

# Expression of 92-kD Type IV Collagenase/Gelatinase (Gelatinase B) in Osteoarthritic Cartilage and Its Induction in Normal Human Articular Cartilage by Interleukin 1

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## Abstract

We report here that a 92-kD gelatinolytic metalloproteinase is expressed as protein and mRNA in human osteoarthritic cartilage, but not in normal adult articular cartilage. Western immunoblotting demonstrated that the 92-kD gelatinolytic activity corresponded to 92-kD type IV collagenase/gelatinase (gelatinase B); mRNA for gelatinase B was identified by Northern blotting. Chondrocytes from normal cartilage also exhibited mRNA for 72-kD type IV collagenase/gelatinase (gelatinase A), tissue collagenase, and stromelysin-1, and these mRNAs were increased in osteoarthritic cartilage. Regional analysis of osteoarthritic cartilage samples from four individuals revealed that gelatinase B mRNA was expressed in grossly fibrillated areas; two of four nonfibrillated cartilage samples failed to exhibit the mRNA, but did have increased levels of mRNA for other neutral metalloproteinases. IL-1 $\alpha$  treatment of normal human cartilage explants or isolated chondrocytes induced increased levels of gelatinase B and increased mRNA for tissue collagenase and stromelysin-1. Under identical conditions, mRNA levels for gelatinase A were not increased indicating that regulation of this enzyme in human articular chondrocytes is distinct from that of other metalloproteinases. Our data showing expression of gelatinase B in fibrillated cartilage suggest that it is a marker of progressive articular cartilage degradation in osteoarthritis. (*J. Clin. Invest.* 1993. 92:179-185.)  
Key words: gelatinase • human articular cartilage • interleukin 1 • metalloproteinase • osteoarthritis

## Introduction

Osteoarthritis (OA)<sup>1</sup> is a disease of complex etiology that results in articular cartilage breakdown. Current experimental evidence suggests that, in addition to mechanical factors, pro-

teolytic pathways contribute to loss of cartilage matrix in OA (1, 2). Many classes of proteases are capable of degrading components of cartilage, but the matrix metalloproteinases, active at neutral pH in the extracellular matrix, are considered to play a particularly important role (3).

The neutral metalloproteinase family contains at least two distinct interstitial collagenases (tissue collagenase and neutrophil collagenase) (4, 5), two types of stromelysin (stromelysin 1 and 2) (6-10), a small proteinase PUMP-1 (10), and the 72-kD (11-13) and 92-kD (12, 14-20) type IV collagenases/gelatinases. Structural analysis of the genes for these metalloproteinases demonstrates an evolutionary relationship between these enzymes (3). The neutral metalloproteinases degrade various components of the extracellular matrix, but vary with respect to substrate specificity. The metalloproteinases share a zinc-binding site, are secreted as proenzymes, and require activation in vitro by the action of proteinases (plasmin, kallikrein, mast cell tryptase, and trypsin) or nonproteolytic agents, such as organomercurial compounds, *N*-ethylmaleimide, oxidized glutathione, and hypochlorous acid. The activating agents perturb the interaction of a critical cysteine residue in a highly conserved propeptide domain of the metalloproteinases with the catalytic zinc atom (3). However, it remains unclear whether a unique agent is responsible for metalloproteinase activation in vivo.

With respect to neutral metalloproteinase activity in cartilage, chondrocyte-derived collagenase (21-23) and stromelysin (24, 25) levels in OA are well characterized; secretion of these enzymes confers upon the chondrocytes the capability of degrading many components of the extracellular matrix, including type II collagen, fibronectin, laminin, and proteoglycans. Other enzymes, such as type IV collagenases/gelatinases, may influence the breakdown of cartilage in OA by accelerating degradation of matrix components or by digesting collagenase- or stromelysin-resistant matrix molecules, such as collagen type XI (26, 27). Although type IV collagenase/gelatinase activities are reported in rabbit cartilage (28, 29), little information exists on expression of the type IV collagenase/gelatinases in human articular cartilage in normal or pathological conditions.

In the present study, we demonstrate for the first time that nonfibrillated and fibrillated osteoarthritic cartilage produces a 92-kD type IV collagenase/gelatinase (gelatinase B; EC 3.4.24.35) that is not secreted by normal articular cartilage. A 72-kD type IV collagenase/gelatinase (gelatinase A; EC 3.4.24.24) is produced by both normal and osteoarthritic cartilage. Expression of gelatinase B is inducible in normal human articular cartilage and in isolated chondrocytes by addition of

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1. Abbreviations used in this paper: APMA, 4-aminophenylmercuric acetate; OA, osteoarthritis; TPA, 12-*O*-tetradecanoylphorbol acetate.

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the cytokine, IL-1 $\alpha$ , which also stimulates synthesis of tissue collagenase and stromelysin-1 (30, 31). Our data suggest that gelatinase B may be a marker of progressive articular cartilage degradation in OA.

