A SYNTHETIC PEPTIDE OF TYPE II COLLAGEN CAN CAUSE TYPE II COLLAGEN CLEAVAGE BY COLLAGENASE THROUGH IL-1- AND TNF-DEPENDENT PATHWAYS IN HUMAN ARTICULAR CARTILAGE

* Koabayashi, M; * Yasuda, T; * Kojima, T; * Tchetina, E; ** Feige, U; ** Poole, A Robin (E-Shriners Hospitals for Children, Medical Research Council of Canada, Canadian Arthritis Network, National Institute of Health, and Amgen Inc.)

++ Joint Diseases Laboratory, Shriners Hospitals for Children, Departments of Surgery and Medicine, McGill University, Montreal, Canada. 1529 Cedar Avenue, Montreal, Quebec, Canada H3G 1A6, 514-849-6208, Fax: 514-849-9684, rpoole@shriners.mcgill.ca

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 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 in articular cartilage is a feature of osteoarthritis (OA) and rheumatoid arthritis (RA) cartilages. Tumour necrosis factor (TNF) and interleukin-1 (IL-1) play important roles in the pathogenesis of arthritis which include inflammation (TNF) and cartilage degradation (erosion) (TNF and IL-1). Matrix metalloproteinases (MMPs) such as collagenase-3 are involved in increased cleavage of type II collagen fibrils in human articular cartilage in OA (1-4). We have discovered that degradation products of type II collagen specifically a single peptide of type II collagen, can cause cartilage cartilage breakdown in adult bovine explant cultures promoting the cleavage of type II collagen by collagenase. The aim of this study was to determine whether the synthetic peptide of type II collagen can enhance collagenase activity and whether this involves an IL-1- and/or TNF-dependent pathways in normal human articular cartilage.

Hypothesis
1) That a synthetic peptide of type II collagen can induce type II collagen degradation by collagenase in explant culture of 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) was synthesized on a 0.25 mmol scale, using standard Fmoc chemistry and purified by reverse-phase chromatography. A collagenase-3 preferential inhibitor (RS102,481) was obtained from Roche Bioscience (Palo Alto, CA). Recombinant human IL-1 receptor antagonist (IL-1ra) and recombinant human soluble TNF receptor type I (sTNF-R1) (Amgen Inc) were used with human normal articular cartilages obtained at autopsy within 16 hours of death from 7 adults without arthritis. The cartilage samples were cut into small pieces and were cultured as explants for up to 16 days in serum-free medium with the peptide at up to 10 µM with or without RS102,481 (10 nM), IL-1ra (20 or 100 ng/ml), or sTNF-R1 (100, 500, or 1000 ng/ml). In some experiments, aminophenyl mercuric acetate (APMA) was added with SP and RS102,481, IL-1ra, or sTNF-R1 on day 4. An immunooassay (COL2-3/4C short, or Cs) was used to measure a collagenase-generated type II collagen cleavage neoepitope in type II collagen and its release into media. Proteoglycan glycosaminoglycan (GAG), which mainly reflects aggrecan, was also assayed by the dimethylmethylene blue method (5). Cartilage wet weight was used for normalizing the results.

Results
Effects of SP on type II collagen degradation
Compared with the control explant, SP at 1, 5 and 10 µM caused an increase in Cs content both in media and cartilage tissue in some but not all explants on day 8 and 12 in a dose-dependent manner.

Inhibitory effects of RS102,481, IL-1ra and sTNF-R1 on type II collagen degradation
The increased collagenase activity caused by SP could be inhibited by RS102,481, IL-1ra, or sTNF-R1. The strongest inhibition was seen when both IL-1ra and sTNF-R1 were added to the culture media. To detect latent pro-MMPs including pro-collagenases induced by SP added from day 0, APMA was added at 1 mM on day 4. When total Cs content both in cartilage and media was assayed on day 6, APMA caused a significant increase of Cs in the cultures treated with SP. Furthermore, this increased amount of latent pro-MMPs was inhibited by adding either RS102,481, IL-1ra, or sTNF-R1 from day 0.

Effects of SP on GAG content
SP with or without RS102,481, IL-1ra, or sTNF-R1 caused no significant effect on GAG content in cartilage tissue and its release into media.

Discussion
In normal human articular explants, we have demonstrated, for the first time, that a single peptide of type II collagen can cause in some human articular cartilage increased type II collagen cleavage by collagenase(s) and that this increased collagenase activity could be inhibited by either a collagenase-3 preferential inhibitor, IL-1ra, or sTNF-R1. MMPs such as collagenase-3 are known to be upregulated in OA cartilages. Thus, degradation products of type II collagen generated by collagenase and other MMPs may “feed-back” and activate MMP expression (mainly collagenase-3) through a cell surface receptor-mediated mechanism and also through IL-1- and TNF-mediated pathways, and may stimulate the degradation of intact type II collagen fibrils.

The identification of the true mechanism whereby a peptide of type II collagen can induce collagen cleavage in culture and not in other case is now under investigation. Our findings may provide a new model for understanding the mechanism involved in type II collagen turn-over in normal and OA cartilages, and also provide new targets for the therapeutic control of matrix breakdown in the treatment of OA and RA.

Conclusion
1) A peptide of type II collagen can induce type II collagen cleavage by collagenase in human articular cartilage explant cultures. This process may play an important role in cartilage degradation in OA where collagen cleavage products may “feed-back” and maintain upregulation of collagenase activity.
2) Generation of this increased collagenase activity involves the activities of IL-1 and TNF generated by chondrocytes. Inhibitors of these cytokines may be of value in the control of cartilage degradation in OA.

Acknowledgments
This study was funded by Shriners Hospitals for Children, Medical Research Council of Canada, Canadian Arthritis Network, and National Institute of Health.

References

** Amgen Inc, Thousand Oaks, CA.