Hyaline cartilage is a highly complex connective tissue that resists compression in articular joints and protects bones from friction. Healthy functional cartilage is characterized by a balance between anabolic and catabolic factors. Disturbance of this balance may result in excessive inflammation that ultimately leads to the breakdown of cartilage. This process has been implicated in the pathogenesis of osteoarthritis.

OBJECTIVE
To determine effects of transforming growth factor (TGF)-β and interleukin (IL)-1β on inflammatory markers in cultured canine chondrocytes to clarify the role of these cytokines in osteoarthritis of dogs.

SAMPLE
Pooled chondrocytes isolated from the stifle joints of 4 adult dogs.

PROCEDURES
Chondrocytes were isolated, cultured, and frozen at −80°C. Pooled cells were incubated in medium with or without TGF-β (1 or 10 ng/mL) and subsequently stimulated with IL-1β (10 ng/mL). Concentrations of nitric oxide (NO) and prostaglandin (PG) E were measured in culture supernatants. Gene expression of matrix metalloproteinase (MMP)-3, tissue inhibitor of metalloproteinase (TIMP)-2, inducible NO synthase (iNOS), and cyclooxygenase (COX)-2 was quantified by use of real-time quantitative PCR assays.

RESULTS
Stimulation with IL-1β increased gene expression of all inflammatory markers, except for TIMP-2. Incubation with TGF-β resulted in a significant decrease in MMP-3 and TIMP-2 mRNA concentrations but had no effect on PGE and NO concentrations. For cells treated with TGF-β followed by IL-1β, concentrations of PGE and NO were lower, compared with concentrations for IL-1β control cells. Furthermore, IL-1β–induced gene expression of iNOS, MMP-3, and COX-2 was downregulated. However, the IL-1β–induced downregulation of TIMP-2 gene expression was partially restored by pretreatment with TGF-β.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that IL-1β increased the expression of inflammatory genes and mediators, and TGF-β largely attenuated the IL-1β–mediated inflammatory response. Therefore, TGF-β might be a novel target for use in the prevention and treatment of cartilage breakdown in dogs with osteoarthritis. (Am J Vet Res 2017;78:1264–1272)

Osteoarthritis is a common condition in dogs. Presumably > 20% of middle-aged dogs in the United States have osteoarthritis. Similar to the condition in humans, affected dogs have signs of joint pain and restricted mobility; thus, they often have a diminished quality of life. Methods have been used to mimic osteoarthritis in dogs for in vitro evaluation of this inflammatory disease.

Several factors derived from chondrocytes and synoviocytes play a major role in the initiation and progression of osteoarthritis. Among them, IL-1β is considered one of the most important. Once IL-1β is bound to the IL-1 receptor, various catabolic pathways are induced, which include the expression of proteolytic enzymes (eg, MMPs and aggrecanases). Major components of the extracellular matrix are broken down and mediators (eg, NO and PGE) are produced, which leads to inflammation in the joint. Furthermore, anabolic activity of the cells is suppressed, which leads to delayed healing of the
resulting cartilage defects.\textsuperscript{15,16} Interleukin-1\(\beta\) also has the potential to induce a variety of chemokines that attract other cells to the joint. These cells generate additional inflammatory and proteolytic mediators,\textsuperscript{17,18} which may result in total cartilage destruction.

Transforming growth factor-\(\beta\) is a supplement commonly added to chondrocyte cell cultures and has been used as a trigger for chondrogenic differentiation of mesenchymal stem cells.\textsuperscript{19,20} However, the actions of TGF-\(\beta\) on chondrocytes remain controversial because it has been found that TGF-\(\beta\) provides both stimulatory and inhibitory effects on cartilage metabolism. On the one hand, TGF-\(\beta\) promotes the production of matrix components in chondrocytes\textsuperscript{21} and plays a key role in chondrogenesis\textsuperscript{22} and cartilage repair.\textsuperscript{23} Furthermore, TGF-\(\beta\) attenuates IL-1\(\beta\)-stimulated NO production\textsuperscript{24} and has proven beneficial for matrix protection because it modulates secretion of TIMPs\textsuperscript{25} and decreases the synthesis of MMPs.\textsuperscript{26,27} On the other hand, TGF-\(\beta\) has a number of effects that promote cartilage breakdown, which include the synthesis of matrix-degenerating enzymes,\textsuperscript{28,29} the development of osteophytes,\textsuperscript{30} and the initiation of fibrosis.\textsuperscript{31} In vivo experiments have revealed that prolonged administration of high doses of TGF-\(\beta\) results in osteoarthrisis-like cartilage defects.\textsuperscript{31} However, moderate doses of TGF-\(\beta\) may be a potential treatment for osteoarthrisis.\textsuperscript{32}

