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Analysis for the major contributor of collagenase to the primary cleavage of type II collagens in cartilage degradation

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Abstract Degradation of type II collagen is a central process in cartilage destruction seen in osteoarthritis and rheumatoid arthritis. Primary cleavage of type II collagen at the collagenase site is rate-limiting and is, therefore, a critical step for its degradation. The major contributor to this cleavage was identified in three isozymes of collagenase in human cartilage. Primary cultured human chondrocytes were used for the study. The production of collagenase-1 was major in total production for three isozymes of collagenase after stimulations with any concentration of tumor necrosis factor-α and/or interleukin-1 at 48 and 72 h, comprising 98% or greater of the total collagenase. When the production of collagenase-1 was specifically suppressed by the transfection with duplexes of 21-nucleotide small interfering ribonucleic acid into the cells, the activity of type II collagen cleavage was linearly decreased at neutral pH after activation. The relative contribution of collagenase-1 to the primary cleavage of type II collagen was determined to be 85%–93%. These findings suggest that collagenase-1 is a major contributor to the primary cleavage of type II collagens in human cartilage and is a potential therapeutic target for osteoarthritis and rheumatoid arthritis.

Key words Cartilage degradation · Collagenase · RNA interference · Type II collagen

Introduction

Type II collagen is a major constituent of cartilage that forms a frame network essential for the maintenance of the architecture and tensile property of cartilage. Degradation of type II collagen is a central process in the destruction of cartilage in osteoarthritis (OA) and rheumatoid arthritis (RA).[1–5] At neutral pH, type II collagen is initially cleaved by a collagenase, a specific breaking enzyme, at a specific site in its α1 chain. This creates a fragment containing the N-terminal three-quarters of the molecule and a fragment containing the C-terminal one-quarter. After the cleavage, the fragments are denatured and subsequently degraded with gelatinases and other proteases. The primary cleavage at the collagenase site is rate-limiting[6,7] and is, therefore, a critical step in the cartilage destruction seen in OA or RA. Three distinct collagenases, collagenase-1 or matrix metalloproteinase-1 (interstitial collagenase), collagenase-2 or matrix metalloproteinase-8 (neutrophil collagenase), and collagenase-3 or matrix metalloproteinase-13, are expressed at both the message level and the protein level in the cartilage of patients with OA or RA.[8–13] These collagenases are also produced by human chondrocytes in primary culture that have been stimulated with tumor necrosis factor-α (TNF-α) or interleukin-1 (IL-1), which are major mediators of arthritis.[14–18] However, it is not clear which of these three isozymes is the major contributor to the primary cleavage of type II collagen in cartilage. The major contributor would potentially be a target for therapeutic intervention in OA or RA.

In the present study, we examined the expression of collagenases produced from primary cultured human chondrocytes after stimulation with various concentrations of TNF-α and/or IL-1. We also made duplexes of 21-nucleotide siRNA (small interfering ribonucleic acid) with symmetric 2 nucleotide 3′ overhangs in order to specifically suppress the production of collagenase-1 through RNA (ribonucleic acid) interference.[19] We then determined the relative contribution of this isozyme to the primary cleavage of type II collagens by measuring the reduction in the activity of type II collagen cleavage in media conditioned by cultured human chondrocytes transfected with siRNA duplexes.

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Materials and methods

Reagents

Dulbecco's modified Eagle's medium (DMEM) was purchased from Life Technologies (Gaithersburg, MD, USA). Fetal bovine serum was purchased from Cytosystems (Castle Hill, Australia). Interleukin-1 and TNF-α were from R&D Systems (Minneapolis, MN, USA). Bovine testes hyaluronidase was from Sigma (St. Louis, MO, USA). The Human Matrix Metalloproteinase-1, -8, and -13 Biotrak Enzyme-linked Immunosorbent Assay systems and the Human Tissue Inhibitor of Metalloproteinase-1 and -2 Biotrak Enzyme-linked Immunosorbent Assay systems were from Amersham Pharmacia Biotech (Buckinghamshire, UK). DNeasy Tissue Kits was from QIAGEN (Valencia, CA, USA). GenePORTER transfection reagent was from Gene Therapy Systems (San Diego, CA, USA). Rapid Collagenase Assay Kit was from Chondrex (Redmond, WA, USA). Gene-specific sense and antisense 21-nucleotide RNA oligomers labeled with or without fluorescein isothiocyanate were from Takara Bio (Shiga, Japan).

