In vitro characterization of chondrocytes isolated from naturally occurring osteochondrosis lesions of the humeral head of dogs

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Objective—To characterize chondrocytes from naturally occurring osteochondrosis (OC) lesions of the humeral head of dogs.

Sample Population—15 cartilage specimens from 13 client-owned dogs with humeral head OC and 10 specimens from the humeral head of healthy dogs (controls).

Procedure—Chondrocytes were isolated and cultured in a 3-dimensional system. On days 7, 10, 15, 20, and 25, glycosaminoglycan and hydroxyproline content and cytologic characteristics were evaluated. Expression of collagen types I, II, and X was assessed by use of immunohistochemistry.

Results—Chondrocytes from OC lesions were less viable, compared with control chondrocytes. Glycosaminoglycan content in the OC group was significantly less than in the control group on all days except day 20. Hydroxyproline content was also significantly less in the OC group on days 10, 20, and 25. Expression of collagen type II was significantly less in the OC group, compared with the control group on all days, whereas expression of collagen type I was significantly greater in the OC group on days 20 and 25. Expression of collagen type X was significantly less in the OC group on all days except day 25.

Conclusions and Clinical Relevance—Chondrocytes from naturally occurring OC lesions of the humeral head of dogs cultured in a 3-dimensional system were less viable and less capable of producing appropriate extracellular matrix molecules than chondrocytes from unaffected dogs. Alterations in the synthetic capabilities of chondrocytes from OC-affected cartilage may be a cause or an effect of the disease process. (*Am J Vet Res* 2002;63:186–193)

Osteochondrosis (OC) is a developmental orthopedic disease commonly diagnosed in humans, dogs, horses, and other domestic animals.[1,2] It is characterized as a disturbance or abnormality of endochondral ossification in articular-epiphyseal and physeal cartilage. Using this definition, more than a dozen disorders can be included within this disease complex.[3] However, in small animal surgery, this term is generally reserved for OC of the articular surfaces of the shoulder, elbow, stifle, and tarsal joints. Failure of normal endochondral ossification results in thickening of the articular cartilage. Thickened cartilage is less tolerant to biomechanical forces and less likely to receive adequate nutrition via diffusion through the synovial fluid.[1] Cartilage in the deeper zones may become necrotic and develop fissures or cracks, resulting in osteochondritis dissecans. Osteochondrosis is a common cause of secondary osteoarthritis in domestic animals, and OC of the shoulder joint is a common cause of lameness in young large- and giant-breed dogs.[4,5]

The etiopathogenesis of OC is not fully understood. Factors that promote rapid growth and weight gain, such as nutritional, genetic, and hormonal influences, are thought to play a role in the development of OC.[1,6-14] Biomechanical forces and ischemia have been reported as possible etiologic factors.[15-17] Consequently, OC is thought to have a multifactorial etiopathogenesis. However, the disease mechanisms of OC have not been fully elucidated, and this lack of knowledge limits its prophylactic and therapeutic strategies for addressing this disorder.

Data from our laboratory revealed differences in **glycosaminoglycan (GAG)** content and expression of collagen types I, II, and X between OC-affected and normal articular cartilage.[5] Alterations in the biochemical characteristics of cartilage **extracellular matrix (ECM)** in OC lesions have also been reported in other species.[18-22] Results of these previous studies suggest that alterations in the biochemical characteristics of ECM may be involved in the multifactorial etiopathogenesis of OC and provoked further investigation regarding the ability of chondrocytes within OC lesions to produce and maintain appropriate ECM molecules.

Chondrocytes are the only cell type in articular cartilage, and for endochondral ossification to proceed, these cells must produce ECM molecules in the appropriate amount, order, size, location, and arrangement while they progress through resting, proliferation, hypertrophic, and calcification zones. As reported in a previous study,[5] abnormalities in the ECM of OC-affected cartilage may be the result of decreased production of integral matrix molecules, increased loss of these molecules, or both. By determining the synthetic capabilities of chondrocytes in OC-affected cartilage, the causes of ECM abnormalities in OC can begin to be delineated. Maintenance of viability and differentiation of chondrocytes cultured from OC lesions, as assessed by production of appropriate ECM molecules, would implicate increased chondrocyte loss as the source of abnormal ECM in affected cartilage. This finding would suggest that phenotype and synthetic activity

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are maintained by chondrocytes within OC lesions. Extrinsic factors, including mechanical forces, nutrition, and hormonal influences, would thus deserve more focus as primary factors in the etiopathogenesis of OC. In addition, chondrocytes cultured from OC-affected cartilage could then be considered for use in cellular autografts for resurfacing the original articular cartilage defect. On the other hand, inappropriate or lack of production of ECM molecules by chondrocytes cultured from OC-affected cartilage would suggest alterations in cell phenotype and synthetic capabilities as a major factor in the pathogenesis of OC.