## Methods

**Osteoarthritic cartilage organ culture.** Osteoarthritic cartilage samples were obtained from arthritic patients undergoing total joint arthroplasty. In each case, full-thickness cartilage slices (40–50 mg wet weight) were made, and cultured in serum-free medium which consisted of DME/F12 (1:1) medium, 50  $\mu$ g/ml of gentamicin,  $2 \times 10^{-8}$  M selenium, and a lipid supplement (32) in 24-cluster microwell culture plates (Costar, Cambridge, MA) for 3 d. The medium was renewed on the second day of culture; the renewed culture medium was harvested on the fourth day of culture, and kept frozen at  $-20^{\circ}\text{C}$  until use.

**Normal human articular cartilage organ culture.** Normal human articular cartilage was obtained at autopsy within 24 h of death and processed immediately. Knee cartilage from four adults (ages 35–65 yr) were studied. All normal cartilage used had a normal macroscopic appearance. None of these knees showed any overt signs of cartilage degeneration. None of the patients surveyed had any clinical history of chemotherapy or radiotherapy. Full-thickness cartilage slices (40–50 mg wet weight), similar in size to the OA cartilage slices, were distributed in 24-cluster microwell culture plates and cultured in the serum-free medium for 3 d. The medium was renewed on the second day of culture, and normal cartilage ( $n = 4$ ) slices from four individuals were challenged with IL-1 $\alpha$  (100 U/ml, Genzyme Corp., Boston MA) for 2 d.

**Cell culture.** Normal human articular chondrocytes were isolated from the samples described above by sequential digestion at  $37^{\circ}\text{C}$ , with 1 mg/ml of trypsin (1:250, Gibco, Grand Island, NY) for 1 h and 2 mg/ml of each collagenase (CLS-II and CLS-IV; Worthington Biochemical Corp., Freehold, NJ) for 12–16 h. Cells were plated at  $1.5 \times 10^5$  cells/cm $^2$  on poly-L-lysine-coated dishes and cultivated in the serum-free medium. After 3 d, the medium was replaced, followed by culture in the presence or absence of IL-1 $\alpha$  (100 U/ml) in the serum-free medium for 48 h. Human keratinocytes of neonatal foreskins were a generous gift of Dr. T. Iwasaki (Department of Dermatology, Stanford University). The cells were cultured in serum-free MCDB 153 medium with 0.1 mM calcium, 30  $\mu$ g/ml of bovine pituitary extract and supplements (Clonetics Corp., San Diego, CA). The cells were treated with 50 ng/ml of 12-*O*-tetradecanoylphorbol acetate (TPA) for 24 h.

**Zymographic analysis of metalloproteinases.** Zymography of secreted proteins by electrophoresis in 10% SDS-polyacrylamide gels impregnated with 1 mg/ml type I gelatin from porcine skin (Sigma Chemical Co., St. Louis, MO) was used to demonstrate the presence of gelatinolytic metalloproteinases in medium from cartilage organ culture (33). Aliquots of medium were mixed with Laemmli sample buffer containing 2.5% SDS without  $\beta$ -mercaptoethanol and electrophoresed without boiling under nonreducing conditions at  $4^{\circ}\text{C}$ , constant current 20 mA. After electrophoresis, gels were washed in 2.5% Triton X-100 for 30 min to allow proteins to renature. Gels were then incubated overnight in substrate buffer (50 mM Tris, pH 8.0, 10 mM CaCl $_2$ ) at  $37^{\circ}\text{C}$ . After incubation, gels were stained with Coomassie Blue R250 to reveal zones of lysis within the gelatin matrix. 1, 10 phenanthroline (2 mM), PMSF (1 mM), or pepstatin (1 mM) was added to the substrate buffer to identify which classes of proteinases were responsible for lysis of the substrate in the gels (34). Some of the conditioned medium samples were incubated with 0.5 mM 4-aminophenylmercuric acetate (APMA) for 3 h at  $37^{\circ}\text{C}$  to activate latent protease before loading to the substrate gels (35).

**Immunoblotting.** Supernatants from organ cultures were concentrated using a microconcentrator (Centricon-30; Amicon, Beverly, MA). Protein concentration was measured using a protein assay kit (Bio-Rad Laboratories, Richmond, CA). 20  $\mu$ g of protein was resus-

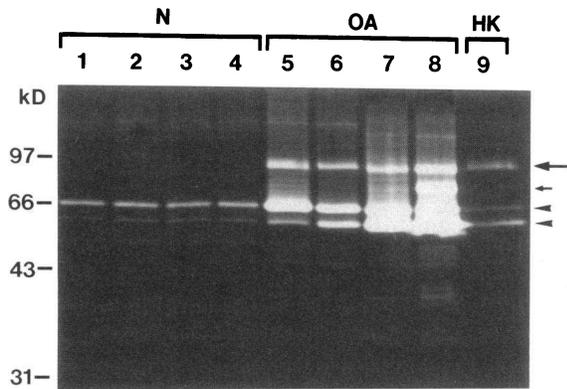
uspended in loading dye (125 mM Tris-HCl [pH 6.8], 4% [wt/vol] SDS, 0.01% bromphenol blue, 20% glycerol), reduced (10%  $\beta$ -mercaptoethanol), heated for 2 min at  $95^{\circ}\text{C}$ , loaded, and electrophoresed on a 10% polyacrylamide gel. After electrophoresis, the proteins were transferred to nitrocellulose membrane (Schleicher & Schuell, Inc., Keene, NH) in electroblotting buffer (20 mM Tris (pH 8.0), 150 mM glycine, 20% MeOH) overnight at 250 mA. Western blots were blocked in a milk solution (5% nonfat dry milk, 150 mM NaCl, 50 mM Tris, 0.05% Tween) at room temperature until solidified. The blots were exposed to a primary antibody against gelatinase B (36) and subsequently developed by using goat anti-rabbit IgG horseradish peroxidase in conjunction with an enhanced chemiluminescence Western blotting detection system (Amersham International, Amersham, UK).