Isolation and primary culture of human chondrocytes

Human articular cartilage was obtained from the lateral femoral condyles of knees of 12 patients with medial type OA (8 women and 4 men, mean age 63 years) when total knee arthroplasty was performed in the authors' institution. Informed consent was given by the patients and the approval was obtained from the ethical committee of the university and the institutional review board. The authors diagnosed OA according to the standard criteria. Pharmacological treatment prior to sampling was limited to nonsteroidal anti-inflammatory drugs. The condition of the obtained cartilage corresponded to grade I of the Outerbridge classification. Full-thickness slices of the articular cartilage were dissected under aseptic conditions and subjected to sequential pronase and collagenase digestion to liberate chondrocytes from the cartilage.[20] Isolated human chondrocytes were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Approximately 3.0×10^6 cells were obtained from each donor, and these primary cultured cells were used for the study.

Preparation of small interfering ribonucleic acid duplexes

The target region for RNA interference was selected from a complementary deoxyribonucleic acid sequence of human collagenase-1 based on its beginning 50–100 nucleotides downstream of the start codon, having a sequence of AA(N19)TT or AA(N19), and having approximately 50% G/C content. The sequences of the sense and antisense RNA oligomers were 5′-GAGCAAGAUGUGGACUUA GUU-3′ (150–168 + UU) and 5′-CUAAGUCCACAUCU UGCUCUU-3′ (168–150 + UU), respectively. The specificity of the sequence was confirmed by a BLAST search against EST (expressed sequence tag) libraries in the National Center for Biotechnology Information database. To obtain siRNA duplexes with symmetric 2 nucleotide 3′ overhangs, sense and antisense RNA oligomers were annealed by incubation in a buffer containing 100 mM potassium acetate, 30 mM HEPES–KOH at pH 7.4, and 2 mM magnesium acetate at a concentration of 20 μM for 1 min at 90°C, followed by 1 h at 37°C. The siRNA duplexes composed of scrambled RNA oligomers were used as controls.

Transfection of small interfering ribonucleic acid duplexes

Isolated human chondrocytes were plated in 35-mm dishes in a monolayer at a concentration of 1.0×10^5 cells/dish. After 12 h, the cells were washed twice with phosphate-buffered saline, pH 7.4, and preincubated with serum-free DMEM containing 1.5 mg/ml of bovine testes hyaluronidase for 1 h. The cells were washed twice and incubated at 37°C for 4 h with 0.5 ml of serum-free DMEM containing a pre-prepared mixture of genePORTER transfection reagent and siRNA duplexes at a final concentration of 0.1 μM. Then, 0.5 ml of DMEM supplemented with 20% fetal bovine serum was added to the medium, and the cells were cultured for a further 72 h for the transfection. Hyaluronidase was added to the media every 24 h during the transfection to a final concentration of 1.5 mg/ml.

Determination of the transfection efficiency

Duplexes of siRNA labeled with fluorescein isothiocyanate were transfected into cells in the manner described above. After 72 h of transfection, the cells were washed three times and fixed with 4% formaldehyde in phosphate-buffered saline for 10 min at room temperature. Then the cells were dehydrated in ethanol (40%, 70%, and then 100%) for 10 min at each concentration on ice and rinsed in phosphate-buffered saline. The number of cells that took up the labeled siRNA duplexes was counted at 10-fold magnification using fluorescence microscopy. Transfection efficiency was determined by counting cells in ten random magnified fields. The samples, of which the transfection efficiencies were less than 30%, were excluded.