To our knowledge, no information has been reported regarding the cellular characteristics of chondrocytes cultured from naturally occurring OC lesions for the humeral head of dogs. The purpose of the study reported here, then, was to characterize the viability, differentiation, and synthetic capability of canine chondrocytes obtained from OC lesions of the humeral head following culture in a **3-dimensional (3-D)** agarose gel system.

Material and Methods

Collection of samples—Detached cartilage (n = 15 specimens) was collected from OC lesions of the humeral head of 13 client-owned dogs (age range, 5 to 12 months; OC group) via arthroscopy. Eight of these dogs were Labrador Retrievers (sexually intact male, 6, castrated male, 1, sexually intact female, 1), 4 were Golden Retrievers (sexually intact male, 2; castrated male, 1; sexually intact female; 1), and 1 was a mixed breed (sexually intact male). Full-thickness articular cartilage specimens (n = 10) were aseptically obtained from the caudocentral portion of the humeral head of 10 dogs (age range, 6 to 24 months; controls) via arthrotomy performed immediately after euthanasia. Control dogs were euthanatized for reasons unrelated to this study and were apparently healthy with grossly normal humeral head cartilage. All cartilage specimens were immediately placed in **Hanks balanced salt solution**[a] **(HBSS)** and transported to a laminar flow hood.

Chondrocyte cultures—Canine chondrocytes were isolated and cultured in monolayer to amplify cell numbers prior to 3-D culture in agarose gel as described.[23-26] When chondrocytes were near confluency in monolayer culture, RPMI-FBS medium (RPMI 1640 medium[a] containing penicillin [100 U/ml], streptomycin [100 µg/ml], and amphotericin B [2.5 µg/ml; RPMI medium) plus 10% **fetal bovine serum [FBS]**) was discarded, and flasks were rinsed. Cells were detached by trypsinization, washed in HBSS, resuspended in RPMI-FBS, and counted. Cell viability, assessed by use of trypan blue exclusion, was > 90% in each sample. Equal volumes of 2% low-melting agarose[b] (gelling temperature, 25 ± 5 C) in PBS solution and double-strength RPMI medium containing 20% FBS were added to yield a cell concentration of 3×10^6 cells/ml. One milliliter of the cell suspension was added to each well of a 24-well tissue culture plate, and plates were placed in a refrigerator (4 C) for 5 minutes. One milliliter of RPMI-FBS was added to each well after the agarose medium gelled to form the 3-D construct. Plates were incubated at 37 C in 5% CO_2 and 95% humidity. Liquid medium was changed every 3 days of 3-D culture.

Sample collection—Three-dimensional constructs were collected on days 7, 10, 15, 20, and 25 of culture. The 3-D construct in each well was divided into 2 portions. One portion was placed in neutral-buffered 10% formalin for histologic processing, and the remaining portion was weighed (wet weight), placed in 1 ml of distilled deionized water, and stored at –80 C for subsequent determination of GAG and **hydroxyproline (HP)** content.

Cytologic evaluation—After routine histologic processing, 5-µm-thick sections were stained with H&E and toluidine blue. Sections were subjectively evaluated in duplicate by 1 investigator (KK) who was unaware of collection day or group (OC or control). Sections were evaluated for cell viability, morphologic characteristics, and **proteoglycan (PG)** staining.

Immunohistochemical evaluation—Unstained 5-µm-thick sections were deparaffinized in xylene and rehydrated in graded ethanol solutions followed by washing with buffer (50 m*M* Tris-HCl with 0.15*M* NaCl). Endogenous peroxidase activity was quenched by immersion in 3% H_2O_2 in methanol for 20 minutes followed by 2 rinses in buffer. Slides were then incubated in 0.1% trypsin solution with 0.1% $CaCl_2$ for 30 minutes at 37 C as a means of unmasking antigens for collagen types-II and -X staining. Slides were steamed in citrate buffer for retrieval of collagen type-I antigens. Nonspecific binding was blocked for 30 minutes, using normal blocking serum prepared from the species in which the secondary antibody was produced. Slides were incubated overnight at 4 C for collagen type-II (antibody, rabbit anti-bovine collagen type II[c]; dilution, 1:400) and collagen type-X (mouse anti-deer collagen type X[d]; 1:600) staining and at 37 C for collagen type-I (goat anti-human collagen type-I[c]; 1:150) staining. The following day, slides were rinsed in buffer, incubated with the appropriate biotinylated secondary antibody,[e] and rinsed again in buffer. Bound primary antibody was detected, using an avidin-horseradish peroxidase method with diaminobenzidine chromogenic substrate.[f] Sections were counterstained with hematoxylin solution[g] and examined subjectively by 2 investigators (KK and JLC) who were blinded to the origin of each section. One hundred cells were counted on each slide, and the number of definitively positive-stained cells was recorded as the percentage of immunoreactive cells for each collagen type.