**Northern blot analysis.** Total RNA was obtained from chondrocytes isolated from normal cartilage or from osteoarthritic cartilage samples, as described above. To evaluate mRNA levels within focal areas of OA, cartilage samples from four individuals were separated and analyzed independently based on the presence or absence of gross fibrillation. The level of fibrillation was determined visually by surface inspection. RNA was extracted from cells using a solution containing 4 M guanidine isothiocyanate, 25 mM sodium citrate, 0.2% (wt/vol) *n*-lauroylsarcosine, and 0.7% (vol/vol)  $\beta$ -mercaptoethanol, followed by centrifugation at 35,000 rpm for 20 h through a 5.6 M cesium chloride cushion. The RNA pellet was solubilized in water, sequentially extracted with 4:1 (vol/vol) chloroform/*n*-butanol, 1:1 (vol/vol) phenol/chloroform, and chloroform. The aqueous phase containing RNA was adjusted to 0.3 M sodium acetate. RNA was precipitated overnight with  $2.5 \times$  vols of absolute ethanol. The concentration of RNA was determined by spectrophotometric absorption at 260 nm. 10  $\mu$ g of total RNA from each sample was separated on a formamide/formaldehyde agarose gel and transferred to a nitrocellulose membrane by capillary blotting overnight. The membranes were air-dried, then baked at  $80^{\circ}\text{C}$  for 2 h. A cDNA for gelatinase A was isolated from the cDNA library of primary human synovial fibroblasts as described before (37). A 900-bp Pst I fragment of gelatinase A, a 2.3-kb Xba I fragment of gelatinase B (17), a 1.5-kb Xba I fragment of tissue collagenase (37), and a 1.8-kb Pst I fragment of stromelysin-1 (37) were  $^{32}\text{P}$ -labeled by random priming (Amersham International). Each probe had a specific activity between 4 and  $6 \times 10^8$  cpm/ $\mu$ g. Blots were hybridized at  $42^{\circ}\text{C}$  overnight and washed at  $55^{\circ}\text{C}$  in  $0.5 \times \text{SSC} + 0.1\%$  SDS for 1 h. The membranes were exposed to Kodak X-Omat films with a Du Pont light intensifying screen at  $-80^{\circ}\text{C}$ . To strip blots, nitrocellulose membranes were put into boiled  $0.1 \times \text{SSC} + 0.1\%$  SDS until the solution cooled.

**Densitometric analysis.** Autoradiographic results generated from Northern blots were quantified using a densitometer (LKB Instruments, Inc., Gaithersburg, MD) to determine relative levels of mRNA expression. The densitometric values obtained for each osteoarthritic cartilage sample, fibrillated and nonfibrillated, were divided by the average value obtained for four normal cartilage samples corresponding to the cDNA being tested. The normal values were normalized to 1.0 for comparison.

## Results

**Arthritic articular cartilage produces a 92-kD gelatinase and a 72-kD gelatinase.** Gelatin zymograms of conditioned culture medium from four arthritic cartilage samples demonstrated 92-kD gelatinase activity in all samples; culture medium from four normal cartilage samples did not exhibit this activity (Fig. 1, *large arrow*). These observations have now been extended to six other patients (data not shown). Some OA samples showed an 82-kD gelatinolytic band, indicative of the active form of the 92-kD enzyme (Fig. 1, lane 8, *small arrow*). The 92-kD gelatinase was shown to be a metalloprotease by the complete loss of enzyme activity when the zymogram was incubated in

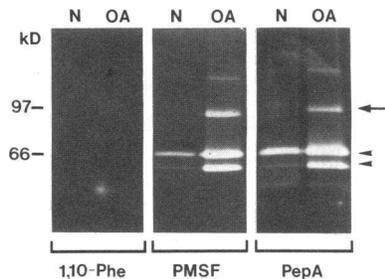


**Figure 1.** Expression of a 92-kD gelatinase by osteoarthritic cartilage. Cartilage slices were cultured in the serum-free medium for 3 d, as described in Methods, and conditioned medium samples were electrophoresed on a gelatin-substrate gel. Zones of clearing represent gelatinolytic activity. Four normal cartilage cultures (*N*, lanes 1–4) did not show the 92-kD gelatinase (*large arrow*), whereas four osteoarthritic cartilage cultures did (*OA*, lanes 5–8). TPA-stimulated human keratinocyte-conditioned medium was run as a positive control for the 92-kD gelatinase (lane 9). 72-kD gelatinase appeared as a doublet of gelatinolytic activity at 66 and 62 kD (*arrowheads*). The 72-kD gelatinase was expressed by all cartilage samples and was increased in osteoarthritic cartilage. One OA sample showed an additional 82-kDa gelatinolytic band, representative of the active form of the 92-kD gelatinase (lane 8, *small arrow*). Molecular mass markers are in the far left lane.

