Chondroitin-sulphate-rich proteoglycan is an essential component of the matrix of cartilage since it enables the tissue to resist compression during load-bearing. Loss of proteoglycan, such as occurs in rheumatoid arthritis, osteoarthritis and other joint diseases, results in severe impairment of the function of cartilage. IL-1 is the only purified cytokine known to cause cartilage to degrade its proteoglycan and to inhibit resynthesis.

Figure 1a shows the amount of proteoglycan (measured as percentage of total chondroitin sulphate) released from porcine articular cartilage during 6 days of culture in the presence of human recombinant TNFα or pure porcine IL-1. The TNFα caused up to 75% of the proteoglycan to be released, although it was less potent than the IL-1, which was significantly active at a 20-fold lower dose (0.5 pM). Figure 1b shows a similar experiment carried out on cartilage from bovine nasal septum which was cultured for a shorter period (48 h): again, the IL-1 was more potent. The time dependence of the release of proteoglycan from bovine cartilage caused by sub-maximal concentrations of the two agents revealed that their effects were additive. Figure 1c shows that 50 pM IL-1 or 290 pM TNFα caused a similar rate of release, and that this was approximately double when the agents were combined. Maximal stimulation of cartilage by IL-1 caused more rapid release of proteoglycan than did TNFα (Fig. 1d): results for two concentrations of each cytokine demonstrate that responses were maximal. Supramaximal doses of the two agents in combination caused a rate of release that was considerably faster than that due to TNFα alone, but was not significantly greater than that seen with IL-1 alone. The failure of TNFα to augment the maximal response to IL-1 may be because the limit of the chondrocytes' ability to degrade their matrix in vitro was being approached.

The enzymatic mechanism by which the proteoglycan is degraded in cartilage is not understood. Normally, cartilage proteoglycans aggregate in a specific manner with hyaluronic acid, and it is thought that the large size of these aggregates causes them to be trapped in the matrix. Cartilage stimulated by IL-1 releases fragments of proteoglycan which, as judged by gel filtration, are smaller than normal proteoglycan monomers and are unable to aggregate with hyaluronic acid. There is no evidence of degradation of their chondroitin sulphate chains. These changes suggest that degradation is by limited proteolysis of the protein core. The fragments of proteoglycan that were released by cartilage stimulated with TNFα behaved similarly on gel filtration to those generated by stimulation with IL-1 (Fig. 2). The bulk of the fragments generated by stimulation with either agent emerged from a Sepharose 2B column at a region between the elution positions of intact proteoglycan and proteoglycan digested with papain (which consists largely of single-chain chondroitin sulphate peptides). Addition of hyaluronic acid to the proteoglycan fragments before chromatography caused little or no formation of aggregates. This suggested that the hyaluronate binding region was blocked or had been lost. When the proteoglycan fragments were chromatographed under dissociative conditions (4 M guanidine-HCl in the chromatographic buffer) the position of the main peak was unchanged. These experiments showed that chondrocytes activated by TNF or IL-1 caused a similar limited proteolysis of the proteoglycans.

In order to study the effect of TNF on the synthesis of proteoglycan, cartilage was stimulated for 48 h, and 35SO4 was added to the culture medium for the last 6 h. In this procedure the isotope becomes incorporated into newly synthesized sulphated glycosaminoglycan (mainly chondroitin sulphate). At the end of the experiment the medium and cartilage were digested with papain, and glycosaminoglycan was precipitated from the digests with cetylpyridinium chloride. The amount of radioactivity present in the precipitate was a measure of chondroitin sulphate (and, by inference, proteoglycan) synthesis. In experiments made with porcine articular (Fig. 3a) or bovine nasal septal (Fig. 3b) cartilages, TNFα caused a marked sup-

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Fig. 1 Stimulation of release of proteoglycan from cartilage by TNFα or IL-1. a, Porcine articular cartilage. The amount of chondroitin sulphate released was expressed as a percentage of the total (the content of the medium and tissue combined). Results are shown as means of quintuplicate cultures ± s.e.m. ⋄, TNF; ○, IL-1; □, no addition. b, Bovine nasal septal cartilage. Symbols as for a, c. Time course for bovine cartilage disks cultured as in b with no addition (□), 50 pM IL-1 (○), 290 pM TNF (●), 50 pM IL-1 and 290 pM TNF in combination (△). Chondroitin sulphate released was measured at the indicated times and results are means ± s.e.m. of eight individual disk cultures. d, Time course for bovine cartilage disks cultured as in c, with no addition (□), 30 nM TNF (●), 90 nM TNF (●), 1.5 nM IL-1 (○), 4.5 nM IL-1 (○) and 90 nM TNF and 4.5 nM IL-1 in combination (△). Methods. a, Pieces of articular cartilage were removed from the metacarpal heads of freshly slaughtered young pigs. Pieces (~4 mg wet weight) were maintained for 48 h at 37°C in CO₂/air 1:19 in culture medium [Dulbecco's modified Eagle's medium (DMEM)] containing 5% normal bovine serum that had been heat-inactivated at 56°C for 30 min. Each was then transferred to a well of a 96-well multititre plate and incubated under the same conditions in 0.2 ml of culture medium, either with no addition, or with human TNFα or porcine IL-1 of pI 5. The medium was changed at 3 days and the culture was terminated after 6 days. Human TNFα was a recombinant protein expressed in Escherichia coli and purified as described previously. Porcine IL-1 was a natural leukocyte protein purified to homogeneity as described elsewhere. The pI 5 form rather than the pI 8 form was used for these experiments. After culture the medium and cartilage were separated. The cartilage was digested completely with papain (see legend to Fig. 3). The chondroitin sulphate content of this digest and culture medium was estimated by use of the metachromatic dye, dimethylmethylene blue (Serva); whale chondroitin sulphate (Sigma) was used as a standard.