Glycosaminoglycan assay—Total sulfated GAG content was quantitated by use of a **dimethyl-methylene blue (DMMB)** assay.[27] Frozen samples of each 3-D construct were thawed and digested overnight at 60 C in 1 ml of papain[g] (0.5 mg/ml;14 U/mg) in 20 m*M* sodium phosphate buffer containing 1 m*M* EDTA. Total GAG content was determined by addition of 240 µl of DMMB solution to a 10-µl aliquot of the digest solution; absorbance was determined spectrophotometrically at a wavelength of 530 nm. Known concentrations of bovine tracheal chondroitin sulfate A[g] were used to construct the standard curve. Results were standardized to correct for differences in sample wet weights, and total GAG content was reported as microgram per milliliter per gram.

Hydroxyproline assay—Total collagen content in 3-D constructs was determined by measuring HP content by use of a colorimetric procedure.[28] The papain digest solution prepared for use in the DMMB assay was also used for the HP assay. A 100-µl aliquot of digest solution was mixed with an equal volume of 4N sodium hydroxide and hydrolyzed by autoclaving at 120 C for 20 minutes. Chloramine T reagent (400 µl) was added to the hydrolysate, mixed gently, and oxidized for 25 minutes at room temperature (approx 25 C). Ehrlich aldehyde reagent (400 µl) was then added to each sample. Samples were incubated at 65 C for 20 minutes to develop a chromophore. Known concentrations of HP[g] were used to construct the standard curve. Absorbance of each sample was read at 550 nm on a spectrophotometer, and HP content was reported as microgram per milliliter per gram.

Statistical analyses—Statistical analyses were performed, using a computer software program.[h] Data from each collec-

tion day in each group were combined, and means ± SEM were determined. Data were compared between 2 groups at each collection time and within each group over time. Differences between groups were analyzed by use of a *t*-test or Mann-Whitney rank sum test, and differences within groups were analyzed by use of ANOVA. Significance was set at $P < 0.05$.

Results

3-D culture system—Chondrocytes were successfully cultured from healthy articular cartilage of 10 dogs and 15 OC lesions from 13 dogs, using a 3-D agarose culture system. Of the 13 dogs with OC, 5 dogs were affected bilaterally, 5 dogs had unilateral lesions on the right humerus, and 3 dogs had unilateral lesions on the left humerus. For dogs with bilateral disease, chondrocytes were cultured from both sides in 2 cases. In the remaining 3 bilateral cases, chondrocytes were cultured from only 1 OC lesion.

Cytologic evaluation—Chondrocytes obtained from the control group maintained characteristics consistent with cell viability and differentiation throughout the study period. Control chondrocytes were uniformly distributed throughout the sections and appeared round to oval with round basophilic nuclei on H&E-stained sections. Proteoglycan production was evidenced by the presence of toluidine blue-stained matrix in slides of 3-D constructs from the control group. In contrast, cells in 3-D structures from the OC group had a wide variety of appearances ranging from characteristics similar to those of control chondrocytes to relatively small numbers of cells with cytologic characteristics consistent with loss of viability (eg, nuclear pyknosis; **Fig 1**). Production of ECM (ie, proteoglycan) was subjectively judged as less in the OC group, compared with the control group, at each time point.