the presence 1,10 phenanthroline (Fig. 2). PMSF, a serine protease inhibitor, and pepstatin A, an acid proteinase inhibitor, had no effect on the 92-kD gelatinase activity under similar conditions.

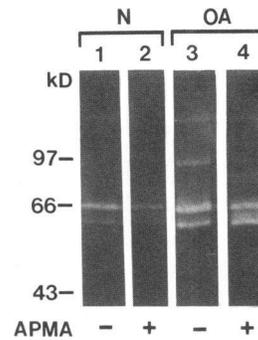
In contrast to the 92-kD enzyme, 72-kD gelatinase was expressed by all cartilage samples and was increased in osteoarthritic cartilage (Fig. 1, *arrowheads*). The 72-kD gelatinase appeared frequently as a doublet of gelatinolytic activity at 66 and 62 kD on SDS-PAGE under nonreduced conditions (12). The 72-kD enzyme was also inhibited by 1,10 phenanthroline (Fig. 2).

Treatment of the medium aliquots with the mercurial, APMA, was carried out to activate the zymogens of matrix metalloproteinases. APMA transformed the 92-kD gelatinase into a 82-kD species (Fig. 3). In contrast, the 72-kD gelatinase became a triplet of gelatinolytic activity at 66, 64, and 62 kD after APMA treatment (Fig. 3).



**Figure 2.** Identification of the gelatinolytic enzymes as metalloproteinases. After electrophoresis of conditioned medium samples from normal (*N*) and osteoarthritic cartilage (*OA*), gelatin substrate gels were incubated in the presence of 2 mM 1,10 phenanthroline (*1,10-Phe*), 1 mM PMSF, 1 mM pepstatin A (*PepA*). All gelatinases, including the 92-kD (*arrow*) and 72-kD gelatinase (*arrowheads*) were inhibited by 1,10 phenanthroline but resistant to inhibition by other proteinase inhibitors.

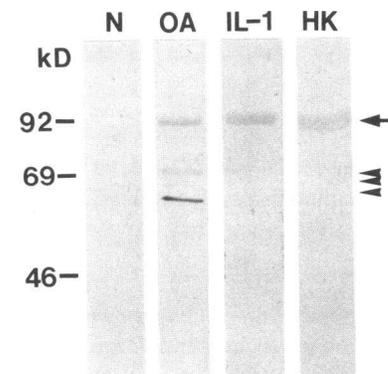
**Figure 3.** Effects of APMA on gelatinases. Before loading, conditioned medium samples were incubated without (lanes 1 and 3) or with 0.5 mM APMA for 3 h at 37°C to activate latent proteases (lanes 2 and 4). APMA transformed the 92-kD gelatinase (*large arrow*) into a 82-kD species (*small arrow*). In contrast, the 72-kD gelatinase became a triplet of gelatinolytic activity at 66, 64, and 62-kD by APMA treatment (*arrowheads*).



The higher molecular weight gelatinase produced by osteoarthritic cartilage comigrated with the 92-kD gelatinase secreted by human foreskin keratinocytes stimulated with TPA (17) (Fig. 1). Western blot analysis was carried out using a polyclonal antibody against gelatinase B to determine the immunologic cross-reactivity between chondrocyte and keratinocyte gelatinases (Fig. 4). The 92-kD band in osteoarthritic cartilage culture medium was recognized by this antibody whereas no such band was present in normal cartilage culture medium (Fig. 4, *arrow*). In addition, in the OA cartilage, two less distinct bands and one prominent band were present at regions near 69 kD (Fig. 4, *arrowheads*). Conditioned medium from TPA-stimulated keratinocyte cultures exhibited the 92-kD gelatinase B and served as a positive control.

