THU0086 INTERLEUKIN 17 RECEPTOR D (IL-17RD) REDUCES INCIDENCE OF COLLAGEN INDUCED ARTHRITIS

M. Molendijk¹, A.M. Mus¹, P.S. Asmawidjaja¹, D. Baeten², E. Lubberts¹. ¹Erasmus MC, Rotterdam; ²AMC, Amsterdam, Netherlands

Background: IL-17RD is a member of the IL-17 receptor family. In contrast to the other IL-17 receptors, IL-17RA, -RB, -RC and -RE, little is known about the ligand and function of IL-17RD. Recently, IL-17RD has been described to negatively regulate a selection of IL-17A responsive genes. IL-17RD is therefore proposed to limit IL-17A signalling.

Objectives: In this study we examined IL-17RD expression in multiple cells types and its role in the development of collagen induced arthritis.

Methods: Human synovial fibroblasts from Rheumatoid Arthritis (RA) patients were stimulated with tumour necrosis factor α (TNF α), interleukin 1 β (IL-1 β) or IL-17A for multiple time points. IL-17RD expression levels were measured via qPCR. Collagen induced arthritis (CIA) was induced in IL-17RD knockout mice and wildtype littermates. At days 1 and 21, mice were immunised intradermally with chicken collagen type II in complete Freund's adjuvant (CFA). Mice were scored 3 times a week for clinical disease defined as swollen joints with a maximum score of 8. Due to ethical reasons, mice were removed from the experiments when they reached a score of 6. CD4⁺ memory T cells, CD8⁺ memory T cells, CD19⁺ B cells and monocytes were isolated from WT spleens and analysed for IL-17RD expression. Blood neutrophil migration assays were performed *in vitro* using WT and IL-17RD deficient (IL-17RD KO) mouse synovial fibroblasts.

Results: Human synovial fibroblasts from RA patients have baseline expression of IL-17RD. Upon stimulation with TNF α a significant downregulation of IL-17RD expression was measured from 24 hours onwards. IL-1 β stimulation had a similar effect as TNF α on IL-17RD expression. Lack of IL-17RD did not result in differences in CIA severity, but the incidence of CIA was reduced. IL-17RD is mainly expressed in synovial fibroblasts. IL-17RD KO synovial fibroblasts attract less neutrophils likely by lower production of neutrophil attractants.

Conclusions: An inflammatory environment causes synovial fibroblasts to down-regulate IL-17RD expression. Lack of IL-17RD reduces the incidence CIA, which is an IL-17-driven model. The decrease in CIA incidence is likely explained via the reduced attraction of neutrophils to the site of inflammation.

Disclosure of Interest: None declared

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THU0087 MICRORNA-1915-3P IN SERUM EXOSOME IS ASSOCIATED WITH DISEASE ACTIVITY OF RHEUMATOID ARTHRITIS IN KOREA

M.-K. Lim¹, J. Song², S.A. Kim³, J. Yoo¹. ¹Internal medicine, School of Medicine, Eulji University; ²Rheumatology, Sun General Hospital; ³Pharmacology, School of Medicine, Eulji University, Daejeon, Korea, Republic of Ireland

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease that is characterised by severe tissue damage and chronic synovial inflammation.¹ Using analysis of gene polymorphism, biochemical assays, and proteomics approaches, several promising biomarkers for treatment response have been proposed, including red blood cell (RBC) MTX polyglutamate levels, as well as serum levels of proteins such as cytokines, growth factors, and autoantibodies.² However, these markers need further development and refinement to attain sufficient sensitivity and specificity.

Objectives: In this study, we used a miRNAarray approach to identify new miRNA in exosome that are related to disease activity in patients with RA who showed inadequate response to treatment. We also examined the relationship between the levels of expression of the RNAs and various serologic parameters of the patients.

Methods: Forty-two RA patients were included in the study. Disease activity was measured using the 28-joint disease activity score with ESR (DAS28-ESR). Patients with RA were stratified according to the following criteria: the clinical remission (CR) group (n=22), DAS28-ESR $\leq$ 2.6; and the non-CR group (n=20), DAS28-ESR $\geq$ 2.6. By exosome preparation, miRNA array, and Reverse Transcription-qPCR reactions, several miRNAs were as potent markers for disease activity.

