INTERLEUKIN-17A, -F, AND -AF INDUCE EXPRESSION OF PRO-INFLAMMATORY GENES IN END-STAGE OSTEOARTHRITIS CHONDROCYTES AND SYNOVIAL FIBROBLASTS

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Purpose: Interleukin (IL)-17 is a family of 6 cytokines from which the homodimers IL-17A and IL-17F, and the heterodimer IL-17AF are most well-studied. These IL-17-cytokines have a pivotal function in host defence, but have also been linked to several autoimmune and chronic inflammatory diseases due to their ability to stimulate resident fibroblasts to release mediators that contribute to inflammation, angiogenesis, and matrix destruction. In osteoarthritis (OA), increased concentration of IL-17A has been found in synovium, and the IL-17A concentration in synovial fluid and serum has been found to correlate with increased pain, decreased function, and increased disease severity. However, it is still unclear how IL-17A, and its family members IL-17F and IL-17AF, can contribute to OA pathophysiology. Therefore, this study aims to investigate the effect of IL-17A, IL-17F, and IL-17AF on gene expression and intracellular signalling pathway activation in fibroblasts derived from patients with end-stage OA.
Methods: Chondrocytes and synovial fibroblasts derived from 6 end-stage knee OA patients were treated with 10 ng/ml IL-17A, IL-17F, or IL-17AF. RNA was isolated and libraries were made for RNA-Seq analysis. Data was analysed using DESeq2 in R. End-stage OA chondrocytes and synovial fibroblasts (n=3) were treated with IL-17A, IL-17F, or IL-17AF for western blot analysis for the activation of ERK1/2, p38, and p65 NF-κB. End-stage OA chondrocytes and synovial fibroblasts were treated with IL-17A with or without the p38 inhibitor SB203580 (n=4), NF-κB inhibitor JSH-23 (n=4), or IL-17A antibody secukinumab (n=5), and gene expression assessed with RT-qPCR.
Results: Treatment with IL-17A, IL-17AF, or IL-17F significantly changed the expression of 856, 188, and 39 genes in chondrocytes and 330, 55, and 17 genes in synovial fibroblasts, respectively ($p<0.05$, log2 fold change +/-1). The three most highly regulated genes by IL-17A were *IL6*, *NOS2*, and *CXCL1* in chondrocytes, and *CSF3*, *CXCL1*, and *CCL20* in synovial fibroblasts. Pathway analyses showed that IL-17A (and to a lesser extent IL-17AF and IL-17F) regulated several OA-related pathways including immune-, matrix-, angiogenesis-, and complement-pathways. Secukinumab significantly inhibited IL-17A-induced gene expression in both chondrocytes and synovial fibroblasts. Western blot results confirmed that IL-17A induced significant activation of p38 and p65 NF-κB. IL-17AF caused significant but less profound activation of ERK1/2, p38, and p65 NF-κB, while IL-17F did not induce activation of these pathways. However, p38 and NF-κB inhibitors were unable to inhibit IL-17A-induced gene expression.
Conclusions: This study shows that IL-17A (and to a lesser extent IL-17F and IL-17AF) induce changes in gene expression in OA-related pathways. The chronic, low-grade inflammation seen in OA fits well with the effects IL-17A has on inflammation; its effects on matrix destruction and angiogenesis further supports a role for IL-17A in OA pathophysiology. The IL-17A antibody secukinumab significantly inhibited IL-17A-induced gene expression, confirming that IL-17A is responsible for these changes in gene expression. Inhibitors of IL-17A-activated signalling pathways p38

and NF-κB did not inhibit IL-17A-induced gene expression, emphasizing the complexity of IL-17 signalling. As there are currently no disease-modifying OA drugs, secukinumab could be investigated as a potential therapeutic option in OA patients.