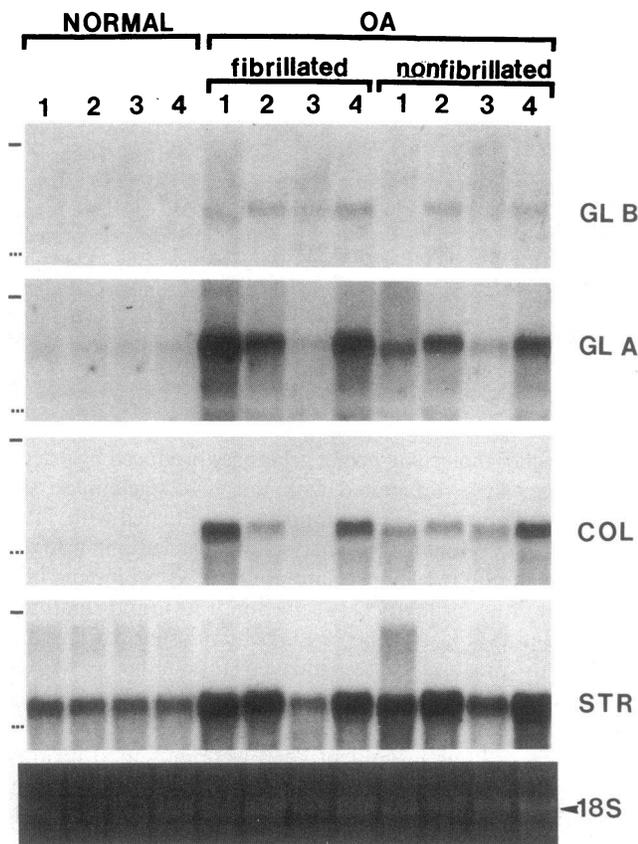
**Northern blot analysis of neutral metalloproteinase mRNA.** Northern blot analysis showed that mRNA for gelatinase B was not expressed in normal chondrocytes, a finding consistent with zymographic and immunoblot results (Fig. 5). Normal and osteoarthritic cartilage chondrocytes expressed mRNA for 72-kD gelatinase A, tissue collagenase, and stromelysin-1. In addition, osteoarthritic chondrocytes showed increased levels of mRNA for each of these enzymes when compared with levels present in normal chondrocytes.

Separation of four osteoarthritic cartilage samples into different morphologic regions showed that the mRNA for gelati-



**Figure 4.** Cross-reactivity of the 92-kD gelatinases from cartilage and TPA-stimulated keratinocytes. Conditioned medium samples from normal cartilage (*N*), osteoarthritic cartilage (*OA*), normal cartilage stimulated with IL-1 $\alpha$  (*IL-1*), and human keratinocytes stimulated with TPA (*HK*) were electrophoresed, transferred to nitrocellulose

membrane, and subjected to immunoblot analysis using a polyclonal anti-gelatinase B. The antibody identified a band at 92 kD (*arrow*) in medium from osteoarthritic cartilage, normal cartilage stimulated with IL-1 $\alpha$ , and keratinocyte cultures but showed no reactivity with medium from normal cartilage. In addition, in the osteoarthritic cartilage, two less distinct bands and one prominent band were present at regions near 69 kD (*arrowheads*).



**Figure 5.** Northern blot analysis of neutral metalloproteinase expression in normal and osteoarthritic articular cartilage. Normal cartilage was from four individuals (*N*, lanes 1–4). Osteoarthritic cartilage samples from four individuals were separated into fibrillated (*OA*, lanes 1–4) and nonfibrillated (*OA*, lanes 1–4) regions that were maintained as paired samples. RNA was extracted from each region separately. 10  $\mu$ g of total RNA from each sample was separated on formamide/formaldehyde agarose gel as described in Methods. Blots were probed sequentially with gelatinase B cDNA (*GL B*), gelatinase A cDNA (*GL A*), tissue collagenase cDNA (*COL*), and stromelysin-1 cDNA (*STR*). Intensity of the 18S RNA staining by ethidium bromide is shown for comparison of total RNA loaded per lane. The location of 28S and 18S ribosomal RNA is shown by the small bars (solid, 28S; and dotted, 18S) to the left of the figure.

nase B was present in chondrocytes from all fibrillated osteoarthritic cartilage samples and in only two of four nonfibrillated osteoarthritic cartilage samples (Fig. 5). Chondrocytes from two nonfibrillated osteoarthritic cartilage samples failed to express mRNA for gelatinase B, although mRNA levels for gelatinase A, tissue collagenase, and stromelysin-1 were elevated relative to the normal cartilage chondrocytes (Fig. 5: *OA* cases 1 and 3).

Densitometric analysis of the Northern blots showed that the relative levels of expression of metalloproteinases associated with osteoarthritic cartilage were elevated in both fibrillated and nonfibrillated cartilage. The levels of mRNA for gelatinase A and tissue collagenase were increased in a range from 2- to 25-fold in fibrillated osteoarthritic cartilage (Fig. 6); the corresponding samples of nonfibrillated *OA* cartilage typically matched the levels in the fibrillated cartilage (Fig. 6, *connecting lines*), although not in every case. Stromelysin-1 mRNA levels were also elevated in three of four samples, but the increases

were only two- to threefold greater than levels in normal cartilage.