Glycosaminoglycan content—Three-dimensional constructs from both groups contained measurable amounts of GAG at all time points. Total GAG content

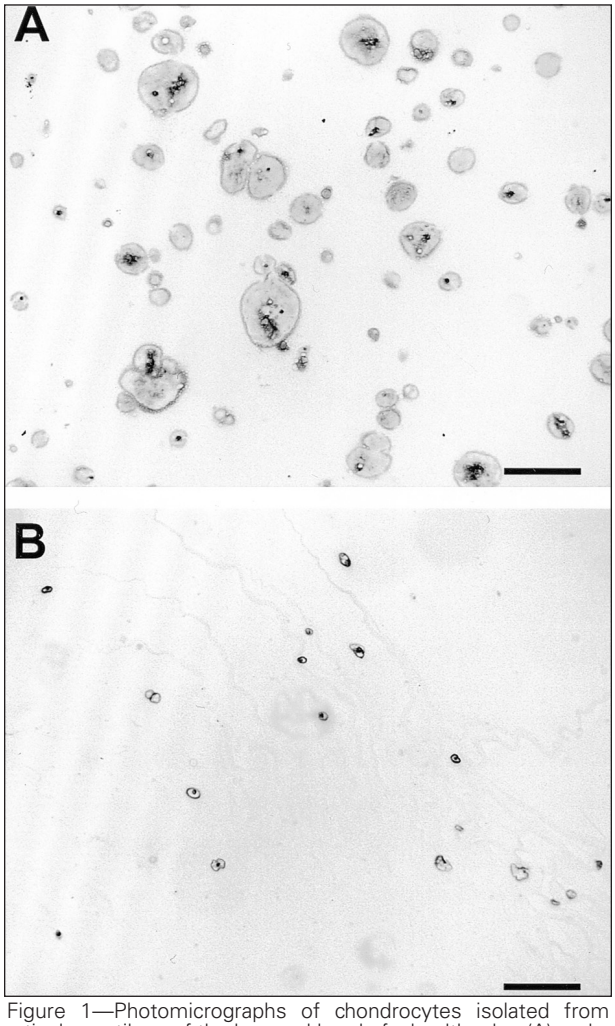

Figure 1—Photomicrographs of chondrocytes isolated from articular cartilage of the humeral head of a healthy dog (A) and a dog with osteochondrosis (OC; B). Chondrocytes were cultured for 25 days in a 3-dimensional (3-D) agarose gel system. Notice that chondrocytes from the healthy dog appear spheroidal and have abundant extracellular matrix (ECM), whereas chondrocytes from the affected dog are small and pyknotic with little to no ECM. Toluidine blue stain; bar = 200 μm.

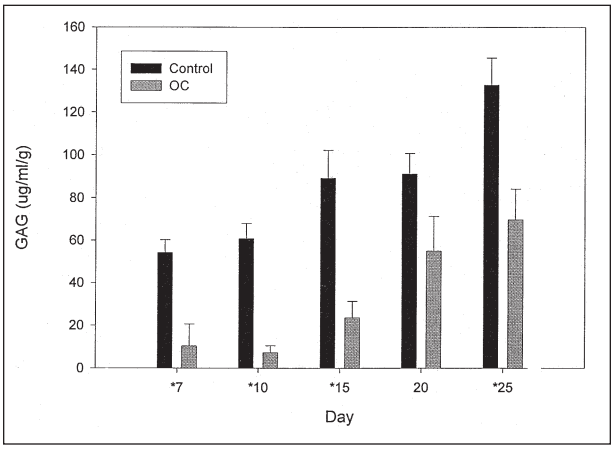

Figure 2—Mean (± SEM) glycosaminoglycan (GAG) content in chondrocytes isolated from articular cartilage specimens of the humeral head of healthy dogs (n = 10; control) and dogs with OC (15). Chondrocytes were cultured for various periods in a 3-D agarose gel system. *Days on which values were significantly ($P < 0.05$) different between groups.

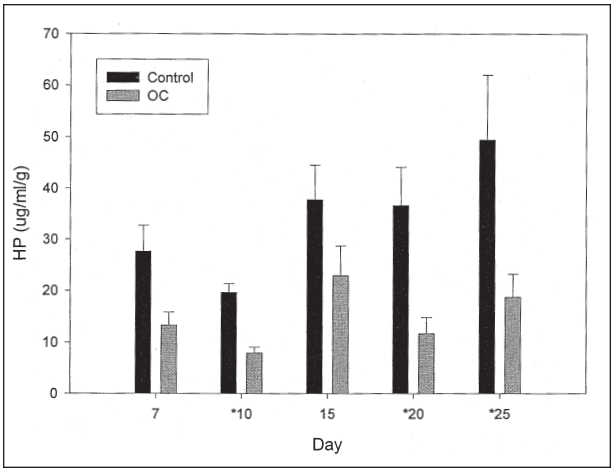

Figure 3—Mean (± SEM) hydroxyproline (HP) content in chondrocytes isolated from articular cartilage specimens of the humeral head of healthy dogs (n = 10; control) and dogs with OC (15). *See* Figure 2 for key.

corresponded well with cytologic findings regarding ECM production. Glycosaminoglycan content increased significantly ($P < 0.001$) over time within each group but was significantly less in the OC group, compared with the control group, on days 7 ($P = 0.001$), 10 ($P < 0.001$), 15 ($P < 0.001$), and 25 ($P = 0.004$; **Fig 2**).

