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Immunology Summit 2015: Blockade of recombinant human IL-6 with tocilizumab inhibits matrix metallo-proteinase-9 in the C 28/ I2 cell line of immortalized human chondrocytes- Charles J Malemud- George Washington University, USA

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In this study, we employed the immortalized human juvenile chondrocyte lines, T/C28a2 and C28/I2, to determine whether recombinant human (RH)-IL-6 caused STAT3 to be phosphorylated (p-STAT3). WHI-P131, a JAK3-selective small molecule inhibitor was used to validate the JAK/STAT response to rhIL-6 since WHI-P131 should decrease p-STAT3 without altering total STAT3 (STAT3). Tocilizumab (TCZ), a monoclonal antibody which neutralizes the interaction between IL-6 and its receptor(s) was also employed to determine if matrix metalloproteinase-9 (MMP-9) production was coupled to the predicted rhIL-6-mediated JAK/STAT response. Western blots revealed that the T/C28a2 and C28/I2 chondrocyte lines produced STAT3 protein. However, constitutive p-STAT3 was detected only in T/C28a2. C28/I2 chondrocytes incubated with rhIL-6 (50 ng/ml) for 30 min increased p-STAT3 which was inhibited by WHI-P131. Furthermore C28/I2 chondrocytes incubated with rhIL-6 increased MMP-9 synthesis. Importantly, the combination of rhIL-6 and TCZ (200 ng/ml) significantly decreased MMP-9 production after 60 min which was sustained after 4 hrs and rhIL-6 plus TCZ significantly reduced the number of MMP-9- positive C28/I2 chondrocytes. Of note, sIL-6R also significantly reduced the number of MMP-9-positive cells compared to rhIL-6 alone. In contrast the combination of rhIL-6 and sIL-6R significantly increased MMP-9 cell positivity. These results indicated that rhIL-6-mediated STAT3 phosphorylation was coupled to MMP-9 production in C28/I2 chondrocytes where MMP-9 production was significantly reduced by TCZ or sIL-6R. These findings also support the view that TCZ likely inhibits rhIL-6-mediated MMP-9 production in C28/I2 chondrocytes by neutralizing all 3 IL-6-mediated-signaling pathways.

Matrix metalloproteinase-9 (MMP-9; gelatinase B; 92 kDa gelatinase; 92 kDa type IV collagenase) is a critical MMP in mediating the progression of various arthritic conditions . MMP-9 has the capacity to degrade several articular cartilage extracellular matrix (ECM) proteins, including aggrecan, link protein, and type II collagen, all of which help maintain normal cartilage biomechanical functions . Importantly, in various types of arthritis, chondrocyte MMP-9 gene expression is significantly up-regulated in response to the elevated levels of pro-inflammatory cytokines in the synovial fluid milieu, exemplified by intereukin-(IL-6), IL-1β, IL-17, and tumor necrosis factor-α (TNF-α) .

To probe the contribution of each of those cytokines to MMP-9 gene expression by articular chondrocytes in vitro would generally require that specific inhibitors for each of them be individually tested. In that regard, the effect of IL-1β or TNF-α blockade on MMP synthesis was previously reported with the results showing that IL-1 receptor antagonist or TNF-α blocking monoclonal antibodies inhibited MMP production. However, the contribution of IL-6 to MMP-9 production by cultured human chondrocytes remains to be fully elucidated. Therefore, to achieve this objective, the extent to which tocilizumab (TCZ), a recombinant fully humanized IgG1(κ) monoclonal antibody that neutralizes the interaction between IL-6 and the IL-6 receptor-α (IL-6Rα) inhibits recombinant human (rh)-IL-6-mediated MMP-9 production was determined in the immortalized human juvenile T/C28a2 and C28/I2 chondrocyte lines. These human chondrocyte lines were employed for this analysis because they had been previously shown to express cartilage-specific extracellular matrix protein genes. T/C28a2 and C28/I2 chondrocytes also expressed several other molecules characteristic of authentic human chondrocytes, most notably the molecular signature SOX9 gene, considered the “master” transcriptional regulator of several cartilage-specific genes as the type II collagen (COL2A1) gene and the aggrecan (AGRN) gene .

The effect of rhIL-6 on the synthesis of chondrocyte-derived neutrophil gelatinase-associated lipocalin (NGAL) was also evaluated. The rationale for analyzing NGAL production by C28/I2 chondrocytes stemmed from our previously reported finding that chondrocytes obtained from human osteoarthritis knee cartilage synthesized NGAL in response to IL-1β. In addition, we showed that NGAL in synovial fluids obtained from patients with end-stage osteoarthritis was found in a complex with MMP-9. Moreover, we proved that the MMP-9/NGAL complex preserved MMP-9 activity by demonstrating that this complex prevented MMP-9 from being degraded, thus preserving MMP-9 activity.

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