*Regulation of gelatinase B expression in normal cartilage and chondrocytes by IL-1 $\alpha$ .* Effects of IL-1 $\alpha$  on production of the gelatinases by normal cartilage explants and normal chondrocytes in high density culture were determined by comparative zymography of culture medium samples (Fig. 7). IL-1 $\alpha$  induced the production of gelatinase B by both normal cartilage and chondrocytes within 48 h (Fig. 7, *large arrow*). In addition to the induction of the 92-kD gelatinase B, IL-1 $\alpha$  also increased levels of other gelatinases (bands at 50–55 kD, Fig. 7, *triangles*), possibly tissue collagenase and stromelysin-1. A band of gelatinolytic activity migrating at 120 kD (Fig. 7, *small arrow*) was also increased by exposure to IL-1 $\alpha$ . The bands located at 66 and 62 kD were relatively unchanged by IL-1 $\alpha$  and likely represent migration of 72-kD gelatinase A species under reduced conditions (12) (Fig. 7, *arrowheads*). Western blot analysis of the culture medium from IL-1 $\alpha$  stimulated normal cartilage showed that the newly produced 92-kD enzyme was recognized by a polyclonal antibody against gelatinase B (see Fig. 4). However, only the proenzyme was visible after IL-1 stimulation by Western blot.

mRNA expression patterns of the gelatinases in response to IL-1 $\alpha$  were also examined by Northern blot analysis (Fig. 8). mRNA for gelatinase B was inducible by IL-1 $\alpha$ , consistent with zymographic and Western blot results. IL-1 $\alpha$  did not increase the expression of gelatinase A, as previously reported for human tumor cells (38). Treatment with IL-1 $\alpha$  increased both tissue collagenase and stromelysin-1 mRNA levels, as reported previously (30, 31).

## Discussion

Our data demonstrated that osteoarthritic cartilage secreted 92-kD gelatinase B, an enzyme not produced by normal adult human articular cartilage. However, IL-1 $\alpha$  induces expression of gelatinase B in normal cartilage. The properties of this enzyme are consistent with its being a neutral metalloproteinase. The 92-kD enzyme was converted to a 82-kD form by APMA treatment and its gelatinolytic activity was completely inhibited by 1,10 phenanthroline, but not by serine- or acid proteinase inhibitors. The enzyme was identified by Western blot as gelatinase B found in other cell lines (12, 14–20). We also showed the presence of mRNA for gelatinase B in osteoarthritic cartilage using Northern blot analysis. The fact that osteoarthritic cartilage produces gelatinase B is, to our knowledge, previously unreported. In addition, the Western blot of osteoarthritic cartilage showed the presence of three identifiable bands in the region of 69 kD. Recent studies of the 92-kD gelatinase B in a human fibrosarcoma cell line show that activation of the proenzyme results in an intermediate form of 83 kD and two active forms of 67 and 64 kD (39). The latter fully activated species results from stromelysin activity and lacks both amino- and carboxy-terminal peptides; this form would be recognized in the Western blotting and may be represented as the prominent staining band in the osteoarthritic cartilage lane. The affinity-purified polyclonal antibody used in this study was generated from a synthetic peptide representing amino acids 397–414 of gelatinase B (36), a region which would be preserved in the 64-kD active form of the enzyme.

The presence of the 92-kD proenzyme and species of active enzyme in *OA* cartilage are consistent with the hypothesis that

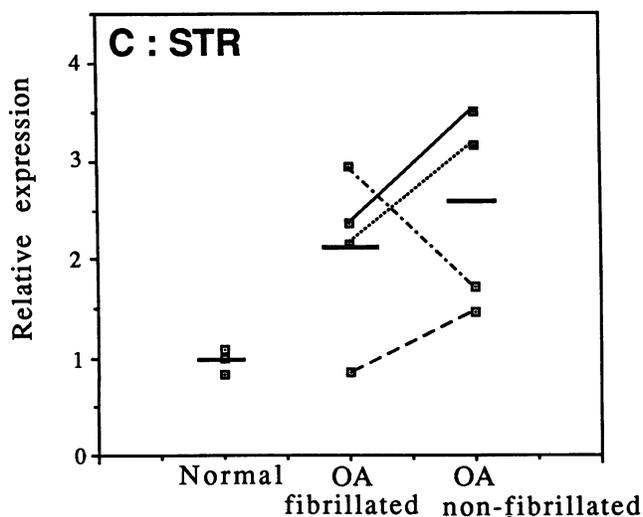
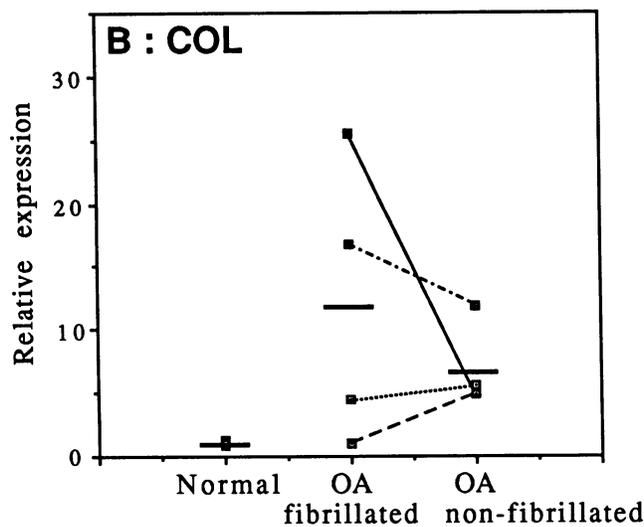
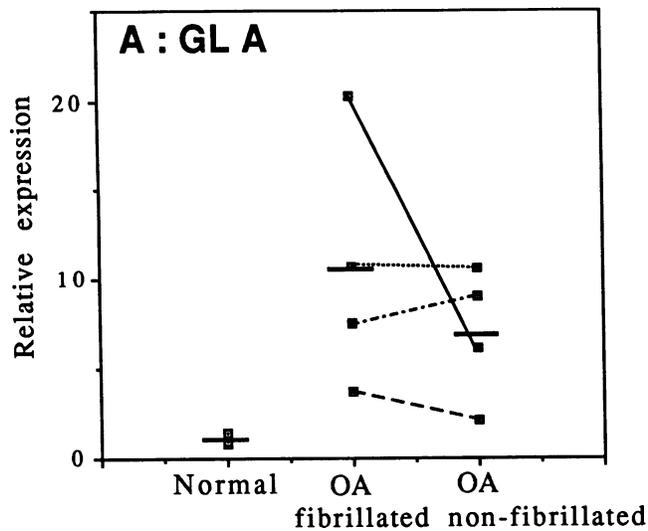