Hydroxyproline content—Three-dimensional constructs from both groups also contained measurable amounts of HP at all time points. Hydroxyproline content was significantly less in the OC group, compared with the control group, on days 10 ($P < 0.001$), 20 ($P = 0.002$), and 25 ($P = 0.012$; **Fig 3**). However, we did not detect significant differences in HP content within each group over time.

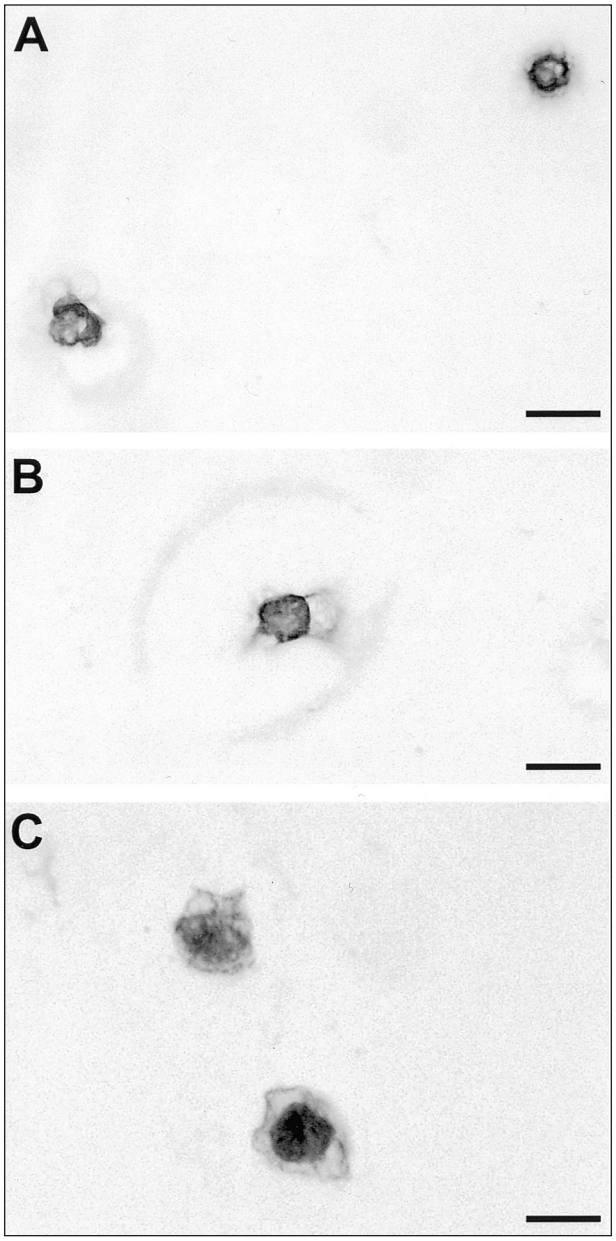

Figure 4—Photomicrographs of sections of chondrocytes after 3-D culture for 25 days. Sections were stained with antibodies against collagen type I (A), collagen type II (B), or collagen type X (C). Avidin-biotin-peroxidase stain; bar = 30 μm.

Collagen immunohistochemistry—Type-I, -II, and -X collagens were detected in 3-D cultured chondrocytes by use of immunohistochemistry (**Fig 4**). Collagen type II was detected in both groups, but the percentage of immunoreactive cells was significantly higher in the control group at all time points, compared with the OC group (day 7, $P = 0.013$; day 10, $P = 0.012$; remaining days, $P < 0.001$; **Fig 5**). Collagen type II was detected in the ECM of control chondrocytes, and expression significantly ($P = 0.036$) increased over time. Few cells in the OC group stained definitively positive for collagen type II, and the percentage of immunoreactive chondrocytes within this group did not increase during the study period. A small number of cells stained for type-I collagen in both groups. Percentage of immunoreactive cells and intensity of staining, however, was greater in the OC group, compared with the control group. On days 20 and 25, percentage of immunoreactive cells for collagen type I was significantly ($P = 0.020$ and 0.023, respectively)

