Effects of interleukin-1β and tumor necrosis factor-α on expression of matrix-related genes by cultured equine articular chondrocytes

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Objective—To determine the effects of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) on expression and regulation of several matrix-related genes by equine articular chondrocytes.

Sample Population—Articular cartilage harvested from grossly normal joints of 8 foals, 6 yearling horses, and 8 adult horses.

Procedure—Chondrocytes maintained in suspension cultures were treated with various doses of human recombinant IL-1β or TNF-α. Northern blots of total RNA from untreated and treated chondrocytes were probed with equine complementary DNA (cDNA) probes for cartilage matrix-related genes. Incorporation of ^{35}S-sulfate, fluorography of ^{14}C-proline labeled medium, zymography, and western blotting were used to confirm effects on protein synthesis.

Results—IL-1β and TNF-α increased steady-state amounts of mRNA of matrix metalloproteinases 1, 3, and 13 by up to 100-fold. Amount of mRNA of tissue inhibitor of metalloproteinase-1 also increased but to a lesser extent (1.5- to 2-fold). Amounts of mRNA of type-II collagen and link protein were consistently decreased in a dose-dependent manner. Amount of aggrecan mRNA was decreased slightly; amounts of biglycan and decorin mRNA were minimally affected.

Conclusions and Clinical Relevance—Treatment of cultured equine chondrocytes with IL-1β or TNF-α resulted in marked alterations in expression of various matrix and matrix-related genes consistent with the implicated involvement of these genes in arthritis. Expression of matrix metalloproteinases was increased far more than expression of their putative endogenous inhibitor. Results support the suggestion that IL-1β and TNF-α play a role in the degradation of articular cartilage in arthritis. (*Am J Vet Res* 2000;61: 624–630)

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The major structural collagen of articular cartilage is type-II collagen.[1,2] The matrix between collagen fibrils in articular cartilage is thought to largely be composed of supramolecular aggregates of hyaluronan coupled to side chains of a large protein termed aggrecan.[3] Aggrecan is noncovalently attached to the hyaluronan backbone through its interaction with a glycoprotein, link protein.[4,5] The aggrecan core protein is modified by multiple side chains of the sulfated glycosaminoglycans, chondroitin 4-sulfate, chondroitin 6-sulfate, and keratan sulfate. These **glycosaminoglycans (GAG)** bind a large volume of water, giving articular cartilage its unique elastic biomechanical behavior. There are also smaller proteoglycans, such as decorin and fibromodulin, that may play a role in type-II collagen fibrillogenesis and in biglycan, which interacts with type-VI collagen in cartilage.[6] Recent studies have demonstrated specific alterations in decorin synthesis with intense exercise[7] and age.[8]

The composition of cartilage matrix is dependent on a critical balance of synthetic and degradative processes. It is generally understood that cytokines such as **interleukin-1β (IL-1β)** and **tumor necrosis factor-α (TNF-α)** play major roles in the pathways that lead to degenerative joint disease and have profound effects on a number of matrix and matrix-related molecules.[9-12] Both have been shown to increase synthesis of **matrix metalloproteinases (MMP)** by chondrocytes and have various effects on other matrix components.[13-18] The purpose of the study reported here was to determine the effects of IL-1β and TNF-α on expression and regulation of several matrix-related genes by equine articular chondrocytes. We used equine-specific **complementary DNA (cDNA)** probes to evaluate steady-state amounts of **messenger RNA (mRNA)** for several matrix-related proteins.

Materials and Methods

Cell culture—Articular cartilage was harvested from grossly normal joints of 8 foals < 1 year old, 6 yearling horses between 14 and 20 months old, and 8 adult horses > 2 years old. The cartilage was minced with a scalpel and digested in 0.5% collagenase[a] overnight.[19] Isolated chondrocytes were counted, and 10×10^6 cells were distributed evenly in 30-mm culture dishes treated with poly(2-hydroxy)ethyl methacrylate to prevent cell adhesion and maintain chondrocyte phenotype.[20] At least 20 dishes of cells from each horse were cultured in parallel for each replication of the experiment. Comparisons were made among dishes of cells from each horse.

Cell cultures were maintained with Dulbecco's modified Eagle medium with 10% fetal calf serum and 50 µg of ascorbic acid/ml for 2 days prior to treatment. Preliminary dose-response experiments were performed to establish dose

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ranges that induced consistent changes in matrix synthesis. The selected doses were 0, 1, 5, 10, and 25 ng of human recombinant TNF-α[b]/ml and 0, 0.5, 1, 5, and 10 ng of human recombinant IL-1β[b]/ml. Medium was collected after 2 days, and **dimethlymethylene blue (DMMB)** assays were performed to measure GAG content.[21] Fresh medium containing the same agents and either ^{14}C-proline (3 µCi/ml) or ^{35}S-sulfate (40 µCi/ml) was added for an additional 18 to 24 hours.

Proteins in the medium were partially characterized by means of gel chromatography and scintillation counting to determine sulfate incorporation, **sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)** and fluorography, and western blotting or zymography.

Sulfate incorporation—After incubation of chondrocytes with medium containing ^{35}S-sulfate, cells and medium were separated by means of gentle centrifugation. The cell pellet was resuspended and washed 3 times in phosphate-buffered saline solution to remove free sulfate. Cells were solubilized in monophasic phenol, and emissions from 50 µl aliquots were counted in a liquid scintillation counter.