Figure 6. Densitometric analysis of the northern blots. The densitometric values obtained for each pair of osteoarthritic cartilage samples, fibrillated and nonfibrillated (see connecting lines), were divided by the average value obtained for four normal cartilage samples

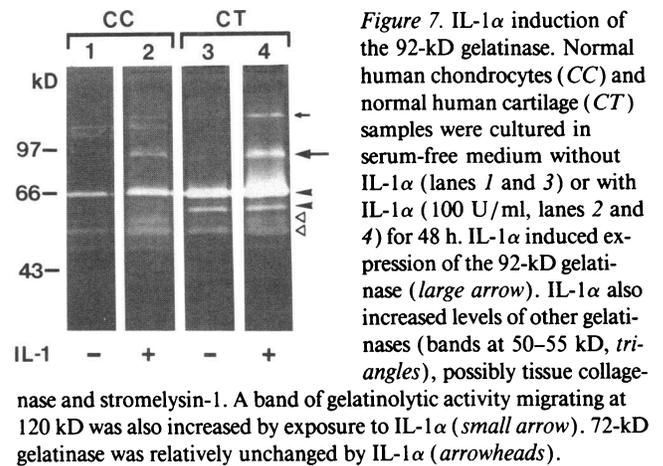


Figure 7. IL-1 $\alpha$  induction of the 92-kD gelatinase. Normal human chondrocytes (CC) and normal human cartilage (CT) samples were cultured in serum-free medium without IL-1 $\alpha$  (lanes 1 and 3) or with IL-1 $\alpha$  (100 U/ml, lanes 2 and 4) for 48 h. IL-1 $\alpha$  induced expression of the 92-kD gelatinase (large arrow). IL-1 $\alpha$  also increased levels of other gelatinases (bands at 50–55 kD, triangles), possibly tissue collagenase and stromelysin-1. A band of gelatinolytic activity migrating at 120 kD was also increased by exposure to IL-1 $\alpha$  (small arrow). 72-kD gelatinase was relatively unchanged by IL-1 $\alpha$  (arrowheads).

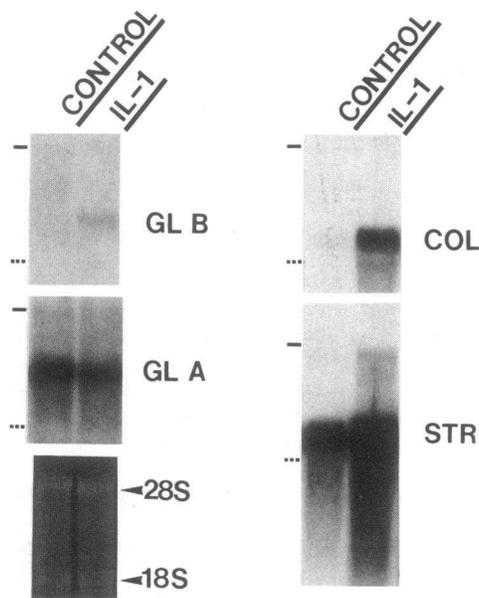
the metalloproteinases contribute importantly to the extracellular matrix degradation. Total endogenous proteoglycan and collagen-degrading activities are elevated in osteoarthritic cartilage relative to normal tissue and are locally increased at the erosion centers (21, 22, 25). In OA, interstitial collagenase may initiate degradation of type II collagen (40) by cleaving at a site within the triple helix resulting in three-quarter and one-quarter fragments (41) that denature spontaneously at body temperature. The resultant gelatin would then be susceptible to other metalloproteinases, such as gelatinase B, that have a high specific activity for denatured collagens (14, 16, 17).

