Assessment of the catabolic effects of interleukin-1β on proteoglycan metabolism in equine cartilage cocultured with synoviocytes

Abigail J. Gregg, BS; Lisa A. Fortier, DVM, PhD; Hussni O. Mohammed, DVM, PhD; Karen G. Mayr, BS; Brian J. Miller, BS; Jennifer L. Haupt, BS

Objective—To evaluate the effects of interleukin (IL)-1β on proteoglycan metabolism in equine cartilage explants when cultured in the presence of synoviocytes.

Sample Population—Samples of cartilage and synovium collected from the femoropatellar joints of three 2- to 3-year-old horses.

Procedures—3 experimental groups were established: cartilage explants only, synoviocytes only, and cartilage explants-synoviocytes in coculture. In each group, samples were cultured with or without IL-1β (10 ng/mL) for 96 hours. Glycosaminoglycan (GAG) content of cartilage and medium samples was measured by use of a spectrophotometric assay; RNA was isolated from synoviocytes and cartilage and analyzed for expression of matrix metalloproteinases (MMP)-3 and -13 (cartilage and synoviocytes), aggrecan (cartilage), collagen type II (cartilage), and 18S as a control (cartilage and synoviocytes) by use of quantitative PCR assays. Cartilage matrix metachromasia was assessed histochemically.

Results—IL-1β-induced GAG loss from cartilage was significantly less in cocultures than in cartilage-only cultures. Cartilage aggrecan gene expression was also significantly less downregulated and synoviocyte MMP-3 expression was less upregulated by IL-1β in cocultures, compared with cartilage- and synoviocyte-only cultures. Histochemical findings supported the molecular and biochemical results and revealed maintenance of matrix metachromasia in cocultured cartilage treated with IL-1β.

Conclusions and Clinical Relevance—Results suggest that synoviocytes secrete 1 or more mediators that preferentially protect matrix GAG metabolism from the degradative effects of IL-1β. Further studies involving proteomic and microarray approaches in similar coculture systems may elucidate novel therapeutic targets for the treatment of osteoarthritis. (Am J Vet Res 2006;67:957–962)

In the development of osteoarthritis, there is a disruption in extracellular matrix homeostasis with an overall balance toward cartilage catabolism. The enzymes of the MMP family deplete the extracellular matrix through decreased synthesis and increased degradation of essential matrix proteins including aggrecan and type II collagen. In a synovial joint, MMPs are synthesized by chondrocytes and synoviocytes. The activity of MMPs is tightly regulated at several levels including transcription, activation of precursor enzymes (zymogens), interaction with extracellular matrix components (collagen type II and aggrecan), and inhibition by tissue inhibitors of metalloproteinases. One of the most potent activators of MMPs in cartilage and synovium is the proinflammatory cytokine IL-1. Although these studies have proven valuable for studying the basic mechanisms involved in matrix degradation and for evaluating treatments, they were not optimally designed to reflect the native articular environment because no synovial membrane or synovial fluid was present in the culture system. Homeostasis of the articular environment depends on interactions between the cartilage, synovial membrane, and synovial fluid and not just the ability of cartilage to respond to molecular or mechanical cues. Therefore, the effects of soluble protein mediators or mechanical stress on cartilage biosynthesis will likely differ depending on the type of culture system employed in each study. For example, cartilage explants have significantly lower synthetic activity when cultured in the presence of synovial fluid versus their activity in tissue culture medium supplemented with fetal bovine serum. The purpose of the study reported here was to evaluate the effects of IL-1β on proteoglycan metabolism in cartilage explants cultured in the presence of synoviocytes. The objective was to examine a coculture system in which cartilage and synoviocytes share a common medium to determine whether the well-established effects of IL-1β on cartilage matrix catabolism were altered by the presence of synoviocytes. Our hypothesis was that the catabolic effects of IL-1β on articular cartilage would be significantly diminished in cultures containing synoviocytes.
Materials and Methods