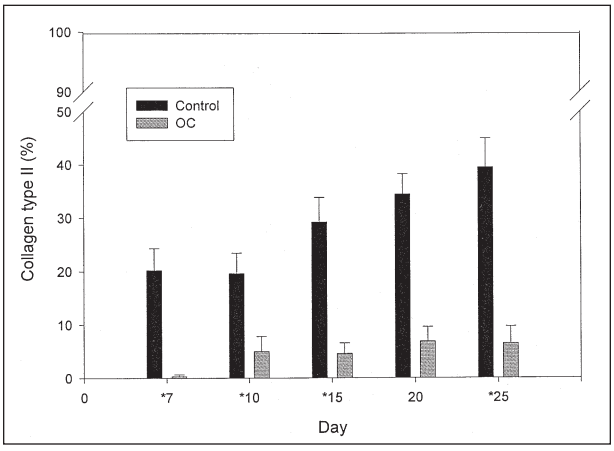

Figure 5—Mean (± SEM) percentage of 3-D cultured canine chondrocytes immunoreactive for collagen type II. Chondrocytes were isolated from OC lesions (n = 15) or full-thickness specimens of articular cartilage from the humeral head of 10 healthy dogs (control). Immunohistochemistry was performed on days 7, 10, 15, 20, and 25 of culture, and 100 cells/slide were counted. *Days on which values were significantly ($P < 0.05$) different between groups.

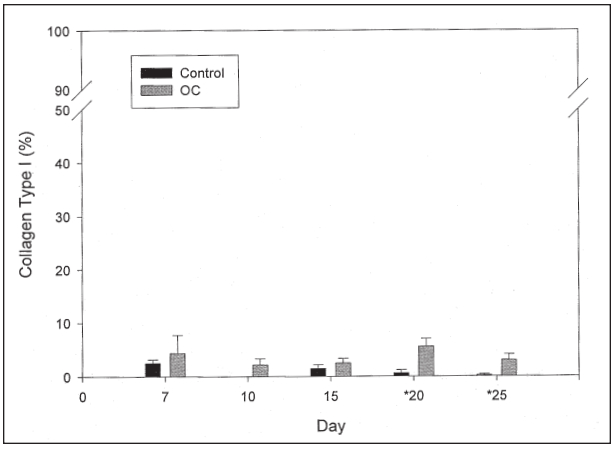

Figure 6—Mean (± SEM) percentage of 3-D cultured canine chondrocytes immunoreactive for collagen type I. *See* Figure 5 for key.

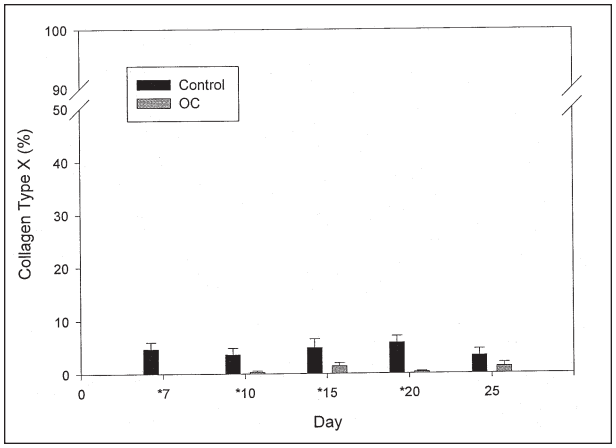

Figure 7—Mean (± SEM) percentage of 3-D cultured canine chondrocytes immunoreactive for collagen type X. *See* Figure 5 for key.

different between groups (**Fig 6**). A small number of cells stained positive for collagen type X in both groups. The mean percentage of cells immunoreactive for collagen type X was significantly higher in the control group, compared with the OC group, on days 7 ($P = 0.042$), 10 ($P = 0.022$), 15 ($P = 0.038$), and 20 ($P < 0.001$; **Fig 7**).

Discussion

The objectives of the present study were to determine the viability and synthetic capability of chondrocytes in naturally occurring OC lesions of the humeral head of dogs. Articular cartilage consists primarily of ECM, with chondrocytes comprising < 10% of tissue volume.[29] This small number of chondrocytes is responsible for production and maintenance of the ECM.[29,30] For articular-epiphyseal cartilage to complete the endochondral ossification process, production of appropriate ECM molecules in the corresponding zone of growing cartilage is essential. To fully understand the pathologic mechanisms of abnormal endochondral ossification in OC, physiologic characteristics of chondrocytes need to be a primary research focus. By determining the cellular events involved in OC or abnormal endochondral ossification, the resultant ECM abnormalities may be better understood.