The effects of TGF-\(\beta\) on canine chondrocytes have not been elucidated. Therefore, the purpose of the study reported here was to use cultured canine chondrocytes to investigate the effects of TGF-\(\beta\) on chondrocytes stimulated with IL-1\(\beta\). We hypothesized that TGF-\(\beta\) would exert positive effects on matrix metabolism and, moreover, inhibit the destructive effects of IL-1\(\beta\).

Materials and Methods

Sample

Full-thickness cartilage slices were aseptically obtained from the stifle joints of 4 dogs (mixed-breed dogs; median age, 12 years) within 24 hours after the dogs were euthanized for reasons unrelated to the present study. Carcasses were stored in a cold room (7\textdegree C) from time of euthanasia until harvest of cartilage slices. Dogs were eligible for inclusion in the study if they had no orthopedic abnormalities in the stifle joints and no current systemic diseases that could have interfered with the growth and viability of chondrocytes.

Chondrocyte isolation and in vitro culture

Cells were cultured and harvested as described elsewhere,\textsuperscript{33} with slight modifications. Briefly, slices of macroscopically normal articular cartilage were digested in an enzyme mixture at 37\textdegree C for 18 hours with constant agitation. The enzyme mixture consisted of collagenase\textsuperscript{a} (1 U/mL), another collagenase\textsuperscript{b} (330 U/mL), and hyaluronidase\textsuperscript{c} (30 U/mL) diluted in Dulbecco modified Eagle medium (high glucose) that was supplemented with 10% fetal calf serum and 1% penicillin-streptomycin. After enzymatic digestion was completed, cells were separated by filtration through a 100-\(\upmu\)m nylon mesh, centrifuged at 400 X \(g\) for 20 minutes, and washed twice with PBS solution. Viability and cell numbers were determined by use of trypan blue dye exclusion in a Neubauer chamber, and cells were plated in culture flasks at a density of 2 X 10\textsuperscript{4} cells/cm\textsuperscript{2}. Freshly isolated cells were maintained in Dulbecco modified Eagle medium (high glucose) with 10% fetal calf serum and 1% penicillin-streptomycin, enriched with 10 ng of human recombinant insulin/mL and 50 \(\mu\)g of phosphoascorbic acid–trisodium salt/mL (basic medium), and incubated at 37\textdegree C, 5% CO\textsubscript{2}, and 95% humidity (passage 0). Culture medium was replaced with basic medium every 2 to 3 days. After confluency was reached, cells were detached with 0.05% trypsin-EDTA, centrifuged at 400 X \(g\) for 20 minutes, and washed once with PBS solution. Cells from each donor were resuspended in antimicrobial-free basic medium with 5% dimethyl sulfoxide at a density of 1 X 10\textsuperscript{6} cells/cryovial and frozen at -80\textdegree C.

Cells harvested from each of the 4 canine cadaver donors were cultured separately (passage 0). They were pooled prior to the respective experiments (passage 1).