Cartilage and synovium were collected from both femoropatellar joints of 3 horses (2 to 3 years old) that were euthanized (via IV injection of an overdose of pentobarbital) for reasons unrelated to lameness or this study. All procedures were approved by the Institutional Animal Care and Use Committee of Cornell University. Three groups were established for samples collected from each of the 3 horses, and all sample groups were cultured in split-well plates specifically designed for coculture of tissues. The experiment was designed with synoviocytes in monolayer on the bottom of the well and cartilage explants suspended in the medium of the same well by use of a low–protein-binding polyester membrane insert (pore size, 3 μm; Figure 1). The insert has gaps in its perimeter to allow free exchange of medium between the 2 compartments. Three groups, each in triplicate, were established: cartilage only (no synoviocytes), synoviocytes only (no cartilage explants), and coculture (cartilage explants with synoviocytes). Two treatments (ie, with or without IL-1β (10 ng/mL)) were applied to each group for each of the 3 horses. Experiments for each of the 3 horses were performed on independent days.

Tissue procurement and culture—Samples of full-thickness cartilage were removed from the trochlear ridges of the femora, and synovial membrane was removed from the proximal portion of the femoropatellar joints. Careful dissection was performed to minimize inclusion of fat and fibrous joint capsule tissue in the synovial membrane samples. The synovium was digested in DMEM, 0.15% collagenase type II, 0.05% papain and the optical density at 595 nm was determined with a spectrophotometer. Mixed-isomer shark chondroitin sulfate was used to construct a standard curve. Cartilage GAG content was expressed per microgram of DNA, which was assessed fluorometrically. Calf thymus DNA was used to construct a standard curve.

RNA isolation and analyses—Four explants from each well were rinsed in PBS solution, snap-frozen in liquid nitrogen, and pulverized in a freezer-mill. Total RNA from cartilage and synoviocytes was isolated by use of a 1,9-dimethyl-methylene blue dye-binding microwell spectrophotometric assay. Media samples were digested (1:1 [vol/vol]) in 0.05% papain. To determine cartilage GAG content, lyophilized samples were digested (10% [wt/vol]) in 0.05% papain and the optical density at 595 nm was determined with a spectrophotometer. Mixed-isomer shark chondroitin sulfate was used to construct a standard curve.

Unauthenticated | Downloaded 08/06/24 08:38 AM UTC
and collagen type II (cartilage only); transcript levels of 18S were assessed as a control. The total RNA was reverse transcribed and amplified by use of a 1-step system with sequence detection software. The primers and dual-labeled fluorescent probes (6-carboxyfluorescein [6-FAM] as the 5′ label [reporter dye] and tetramethylrhodamine [TAMRA] as the 3′ label [quenching dye]) were designed with specialized software and equine sequences published in GenBank, sequenced in our laboratories, or obtained from another research group.

Histochemical analysis with toluidine blue—One cartilage explant from each well was used for histochemical analysis, yielding triplicate samples per treatment group per horse. Explants were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 6 µm. Cartilage sections were stained with 0.5% toluidine blue and assessed for matrix metachromasia via light microscopy.

Statistical analysis—Differences in outcome variables attributable to IL-1β treatment were assessed by use of 1-sample t tests. To assess differences in the magnitude of response to IL-1β treatment between groups, the fold change in outcome variables (attributable to IL-1β treatment) within each group was calculated and then the fold changes between culture groups were compared by use of 2-sample independent-groups t tests. A 2-sample t test analysis was chosen over an ANOVA because the objective was to compare the responses between groups and not to compare all treatments.

Results

GAG content of cartilage and medium—There were no significant (P = 0.13) effects of IL-1β on GAG content in cartilage that was cultured with synoviocytes (Figure 2). As expected, GAG concentration in the cartilage-only group (ie, cartilage cultured without synoviocytes) was significantly (P = 0.001) decreased by IL-1β treatment. Subsequently, when the fold changes in GAG concentration attributable to IL-1β in the cartilage-only and coculture groups were compared, there was significantly (P = 0.04) more GAG loss in the cartilage-only group than in the coculture group. There were no significant (P = 0.14) differences in DNA content within groups in response to IL-1β treatment, allowing for normalization of GAG content to DNA content. Glycosaminoglycan concentration in media samples was increased (P ≤ 0.03) by IL-1β treatment at 48 and 96 hours in cartilage-only and coculture groups, compared with media collected from their respective untreated cultures (Figure 3). After 48 or 96 hours, there was no difference (P > 0.8) in media GAG concentration between the 2 IL-1β-treated cultures; however, GAG content within IL-1β-treated culture groups was significantly (P ≤ 0.04) greater at 96 hours, compared with their respective values at 48 hours.