Our data support such a role for the gelatinase B since the mRNA levels for gelatinase B were elevated in chondrocytes from fibrillated cartilage but not in all samples of nonfibrillated cartilage. Chondrocytes from the nonfibrillated cartilage samples did, however, show elevated levels of mRNA for tissue collagenase and stromelysin-1, enzymes proposed to initiate cartilage degradation and activate other proenzymes (21–25, 39, 42).

In addition to gelatinolytic activity, gelatinase B degrades various components of extracellular matrix, including collagen types IV, V, and XI (14, 16, 17, 43, 44) and elastin (45, 46) that are resistant to collagenase and stromelysin. Therefore, expression of gelatinase B in OA cartilage may contribute importantly to the progressive breakdown of cartilage by degrading minor constituents of the extracellular matrix. Okada et al. (47) demonstrated that MMP-3 (stromelysin-1) is present in osteoarthritic cartilage and synovium and suggested that this enzyme is responsible for proteoglycan degradation. Our data demonstrating the presence of gelatinase B in nonfibrillated and in fibrillated osteoarthritic cartilage may represent a condition in which stromelysin-1 and other enzymes act in concert to degrade the structural backbone of the cartilage matrix.

We also demonstrated that osteoarthritic cartilage exhibits elevated levels of gelatinase A, an enzyme present in normal cartilage. However, the expression patterns of the gelatinase A appear distinct from gelatinase B. Although these enzymes

corresponding to the cDNA being tested. The normal values were set to 1.0 for comparison with OA values; the mean level of mRNA for each of the cartilage samples (normal, fibrillated, and nonfibrillated) are shown by horizontal bars. The relative levels of expression of gelatinase A (A: GLA), tissue collagenase (B: COL), and stromelysin-1 (C: STR) were elevated in both fibrillated and nonfibrillated OA cartilage.



**Figure 8.** Northern blot analysis of neutral metalloproteinase expression in normal articular chondrocytes. Chondrocytes were untreated (control) or treated with 100 U/ml of IL-1 $\alpha$  in serum-free medium for 48 h before RNA extraction. Blots were probed as described for Fig. 5. mRNA for gelatinase B (*GL B*) was induced by IL-1 $\alpha$ , although IL-1 $\alpha$  did not increase expression of gelatinase A (*GL A*). Treatment with IL-1 $\alpha$  increased both tissue collagenase (*COL*) and stromelysin-1 (*STR*) mRNA levels. Intensity of the 28S and 18S RNA staining by ethidium bromide is shown for comparison of total RNA loaded per lane.

have similar substrate specificity profiles *in vitro*, the regulation of the two enzymes is distinct in other tissues. For example, alveolar macrophages and polymorphonuclear leukocytes have been found to secrete gelatinase B but not gelatinase A (17). Human skin fibroblasts secrete gelatinase A but not noticeable amounts of gelatinase B (17). Some transformed cells such as human fibrosarcoma cells HT-1080 secrete both enzymes (12), although secretion of the two enzymes by these cells is not coordinately regulated (17, 48).

Recent characterization of genes for the two enzymes demonstrates that they differ substantially from each other with respect to transcriptional control (49, 50). Our data suggest that gelatinase A may participate in the turnover of normal adult cartilage matrix, whereas gelatinase B may only play a role in the removal of denatured collagen fragments during degradation of cartilage matrix in OA.

Paracrine and/or autocrine mechanisms involving cytokines are proposed to be instrumental in degradation of human osteoarthritic cartilage. Among the cytokines, IL-1 appears to be an important mediator of inflammation and cartilage destruction. IL-1 stimulates the production of procollagenase and prostromelysin in chondrocytes (30, 31) and suppresses the synthesis of aggregating proteoglycan and cartilage-specific collagen types II and IX (51, 52). In the present study IL-1 $\alpha$  added to the culture medium induced gelatinase B production by chondrocytes in normal human articular cartilage. Similar results were obtained from animal models (15, 29). IL-1 $\alpha$  did not increase the production of the 72-kD enzyme by normal human chondrocytes as previously reported for human tumor cells (38). Our data also support the idea that expression of

gelatinase B, as in other tissues, is regulated independently of gelatinase A.

The question remains as to the precise origin of the enzyme detected in the osteoarthritic cartilage. Evidence is available that other tissues such as the inflammatory cells, macrophages and polymorphonuclear leukocytes, secrete neutral metalloproteinases, including gelatinase B (53, 54). In an arthritic joint, the role of cartilage-derived enzymes versus enzymes originating from other tissues remains to be determined. The implication from our study is that chondrocytes retain full capacity for elaboration of gelatinase B and may contribute importantly to the disease process through its release.

In conclusion, chondrocytes in osteoarthritic cartilage secrete a 92-kD gelatinase that is immunologically cross-reactive with gelatinase B secreted by activated keratinocytes. Normal chondrocytes can also produce the enzyme by the action of IL-1 $\alpha$ . Our data showing expression of gelatinase B in all fibrillated cartilage, but not in some nonfibrillated cartilage that has elevated levels of gelatinase A, tissue collagenase, and stromelysin-1, suggests that gelatinase B may facilitate the progressive destruction of articular cartilage in OA.

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