A SYNTHETIC PEPTIDE OF TYPE II COLLAGEN INDUCES UPREGULATION OF COLLAGENASE EXPRESSION AND TYPE II COLLAGEN CLEAVAGE BY COLLAGENASES THROUGH IL-1 AND TNF PATHWAYS IN HUMAN ARTICULAR CARTILAGE

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Relevance to Musculoskeletal Condition
Cellular interactions with type II collagen fragments may be important in regulating collagen metabolism in both normal and osteoarthritic cartilage. This study demonstrates that a single synthetic peptide of type II collagen can induce upregulation of collagenase expression and degradation of type II collagen via interleukin-1 and tumour necrosis factor-mediated pathways in normal human articular cartilage.

Introduction
Excessive degradation of type II collagen is observed in osteoarthritic (OA) and rheumatoid arthritis (RA) articular cartilages. Tumour necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) are believed to play a role in the pathogenesis of arthritis. Matrix metalloproteases (MMPs) such as MMP-1 and –13 are probably both involved in increased cleavage of type II collagen fibrils in human articular cartilage in OA. We have discovered that degradation products of type II collagen, specifically a single peptide of type II collagen, can cause articular cartilage breakdown in adult bovine and normal human explant cultures promoting the cleavage of type II collagen by collagenase(s). The aim of this study was to determine whether the synthetic peptide of type II collagen can upregulate collagenase expression and enhance collagenase activity and whether this involves an IL-1- and/or TNF-dependent pathway in normal human articular cartilage.

Hypothesis
1) That a synthetic peptide of type II collagen can induce upregulation of collagenase expression and type II collagen degradation by collagenase in normal human articular cartilage.
2) That IL-1 and TNF mediate type II collagen degradation in articular cartilage induced by a synthetic peptide of type II collagen.

Materials and Methods
According to the primary sequence of human type II collagen, a synthetic peptide (SP) (24 amino acid residues) without RGD sequence was synthesized. Another peptide (USP) which has also 24 amino acid residues but has sequence totally different from SP was also synthesized. A collagenase-3 preferential inhibitor (RS102,481) was obtained from Rosche Bioscience (Palo Alto, CA). Recombinant human interleukin-1 receptor antagonist (anakinra) (IL-1ra) and recombinant human soluble TNF receptor type I (PEG sTNF-RI) (Amgen Inc) were used with human normal articular cartilages obtained at autopsy within 16 hours of death from 10 adults without arthritis. Explants were cultured for up to 16 days with SP or USP at up to 10 µM with or without RS102,481 (10 nM), anakinra (20 or 100 ng/ml), or PEG sTNF-RI (1 or 10 µg/ml). An immunoassay was used to measure cleavage of type II collagen by collagenase in cartilage and media. Proteoglycan (GAG) (mainly aggrecan) release and content was also assayed by the dimethylmethylene blue method (5). Cartilage wet weight was used for normalizing the results. Gene expression of MMPs was separately assayed by RT-PCR in cultures of isolated chondrocytes incubated with or without SP. Protein expression of MMPs was also investigated in media by Western blotting.

Results
Effects of SP on type II collagen degradation
In 10 specimens tested, SP at 10 µM caused an increase in collagen cleavage. SP at 1, 5, and 10 µM increased cleavage dose-dependently. USP did not induce cleavage.

Inhibitory effects of RS102,481, anakinra (IL-1ra) and PEG sTNF-RI on type II collagen degradation
In 6 out of 8 specimens tested, increased cleavage was inhibited by anakinra. In 3 out of 4 specimens, increased cleavage was arrested by PEG sTNF-RI. The increased cleavage caused by SP could be inhibited by RS102,481.

Effects of SP on GAG content
In 10 specimens tested, SP had no significant effect on GAG release into media.

Effect of SP on MMPs expression
SP up-regulated MMP-1 and –13 gene and protein expressions within 24 h.

Discussion
In normal human articular explants, we have demonstrated that a single peptide of type II collagen can upregulate collagenase gene and protein expressions and cause increased cleavage of type II collagen by collagenase(s). This increased collagenase activity could usually be inhibited by either anakinra (IL-1ra), or PEG sTNF-RI. In pathology, excessive amounts of degradation products of type II collagen generated by collagenase and other MMPs may “feed-back” and activate MMP expression through a cell surface receptor-mediated mechanism involving IL-1- and TNF-mediated pathways, and may stimulate the degradation of intact type II collagen fibrils. Our findings provide new insights into understanding the mechanisms involved in type II collagen turnover in normal and OA cartilages. They also identify new targets for the therapeutic control of matrix breakdown in the treatment of OA and RA.

Conclusion
A peptide of type II collagen can upregulate expression of collagenases leading to induction of type II collagen cleavage by collagenases in human articular cartilage explant cultures. Generation of this increased collagenase activity involves the activities of IL-1 and TNF generated by chondrocytes.

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References

**Amgen Inc., Thousand Oaks, CA.**