Cartilage gene expression—Matrix gene expression in response to IL-1β treatment was different between groups, and aggrecan mRNA was partially protected against the treatment in coculture groups. In cartilage-only groups, IL-1β induced a significant (P ≤ 0.02) decrease in aggrecan and type II collagen gene expression, as expected (Table 1). However, in coculture, aggrecan expression was less affected by IL-1β, and values in the cartilage-only and coculture groups after treatment were significantly (P = 0.03) different from each other. Collagen gene expression in response to IL-1β treatment was not significantly (P = 0.4) different between cartilage-only and coculture groups.

The catabolic genes MMP-3 and -13 were significantly (P ≤ 0.03) increased in response to IL-1β treatment in both culture groups (Table 1). There were no differences (P ≥ 0.07) in cartilage MMP-3 or -13 expression after IL-1β treatment between the cartilage-only and coculture groups.

Synoviocyte gene expression—In synoviocyte-only (ie, no cartilage explants) and coculture groups, expression of MMP-3 and -13 was significantly (P ≤ 0.03) by IL-1β treatment at 48 and 96 hours in cartilage-only and coculture groups, compared with media collected from their respective untreated cultures (Figure 3). After 48 or 96 hours, there was no difference (P > 0.8) in media GAG concentration between the 2 IL-1β-treated cultures; however, GAG content within IL-1β-treated culture groups was significantly (P ≤ 0.04) greater at 96 hours, compared with their respective values at 48 hours.
increased in an attempt at cartilage self-repair.20 Despite this effort to reestablish homeostasis, cartilage degeneration continues with a marked loss of proteoglycan content.21 Treatment of cartilage explants or chondrocytes in monolayer culture with IL-1α or -1β induces a similar loss of aggrecan from the extracellular matrix with a concomitant decrease in proteoglycan synthesis, and such treatments can be used to induce cartilage degradation for osteoarthritis studies.14,16,22,23 In the present study, the results of IL-1β treatment of cartilage explants that were cultured alone were consistent with findings of previous studies,14,16,22,23 but when cartilage explants were cultured with synoviocytes, the catabolic effects of IL-1β were diminished. The protection imparted by the presence of synoviocytes was relatively specific for proteoglycan biosynthesis.

In cartilage-synoviocyte cocultures, there was no significant loss of GAG from cartilage after treatment with IL-1β. This was an unexpected finding because the cocultures were exposed to a relatively high concentration of IL-1β (10 ng/mL) for 96 hours, which should have resulted in considerable GAG loss from cartilage matrix. In contrast, and as expected, approximately 51% of GAG content was lost from cartilage explants cultured without synoviocytes but in the presence of IL-1β. These results were supported by toluidine blue histochemistry—

In cartilage-only cultures, matrix metachromasia was markedly diminished throughout cartilage explants by IL-1β treatment, as anticipated (Figure 4). However, in coculture, matrix metachromasia in cartilage explants was intense and evenly distributed throughout the depth of the tissue and was subjectively increased, compared with findings in cartilage-only groups in the absence of IL-1β. In addition, there was little perceivable loss of metachromasia in cocultured cartilage explants treated with IL-1β, compared with findings in cocultured cartilage explants cultured in the absence of IL-1β, which supported the biochemical and molecular findings.

