IL-6 AND ITS SOLUBLE RECEPTOR AUGMENT AGGRECANASE-MEDIATED PROTEOGLYCAN CATABOLISM IN ARTICULAR CARTILAGE

Introduction
Elevated concentrations of interleukin-6 (IL-6) and soluble IL-6 receptor (sIL-6R) in the synovial fluids and serum of patients with arthritis have been implicated in the joint tissue destruction associated with these conditions, however studies conducted to date on the role and effects of IL-6 in the process of cartilage proteoglycan (aggrecan) catabolism are disparate. Contradictory results have been reported on the effects of IL-6 on cartilage PG catabolism. Previous studies have demonstrated that IL-6 can either protect or enhance IL-1-induced PG catabolism in different culture systems (1,2). However, cellular responses to IL-6 can also be mediated by a variety of other proinflammatory cytokines to further exacerbate articular tissue destruction.

Materials and Methods
Cartilage isolation and culture. Bovine articular cartilage explants (from 1-2 week old calves) were maintained for 4 days in DMEM in the presence or absence of 0.1 ng/ml recombinant human IL-1alpha or 100 ng/ml recombinant human TNF-alpha, and in the presence or absence of 50 ng/ml recombinant human IL-6, 250 ng/ml recombinant human sIL-6R, or both IL-6 and sIL-6R together.

Quantification of proteoglycans. Proteoglycan content in the medium of cartilage explant cultures was measured by DMMB assay. Differences in the release of sulfated GAG (expressed as µg GAG per mg wet weight of cartilage) associated with culture treatment (n=6 separate explants for each culture condition) were assessed using a two-factor analysis of variance with P values 0.05 being considered statistically significant.

Western blot analyses of aggrecanase- and MMP-generated aggrecan catabolites. Portions of conditioned media containing an equivalent quantity of proteoglycan macromolecules (measured as sulfated GAG) were analyzed by SDSPAGE and Western blotting as described (1) using monoclonal antibodies BC-3 or BC-14 (which specifically recognize the aggrecanase- or MMP-generated neoepitopes sequences 374-ARGSV... or 343-FFGVG... on aggrecan metabolites, respectively).

RNA Extraction and RT-PCR analyses. Total RNA was extracted from cultured cartilage explants and analyzed by RT-PCR as described (1) using primers specific for aggrecanase-1 and -2, IL-6, and gp130. The nucleotide sequences of all PCR products were verified.

Results and Discussion
Contradictory results have been reported on the effects of IL-6 on cartilage PG catabolism. Independent studies have reported that IL-6 can either protect against or enhance IL-1-induced PG catabolism in different culture systems (3,4). However, cellular responses to IL-6 can also be mediated by a variety of other proinflammatory cytokines to further exacerbate articular tissue destruction.

In conclusion, IL-6 and sIL-6R are capable of augmenting aggrecan catabolism under conditions of elevated matrix degradation in situ in articular cartilage explants, potentiating the effects of both IL-1 and TNF-α. These data, in association with the observed increases in serum and synovial fluid levels of IL-6 and sIL-6R in arthritic patients, strongly indicate a role for this pleiotropic cytokine in the diseases affecting synovial joints, participating in concert with other proinflammatory cytokines to further exacerbate articular tissue destruction.

References