Sephadex G-50 columns were equilibrated with 4 *M* guanidine hydrochloride, 0.2% Triton, and 0.1 *M* sodium chloride. Medium (1.5 ml) was added, and the void volume was collected. Aliquots (250 µl) were mixed with 3.5 ml of scintillation fluid,[c] and emissions were counted in a liquid scintillation counter.

Fluorography—After chondrocytes were incubated with medium containing ^{14}C-proline, the medium was collected and acidified with glacial acetic acid (2.9% v:v). Pepsin[d] (10 mg/ml) was added, and digestion was allowed to continue for at least 48 hours at 4 C. Samples were exhaustively dialyzed (12,000 to 14,000 molecular weight cutoff). Sodium chloride (2 *M*) was added, and samples were centrifuged for 30 minutes at 12,000 × g. The pellet was resuspended in 0.5 *M* acetic acid and an equal volume of 2X denaturing sample buffer (1X denaturing sample buffer = 50 m*M* Tris hydrochloride [pH 6.8], 2% SDS, 15% glycerol, and 1% mercaptoethanol) and subjected to SDS-PAGE (7.5% gel). Gels were enhanced,[e] dried, and exposed to radiographic film for 7 to 14 days.

Western blotting—After incubation of chondrocytes, proteins in the medium were precipitated by adding 2X volume of ice-cold acetone and freezing at –70 C for 1 hour. Samples were centrifuged at 10,000 × g at 4 C for 30 minutes. The pellet was dissolved in 0.5 *M* acetic acid and added to 1X denaturing sample buffer, and proteins were separated by means of SDS-PAGE (10% gel). Gels were soaked for 10 to 15 minutes in Tris-glycine buffer with 5% methanol to partially remove the SDS and transferred to nitrocellulose membranes[f] by means of electroblotting. Membranes were washed at room temperature (approx 20 C) for 30 minutes in **0.05% Tween 20[g] in Tris-buffered saline solution (TBSS-Tween)**. Blocking was done for at least 1 hour at room temperature, using TBSS-Tween with 3% nonfat milk. Membranes were incubated overnight at 4 C with the primary antibody (1:500 dilution of polyclonal ovine antihuman MMP-3[h]) in TBSS-Tween with 0.5% milk. The following morning, membranes were washed twice (10 minutes each time) with TBSS-Tween, and the secondary antibody (1:5,000 dilution of alkaline phosphatase-conjugated goat antisheep antibody[i]) was added. Membranes were incubated for 60 minutes at room temperature and washed 3 times (10 minutes each time) with TBSS-Tween and 2 times (10 minutes each time) with TBSS, and substrate solution[j] was added.

Zymography—After incubation of chondrocytes, 1 µl of 100 m*M* ***p*-aminophenyl mercuric acetate (APMA)**[k] was added to 100 µl of medium, and the sample was incubated at 37 C for 30 minutes. Proteins in the sample were precipitated by addition of a 2X volume of acetone and freezing at –70 C for 1 hour. The sample was thawed and centrifuged (12,000 × g for 30 minutes at 4 C), and the pellet was dried in a vacuum centrifuge. The pellet was resuspended in water, an equal volume of loading buffer (0.125 *M* Tris hydrochloride [pH 6.8], 20% glycerol, 4% SDS, 0.05% bromophenol blue) was added, and the sample was incubated at room temperature for 10 minutes. Aliquots were applied to gels containing 0.1% gelatin or 0.05% casein.[l] Gels were incubated in renaturing buffer (2.5% Triton X-100) for 30 minutes at room temperature and in developing buffer (10 m*M* Tris base, 40 m*M* Tris hydrochloride, 0.2 *M* NaCl, 5 m*M* CaCl2, 0.02% [wt/vol] Brij 35) for another 30 minutes. Gels were incubated overnight at 37 C in fresh developing buffer, stained with 0.5% Coomassie blue R250 for 30 minutes, destained for 15 minutes in a 45% methanol-7% acetic acid solution, and washed twice for 20 minutes in water. Gels were dried, and images of the gels were digitized. Cleared areas representing proteinase activity were quantitated by use of imaging software. Matching samples were prepared without APMA activation.

Northern blotting—Following incubation, cells were solubilized in monophasic acid phenol,[m] and aliquots were saved for scintillation counting. Total RNA was extracted from the remaining cells and separated in denaturing 1% agarose gels (10 µg/lane). Gels were capillary blotted to charged nylon membranes.[n] Probes were prepared by [^{32}P]dCTP random primed labeling of equine cDNA inserts for type-II collagen,[1] aggrecan, biglycan, decorin, link protein, **tissue inhibitor of metalloproteinase-1 (TIMP-1)**, and MMP 1, 3, and 13 (interstitial collagenase, stromelysin-1, and collagenase-3, respectively). Each of these equine cDNA inserts were cloned from a cDNA library prepared from equine chondrocyte mRNA. Their full or partial sequences have been deposited with GenBank. Prehybridization and hybridization were performed at 65 C for 30 minutes and 90 minutes, respectively, using a commercial hybridization buffer.[o] Blots were washed 3 times (20 minutes each time) at 55 C with 0.5X, 0.25X, and 0.1X SSC (saline sodium citrate buffer solution) and 0.1% SDS and exposed to radiographic film[p] for 6 to 24 hours at –70 C. The resulting images were quantified by use of computer-integrated densitometry. Variations in gel loading were corrected by means of densitometry of ribosomal bands in photographic negatives of the ethidium-stained gels.