Quantification of the three isozymes of collagenases in conditioned media

Isolated human chondrocytes were plated in 35-mm dishes in a monolayer at a concentration of 2.0×10^5 cells/dish and cultured for 12 h. Primary cultured cells or cells transfected with siRNA duplexes were washed twice with phosphate-buffered saline and incubated with 1.5 ml DMEM containing 0.1% fetal bovine serum and 15 mM HEPES at pH 7.4 in the presence or absence of IL-1 or TNF-α at a

concentration of 0.5 ng/ml, 5.0 ng/ml, or 50.0 ng/ml, or in the presence of both IL-1 and TNF-α at concentrations of 50 ng/ml. Conditioned media were collected after 48 and 72 h of incubation just after shaking the dish well. In some samples, after removal of the conditioned media the cell layers were incubated with 1 ml of phosphate-buffered saline containing 10 mM ethylenediaminetetraacetic acid and 3.0 mg/ml bovine testes hyaluronidase for 10 min at 37°C, and then were collected. After the cells were precipitated by centrifugation, the supernatants were obtained as samples of cell layer matrices. Total collagenase-1, -2, or -3 including the active form, latent form, and binding form with tissue inhibitor of metalloproteinase in media or in cell layer matrices were quantified immediately using Human Matrix Metalloproteinase Biotrak Enzyme-linked Immunosorbent Assay systems according to the manufacturer's instructions. Standards or samples were diluted 2-fold to 100-fold or concentrated 20-fold to 40-fold for the assay. The concentrations of the collagenases in the samples were determined by interpolation from each standard curve. The detection limits for collagenase-1, -2, and -3 were 6.25, 0.25, and 0.094 ng/ml, respectively. The amounts of the collagenases were expressed as pmol/μg of DNA of cells after normalization to cellular DNA content. Triplicate samples were examined.

Quantification of the activity for the cleavage of type II collagen

Sample media were collected 48 h after stimulation with cytokines. After activation of collagenases with *p*-aminophenylmercuric acetate, the activity for the cleavage of type II collagens in the conditioned media was measured using the Rapid Collagenase Assay Kit for 1 h incubation according to the manufacturer's instructions. The activity was expressed as pmol of cleaved type II collagen per microgram of cellular DNA per hour. Triplicate samples were examined.

Determination of the relative contribution of collagenase-1 to the primary cleavage of type II collagen

Productions of collagenase-1 and activities of type II collagen cleavage were simultaneously quantified at 48 h after stimulation with 50 ng/ml IL-1 or TNF-α for the cells transfected with siRNA designed to the suppression of collagenase-1 production or with scramble control siRNA. The means of the suppression ratios for the production of collagenase-1 and for the activity of type II collagen cleavage were determined. The relative contributions of collagenase-1 to the cleavage of type II collagen were calculated using the formula: relative contribution = the mean of suppression ratio for the activity of type II collagen cleavage divided by the mean of suppression ratio for collagenase-1 production.

Quantification of tissue inhibitor of metalloproteinases in conditioned media

Tissue inhibitor of metalloproteinase-1 and -2 were quantified in media using the Human Tissue Inhibitor of Metalloproteinase-1 and -2 Biotrak Enzyme-linked Immunosorbent Assay systems at 48 h after stimulation with cytokines.

Measurement of deoxyribonucleic acid in cells

Genomic DNA (deoxyribonucleic acid) was purified from the cells using the DNeasy Tissue Kit according to the manufacturer's instructions, and the amounts of obtained DNA were measured by spectrophotometry.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical significance was assessed with Student's *t*-tests. A probability value of less than 0.001 was considered statistically significant.