**Discussion**

In osteoarthritis, aggrecan synthesis is initially increased in an attempt at cartilage self-repair.20 Despite this effort to reestablish homeostasis, cartilage degeneration continues with a marked loss of proteoglycan content.21 Treatment of cartilage explants or chondrocytes in monolayer culture with IL-1α or -1β induces a similar loss of aggrecan from the extracellular matrix with a concomitant decrease in proteoglycan synthesis, and such treatments can be used to induce cartilage degradation for osteoarthritis studies.14,16,22,23 In the present study, the results of IL-1β treatment of cartilage explants that were cultured alone were consistent with findings of previous studies,14,16,22,23 but when cartilage explants were cultured with synoviocytes, the catabolic effects of IL-1β were diminished. The protection imparted by the presence of synoviocytes was relatively specific for proteoglycan biosynthesis.

In cartilage-synoviocyte cocultures, there was no significant loss of GAG from cartilage after treatment with IL-1β. This was an unexpected finding because the cocultures were exposed to a relatively high concentration of IL-1β (10 ng/mL) for 96 hours, which should have resulted in considerable GAG loss from cartilage matrix. In contrast, and as expected, approximately 51% of GAG content was lost from cartilage explants cultured without synoviocytes but in the presence of IL-1β. These results were supported by toluidine blue histochemistry—

**Table 1**—Cartilage matrix and synoviocyte gene expression in equine cartilage-only or synoviocyte-only cultures and cartilage-synoviocyte cocultures.

<table>
<thead>
<tr>
<th>mRNA analysis</th>
<th>Culture group</th>
<th>Cartilage or synoviocytes only</th>
<th>Cartilage with synoviocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without IL-1β</td>
<td>With IL-1β</td>
<td>Without IL-1β</td>
</tr>
<tr>
<td>Cartilage aggrecan</td>
<td>27.8 ± 10^5</td>
<td>11.1 ± 10^5 *</td>
<td>26.6 ± 10^5</td>
</tr>
<tr>
<td>Cartilage Col IIB</td>
<td>1.26 ± 10^5</td>
<td>0.81 ± 10^5 *</td>
<td>1.16 ± 10^5</td>
</tr>
<tr>
<td>Cartilage MMP-3</td>
<td>0.36 ± 10^5</td>
<td>6.1 ± 10^5 *</td>
<td>0.39 ± 10^5</td>
</tr>
<tr>
<td>Cartilage MMP-13</td>
<td>0.11 ± 10^5</td>
<td>3.0 ± 10^5 *</td>
<td>0.11 ± 10^5</td>
</tr>
<tr>
<td>Synoviocyte MMP-3</td>
<td>283 ± 10^5</td>
<td>484 ± 10^5 *</td>
<td>133 ± 10^5</td>
</tr>
<tr>
<td>Synoviocyte MMP-13</td>
<td>0.12 ± 10^5</td>
<td>0.32 ± 10^5 *</td>
<td>0.21 ± 10^5</td>
</tr>
</tbody>
</table>

Data are presented as mean (SE) of (copy number of gene/ng of RNA)/copy of 18S from 3 horses in 3 independent experiments.

*Value significantly (P < 0.05; 1-sample t test) different from that of the culture that was not treated with IL-1β in the same group. **Value significantly (P < 0.05; 2-sample t test) different from that of the cartilage-only or synoviocyte-only culture treated with IL-1β.

Col IIB = Collagen type IIB.
dine blue histochemical assessment of matrix metachromasia. Analysis of medium GAG content in cartilage-only and cartilage-synoviocyte cultures with and without IL-1β revealed that IL-1β treatment resulted in a significant increase in medium GAG content in both groups. This suggests that the lack of GAG loss from the cartilage matrix in response to IL-1β is a result of protected or increased GAG synthesis rather than diminished loss of GAG from cartilage in cocultures. Glycosaminoglycan synthesis analysis by use of radiolabeled sulfur incorporation methods was not performed in the present study in lieu of our goal to assess mRNA expression of genes with known roles in matrix synthesis and degradation.