Data analysis—Because of the considerable variability in baseline expression of cartilage matrix proteins among individual horses, all results were expressed as a percentage of the value for untreated control samples from the same horse. Two-way ANOVA followed by the Student-Newman-Keuls test was used to test for differences between treated and control samples in regard to results of northern blotting, sulfate incorporation, and GAG content. Values of $P < 0.05$ were considered significant.

Results

Cell culture—Collagenase digestion of articular cartilage yielded 10 to 15 × 10^6 cells/g of wet weight for cartilage harvested from the adult horses, 20 to 30 × 10^6 cells/g of wet weight for cartilage harvested from the yearling horses, and 30 to 60 × 10^6 cells/g of wet weight for cartilage harvested from the foals. Viability, assessed by use of the trypan blue exclusion test, was consistently > 95%. In the culture plates, the chondrocytes maintained a round shape (**Fig 1**), because they were prevented from adhering to the

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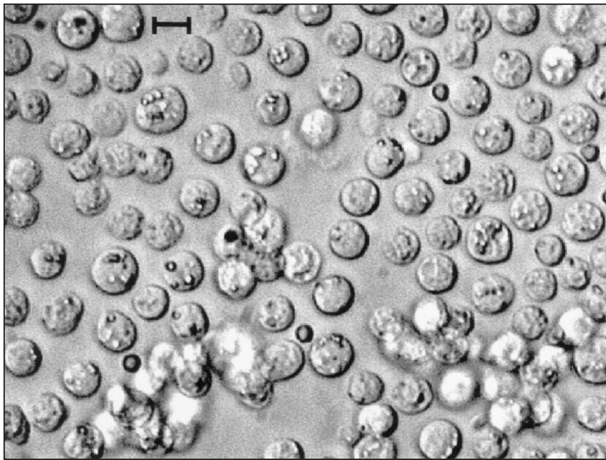

Figure 1—Photomicrograph of cultured equine chondrocytes maintained in dishes coated with poly(2-hydroxy)ethyl methacrylate so that they will not adhere to the dish and will maintain a spherical phenotype for an extended period. Bar = 20 µm.

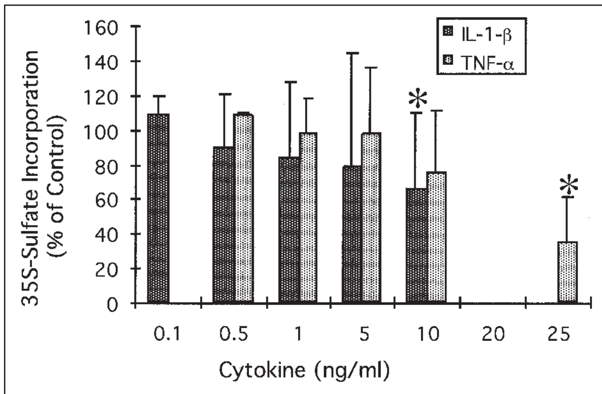

Figure 2—Incorporation of ^{35}S-sulfate by proteins in the medium of equine chondrocytes incubated with various concentrations of the cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). *Significantly ($P < 0.05$) different from incorporation for control cultures.

plates. Because experiments were short term (< 1 week of cell culture), chondrocytes did not accumulate enough matrix to make cell solubilization or RNA extraction difficult.

Glycosaminoglycan content—Compared with medium from control cell cultures, addition of IL-1β or TNF-α to the culture medium did not significantly alter GAG content of the medium.

Sulfate incorporation—Incorporation of ^{35}S-sulfate by cultured chondrocytes and their associated matrix was not significantly changed by addition of IL-1β or TNF-α to the culture medium. A significant decrease in amount of ^{35}S-sulfate-labeled proteins in the culture medium was detected when IL-1β or TNF-α was added at the highest doses used (**Fig 2**).

Fluorography—Fluorography of medium from chondrocytes cultured with various concentrations of IL-1β or TNF-α indicated a generalized decrease in ^{14}C-proline-labeled proteins as concentration of IL-1β or TNF-α increased. Specifically, a decrease in the amount of pepsin-resistant collagens, identified as

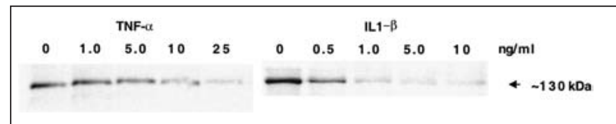

Figure 3—Representative fluorogram of medium from equine chondrocytes cultured with various concentrations of IL-1β or TNF-α and ^{14}C-proline illustrating a dose-dependent decrease in amount of pepsin-resistant collagens.

