the intensity of Sox5 3'UTR. To determine whether Sox5 mediates the roles of miR-15a/16 in cell migration and invasion, we constructed recombinant Adenovirus SOX5 recombinant Adenovirus SOX5 overexpressing vector. We then found that overexpression of Sox5 restored the cell invasion, and migration in MH7A that were inhibited by and miR-15a/16.

Conclusions: In conclusion, our data indicate that MiR-15a/16 play an important role in inflammatory response and cell invasion and migration in RA-FLS by directly targeting Sox5, and imply that these miRNAs have potential as therapeutic targets for RA.

Disclosure of Interest: None declared

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SAT0023

CONTROL OF CYTOKINE MRNA DEGRADATION BY THE HISTONE DEACETYLASE INHIBITOR ITF2357 IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Control of cytokine mRNA degradation acts as an essential checkpoint to limit the overproduction of inflammatory proteins.¹ In rheumatoid arthritis (RA), altered expression of the mRNA-degrading protein TTP (tristetraprolin, ZFP36) has been recently reported in synovial tissue, possibly contributing to the perpetuating inflammatory loop in the synovium.² Histone deacetylase inhibitors (HDACi) are small molecule drugs suppressing cytokine production *in vitro* and *in vivo* and displaying initial safety and efficacy in the treatment of systemic onset juvenile idiopathic arthritis.^{3,4} However, their transcriptional and post-transcriptional mechanisms of action are not yet completely characterised.

Objectives: We aimed to investigate the mRNA degrading properties of the HDACi ITF2357 on a panel of inflammatory mediators in RA fibroblast-like synoviocytes (FLS).

Methods: The effects of ITF2357 on the expression and mRNA stability of IL-1 β -inducible genes in FLS were analysed using array-based qPCRs and Luminescence. The expression of primary and mature cytokines transcripts, the mRNA levels of TTP and other AU-rich element binding proteins (ARE-BP) and the cytokine profile of fibroblasts derived from ZFP36^{+/+} and ZFP36^{-/-} mice was measured by qPCR. ARE-BP silencing was performed by siRNA-mediated knockdown, and TTP post-translational modifications analysed by immunoblotting.

Results: ITF2357 reduced the expression of 85% of the analysed IL-1 β -inducible transcripts, including cytokines (IL6, IL8), chemokines (CXCL2, CXCL5, CXCL6, CXCL10), matrix-degrading enzymes (MMP1, ADAMTS1) and other inflammatory mediators. Analyses of mRNA stability demonstrated that ITF2357 accelerates IL6, IL8, PTGS2, and CXCL2 mRNA degradation, a phenomenon associated with the enhanced transcription of TTP, but not other ARE-BP, and the altered post-translational status of TTP protein. TTP knockdown potentiated cytokine production in RA FLS and murine fibroblasts.

Conclusions: Our study identifies that regulation of cytokine mRNA stability is a predominant mechanism underlying ITF2357 anti-inflammatory properties, occurring via regulation of TTP. These results highlight the therapeutic potential of ITF2357 in the treatment of RA.

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SAT0026

DIFFERENTIAL LEVELS OF IL-7 EXPRESSION IN ADVENTITIA OF NON-RA AND RA PATIENTS WITH CORONARY ARTERY DISEASE

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Background: Rheumatoid Arthritis (RA) patients have increased cardiovascular risk due to accelerated atherosclerosis (ATS), which significantly contributes to excess mortality in RA¹. The increased cardiovascular risk cannot be fully explained by traditional risk factors and systemic chronic inflammation appears to play a crucial role. Interestingly, IL-7, a proinflammatory cytokine involved in RA pathogenesis, appears to play a role also in atherosclerosis² but its effect on cardiovascular disease (CVD) in RA has not been studied yet.

Objectives: To examine serum IL-7 levels and expression of IL-7, IL-7R, CD3 and CD20 in aortic adventitia of RA and non-RA patients with coronary artery disease (CAD) and to search for relationships between systemic IL-7 levels and expression of vascular markers, cardiovascular risk factors including metabolic and inflammatory markers.

Methods: We examined 19 RA patients and 20 non-RA patients undergoing coronary artery bypass graft surgery included in the Feiring Heart Biopsy Study. Serum IL-7 levels were measured by chemiluminescence (MSD). Biopsies from the adventitia of thoracic aorta from a subset of patients (12 RA and 14 non-RA) were stained for IL-7, IL-7R, CD3 and CD20 by immunohistochemistry and scored per mm² of tissue.