Fig. 2 Gel chromatography of proteoglycans released from stimulated cartilage. Samples of culture supernatants from stimulated bovine nasal cartilage were chromatographed on a column (930 × 6.5 mm) of Sepharose 2B (Pharmacia) eluted with 0.5 M acetate buffer pH 5.8. Fractions (0.4 ml) were collected. Proteoglycan was detected as chondroitin sulphate by the dye dimethylmethylene blue (see Fig. 1) and is shown as A535. Culture supernatant from bovine nasal cartilage cultured as in Fig. 1 in the presence of 5 nM TNF; ----, the same supernatant to which hyaluronic acid (2%) had been added before chromatography; ----, culture supernatant from cartilage stimulated with 0.5 nM porcine IL-1, and to which hyaluronic acid had been added before chromatography; ----, a papain digest of proteoglycan extracted from fresh cartilage by 4 M guanidine-HCl. ----, proteoglycan (+2% hyaluronic acid) extracted by guanidine-HCl (4 M) from unstimulated cartilage that had been biosynthetically labelled with 35SO₄ for 24 h, measured as c.p.m. Arrows are: V₀, void volume; M, elution position of 35SO₄-labelled proteoglycan without addition of hyaluronic acid.
these experiments the effects may be additive. Synthesis of new proteoglycan. Exposure of cartilage to either loss of proteoglycan and impairment of function; furthermore, a similar action to IL-1 on chondrocytes. Degradation proteoglycan by limited proteolysis and inhibits their may be due to species differences.

Pigs, like humans, have two different IL-1 proteins: for these experiments the p15 IL-1 was used rather than the p18 form. The IL-1s are equipotent on cartilage and compete for the same receptors on porcine synovial fibroblasts: TNFα at 400 times excess over IL-1 did not compete for these receptors (T. A. Bird and J. S., in preparation). The augmentation of the effect of maximal doses of TNFα by IL-1 reported here is consistent with there being different receptors on chondrocytes for the two cytokines. Since they are apparently not homologous, IL-1 and TNFα would be expected to combine with different receptors. These considerations suggest that chondrocytes (and probably other connective tissue cells) could have two distinct types of receptor (one for IL-1 and one for TNF) whose interaction with ligand promotes resorption of matrix polymers while inhibiting their synthesis. Such a possibility could have important implications for the pharmacological control of inflammatory tissue destruction.

Following submission of this manuscript, Bertolini et al. have reported that human TNFs, like IL-1, stimulated CA release from fetal rat bones.

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Methods. a, Pieces of pig articular cartilage were dissected, preincubated and then stimulated either with TNFα or porcine IL-1 for 48 h exactly as described for Fig. 1, except that the culture medium (DMEM) contained 1% normal bovine serum (heat inactivated) during the stimulation period. For the last 6 h the medium was replaced with SO4-free culture medium (still containing TNFα or IL-1) to which was added 2.5 μCi ml−1 of 35SO4 (25–40 Ci mg−1; Amersham). After culture, the cartilage pieces were separated from the medium, briefly blotted to remove excess medium, and then weighted. Each piece was digested at 65°C for 2 h in 0.2 ml of 0.05 M sodium phosphate buffer pH 6.5 containing 1 mM EDTA, 2 mM N-acetylcysteine, 28 μg ml−1 papain (Sigma Type III). Samples of medium (0.2 ml) were also digested with 0.1 ml of the papain solution under the same conditions. Chondroitin sulphate (0.1 ml of 2 mg ml−1) was added to all the samples followed by 0.1 ml of dimethylpyridinium chloride (10% w/v). Samples were centrifuged, the precipitates were washed twice in 3% dimethylpyridinium chloride, then dissolved in 0.5 ml of formic acid and added to 5 ml of a scintillation mixture (Pico-fluor-30, Packard) and counted in a liquid scintillation counter. The radioactivity of the digests of tissue and medium were added together and the results expressed as d.p.m. per mg wet weight of cartilage. For a, except that disks of bovine nasal septal cartilage were used (see Fig. 1), and the culture medium contained 5% normal bovine serum throughout the experiment.

Post-translational insertion of a fragment of the glucose transporter into microsomes requires phosphoanhydride bond cleavage

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