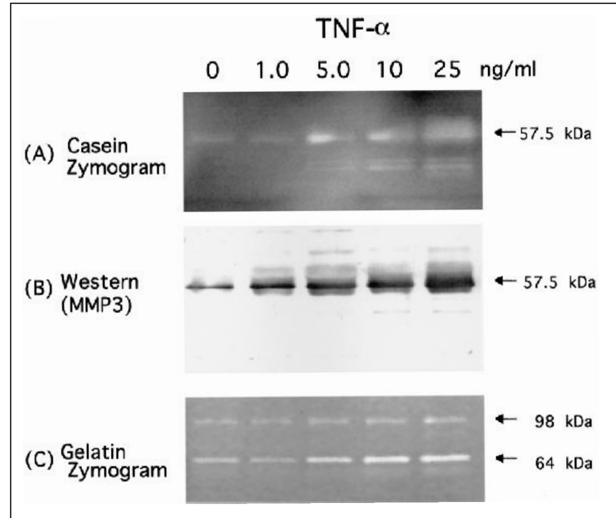

Figure 4—Representative zymograms and western blots of medium from equine chondrocytes incubated with various concentrations of TNF-α. A—Zymography with gels containing casein revealed a dose-dependent increase in a caseinase of approximately 57 kD, presumably matrix metalloproteinase (MMP)-3. B—Western blotting with ovine anti-human MMP-3 of spent medium revealed a similar dose-dependent increase in a primary band of about 57 kD. C—Zymography with gels containing gelatin revealed a dose-dependent increase in a band of approximately 64 kD that presumably was MMP-2 and a second band of approximately 98 kD that presumably was MMP-9 and did not increase in response to an increase in dose of TNF-α.

type-II collagen, in the medium was detected when IL-1β or TNF-α was added at highest doses used (**Fig 3**).

Western blotting and zymography—Zymography revealed a major active caseinate of approximately 57 kD as well as numerous smaller active fragments representing multiple cleaved forms of MMP-3. The amount of active caseinate increased as amount of IL-1β or TNF-α added to the culture medium increased (**Fig 4**). Western blot analysis using ovine antihuman MMP-3 revealed a primary band of about 57 kD with multiple smaller bands. Zymography with gels containing gelatin revealed a band of approximately 64 kD that presumably was MMP-2[22]; intensity of the band increased as dose of IL-1β or TNF-α added to the culture medium increased. A larger band of approximately 98 kD that presumably was MMP-9[22] was also seen, but intensity of this band did not increase as dose of IL-1β or TNF-α increased.

Northern blotting—Addition of IL-1β or TNF-α to the culture medium increased steady-state amounts of mRNA of MMP-1, MMP-3, and MMP-13 in a dose-dependent manner (**Fig 5 and 6**). The MMP mRNA was increased 5- to 100-fold with addition of IL-1β or TNF-α at the highest doses used.

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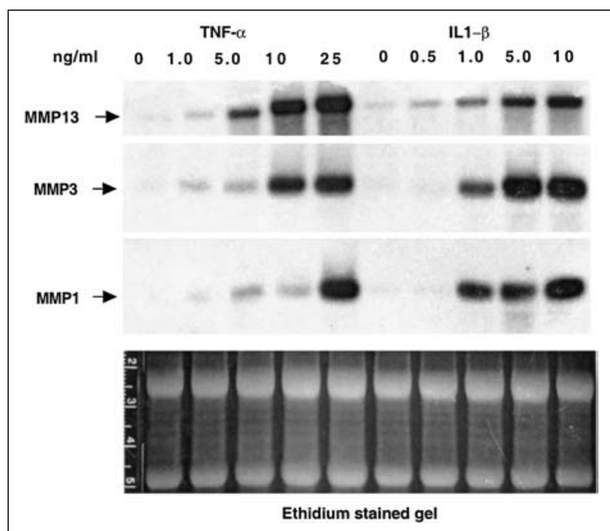

Figure 5—Representative northern blots indicating dose-dependent increases in mRNA for MMP-1, MMP-3, and MMP-13 in cultured equine chondrocytes in response to addition of IL-1β or TNF-α to the culture medium.

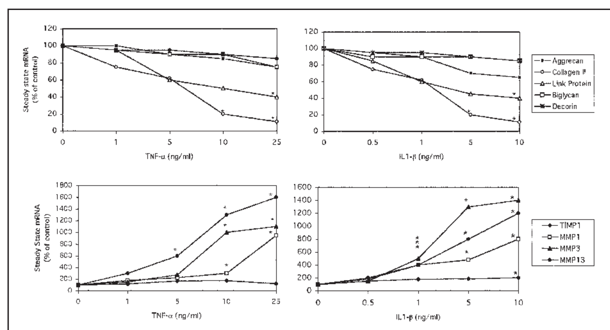

Figure 6—Change in steady-state amounts of mRNA of various matrix and matrix-related constituents in cultured equine chondrocytes incubated with various concentrations of IL-1β or TNF-α. Data points represent the mean values for all horses at each concentration. *Significantly ($P < 0.05$) different from value for control chondrocyte cultures.

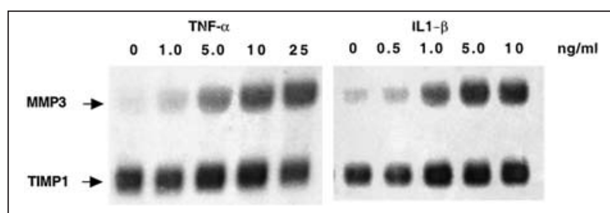

Figure 7—Representative northern blots illustrating change in expression of mRNA of MMP-3 and tissue inhibitor of metalloproteinase-1 (TIMP1) in equine chondrocytes incubated with various concentrations of IL-1β or TNF-α.