Results: Non-RA patients had lower IL-7 serum levels than RA (3.4±3.3 vs. 6.7±3.5, p<0.05). Independently of RA diagnosis, IL-7 significantly correlated with CRP (rho=0.450, p=0.008), triglycerides (TG, rho=0.566, p=0.005), glucose (rho=0.642, p=0.001) and hypertension (p=0.036). Levels of IL-7 were associated with New York Heart Association class (rho=0.429, p=0.014) and this was stronger in non-RA patients (rho=0.577, p=0.010). No associations were found with smoking or markers of CVD severity (i.e. numbers of arteries with significant stenosis or number of previous myocardial infarcts (MI)).

The number of IL-7 + and IL-7R+cells/mm² in adventitia were significantly higher in RA (134.2±45.5 and 144±49.9 respectively) than non-RA patients (46.9±22.8 and 54.4±20.2, p<0.005) and were associated with serum IL-7 levels (rho=0.551 and rho=0.588, p<0.01). Both IL-7 + and IL-7R+cells were associated with a positive history of MI (p=0.047 and p=0.005) and IL-7R+cells with the number of previous MIs (rho=0.408, p=0.038). Only in RA patients, IL-7R+cells showed a trend for correlation with TG (rho=0.771, p=0.072). IL-7 + and IL-7R +cells correlated with CD3 (rho=0.688, p=0.013 and rho=0.630 p=0.028), but no correlation was found with CD20. Cholesterol and HDL levels were associated with IL-7 +cells only in non-RA patients (rho=0.729 p=0.04 and rho=0.733, p=0.038).

Conclusions: Among patients with CAD, those with RA had higher serum IL-7 and a greater expression of both IL-7/IL-7R in aortic adventitia. Systemic levels of IL-7 were related to its vascular expression. Thus, the IL-7/IL-7R axis may play a role in the accelerated atherogenesis observed in RA; further studies are needed to elucidate the precise role of IL-7 and impact of potential IL-7R blockade in CV risk in RA.

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SAT0027

CO-EXPRESSION OF RECEPTORS TO TNFA AMONG NAIVE T-CELLS AND MEMORY T-CELLS IS ALTERED IN RHEUMATOID ARTHRITIS AND THE CHANGES CORRELATE WITH DISEASE ACTIVITY

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Background: Changing the ratio of TNFα receptors of different types can lead to shift in the balance between pro-apoptotic and proliferative signalling pathways which is crucial for rheumatoid arthritis (RA) inflammatory processes.

Objectives: To study the level of expression of type 1 and 2 receptors for TNFα (TNFR1/TNFR2) on individual subpopulations of peripheral blood actively involved in immunopathological processes in RA

Methods: The study included 20 healthy donors aged 18–60 years (11 men (55%)) and 36 RA patients with high (n=14), medium (n=15) and low (n=7) disease activity at the age of 22–77 years (31 women (86, 1%)). Co-expression and number of type 1 and 2 receptors for TNFα were calculated for monocytes, B-cells, T-cells, as well as among: cytotoxic T-cells (CD8+), T-helper cells (CD4+), activated CD8 +cells, activated CD4 +cells, memory T-cells (CD45RO+) and naive T-cells (CD45RA+) from cytotoxic and T-helper cells, and T-regulatory cells (CD4 +CD25highCD127low) by flow cytometry analysis (BD FACSVerse, USA).

Results: Seven indicators of receptor expression were revealed, which differed in patients with RA with high disease activity in comparison with HD, and didn't differ between HD and patients with low disease activity. These were 4 indicators on the number of receptors on cells and 3 cell percentages with specific combinations of expressed. The association between disease severity and activity indexes (DAS-28 index, disease duration, X-ray stage, activity stage, RF level, ASCP and C-RB, systemic manifestations and erosive arthritis) and parameters of TNFα receptor expression on immunocompetent cell subpopulations were studied. The following associations were revealed: RF level positively correlated with the percentage of double-positive TNFR1 +TNFR2+cells among naive and memory T-cells for both T-helpers and cytotoxic T-cells (r levels from 0.62 to 0.68 with p<0.05); the level of C-reactive protein positively correlated with the percentage of cells carrying only receptor type 1 among all the T-lymphocyte subpopulations studied (r levels from 0.73 to 0.9 with p<0.05); the presence of systemic manifestations is negatively correlated with the number of receptors of both types 1 and 2 on naive T-helper and cytotoxic cells (r levels from –0.63 to –0.74 with p<0.05).

Conclusions: The active inflammatory process in rheumatoid arthritis with DAS28 >5.1 is accompanied by a change in the ratio of cells with different variants of co-expression of receptors to TNFα among populations actively involved in the pathological process (naive T-cells and memory T-cells among T-helpers and cytotoxic T-cells). The selected parameters will be used to construct and verify the predictive models of response to therapy of different subclasses

Disclosure of Interest: None declared

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