Results

Production of three isozymes of collagenase by human articular chondrocytes

The production of the three isozymes of collagenase varied among individual 12 samples and was not correlated with the age or gender of the patients (Table 1). The data for collagenase-2 production is not included in Table 1 because much less collagenase-2 was produced than collagenase-1 and -3. Production of collagenase-2 ranged from 0.01 to 0.02 fmol/μg of DNA 48 or 72 h after stimulation with IL-1 at 50 ng/ml, and was too low to quantify after any other stimulation. Production of collagenase-1 and -3 was induced in a dose-dependent manner by TNF-α or IL-1 between 0.5 and 50 ng/ml (Table 1). A combination of TNF-α and IL-1 synergistically induced the greatest production of collagenase-1 and -3 (Table 1). The amount of collagenase-1 in conditioned media increased until 72 h after stimulation with the cytokines, whereas the amounts of collagenase-3 peaked 48 h after stimulation in all samples (Table 1). Collagenase-1 was present in greater amounts than collagenase-3 in all samples after any stimulation with any concentration of stimulant, comprising approximately 98% of total collagenase at 48 h after stimulation and even more at 72 h (Table 1). The amounts of the collagenases in cell layer matrices were too low to quantify, even after the samples were concentrated 40-fold for enzyme-linked immunosorbent assay.

Table 1. Production of collagenase-1 and collagenase-3 from primary cultured human chondrocytes

Cytokine	Concentration (ng/ml)	Production of collagenase (pmol/μg of DNA)			
		48 h after stimulation		72 h after stimulation	
		Collagenase-1	Collagenase-3	Collagenase-1	Collagenase-3
No cytokine		0.32 ± 0.02	0.01 ± 0.001	0.41 ± 0.03	0.01 ± 0.002
TNF-α	0.5	6.38 ± 0.78	0.10 ± 0.01	12.69 ± 1.35	0.11 ± 0.01
	5.0	11.58 ± 1.24	0.18 ± 0.02	22.88 ± 2.37	0.20 ± 0.02
	50.0	18.14 ± 1.97	0.32 ± 0.03	36.01 ± 3.74	0.33 ± 0.03
IL-1	0.5	6.40 ± 0.72	0.11 ± 0.01	12.33 ± 1.41	0.10 ± 0.01
	5.0	14.84 ± 1.68	0.26 ± 0.03	29.01 ± 3.03	0.26 ± 0.03
	50.0	20.66 ± 2.32	0.37 ± 0.04	40.43 ± 4.22	0.38 ± 0.04
TNF-α + IL-1	50.0	21.70 ± 2.24	0.42 ± 0.04	43.07 ± 4.41	0.41 ± 0.04
		*		*	

TNF, tumor necrosis factor; IL, interleukin
* $P < 0.001$

Activities of the cleavage for type II collagens in the conditioned media

After activating the collagenases with *p*-aminophenylmercuric acetate, we measured the total cleavage of the type II collagens at neutral pH in 5 samples derived from 5 (4 women and 1 man) of 12 patients 48 h after stimulation with 50 ng/ml IL-1 or TNF-α. The activities differed between individual samples (Figs. 1 and 2). The activities were positively correlated with the amounts of collagenase-1 in the media (Fig. 3). In nonactivated samples, the activities were too low to quantify.

Specific suppression of collagenase-1 production by transfection of small interfering ribonucleic acid duplexes

The efficiency with which siRNA duplexes were transfected into human chondrocytes varied among the individual 5 samples from 30% to 46%. Induction of collagenase-1 by cytokines was specifically suppressed by the transfection of siRNA duplexes designed to silence collagenase-1 gene expression in all five samples ($P < 0.001$) (Figs. 1 and 2). Transfection with these siRNA duplexes had no effect on the production of collagenase-3 or tissue inhibitor of metalloproteinase-1 or -2 in these conditions and it induced no cytotoxicity in all five samples (Table 2). The suppression efficacy was slightly reduced 72 h after stimulation compared with 48 h after stimulation in all five samples, and transfection of control siRNA duplexes did not affect the production of collagenase-1, -2, and -3 or tissue inhibitor of metalloproteinase-1 and -2 (data not shown).