Amount of TIMP-1 mRNA also increased as dose of IL-1β or TNF-α increased, but to a lesser extent (1.5- to 2-fold increase) and, in some experiments, amount of TIMP-1 mRNA decreased when TNF-α was added at the highest dose (**Fig 7**). Baseline amounts of TIMP-1 mRNA were typically highest for chondrocytes from adult horses.

Amounts of mRNA of type-II collagen and link protein were most consistently decreased in a dose-dependent manner by addition of IL-1β or TNF-α to the culture medium (Fig 6 and **8**). Amounts of mRNA

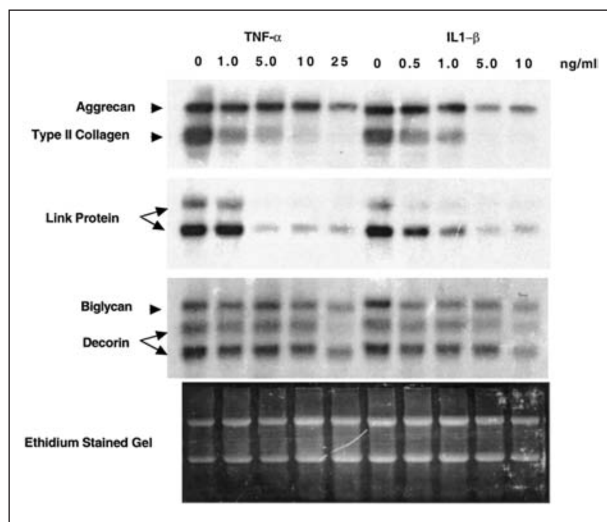

Figure 8—Representative northern blots indicating changes in expression of mRNA of various matrix and matrix-related constituents in equine chondrocytes incubated with various concentrations of IL-1β or TNF-α.

of aggrecan, biglycan, and decorin were not uniformly affected by addition of IL-1β or TNF-α. Amount of mRNA of aggrecan was decreased slightly by addition of IL-1β or TNF-α at the highest doses, but amounts of mRNA of biglycan and decorin were minimally affected at doses that had obvious effects on the other matrix constituents.

Control chondrocytes from adult horses had greater amounts of MMP mRNA than chondrocytes from foals. Thus, relative increases were always greater for chondrocytes from foals than for chondrocytes from adult horses.

Discussion

In the present study, addition of IL-1β and TNF-α decreased steady-state amounts of type-II collagen mRNA in cultured equine chondrocytes and had a parallel effect on the secreted propeptide in the culture medium. Because this cytokine-induced decrease in synthesis was coupled with a marked increase in expression of MMP-1 and MMP-13, a net loss of collagen content of cartilage is a long-term consequence of conditions that result in an excess of IL-1β or TNF-α. The amount of type-II collagen mRNA in cultured chondrocytes from older horses was markedly less than that in cultured chondrocytes from younger horses, and the relative decrease in amount of type-II collagen mRNA induced by addition of IL-1β or TNF-α was less pronounced. It is generally acknowledged that younger animals have a higher potential for healing of articular cartilage defects, and the higher biosynthetic activity of type-II collagen by chondrocytes from younger horses may be related to this enhanced capability for healing.

In the present study, we found a dose-dependent decrease in amount of link protein mRNA in cultured equine chondrocytes following addition of IL-1β or TNF-α. Link protein is an extracellular matrix glycoprotein that functions in cartilage to bind the globular domain of aggrecan to hyaluronan.[5] It is, therefore, an essential element in the formation of the supramolecu-

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lar aggregates that bind large volumes of water and provide the elasticity of cartilage. In arthritic cartilage and cartilage treated with cytokines, there is a loss of fully aggregated proteoglycans that may be related to assembly of these supramolecular aggregates.[23] A decrease in synthesis of link protein could play a role in the loss of normally aggregating proteoglycans in diseased cartilage.[24] Such a decrease in aggregability of newly synthesized proteoglycans has been reported for cultured chondrocytes exposed to IL-1β, and similar transcriptional effects on amounts of link protein mRNA have been documented by use of a reverse-transcription polymerase chain reaction assay.[25]

Effects of IL-1β or TNF-α in this study were disparate and complex. Although expression of the large aggregating protein of cartilage, aggrecan, was downregulated by addition of IL-1β or TNF-α, expression of the smaller dermatan sulfate-containing proteoglycans, biglycan and decorin, was less affected, and little alteration in the expression of biglycan or decorin was seen even with the highest doses of these cytokines. These results differ from those of von den Hoff et al,[26] who reported 23 and 27% decreases in biglycan and decorin synthesis, respectively, following IL-1β treatment of bovine cartilage explants. However, Qwarnstrom et al[27] found that IL-1β had minimal effects on steady-state amounts of decorin mRNA in human fibroblasts, but expression of versican was markedly downregulated. In another study,[28] biglycan and decorin expression in cultured rabbit chondrocytes were little affected by IL-1β. The roles of biglycan and decorin in cartilage degeneration in vitro and natural disease are unclear. Decorin has been shown to have a role in the process of collagen fibrillogenesis,[6] and amounts increase in response to mechanical stresses.[7,29-31] Decorin has also been shown to be diminished on the surface of damaged cartilage in an avian model of osteoarthritis.[32] There is evidence that decorin is preferentially localized to the largest-diameter collagen fibrils in adult articular cartilage, and this, in turn, may be related to the mechanical behavior of the collagenous network.[33,34] Decorin's role in the mechanical function of cartilage is further evidenced by its localization to the surface of the temporomandibular disk in rats.[35] Roughley et al[36] reported that expression of decorin increases in older humans. In the horses in the present study, it appeared that steady-state amount of decorin mRNA was greater than steady-state amounts of type-II collagen and aggrecan mRNA in older animals. This is probably more a result of a decrease in type-II collagen and aggrecan than to upregulation of decorin expression in older horses.[1]

