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

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Background: IL-17RD is a member of the IL-17 receptor family. In contrast to the other IL-17 receptors, IL-17A/IL-17B/IL-17C/IL-17D, IL-17RD binds IL-17A, IL-17C, and IL-17D, and the level of IL-17RD expression is related to the risk of developing collagen induced arthritis.

Methods: Human synovial fibroblasts from Rheumatoid Arthritis (RA) patients with a history of disease activity were stimulated with tumour necrosis factor (TNF)α, interleukin 1β (IL-1β), and interleukin 6 (IL-6) for 24 hours. Supernatants were collected and analysed for IL-17RD expression. Blood neutrophil migration assays were performed in vitro using TNFα and IL-17RD deficient (IL-17RD KO) mouse synovial fibroblasts.

Results: Human synovial fibroblasts from RA patients have a baseline expression of IL-17RD. Upon stimulation with TNFα, a significant downregulation of IL-17RD expression was observed. IL-17RD expression in the CR group was significantly lower than in the non-CR group (p=0.03). The expression of IL-17RD in the CR group was also lower than in healthy controls (p=0.03).

Conclusions: IL-17RD is a promising biomarker for the diagnosis and monitoring of RA. Further studies are needed to determine the role of IL-17RD in the pathogenesis of RA.

Disclosure of Interest: None declared

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MONOCYTE DOWNREGULATION OF MITOCONDRIAL TRANSCLOCATOR PROTEIN MAY BE A CONTRIBUTORY MECHANISM TO INFLAMMATION IN RA


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 monocytes in both healthy and RA donors. MDM were generated from both healthy and RA donors, exhibiting a significant reduction in expression of TSPO 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, TG-β or glucocorticoid, followed by quantification of TSPO mRNA utilising real-time PCR, and TSPO protein, utilising western blotting and radioligand binding.

Results: Our findings indicate 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±0.24, p=0.001 for RA monocytes and mean TSPO optical densitometry of 1.01±0.10 for healthy monocytes and 0.85±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.

REFERENCES:


IL-17A+GM-CSF+ neutrophils are the major infiltrating cells in interstitial lung disease in an autoimmune arthritis model

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Background: Intestinal lung disease (ILD) is a common extra-articular manifestation of rheumatoid arthritis (RA). Discrepancy in the effect of biologic agents on synovial and lung inflammation exists, indicating that the nature of inflammation in the synovium and lung may be different in RA.

Objectives: To gain a better understanding of the pathogenesis of rheumatoid arthritis-associated interstitial lung disease (ILD), we sought to identify the characteristics of lung-infiltrating cells in SKG mice with ILD.

Methods: We injected curdled in SKG mice at 8 weeks of age, and identified the presence of ILD by PET-MRI at 20 weeks post-injection and histological analysis at 22 weeks post-injection. Lung-infiltrating cells were examined by flow cytometry. Analysis of serum cytokines by the Luminex multiplex cytokine assay was performed at 14 and 22 weeks post-injection, and cytokine profiles before and after the development of ILD were compared. Opal multiplexed immunofluorescent staining of lung tissue was also performed.

Results: At 20 weeks post-injection, curdled-treated SKG mice developed not only arthritis but also lung inflammation combined with fibrosis, which was identified by PET-MRI and histological analysis. The majority of inflammatory cells that accumulated in the lungs of curdled-treated SKG mice were CD11b+Gr1+ neutrophils, which co-express IL-17A and GM-CSF, rather than TNF-α and IL-7Rα, and IL-7Rα had increased at 22 weeks post-injection, whereas those of IFN-γ, IL-22, IL-6, and TNF-α remained unchanged. Furthermore, IL-23, CXCL5, IL-17A, and GM-CSF, but not TNF-α, were observed in immunofluorescent-stained lung tissue.

Conclusions: We found that IL-17A+GM-CSF+ neutrophils represented the major inflammatory cells in the lungs of curdled-treated SKG mice. In addition, GM-CSF and IL-17A appear to play a more important role than TNF-α in ILD development.

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TIE2 INDUCES AN INFLAMMATORY PHENOTYPE IN RHEUMATOID ARTHRITIS AND PSORIATIC ARTHRITIS PATIENTS DRIVEN BY ANGIOPEPTIN-2

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Background: Several studies have shown that angiopetitin signalling to Tie2 may play a role in the initiation and perpetuation of disease rheumatoid arthritis (RA) and psoriatic arthritis (PsA). However, the cell type(s) involved in this process, as well as the specific role of Tie2 signalling in the synovium of arthritis patients, remain unclear.

Objectives: To examine the role Tie2 signalling in macrophages and fibroblast-like synoviocytes (FLS) within the context of the synovial microenvironment of arthritic patients.

Methods: Peripheral blood (PB) monocytes from healthy donors (HD) were differentiated with synovial fluid (SF) of RA and PsA patients. PB and SF monocytes from RA and PsA patients were differentiated into pro-inflammatory macrophages in vitro. Tie2 expression was analysed by flow cytometry and quantitative PCR. Macrophages, RA FLS and synovial tissue explants were stimulated with Ang-1 or Ang-2 (200 ng/ml) alone or in combination with TNF (10 ng/ml) for 4 or 24 hour. mRNA and protein expression of inflammatory mediators was analysed by quantitative PCR and ELISA and luminex, respectively. Arthritis was induced in wild type (WT) and Tie2 over-expressing (Tie2-TG) mice by intraperitoneal injection of 100 ml of K/BxN serum on day 0 and day 2. Mice were sacrificed on day 14 after serum transfer.

Results: Tie2 expression was observed IFN-γ-differentiated macrophages, from RA and PsA patients, as well as HD macrophages differentiated with RA and PsA SF. Tie2 expression was associated with enhanced synovial expression of IL-6, IL-12B, TNF, IL-23, IL-22 and CCL-3 in the synovial tissue explants of RA FLS. The clinical severity of serum-induced arthritis was significantly higher in Tie2-TG mice compared to WT mice, associated with enhanced synovial expression of IL-6, IL-12B, IL-23, CCL-2 and CCL-3.

Conclusions: These results suggest that Tie2 signalling, even within the complex microenvironment of affected synovial tissue, has an important pro-