Experimental design

Canine IL-1\(\beta\)\textsuperscript{4} was obtained as a solid lyophilized powder, reconstituted in sterile distilled water to a concentration of 50 \(\mu\)g/mL, and placed in aliquots and frozen at -20\textdegree C until usage. Frozen cells pooled from all 4 canine cadaver donors were thawed, diluted with basic medium, and centrifuged at 1,200 X \(g\) for 6 minutes. The cell pellet was resuspended in basic medium (with 5% fetal calf serum), and cells were counted and seeded onto 6-well plates at a density of 1.8 X 10\textsuperscript{5} cells/well (passage 1). During incubation, plates were maintained at 37\textdegree C with 5% CO\textsubscript{2} and 95% humidity. To investigate the effects of TGF-\(\beta\), cells were incubated in basic medium alone or basic medium containing 1 or 10 ng of canine TGF-1\(\beta\)/mL. Cultures were incubated for 48 hours, and cells then were incubated with (stimulated) or without 10 ng of IL-1\(\beta\)/mL for another 24 hours in basic medium supplemented as described previously. The incubation time of 24 hours was chosen on the basis of a previous study\textsuperscript{5} in which investigators found that the first peak of inflammatory marker release in osteoarthritis models occurs after cells have been incubated for 24 hours. After cultures were incubated for 72 hours, cells and culture supernatants were collected and stored at -80\textdegree C for subsequent assessment of PGE and NO concentrations and gene expression. All samples were prepared in triplicate. At the end of the experiment, the total yield of chondrocytes was 4 X 10\textsuperscript{5} cells/well.

Histologic examination of extracellular matrix

Cells were examined daily by use of phase-contrast microscopy during in vitro culture. Extracellular matrix production was visible by use of Alcian blue staining for proteoglycan and alcian blue. Cells were cultured and harvested as described elsewhere,\textsuperscript{35} with slight modifications. Briefly, slices of macroscopically normal articular cartilage were digested in an enzyme mixture at 37\textdegree C for 18 hours with constant agitation. The enzyme mixture consisted of collagenase\textsuperscript{a} (1 U/mL), another collagenase\textsuperscript{b} (330 U/mL), and hyaluronidase\textsuperscript{c} (30 U/mL) diluted in Dulbecco modified Eagle medium (high glucose) that was supplemented with 10% fetal calf serum and 1% penicillin-streptomycin. After enzymatic digestion was completed, cells were separated by filtration through a 100-\(\upmu\)m nylon mesh, centrifuged at 400 X \(g\) for 20 minutes, and washed twice with PBS solution. Viability and cell numbers were determined by use of trypan blue dye exclusion in a Neubauer chamber, and cells were plated in culture flasks at a density of 2 X 10\textsuperscript{4} cells/cm\textsuperscript{2}. Freshly isolated cells were maintained in Dulbecco modified Eagle medium (high glucose) with 10% fetal calf serum and 1% penicillin-streptomycin, enriched with 10 ng of human recombinant insulin/mL and 50 \(\mu\)g of phosphoascorbic acid–trisodium salt/mL (basic medium), and incubated at 37\textdegree C, 5% CO\textsubscript{2}, and 95% humidity (passage 0). Culture medium was replaced with basic medium every 2 to 3 days. After confluency was reached, cells were detached with 0.05% trypsin-EDTA, centrifuged at 400 X \(g\) for 20 minutes, and washed once with PBS solution. Cells from each donor were resuspended in antimicrobial-free basic medium with 5% dimethyl sulfoxide at a density of 1 X 10\textsuperscript{6} cells/cryovial and frozen at -80\textdegree C.

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Histologic examination of extracellular matrix

Cells were examined daily by use of phase-contrast microscopy during in vitro culture. Extracellular matrix production was visible by use of Alcian blue stainin
stain. Cells of passage 0 and passage 1 were grown in monolayer, as described previously. After confluence was reached, cells were fixed with 10% formaldehyde for 15 minutes, incubated in 3% acetic acid for 5 minutes, and then incubated with 1% Alcian blue salt solution for 3 hours. Phase-contrast microscopy of stained cells was performed, and photographs were obtained of representative specimens.