The function of biglycan in cartilage is also not understood, although it probably involves interactions with collagens and other matrix macromolecules, including hyaluronan.[6,36,37] In the present study, doses of IL-1β or TNF-α that induced large relative changes in MMP and type-II collagen transcription caused a small decrease in amount of biglycan mRNA. This is different from results reported following TNF-α treatment of cultured human endothelial cells, in which biglycan and perlecan, a large heparan sulfate proteoglycan, were apparently downregulated[38] but comparable to findings for rabbit chondrocytes treated with IL-1β.[39]

The actions of the MMP are more specific to certain substrates, particularly collagens. For instance, MMP-1, or interstitial collagenase, is capable of cleaving the triple helical region of fibrillar collagens, such as type-II collagen, unique to cartilage. It has been shown that MMP-13 may have a high affinity for type-II collagen, further supporting the importance of MMP-13 in the destructive processes of arthritis.[33-36,40-43] Caron et al[16] have shown that amount of MMP-13[16] mRNA in equine chondrocytes increases with addition of IL-1β.

There is still controversy concerning the relative importance of the known MMP in the degradation of articular cartilage matrix. Although aggrecan can clearly be cleaved by known MMP at amino acids Asn_{341}–Phe_{342} in its globular (G1) domain, aggrecan is also found in diseased tissue to be cleaved at a novel site (Glu_{373}–Ala_{374}) by a newly described enzyme, aggrecanase.[44-47] Although it is known that IL-1β[48] and TNF-α[49] will increase aggrecanase activity, as determined by detecting its cleavage products, the specific protein cleaving efficiently at that site has just been characterized.[46,47] Aggrecanase was believed to be of the metalloproteinase family because of its inhibition by MMP inhibitors, and its recent cloning confirms its identity as an MMP. The regulatory elements in the known MMP are similar, so it is likely that the active MMP in cartilage are similarly upregulated by IL-1β or TNF-α. In the present study, we found similar changes in transcription of MMP-1, MMP-3, and MMP-13 in response to addition of IL-1β or TNF-α to the culture medium. We did not have cDNA probes for the gelatinases, MMP-2 and MMP-9, but results of zymography in the present study were consistent with those reported.[22,50]

In the present study, the DMMB assay failed to demonstrate an effect of the cytokines on GAG content of the secreted proteoglycans. This likely was a consequence of the complex effects of these cytokines on cartilage degradation and synthesis. Additionally, there could be a large amount of proteoglycan sequestered or organized with cell-associated matrix, and the latter was not specifically analyzed in these studies. The lack of a significant difference in incorporation of ^{35}S-sulfate by the cultured chondrocytes was probably attributable to the presence of bound and unbound sulfate. Furthermore, if there was a combination of decreased synthesis of GAG coupled with increased loss from the pericellular matrix into the medium, the measured content of GAG in the medium would be relatively unchanged. The decrease in ^{35}S-sulfate incorporation by proteins in the medium from cells treated with high doses of the cytokines may more accurately reflect the effects of these agents, because chromatographic separation allows measurement of labeled proteoglycans rather than bound and unbound GAG.

Understanding how IL-1β and TNF-α modulate expression of the mRNA of these matrix-related genes may shed light on the ability of these cytokines to induce cartilage matrix loss. Expression of key components of articular cartilage matrix, including type-II collagen, link protein, and aggrecan, was decreased in a dose-dependent manner by both cytokines. Steady-state amounts of mRNA of the matrix-degrading MMP

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was increased dramatically at the same time that expression of their natural inhibitor was essentially unchanged. This suggests a net imbalance of synthesis and degradation that could accelerate loss of cartilage matrix and degenerative joint disease. Although in vitro results of treating cultured chondrocytes with single cytokines may not always accurately reflect the complex interaction of the multiple bioactive substances that play roles in the disease process in vivo, it is necessary to first examine individual effects. Future investigations studying specific regulation of each gene by more complex stimuli should help in the development of better strategies for preventing degradation of cartilage matrix.

[a]Collagenase D, Boehringer Mannheim, Indianapolis, Ind.
[b]Collaborative Biomedical Products, Becton-Dickinson, Franklin Lakes, NJ.
[c]Scintiverse BD, Fisher Scientific, Pittsburgh, Pa.
[d]P6887, Sigma Chemical Co, St Louis, Mo.
[e]EN3HANCE-NEN, Life Sciences, Boston, Mass.
[f]NitroPure, Micron Separations Inc, Westborough, Mass.
[g]Tween 20, Fisher Scientific, Pittsburgh, Pa.
[h]Donated by E. Arner, DuPont Merck Pharmaceutical Co, Wilmington, Del.
[i]Sigma Chemical Co, St Louis, Mo.
[j]BCIP/NBT color development substrate, Promega, Madison, Wis.
[k]A 9563, Sigma Chemical Co, St Louis, Mo.
[l]Novex, San Diego, Calif.
[m]TRIzol, GIBCO BRL Life Technologies, Gaithersburg, Md.
[n]Hybond N+, Amersham, Arlington Heights, Ill.
[o]Rapid-hyb, Amersham, Arlington Heights, Ill.
[p]Reflection, NEN Life Sciences, Boston, Mass.