Relative contribution of collagenase-1 to the activity of the cleavage for type II collagens

When production of collagenase-1 was specifically suppressed by the transfection of siRNA duplexes, the activity of the cleavage for type II collagens in the media was significantly reduced compared to the activity in the media of cells transfected with control siRNA duplexes at 48 h after stimulations with cytokines in all five samples ($P < 0.001$) (Figs. 1 and 2). The amount of reduction of the cleavage activity was positively correlated with the reduction in collagenase-1 production in all five samples (data not shown). The relative contribution of collagenase-1 to the activity for type II

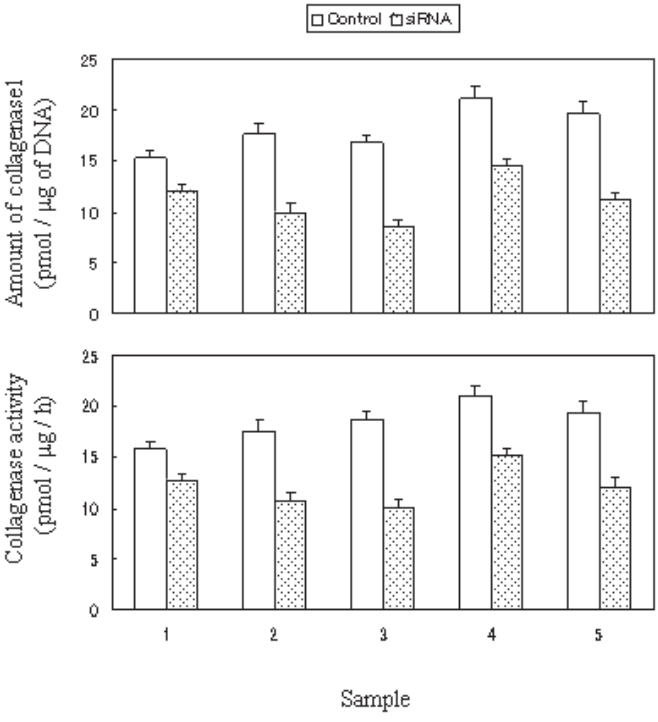

Fig. 1. Production of collagenase-1 from primary cultured human chondrocytes (*upper graph*) and the cleavage activity of type II collagen in media (*lower graph*) after 48 h stimulation with tumor necrosis factor-α (TNF-α). Primary cultured human chondrocytes were stimulated with TNF-α at a concentration of 50 ng/ml. After 48 h, the amount of collagenase-1 and the cleavage activity of type II collagen in media were quantified in five samples, of which the transfection efficiencies were more than 30%, by the methods described in Materials and methods. Duplexes of small interfering RNA pretransfected into the cells significantly suppressed the production of collagenase-1 ($P < 0.001$) (*upper graph*) and simultaneously suppressed the cleavage activity of type II collagen in media ($P < 0.001$) (*lower graph*) in all five samples. The *lines outside the bars* represent the standard deviation