**Gene expression analysis**

Total RNA was extracted from the cells with a commercially available kit in accordance with the manufacturer’s instructions. Contaminating residual genomic DNA was removed with an endonuclease. Purity and concentration of RNA were determined by absorbance measurement at 260 and 280 nm. For each sample, 500 ng of RNA was reverse transcribed into cDNA by use of a commercial kit and thermal cycler. Relative gene expression of MMP-3, TIMP-2, iNOS, and COX2 and the reference genes TBP and GAPDH was analyzed by use of a commercially available PCR assay kit and real-time detection system. Final volume of reaction samples was 20 µL, which consisted of 10 µL of master mix, 400 nM of forward primer, 400 nM of reverse primer, and 1 µL of cDNA diluted 1:10 in PCR-grade water. Previously validated primers for target and reference genes were used. The amplification efficiency for each gene was determined from the slope of a standard curve generated by performing qPCR assays with a logarithmic dilution of the template DNA. All reactions were performed in triplicate by use of the following cycling conditions: initial denaturation at 95°C for 3 minutes, which was followed by 40 cycles of denaturation at 95°C for 3 seconds, annealing at the appropriate temperature for 20 seconds, and extension at 72°C for 1 second. Control samples (no reverse transcriptase and no template) were included in each assay. To verify specificity of the PCR assay products, a melting curve analysis was performed by heating the samples from 55°C to 99°C in 1°C increments with continuous measurement of fluorescence. Relative expression was obtained by normalizing target gene expression to that of the reference genes TBP and GAPDH. The change in gene expression for each sample was calculated by use of computer software.

**Assay of NO concentrations**

Nitric oxide is a short-lived free radical that is rapidly metabolized to nitrite. Therefore, the nitrite concentration was quantified by use of a calorimetric assay and used as an indicator of NO production. Briefly, 50 µL of Griess reagent (1:1 mixture of 0.1% sulfanilamide in 5% phosphoric acid and 0.1% N-naphthyl-ethylenediamine dihydrochloride) was added to 50 µm of cell culture supernatant and allowed to incubate for 10 minutes. Absorbance then was measured at 540 nm on a plate reader. A standard curve was generated from solutions containing 0 to 6 µg of sodium nitrite/mL.

**Statistical analysis**

Data for the real-time qPCR assay were analyzed as logarithmic (base 2) ratios of expression. The study was conducted as 3 separate experiments in duplicate for PGE and NO concentrations and in triplicate for gene expression analysis by use of pooled cells from the 4 canine cadaver donors. To compare the effects of TGF-β and IL-1β, all data were analyzed with a 2-factor ANOVA by use of a statistical program. When there was a significant main effect of TGF-β or IL-1β or an interaction between TGF-β and IL-1β, a Bonferroni post hoc test was performed to detect differences between means. Significance for the ANOVA and post hoc test was set at α = 0.01.
Results

Chondrocyte in vitro culture

Examination after application of trypan blue dye revealed that viability of the isolated chondrocytes was > 90%. Cells adhered to the bottom of the flasks within 24 to 72 hours after seeding. There was no appreciable variation of viability between cultured cells isolated from the various donors. Cells in monolayer appeared small and had a polygonal shape, although clusters of round cells remained. Cells in clusters were encapsulated in metachromatic material that consisted of glycosaminoglycans, as indicated by a positive response to Alcian blue stain (Figure 1).

Gene expression of MMP-3 and TIMP-2

To determine the effect of TGF-β and IL-1β on cartilage matrix degradation, gene expression of MMP-3 and its antagonist TIMP-2 was examined (Figure 2). For cells stimulated with IL-1β alone, there was a 51-fold increase in MMP-3 gene expression, whereas there was a 2-fold decrease in TIMP-2 gene expression, compared with results for cells incubated in the control medium. For cells treated with TGF-β alone, there was a substantial decrease in expression for both genes. Gene expression of MMP-3 was significantly lower in chondrocytes exposed to TGF-β and stimulated with IL-1β, compared with results for cells stimulated with IL-1β alone. Gene expression analysis revealed a 4-fold downregulation for 1 ng of TGF-β/mL and a 12-fold downregulation for 10 ng of TGF-β/mL. In contrast, gene expression of TIMP-2 was only significantly increased after addition of 1 ng of TGF-β/mL in combination with IL-1β. Expression of TIMP-2 in cells treated with 1 ng of TGF-β/mL in combination with IL-1β was slightly upregulated, compared with the response for cells treated with IL-1β alone. However, 10 ng of TGF-β/mL in combination with IL-1β had no significant effect on TIMP-2 gene expression.