References

1. Richardson D, Dodge G. Cloning of equine type II procollagen and the modulation of its expression in cultured equine articular chondrocytes. *Matrix Biol* 1997;16:59–64.
2. Todhunter RJ, Wootton JAM, Lust G, et al. Structure of equine type I and type II collagens. *Am J Vet Res* 1994;55:425–431.
3. Hardingham TE, Fosang AJ, Dudhia J. The structure, function and turnover of aggrecan, the large aggregating proteoglycan from cartilage. *Eur J Clin Chem Clin Biochem* 1994;32:249–257.
4. Bonnet F, Dunham DG, Hardingham TE. Structure and interactions of cartilage proteoglycan binding region and link protein. *Biochem J* 1985;228:77–85.
5. Dudhia J, Platt D. Complete primary sequence of equine cartilage link protein deduced from complementary DNA. *Am J Vet Res* 1995;56:959–965.
6. Roughley PJ, Lee ER. Cartilage proteoglycans: structure and potential functions. *Microsc Res Tech* 1994;28:385–397.
7. Little CB, Ghosh P, Rose R. The effect of strenuous versus moderate exercise on the metabolism of proteoglycans in articular cartilage from different weight-bearing regions of the equine third carpal bone. *Osteoarthritis Cartilage* 1997;5:161–172.
8. Roughley PJ, Melching LI, Recklies AD. Changes in the expression of decorin and biglycan in human articular cartilage with age and regulation by TGF-beta. *Matrix Biol* 1994;14:51–59.
9. Pelletier JP, Roughley PJ, DiBattista JA, et al. Are cytokines involved in osteoarthritic pathophysiology? *Semin Arthritis Rheum* 1991;20:12–25.
10. Shinmei M, Masuda K, Kikuchi T, et al. Production of cytokines by chondrocytes and its role in proteoglycan degradation. *J Rheumatol* 1991;27(suppl):89–91.
11. MacNaul KL, Chartrain N, Lark M, et al. Differential effects of IL-1 and TNF alpha on the expression of stromelysin, collagenase and their natural inhibitor, TIMP, in rheumatoid human synovial fibroblasts. *Matrix Suppl* 1992;1:198–199.
12. Miller VE, Rogers K, Muirden KD. Detection of tumour necrosis factor alpha and interleukin-1 beta in the rheumatoid osteoarthritic cartilage-pannus junction by immunohistochemical methods. *Rheumatol Int* 1993;13:77–82.
13. Morris EA, Treadwell BV. Effect of interleukin 1 on articular cartilage from young and aged horses and comparison with metabolism of osteoarthritic cartilage. *Am J Vet Res* 1994;55:138–146.
14. MacDonald MH, Stover SM, Willits NH, et al. Regulation of matrix metabolism in equine cartilage explant cultures by interleukin 1. *Am J Vet Res* 1992;53:2278–2285.
15. May SA, Hooke RE, Lees P. Interleukin-1 stimulation of equine articular cells. *Res Vet Sci* 1992;52:342–348.
16. Caron JP, Tardif G, Martel-Pelletier J, et al. Modulation of matrix metalloprotease 13 (collagenase 3) gene expression in equine chondrocytes by interleukin 1 and corticosteroids. *Am J Vet Res* 1996;57:1631–1634.
17. Billinghurst RC, Fretz PB, Gordon JR. Induction of intra-articular tumour necrosis factor during acute inflammatory responses in equine arthritis. *Equine Vet J* 1995;27:208–216.
18. Hawkins DL, MacKay RJ, Gum GG, et al. Effects of intra-articularly administered endotoxin on clinical signs of disease and synovial fluid tumor necrosis factor, interleukin 6, and prostaglandin E_2 values in horses. *Am J Vet Res* 1993;54:379–386.
19. Glade MJ, Kanwar YS, Hefley TJ. Enzymatic isolation of chondrocytes from immature rabbit articular cartilage and maintenance of phenotypic expression in culture. *J Bone Miner Res* 1991;6:217–226.
20. Reginato AM, Iozzo RV, Jimenez SA. Formation of nodular structures resembling mature articular cartilage in long-term primary cultures of human fetal epiphyseal chondrocytes on a hydrogel substrate. *Arthritis Rheum* 1994;37:1338–1349.
21. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim Biophys Acta* 1986;883:173–177.
22. Clegg PD, Burke RM, Coughlan AR, et al. Characterisation of equine matrix metalloproteinase 2 and 9; and identification of the cellular sources of these enzymes in joints. *Equine Vet J* 1997;29:335–342.
23. Chandrasekhar S, Phadke K. Interleukin-1-induced alterations in proteoglycan metabolism and matrix assembly. *Arch Biochem Biophys* 1988;265:294–301.
24. Tang LH, Buckwalter JA, Rosenberg LC. Effect of link protein concentration on articular cartilage proteoglycan aggregation. *J Orthop Res* 1996;14:334–339.
25. Bolton MC, Dudhia J, Bayliss MT. Quantification of aggrecan and link-protein mRNA in human articular cartilage of different ages by competitive reverse transcriptase-PCR. *Biochem J* 1996;319:489–498.
26. von den Hoff H, de Koning M, van Kampen J, et al. Interleukin-1 reversibly inhibits the synthesis of biglycan and decorin in intact articular cartilage in culture. *J Rheumatol* 1995;22:1520–1526.
27. Qwarnstrom EE, Jarvelainen HT, Kinsella MG, et al. Interleukin-1 beta regulation of fibroblast proteoglycan synthesis involves a decrease in versican steady-state mRNA levels. *Biochem J* 1993;294:613–620.
28. Demoor-Fossard M, Redini F, Boittin M, et al. Expression of decorin and biglycan by rabbit articular chondrocytes. Effects of cytokines and phenotypic modulation. *Biochim Biophys Acta* 1998;1398:179–191.
29. Visser NA, Vankampen GP, Dekoning MH, et al. The effects of loading on the synthesis of biglycan and decorin in intact mature articular cartilage in vitro. *Connect Tissue Res* 1994;30:241–250.
30. Little CB, Ghosh P, Bellenger CR. Topographic variation in biglycan and decorin synthesis by articular cartilage in the early stages of osteoarthritis: an experimental study in sheep. *J Orthop Res* 1996;14:433–444.
31. Visser NA, de Koning MH, Lammi MJ, et al. Increase of decorin content in articular cartilage following running. *Connect Tissue Res* 1998;37:295–302.
32. Masse PG, Carrino DA, Morris N, et al. Loss of decorin from the surface zone of articular cartilage in a chick model of osteoarthritis. *Acta Histochemica* 1997;99:431–444.
33. Kuc IM, Scott PG. Increased diameters of collagen fibrils