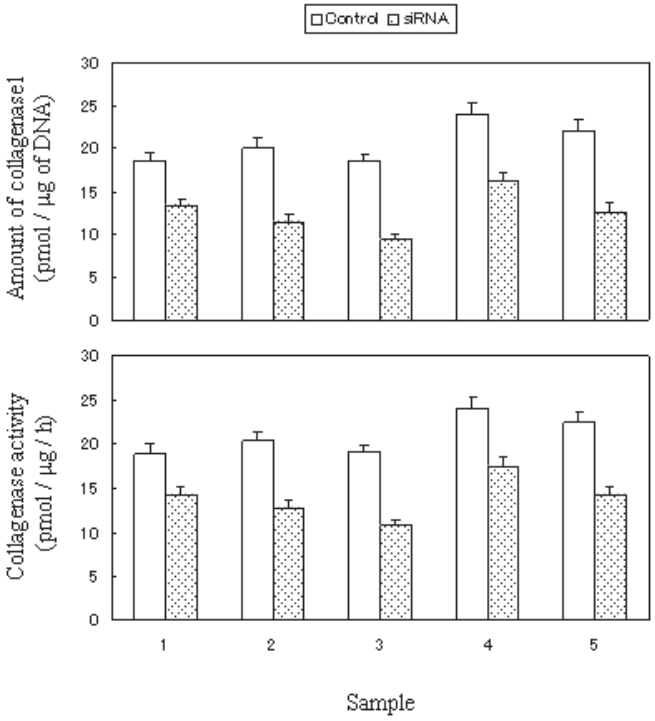

Fig. 2. Production of collagenase-1 from primary cultured human chondrocytes (*upper graph*) and the cleavage activity of type II collagen in media (*lower graph*) after 48 h stimulation with interleukin-1 (IL-1). Primary cultured human chondrocytes were stimulated with IL-1 at the concentration of 50 ng/ml. After 48 h, the amount of collagenase-1 and the cleavage activity of type II collagen in media were quantified in five samples, of which the transfection efficiencies were more than 30%, by the methods described in Materials and methods. Duplexes of small interfering RNA pretransfected into the cells significantly suppressed the production of collagenase-1 ($P < 0.001$) (*upper graph*) and simultaneously suppressed the cleavage activity of type II collagen in media ($P < 0.001$) (*lower graph*) in all five samples. The *lines outside the bars* represent the standard deviation

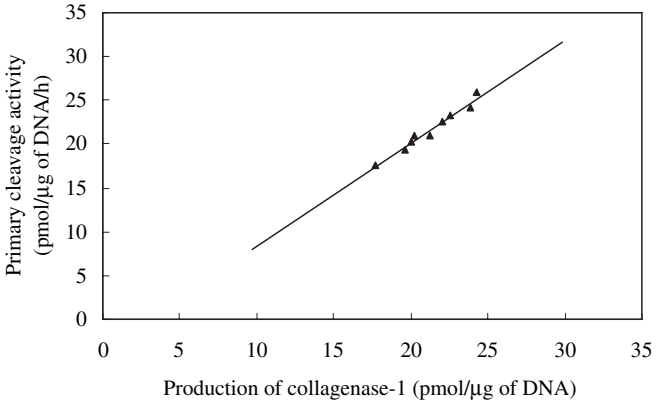

Fig. 3. Correlation between the productions of collagenase-1 from primary cultured human chondrocytes and the cleavage activity of type II collagen in cultured condition media. Production of collagenase-1 from primary cultured human chondrocytes and cleavage activities of type II collagens in cultured condition media were simultaneously quantified by the methods described in Materials and methods after stimulations with TNF-α and/or IL-1 at various concentrations. The production of collagenase-1 positively correlated with the cleavage activity of type II collagens in cultured condition media

collagen cleavage at neutral pH was determined to be approximately 85%–93% at 48 h after stimulation with cytokines (Table 3).

Discussion

Collagenases play a crucial role in cartilage degradation in OA or RA.[1–7] Here we showed that human chondrocytes produce approximately 50- to 65-fold more collagenase-1 than collagenase-3 at 48 h after stimulation with various doses of TNF-α and/or IL-1, and 105- to 125-fold at 72 h. Collagenase-1 accounted for more than 85% of the primary cleavage of type II collagens at neutral pH 48 h after stimulation with cytokines. These findings suggest that collagenase-1 is the major contributor to the primary cleavage of type II collagens during the initial period of cartilage destruction in OA or RA and is a potential therapeutic target for osteoarthritis and rheumatoid arthritis.

Production of collagenase-3 was much lower than that of collagenase-1 in molecular number (less than 2%), and the expression of collagenase-3 was transient after stimulation with cytokines. It has generally been thought that the mechanism of activation of collagenase-3 is the same as that of collagenase-1. This suggests that the contribution of collagenase-3 to the primary cleavage of type II collagens in cartilage is much lower than that of collagenase-1, although the cleavage activity of collagenase-3 for type II collagens has been shown to be approximately 7-fold greater than that of collagenase-1 at neutral pH.[21] We speculate that the main role of collagenase-3 is the cleavage of other constituent molecules in cartilage or the activation of proteinases expressed in cartilage.

Ribonucleic acid interference is the process of sequence-specific post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA that is homologous in sequence to the silenced gene. The mediators of sequence-specific messenger RNA degradation are 21- and 22-nucleotide small interfering RNAs generated by ribonuclease III cleavage from longer double-stranded RNAs. It has been reported that exogenously added siRNA duplexes can specifically suppress the expression of endogenous genes in mammalian cells.[19] Duplexes of 21-nucleotide RNAs mediate efficient sequence-specific messenger RNA degradation, although the mechanism of the interference process remains to be discovered in mammalian cells. In the present study, the siRNA duplexes were effective in suppressing the production of collagenase-1, although the transfection efficiency was not high. Hence, a system of RNA interference is very useful for the discover of protein function or for the analysis of contribution of the protein to the reaction in metabolism.