Gene expression of iNOS and NO concentration

Gene expression of iNOS (Figure 3) and the concomitant NO concentration (Figure 4), which is an important inflammatory mediator in osteoarthritis, were determined. Cells stimulated with IL-1β alone had significantly higher gene expression of iNOS and a significantly higher NO concentration than did chondrocytes incubated in control medium. Gene expression of iNOS
and generation of NO were not affected by TGF-β alone.

In IL-1β-stimulated cells treated with the 2 concentrations of TGF-β, IL-induced iNOS gene expression and NO concentration were decreased in a dose-dependent manner. There was a 3-fold downregulation of iNOS gene expression for 1 ng of TGF-β/mL and a 6-fold downregulation for 10 ng of TGF-β/mL, compared with results for cells treated with IL-1β alone.

**Gene expression of COX-2 and PGE concentration**

Effects on PGE, which is another important inflammatory mediator, were determined by gene expression analysis of the PGE-generating enzyme COX-2 (Figure 5) and by measuring the PGE concentration (Figure 6). When cells were stimulated with IL-1β alone, there was a significant increase in COX-2 gene expression, which was accompanied by a high PGE concentration. For chondrocytes incubated with TGF-β alone, no significant changes in PGE concentration were found in the cell culture supernatant. In contrast, COX-2 gene expression was approximately twice as high for cells incubated with 10 ng of TGF-β/mL. When cells were stimulated with IL-1β, 10 ng of TGF-β/mL significantly reduced gene expression of COX-2, compared with results for cells stimulated with IL-1β alone. This change in gene expression was associated with significantly lower PGE concentrations in cells treated with TGF-β in combination with IL-1β.

**Discussion**

For the study reported here, we chose to use a 2-D technique, similar to the one used in another study. Effects in monolayer cultures can only be attributable to cell metabolism and are not affected by surrounding 3-D structures. Additionally, all cells in a monolayer culture have the same access to cell culture media components and stimulants. A major disadvantage is that chondrocytes grown in monolayer tend to lose their phenotype and become fibroblastoid. However, because the cells used in the present study formed clusters encapsulated in extracellular matrix, we believed that the cell culture consisted primarily of differentiated chondrocytes.

It is known that IL-1β is a major trigger in osteoarthritis and stimulates catabolic changes, suppresses anabolic pathways, and decreases matrix synthesis. In chondrocyte culture, it is also known that IL-1β initiates an inflammatory cascade comparable to that...
of naturally occurring osteoarthritis.4–7 In the present study, the addition of IL-1β led to higher expression of all genes and concentrations of inflammatory mediators selected for analysis, compared with results for nonstimulated control cells. Analysis of data for the real-time qPCR assay revealed a ≥ 35-fold upregulation for expression of the catabolic enzymes iNOS, COX-2, and MMP-3, whereas the matrix-protective enzyme TIMP-2 was slightly downregulated. The cells were more responsive to stimulation to induce osteoarthritic changes than has been reported for other studies on canine chondrocytes in 2-D or 3-D culture, even when higher IL-1β concentrations6,7 or a combination of several cytokines4,5 were used. Therefore, we concluded that the addition of 10 ng of IL-1β/mL for 24 hours was sufficient for canine chondrocyte cultures in monolayer to evoke a subset of events similar to those of naturally occurring osteoarthritis. The canine cell culture described here served as a simple in vitro technique to mimic osteoarthritis in dogs. This technique may be used to investigate the mechanisms underlying osteoarthritis in dogs and to explore new treatment options for this degenerative joint disease.

To our knowledge, the study reported here was the first in which in vitro evaluation of the impact of TGF-β on chondrocytes are rather complex. Although members of the TGF family are important for chondrocyte differentiation22 and the maintenance of healthy cartilage,40 some authors have detected a negative impact,28,29 which suggests that TGF-β may also contribute to the pathogenesis of osteoarthritis. In the present study, we found no evidence for detrimental effects of TGF-β.