In our study, results of the analysis of aggregan gene expression supported the GAG biochemical data. Although aggregan mRNA expression was significantly decreased by IL-1β in the cartilage-only and coculture groups, the effect was significantly less (approx 90% less) in the cocultures than in cartilage-only cultures. Taken together with the cartilage GAG content data, the results imply that in the presence of synoviocytes, aggregan biosynthesis in cartilage is protected or maintained rather than stimulated. Further studies to determine mRNA half-life and GAG biosynthetic rate would be required to fully evaluate the mechanism by which synoviocytes protect GAG synthesis in cartilage from the effects of IL-1β. This protective effect of synoviocytes on cartilage gene expression was not evident for collagen type IIβ, MMP-3, or MMP-13. In assessments of these variables, IL-1β induced alterations in gene expression patterns that were anticipated (ie, decreased collagen type IIβ and increased MMP-3 and -13 expressions).

Synoviocyte mRNA expression subsequent to IL-1β treatment was also disparately affected by culture-group type. In synoviocyte-only culture and coculture, synoviocyte MMP-3 and -13 mRNAs were significantly increased in response to IL-1β exposure, as would be expected. However, compared with the synoviocyte-only group, synoviocyte MMP-3 mRNA was increased by IL-1β to a significantly lesser extent in coculture. It is well known that IL-1α and -1β upregulate MMP-3 expression in articular chondrocytes and synoviocytes and that MMP-3 induces GAG loss from cartilage. Interestingly, hyaluronate can inhibit IL-1β–induced upregulation of MMP-3. Hyaluronate was not measured in the medium samples collected in our study, but if it were a mechanism to explain our findings, then presumably synoviocytes, cartilage, or both in cocultures would have to secrete hyaluronate to a greater extent than synoviocytes alone because the amelioration of IL-1β–induced upregulation of synoviocyte MMP-3 transcription was greater in the coculture group than it was in the synoviocyte-only group. Alternatively, an unidentified molecule may be released from cartilage, which reduces the impact of IL-1β on synoviocyte MMP-3 transcription.

Identification and characterization of mediators secreted from synoviocytes and cartilage in cocultures treated with IL-1β may elucidate novel therapeutic targets for patients with rheumatoid or osteoarthritis. Microarray and proteomic approaches to identify such target molecules are under investigation. Similar experiments using IL-1β–conditioned medium from synoviocyte cultures to identify novel cytokines involved in cartilage catabolism have been performed. The results of the present study suggest that by use of a coculture system, mediators that protect cartilage from IL-1β–induced catabolism may also be identified. Interleukin-1 receptor antagonist protein is a naturally occurring antagonist to IL-1β and has proven effective at protecting cartilage from IL-1–induced catabolism. However, by use of a coculture system similar to that used in the present study, Haupt et al did not detect an IL-1β–induced increase in IL-1 receptor antagonist protein or mRNA synthesis in synoviocytes. This suggests that there are mediators in addition to IL-1 receptor antagonist protein that are capable of protecting cartilage from IL-1β–induced proteoglycan loss.

References
the interleukin-1beta-induced expression of matrix metallopro-

14. Arner EC. Effect of animal age and chronicity of interleukin-

15. Balkman CE, Nixon AJ. Molecular cloning and cartilage

16. Kuroki K, Stoker AM, Cook JI. Effects of proinflammatory
cytokines on canine articular chondrocytes in a three-dimensional

17. Martin JA, Wilkey AL, Brand RA. Cartilage extracellular

18. Chandrasekhar S, Esterman MA, Hoffman HA. Microdetermination of proteoglycans and glycosaminoglycans in


20. Sandy JD, Adams ME, Billingham ME, et al. In vivo and in

21. Sandell LJ, Aigner T. Articular cartilage and changes in

22. Takafuji VA, McIlwraith CW, Howard RD. Effects of equine
recombinant interleukin-1alpha and interleukin-1beta on proteogly-
can metabolism and prostaglandin E2 synthesis in equine articular

mediates interleukin-1beta-induced dedifferentiation but not

24. Kobayashi M, Squires GR, Mousa A, et al. Role of inter-
leukin-1 and tumor necrosis factor alpha in matrix degradation of

of chondrocytes: evidence for complex cytokine interactions involving a


27. Haupt JL, Frisbie DD, McIlwraith CW, et al. Dual transduc-
tion of insulin-like growth factor-I and interleukin-1 receptor antag-
onist protein controls cartilage degradation in an osteoarthritic cul-