We conducted our assays at neutral pH, since all collagenases lose their activity at acidic pH. However, cathepsin K, one of the cysteine proteinases, cleaves type II collagens at multiple sites in the N-terminal region (not at the collage-

Table 2. Production of collagenase-3 and tissue inhibitor of metalloproteinase-1 and -2 after transfection with duplexes of small interfering ribonucleic acid designed for gene silence of collagenase-1

Sample	Cytokine	Small interfering ribonucleic acid	Collagenase 3 (pmol/μg of DNA)	Tissue inhibitor of metalloproteinase (ng/μg of DNA)	
				1	2
1	TNF-α	Control	0.27 ± 0.02	437.3	4.0
		Collagenase-1	0.27 ± 0.02	433.5	3.9
	IL-1	Control	0.29 ± 0.02	492.1	4.3
		Collagenase-1	0.30 ± 0.02	489.7	4.2
2	TNF-α	Control	0.27 ± 0.01	485.5	4.2
		Collagenase-1	0.27 ± 0.01	487.2	4.3
	IL-1	Control	0.36 ± 0.02	543.8	4.5
		Collagenase-1	0.37 ± 0.03	540.2	4.5
3	TNF-α	Control	0.32 ± 0.02	415.7	3.9
		Collagenase-1	0.32 ± 0.02	417.3	3.9
	IL-1	Control	0.34 ± 0.03	465.6	4.3
		Collagenase-1	0.34 ± 0.02	462.3	4.3
4	TNF-α	Control	0.40 ± 0.02	495.0	4.3
		Collagenase-1	0.40 ± 0.03	493.7	4.3
	IL-1	Control	0.44 ± 0.02	554.1	4.4
		Collagenase-1	0.44 ± 0.03	555.2	4.5
5	TNF-α	Control	0.34 ± 0.02	451.9	4.1
		Collagenase-1	0.34 ± 0.03	447.8	4.1
	IL-1	Control	0.43 ± 0.03	506.2	4.4
		Collagenase-1	0.42 ± 0.03	503.5	4.4

Table 3. Relative contribution of collagenase-1 to the activity for primary cleavage of type II collagen

Sample	Cytokine	Contribution of collagenase-1 to the cleavage activity (%)
1	TNF-α	91.5
	IL-1	86.5
2	TNF-α	90.1
	IL-1	88.1
3	TNF-α	93.1
	IL-1	88.5
4	TNF-α	88.7
	IL-1	85.0
5	TNF-α	88.5
	IL-1	85.8

nase site) at pHs between 5.0 and 5.5.[22] Furthermore, cathepsin B has been implicated in the cartilage degradation of OA,[23] although it is not known whether cathepsin B participates in the primary cleavage of type II collagens. We did not examine these enzymes in the present study, nor did we assess primary cleavage in the N-terminal region. However, if the cartilage matrix were to become more acidic during the process of cartilage degradation, cathepsin enzymes might be major contributors to type II collagen degradation. It is not known whether such a pH shift occurs in OA or RA.