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precipitated in vitro in the presence of decorin from various connective tissues. *Connect Tissue Res* 1997;36:287–296.

34. Hagg R, Bruckner P, Hedbom E. Cartilage fibrils of mammals are biochemically heterogeneous: differential distribution of decorin and collagen IX. *J Cell Biol* 1998;142:285–294.

35. Mizoguchi I, Scott PG, Dodd CM, et al. An immunohistochemical study of the localization of biglycan, decorin and large chondroitin-sulphate proteoglycan in adult rat temporomandibular joint disc. *Arch Oral Biol* 1998;43:889–898.

36. Roughley PJ, Rodriguez E, Lee ER. The interactions of 'non-aggregating' proteoglycans. *Osteoarthritis Cartilage* 1995;3:239–248.

37. Schonherr E, Witsch-Prehm P, Harrach B, et al. Interaction of biglycan with type I collagen. *J Biol Chem* 1995;270:2776–2783.

38. Ramasamy S, Lipke DW, McClain CJ, et al. Tumor necrosis factor reduces proteoglycan synthesis in cultured endothelial cells. *J Cell Physiol* 1995;162:119–126.

39. Noyori K, Takagi T, Jasin HE. Characterization of the macromolecular components of the articular cartilage surface. *Rheumatol Int* 1998;18:71–77.

40. Knauper V, Lopez-Otin C, Smith B, et al. Biochemical characterization of human collagenase-3. *J Biol Chem* 1996;271:1544–550.

41. Reboul P, Pelletier JP, Tardif G, et al. The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes. A role in osteoarthritis. *J Clin Invest* 1996;97:2011–2019.

42. Stahle-Backdahl M, Sandstedt B, Bruce K, et al. Collagenase-3 (MMP-13) is expressed during human fetal ossification and re-expressed in postnatal bone remodeling and in rheumatoid arthritis. *Lab Invest* 1997;76:717–728.

43. Billinghurst RC, Dahlberg L, Ionescu M, et al. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. *J Clin Invest* 1997;99:1534–1545.

44. Lohmander LS, Neame PJ, Sandy JD. The structure of aggrecan fragments in human synovial fluid. Evidence that aggrecanase mediates cartilage degradation in inflammatory joint disease, joint injury, and osteoarthritis. *Arthritis Rheum* 1993;36: 1214–1222.

45. Lark MW, Bayne EK, Flanagan J, et al. Aggrecan degradation in human cartilage. Evidence for both matrix metalloproteinase and aggrecanase activity in normal, osteoarthritic, and rheumatoid joints. *J Clin Invest* 1997;100:93–106.

46. Arner EC, Pratta MA, Trzaskos JM, et al. Generation and characterization of aggrecanase. A soluble, cartilage-derived aggrecan-degrading activity. *J Biol Chem* 1999;274:6594–6601.

47. Tortorella MD, Burn TC, Pratta MA, et al. Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. *Science* 1999;284:1664–1666.

48. Arner EC, Dimeo TM, Pratta MA, et al. Interleukin-1 induces aggrecanase-mediated cleavage in human articular cartilage without up-regulating stromelysin or glycosaminoglycan release. *Acta Orthop Scand* 1995;66:153–154.

49. Buttle DJ, Fowles A, Ilic MZ, et al. "Aggrecanase" activity is implicated in tumour necrosis factor alpha mediated cartilage aggrecan breakdown but is not detected by an in vitro assay. *Mol Pathol* 1997;50:153–159.

50. Clegg PD, Coughlan AR, Riggs CM, et al. Matrix metalloproteinases 2 and 9 in equine synovial fluids. *Equine Vet J* 1997;29:343–348.

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