To investigate combined actions of TGF-β and IL-1β on chondrocyte-specific gene expression and mediator release, both cytokines were added alone but also in combination to the cells after pretreatment with TGF-β. Different concentrations have been used to detect potential synergistic41,42 or antagonistic effects.24,27 In fact, TGF-β in the present study could have attenuated signs of cartilage degradation induced by IL-1β. Only a few in vivo43,44 and in vitro5,7 studies have been conducted to examine the role of MMP-3 in osteoarthritic dogs. The MMPs degrade type II collagen,45 which is a major component of the extracellular matrix. In particular, MMP-3 is a useful marker of joint disease in dogs.44 In healthy joints, the destructive actions of MMPs are balanced by their inhibitor TIMPs, which act by blocking MMPs in a ratio of 1 to 1.46,47 Therefore, protective actions on the extracellular matrix are mediated by a higher expression of TIMPs or a lower expression of MMPs. In the study reported here, TGF-β alone reduced MMP-3 gene expression but decreased the expression of its inhibitor TIMP-2. However, the ratio of MMP-3 to TIMP-2 was lower in cells treated with TGF-β, which indicated that TGF-β alone significantly inhibited matrix degradation in healthy cartilage. When TGF-β was applied in combination with IL-1β, 1 ng of TGF-β/mL caused an effective dose-dependent downregulation of MMP-3 gene expression that was accompanied by a slight increase in TIMP-2 gene expression. Because the ratio of MMP-3 to TIMP-2 decreased in cells treated with TGF-β applied in combination with IL-1β, compared with result for IL-1β-stimulated control cells, we concluded that TGF-β applied in low concentrations had a protective effect on cartilage matrix. Whether expression of other members of the TIMP family would be increased or other members of the MMP family would be decreased, which therefore may have contributed to the favorable response, would need to be investigated in additional studies.

Nitric oxide exerts a number of detrimental effects on cartilage, including enhancement of matrix destruction, induction of inflammatory mediators, and apoptosis of chondrocytes.48,49 Incubation with TGF-β followed by stimulation with IL-1β suppressed NO concentrations and iNOS gene expression in a dose-dependent manner. These results indicated that the strong decrease in NO concentration was, at least in part, associated with the reduction in gene expression of the NO-generating enzyme iNOS. Overall, results of the present study confirmed that TGF-β rapidly attenuated IL-1β-induced NO production in canine chondrocytes. In accordance with results of other studies, TGF-β alone did not alter iNOS mRNA or NO concentrations, whereas IL-1β alone effectively induced iNOS gene expression and higher concentrations of NO. This pattern of responsiveness is known to be linked to highly differentiated cells14 and therefore also suggested that the cells used in the present study had many features of differentiated chondrocytes.

Depending on the concentration, PGE can exert anabolic or catabolic actions in cartilage. At higher concentrations, it is considered a major component of the pathological mechanisms that cause pain and joint inflammation.60 whereas at lower concentrations, it has been found to have protective effects on the extracellular matrix.51 Because there was no PGE assay commercially available that accurately discriminated between PGE₂ and PGE₁, total PGE concentration was measured in the supernatants. However, it has been found that in vivo concentrations of PGE₂ are much lower in dogs than are PGE concentration (ratio, approx 1 to 500).52 Therefore, we assumed that the significant changes in total PGE concentration were mainly attributable to PGE₂ and not to PGE₁. Analysis of the results for the present study indicated that TGF-β reduced the IL-dependent increase in PGE concentration in a dose-dependent manner, whereas only 10 ng of TGF-β/mL significantly decreased COX-2 gene expression. In cells exposed to TGF-β alone, PGE concentration was not affected, whereas COX-2 mRNA was slightly more abundant. Changes in COX-2 expression often were not consistent with PGE concentration in the cell culture medium. Therefore, we assumed that COX-2 gene expression may not have
been the predominant pathway for altering PGE concentrations in canine chondrocytes. Taken together, these results suggested that TGF-β can decrease IL-induced PGE production, probably by allowing a shift from the deleterious effect of high concentrations of PGE to the rather chondroprotective effects at lower concentrations of PGE.