Chondrocytes in 3-D culture provide a well recognized in vitro model for diarthrodial joints.[23-26] Although any in vitro model is inherently nonphysiologic, the 3-D agarose gel culture system used in the present study has been used by others[31-33] to investigate characteristics of articular chondrocytes. This system permits maintenance of cell viability, differentiation, and appropriate ECM production. Results of the present study indicate that the 3-D culture system facilitates the in vitro investigation of naturally occurring arthropathies of dogs.

Articular cartilage ECM consists primarily of PG and collagens. Proteoglycan aggrecan is composed of a core protein with covalently bound GAG, predominantly chondroitin sulfate and keratan sulfate.[34] The N-terminal G1 domain of the core protein binds to hyaluronate with stabilization by link proteins, forming larger aggregate PG.[29,34] Glycosaminoglycan and PG production by 3-D cultured chondrocytes increases in a time-dependent manner,[24,33] and data from the present study are consistent with these previous findings. The less intense PG staining that we detected in chondrocytes from the OC group suggests that the ability of chondrocytes from OC lesions to produce PG is less than that of chondrocytes in unaffected articular cartilage. The significant differences in GAG content between groups verified the results of PG staining. Taken together, results of histochemical and biochemical analyses in the present study suggest that chondrocytes in OC-affected cartilage have less capability to produce GAG than chondrocytes in healthy cartilage. Although our data do not refute the role of genetic, nutritional, hormonal, or traumatic factors as causes for alterations in ECM production by chondrocytes from OC-affected cartilage, these data do support results of previous studies[5,18-21,35] in which PG metabolism and GAG content were altered in OC lesions from various species. Furthermore, our data provide evidence that these alterations could be the result of alterations in cellular (ie, chondrocyte) metabolism and phenotype. Recently, Semevolos et al[36] reported that PG staining in OC-affected cartilage from horses was less intense than in normal cartilage but aggrecan mRNA expression was similar between groups. Results of an ex vivo study of equine cartilage suggest that chondrocytes in OC lesions are less viable and have a corresponding decrease in PG metabolism, compared with chondrocytes from healthy articular cartilage.[37] The hydrophilic and negatively charged character of GAG contributes to the hydrodynamic properties of articular cartilage by providing compressive stiffness.[30] Lower GAG content in the ECM of chondrocytes from OC lesions may make affected cartilage more susceptible to biomechanical stresses, even at physiologic levels. Although decreased GAG content in OC-affected cartilage has been reported in dogs, pigs, and horses,[5,21,22] it is not yet known whether alterations in GAG content were the result of increased loss, decreased production, or both. Data from the present study suggest that decreased GAG production by chondrocytes from OC lesions may be responsible, at least in part, for the low GAG content consistently detected in OC lesions. However, the definitive cause or timing of decreases in GAG synthesis were not elucidated by our results.

Collagen type II provides articular cartilage with tensile strength and comprises > 90% of the collagen in normal articular cartilage.[29] Collagen type I is a primary collagen in many tissues, including fibrocartilage, bone, tendon, and skin. In articular cartilage, it is produced early in chondrogenesis and becomes undetectable later during development.[38] However, type-I collagen is found in remodeling and osteoarthritic cartilage.[39,40] Collage type X is predominantly produced by hypertrophic chondrocytes in the zone of hypertrophy during endochondral ossification and is thought to be associated with the process of calcification.[41] In the 3-D agarose culture system used in the present study, chondrocytes from normal articular cartilage can maintain a rounded phenotype accompanied by production of primarily type-II collagen.[31-33] Benya et al[31] reported that < 5% of the collagen peptides in rabbit articular

chondrocytes grown in 3-D culture for 14 days were type-I collagen. Collagen type X is expressed by chick chondrocytes undergoing terminal differentiation in a 3-D agarose culture system.[42] Therefore, it was expected that canine articular chondrocytes cultured in this 3-D system would be a heterogeneous population with regards to collagen expression. As expected, chondrocytes from healthy cartilage produced predominantly type-II collagen with little expression of type-I or -X collagen. Chondrocytes from OC lesions produced significantly less type-II and -X collagen but more type-I collagen, compared with control chondrocytes. These data agree with results of a previous report from our laboratory[5] indicating that amounts of type-II and -X collagen are significantly decreased and amount of type-I collagen significantly increased in articular cartilage from dogs with naturally occurring OC, compared with articular cartilage from unaffected dogs.