Results: After data processing for relative quantification of miRNA in exosome between the CR and non-CR groups, we identified 47 miRNAs with a relative fold change (non-CR/CR) $>$ 2. The expression levels of 37 miRNAs were found decreased in non-CR group, while 10 miRNAs increased in non-CR group. To validate these results, five miRNAs were selected (hsa-miR-1915-3 p, hsa-miR-4516, hsa-miR-6511b-5p, hsa-miR-3665, hsa-miR-3613) showing at least 2-fold change between the CR and non-CR groups. Both levels of hsa-miR-1915-3 p and hsa-miR-6511b-5p were significantly increased in CR group; hsa-miR-1915-3 p was 43.75 in the CR group and 24.68 in the non-CR group (p=0.004), and

hsa-miR-6511b-5p was 3.02 in the CR group and 2.45 in the non-CR group (p=0.03).

Conclusions: hsa-miR-1915-3 p showed promise as additional markers for evaluating disease activity in patients with RA. Prospective investigation of hsa-miR-1915-3 p may facilitate development of new diagnostic tools to assess disease activity and prognosis in RA and other autoimmune diseases.

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THU0088 MONOCYTE DOWNREGULATION OF MITOCHONDRIAL TRANSLOCATOR PROTEIN MAY BE A CONTRIBUTORY MECHANISM TO INFLAMMATION IN RA

N. Narayan, H. Mandhair, T. Sekine, A. Sabokbar, P.C. Taylor. Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK

Background: The translocator protein is an 18 kDa mitochondrial transporter, increasingly thought to play a critical role in cholesterol efflux in macrophages. Recent work demonstrates that macrophages engineered to over-express TSPO, exhibit increased cholesterol efflux, and reduced ability to form a pro-inflammatory ('M1') phenotype, with significant reduction in the ability to produce TNF- α . Additionally, there is growing data to demonstrate a difference in TSPO expression in monocytes in those with inflammatory disease compared with healthy, as exemplified by studies of multiple sclerosis, suggesting a role for TSPO in the generation of inflammation.

Objectives: In this study, we investigate the expression of TSPO in healthy and RA peripheral blood monocytes, and in monocyte derived macrophages (MDM), differentiated to an M1 (pro-inflammatory), and M2 (reparative) phenotype.

Methods: Using positive magnetic-activated cell sorting, we use peripheral blood mononuclear cells from 24 RA patients with active disease (as determined by clinical examination, and DAS28 CRP score), and 24 healthy controls, to isolate peripheral blood monocyte mRNA and protein, to ascertain any differences in TSPO expression at monocyte level. MDM were generated *in vitro* through differentiation of monocytes with 100 ng/ml M-CSF for 7 days, followed by activation to an M1 phenotype using LPS and IFN- γ , and a reparative 'M2' phenotype using IL-4, TGF- β or glucocorticoid, followed by quantification of TSPO mRNA utilising real-time PCR, and TSPO protein, utilising western blotting and radioligand binding.

Results: Our data establishes that both healthy and RA peripheral blood monocyte derived macrophages (MDM) exhibit a statistically significant downregulation of TSPO at mRNA and protein level, when activated to a pro-inflammatory 'M1' macrophage phenotype, with no change in TSPO expression in MDM activated to a reparative 'M2' phenotype. Our mRNA data also suggests that M1 macrophages from both healthy and RA donors, exhibit a significant reduction in expression of key cell components promoting cholesterol efflux in macrophages, including CYP27A1, and ABCA1. Our data additionally demonstrates a significant reduction in expression of TSPO between healthy and RA monocytes, at both mRNA and protein level (mean fold change TSPO mRNA of 1.00 for healthy monocytes, and 0.47 $\pm$ 0.24, p<0.001 for RA monocytes and mean TSPO optical densitometry of 1.01 $\pm$ 0.10 for healthy monocytes and 0.85 $\pm$ 0.02 p<0.05 for RA monocytes relative to β -actin).

Conclusions: Our findings indicate that pro-inflammatory activation of both healthy and RA monocyte-derived macrophages downregulates TSPO, and is also associated with reduction in key components of the cholesterol efflux pathway, in line with pre-existing studies of TSPO silencing and over-expression in human macrophages. Furthermore, we demonstrate that RA peripheral blood monocytes themselves may have a predisposition to a pro-inflammatory phenotype through downregulation of TSPO expression, which could indicate an as yet uninvestigated cellular mechanism contributing to synovial inflammation in RA.

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