It is known that low concentrations of TGF-β decrease the matrix-degrading activity of IL-1β and stimulate matrix synthesis. In contrast, higher concentrations of TGF-β have catabolic effects on extracellular matrix, including upregulation of matrix-degrading enzymes. The diverse actions of TGF-β could be attributed to a dose-dependent modulation of signaling pathways. Although the canine chondrocytes of the present study were exposed to a maximum of 10 ng of TGF-β/mL over 72 hours, mostly beneficial effects on matrix metabolism were detected, even when used in combination with the catabolic mediator IL-1β. It was most probable that IL-1β signaling was rapidly altered by TGF-β in a dose-dependent manner. Studies on rabbit chondrocytes have revealed that TGF-β suppresses the production of IL-1β by downregulating the expression of the IL-1 receptor. The present study provided evidence that this may be the case for canine chondrocytes as well.

Changes in TGF-β signaling during maturation have been assessed in murine, bovine, and human cartilage samples. Results of those studies revealed an age-related decrease in TGF-β responsiveness and the loss of chondroprotective properties in mature cartilage. Although chondrocytes in the present study were obtained from older dogs, they were highly responsive to TGF-β. Therefore, we concluded that age-related changes were masked and TGF-β remained effective as a protective agent in cultured canine chondrocytes.

Analysis of the results for the present study suggested that TGF-β might be an important factor for limiting cartilage damage in dogs with osteoarthritis. Therefore, TGF-β should be considered as a therapeutic target for osteoarthritis in dogs.

Acknowledgments

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The authors thank Bastian Kaiser for assistance with the gene expression analysis.

Footnotes

a. Collagenase P, Roche Diagnostic Deutschland GmbH, Mannheim, Germany.
b. Collagenase CLS, Biochrom AG, Berlin, Germany.
c. Sigma-Aldrich Chemie GmbH, Steinheim, Germany.
d. Life Technologies, Carlsbad, Calif.
e. Sino Biological Inc, BeiJing, China.
f. InvITrap spin cell RNA mini kit, Stratec Molecular GmbH, Berlin, Germany.
g. DNase I, RNase-free, Thermo Scientific Inc, Waltham, Mass.
i. Maxima First Strand cDNA synthesis kit for RT-qPCR, Thermo Scientific Inc, Waltham, Mass.
j. TI thermocycler 96, Biometra GmbH, Göttingen, Germany.
k. KAPA Sybr Fast qPCR kit master mix universal, Kapa Biosystem Ltd, Cape Town, Republic of South Africa.
l. RotorGene 6000, Qiagen GmbH, Hilden, Germany.
m. Relative expression software tool, version 2.0.13, Qiagen GmbH, Hilden, Germany.
n. SpectraMax 140 PC, Molecular Device, Munich, Germany.
o. Carl Roth GmbH + Co KG, Karlruhe, Germany.
q. Victor2 1420 multilabel counter, PerkinElmer, Waltham, Mass.
r. GraphPad Prism 4 software, GraphPad Software Inc, La Jolla, Calif.

References


### Appendix

Oligonucleotide primers used for real-time qPCR assays performed to analyze changes in gene expression in canine chondrocytes treated with IL-1β, TGF-β, or a combination of both.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplicon size (bp)</th>
<th>TA (°C)</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>MMP-3</td>
<td>NM_001002967</td>
<td>F ATGGCATCCAGTCCCCGTAT &lt;br&gt;R AAAGAACAGGAACTCTCCC &lt;br&gt;CAACCGGGAGCTAGTGTATTA &lt;br&gt;TTCCGCGAATGAGATCTCC</td>
<td>161</td>
<td>53</td>
<td>34</td>
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<tr>
<td>TIMP-2</td>
<td>NM_001003082.1</td>
<td>F CAACCGGGAGCTAGTGTATTA &lt;br&gt;TTCCGCGAATGAGATCTCC</td>
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<td>53</td>
<td>35</td>
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<tr>
<td>COX-2</td>
<td>NM_001003354</td>
<td>F GCCCTTAACAGTTGATGGA &lt;br&gt;AGCCTAAAGCGTTTGGCATA</td>
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<td>31</td>
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<tr>
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<td>TBP</td>
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<td>37</td>
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<tr>
<td>GAPDH</td>
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<td>153</td>
<td>52</td>
<td>38</td>
</tr>
</tbody>
</table>

Target genes were normalized to expression of the reference genes TBP and GAPDH. F = Forward. R = Reverse. TA = Annealing temperature.