References

1. Hollander AP, Heathfield TF, Webber C, Iwata Y, Bourne R, Rorabeck C, et al. Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. J Clin Invest 1994;93:1722–32.
2. Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, et al. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J Clin Invest 1997;99: 1534–45.
3. Freemont AJ, Byers RJ, Taiwo YO, Hoyland JA. In situ zymographic localization of type II collagen degrading activity in osteoarthritic human articular cartilage. Ann Rheum Dis 1999;58: 357–65.
4. Poole AR. Cartilage in health and disease in Arthritis and allied conditions. In: Koopman WJ, editor. Textbook of rheumatology. 14th ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 2226–84.
5. Kojima T, Mwale F, Yasuda T, Girard C, Poole AR, Laverty S. Early degradation of type IX and type II collagen with the onset of experimental inflammatory arthritis. Arthritis Rheum 2001;44: 120–7.
6. Mort JS, Dodge GR, Roughley PJ, Liu J, Finch SF, DiPasquale G, et al. Direct evidence for active metalloproteinases mediating matrix degradation in interleukin-1 stimulated articular cartilage. Matrix 1993;13:95–102.
7. Kozaci LD, Buttle DJ, Hollander AP. Degradation of type II collagen, but not proteoglycan, correlates with metalloproteinase activity in cartilage explant cultures. Arthritis Rheum 1997;40:164–74.
8. Shlopov BV, Lie W-R, Mainardi CL, Cole AA, Chubinskaya S, Hasty KA. Osteoarthritic lesions: involvement of three different collagenases. Arthritis Rheum 1997;40:2065–74.
9. Freemont AJ, Hampson V, Tilman R, Goupille P, Taiwo YO, Hoyland JA. Gene expression of matrix metalloproteinase 1, 3, and 9 by chondrocytes in osteoarthritic human knee articular cartilage is zone and grade specific. Ann Rheum Dis 1997;56:542–9.
10. Tetlow LC, Woolley DE. Comparative immunolocalization studies of collagenase 1 and collagenase 3 production in the rheumatoid

lesion, and by human chondrocytes and synoviocytes in vitro. Br J Rheumatol 1998;37:64–70.
11. Fernandes JC, Martel-Pelletier J, Lascau-Coman V, Moldovan F, Jovanovic D, Raynaud JP, et al. Collagenase-1 and collagenase-3 synthesis in normal and early experimental osteoarthritic canine cartilage: an immunohistochemical study. J Rheumatol 1998;25: 1585–94.
12. Huebner JL, Otterness IG, Freund EM, Caterson B, Kraus VB. Collagenase 1 and collagenase 3 expression in a guinea pig model of osteoarthritis. Arthritis Rheum 1998;41:877–90.
13. Tetlow LC, Adlam DJ, Woolley DE. Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: associations with degenerative changes. Arthritis Rheum 2001;44:585–94.
14. Cole AA, Chubinskaya S, Schumacher B, Huch K, Cs-Szabo G, Yao J, et al. Chondrocyte matrix metalloproteinase-8: human articular chondrocytes express neutrophil collagenase. J Biol Chem 1996;271:11023–6.
15. Borden P, Solymar D, Sucharczuk A, Lindman B, Cannon P, Heller R. Cytokine control of interstitial collagenase and collagenase-3 gene expression in human chondrocytes. J Biol Chem 1996; 271:23577–81.
16. Chubinskaya S, Huch K, Mikecz K, Szabo C, Hasty K, Kuettner K, et al. Chondrocyte matrix metalloproteinase-8: up-regulation of neutrophil collagenase by interleukin-1β in human cartilage from knee and ankle joints. Lab Invest 1996;74:232–40.
17. Tardif G, Pelletier J-P, Dupuis M, Geng C, Cloutier J-M, Martel-Pelletier J. Collagenase 3 production by human osteoarthritic chondrocytes in response to growth factors and cytokines is a function of the physiological state of the cells. Arthritis Rheum 1999;42:1147–58.
18. Mengshol JA, Vincenti MP, Coon CI, Barchowsky A, Brinckerhoff CE. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-JUN N-terminal kinase, and nuclear factor κB. Arthritis Rheum 2000; 43:801–11.
19. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001;411:494–8.
20. Aydelotte MB, Kuettner KE. Differences between sub-populations of cultured bovine articular chondrocytes. I. Morphology and cartilage matrix production. Connect Tissue Res 1988;18:205–22.
21. Knäuper V, López-Otin C, Smith B, Knight G, Murphy G. Biochemical characterization of human collagenase-3. J Biol Chem 1996;271:1544–50.
22. Kafienah W, Bromme D, Buttle DJ, Croucher LJ, Hollander AP. Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. Biochem J 1998;331:727–32.
23. Lang A, Horler D, Baici A. The relative importance of cysteine peptidases in osteoarthritis. J Rheumatol 2000;27:1970–9.