CHONDROCYTE DERIVED IL-1 AND TNF ARE INVOLVED IN MATRIX DEGRADATION OF HUMAN OSTEOARTHRITIC CARTILAGE IN EXPLANT CULTURE

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Relevance to Musculoskeletal Condition
Both tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) play important roles in mediating and controlling inflammation in arthritis. This study shows involvement of chondrocyte generated TNF-alpha and IL-1 in the degradation of human osteoarthritic (OA) cartilage matrix, and thereby identifies possible therapeutic targets in the treatment of OA.

Introduction
Excessive degradation of type II collagen is commonly observed in osteoarthritic (OA) and rheumatoid arthritic (RA) articular cartilages. Our recent studies have demonstrated increased cleavage and denaturation of type II collagen in human articular cartilage in OA (1-4). It is known that TNF-alpha and IL-1 play important roles in the pathogenesis of inflammation in arthritis and can each cause damage to cartilage, which is mediated by chondrocytes. There have been reports showing that both TNF inhibitors including antibodies to TNF-alpha or a soluble TNF receptor type II (soluble TNF-RII) and IL-1 receptor antagonist (IL-1ra; Anakinra) can control chronic inflammation and erosion in RA. Cytokines, including IL-1, activate synthesis and release of matrix metalloproteinases such as MMP-13 (collagenase-3) leading to cartilage matrix degradation. We have already reported that treatment with PEGylated soluble TNF receptor type I (PEG sTNF-R1) and/or IL-1ra inhibited increased type II collagen cleavage by collagenases and proteoglycan glycosaminoglycan (GAG) release in a majority of cartilage explants, and that this treatment enhanced GAG content in some specimens (5).

The purpose of this study was to determine if soluble TNF-R1 and/or IL-1ra can inhibit gene expression of MMPs and cytokines, and if these anti-cytokines can enhance gene expression of type II collagen and aggrecan in human OA cartilage explants.

Hypothesis
1) That chondrocyte derived TNF-alpha and IL-1 are involved in cartilage resorption in human articular cartilage in OA.
2) That soluble TNF-R1, IL-1ra or their combination can inhibit gene expression of MMPs and cytokines, and can enhance gene expression of type II collagen and aggrecan.

Materials and Methods
Both recombinant human IL-1ra (Anakinra) and PEG sTNF-R1 were cultured with OA cartilage explants of nine specimens from total knee arthroplasty for 24h. Gene expression of MMP-1, MMP-13, IL-1beta, TNF-alpha, COL2A1, and aggrecan was analyzed by semi-quantitative RT-PCR using RNA directly extracted from nine cartilage explants. Expression for these genes was also analyzed by RT-PCR using RNA extracted from eight specimens of OA cartilage without culture.

Results
RT-PCR analyses revealed that IL-1ra (6/9), PEG sTNF-R1 (3/9) or combination (7/9) down-regulated MMP-1 gene expression. IL-1ra (3/7), PEG sTNF-R1 (3/7), or the combination (6/7) down-regulated MMP-13 gene expression. IL-1 beta gene expression was down-regulated by IL-1ra (4/9) or PEG sTNF-R1 (3/9). The combination suppressed 3 of 9. Gene expression of TNF-alpha was suppressed by IL-1ra (4/8) or PEG sTNF-R1 (3/8). The combination suppressed 3 of 8. In a proportion of the specimens, IL-1ra (2/8), PEG sTNF-R1 (3/8), or the combination (4/8) up-regulated COL2A1 expression, and IL-1ra (3/9), PEG sTNF-R1 (2/9) and its combination (5/9) up-regulated gene expression of aggrecan.

Quantitative analyses of all samples using NIH image software revealed that MMP-1 gene expression was significantly down-regulated by IL-1ra or the combination (Fig. 1-A), and that MMP-13 gene expression was significantly inhibited by the combination (Fig. 1-B). However, these anti-cytokines had no significant effect on inhibition of gene expression for IL-1beta, TNF-alpha, COL2A1, and aggrecan.

Discussion and Conclusion
In OA cartilage MMP-1 and MMP-13 expressions are significantly down-regulated by combination of IL-1 and TNF blockade. MMP-1 is significantly down-regulated by IL-1ra alone and MMP-13 is reduced but not significantly. The results suggest that TNF-alpha and IL-1 often play important roles in matrix degradation in OA. Thus, treatment with PEG sTNF-R1 and/or IL-1ra (Anakinra) may offer an opportunity to inhibit type II collagen and aggrecan degradation and stimulate matrix synthesis therapeutically in OA cartilage.

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References

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