Hydroxyproline is present in the Y position of the Gly-X-Y repeating tripeptide of collagen, and determination of HP concentration has been used as a measurement of collagen content in tissue specimens[22,28] and chondrocytes in 3-D culture.[43] In the present study, changes in HP content corresponded well with cytologic results. Together, results of the HP assay and collagen immunohistochemistry indicated that increased expression of collagen type II largely contributed to the increase in collagen content detected over time in both groups. The lower HP content in the OC group, compared with the control group, was largely a result of a decrease in the percentage of chondrocytes immunoreactive for collagen type II. Expression of type-II collagen increased over time in the control group, indicating that cells in the 3-D culture system effectively maintained the ability to differentiate and synthesize appropriate ECM molecules. In contrast, expression of collagen type II was less and expression of collagen type I greater in the OC group, compared with the control group, indicating that cells from OC-affected cartilage may be synthetically altered. Similarly, expression of collagen type X was less in the OC group, suggesting that chondrocytes from OC lesions may have less ability than normal chondrocytes to undergo terminal differentiation to the hypertrophic stage. Others have also reported that expression of type-I collagen in OC-affected cartilage is increased, compared with normal cartilage.[5,22,36] Type-I collagen production by chondrocytes from OC lesions is likely associated with repair processes.

Results have been variable with respect to expression of collagen types II and X. Expression of collagen types II and X was less in OC-affected cartilage of dogs, compared with that in unaffected cartilage.[5] However, expression of mRNA encoding collagen types II and X was similar between OC-affected and unaffected cartilage of horses.[36] In pigs, collagen type X was detected in OC-affected cartilage but not in normal cartilage, and expression of collagen type II was less in affected cartilage, compared with control cartilage.[22] Expression of mRNA encoding type-X collagen was increased but protein content was decreased in OC lesions from fowl.[44] These inconsistencies with regard to expression of collagen types II and X in OC-affected cartilage may be attributable to species differences, differences in disease severity and duration, discrepancies in signaling, transcription, translation, synthesis, and release, or differences in assessment techniques.

Abnormalities in production of ECM molecules by chondrocytes may lead to disorders in endochondral ossification and may, perhaps, initiate OC. In addition, inappropriate production of ECM constituents may make affected cartilage less tolerant to biomechanical forces. However, results of the present study do not provide direct evidence as to whether differences between chondrocytes from OC-affected and -unaffected cartilage are a cause or an effect of the disease process.

Synthesis and deposition of ECM increase in an amplitude-, frequency-, and spatially-dependent manner as a result of dynamic tissue loading both in vitro and in vivo.[43,45-47] More recently, Brama et al[48] reported that the biochemical characteristics of articular cartilage in foals are uniform among joints at birth but become significantly different during adaptation to weight-bearing forces. Cartilage specimens harvested from OC lesions in the present study were detached from the underlying subchondral bone. This lack of normal weight-bearing stimuli on OC-affected cartilage may have altered the synthetic characteristics of chondrocytes.

The effects of OC on cell viability should also be considered. Alterations in the ECM produced by chondrocytes from OC lesions could be the result of a reduction in the number of viable cells. Results of a study evaluating bovine cartilage explants revealed that injurious mechanical compression caused increased chondrocyte apoptosis prior to ECM alterations.[49] If supraphysiologic biomechanical stress is involved in the etiopathogenesis of OC, chondrocytes harvested from OC-affected cartilage may undergo apoptosis, leading to a reduced number of viable cells in the 3-D culture system. The apoptotic rate in osteoarthritic chondrocytes and osteoarthritic cartilage is increased, compared with normal cells and tissue.[50] Therefore, it is also possible that chondrocytes from OC lesions associated with secondary osteoarthritis may undergo apoptosis at an increased rate, resulting in ECM alterations. However, apoptosis of chondrocytes is an essential event in endochondral ossification that allows for intracellular calcium accumulation and release, matrix calcification, matrix resorption, and bone formation.[51] Suppression of apoptosis may cause retained cartilage and OC, but we have no evidence to suggest either an abnormally high or low rate of apoptosis in chondrocytes from OC lesions in dogs. In addition, various cytokines, such as core binding factor α subunit 1, Indian hedgehog, parathyroid hormone-related protein, and vascular endothelial cell growth factor, are thought to play a role in the regulation of endochondral ossification.[52] These factors work reciprocally to induce proliferation, differentiation, and apoptosis of growing chondrocytes. Further studies are required to evaluate the rate of apoptosis in OC-affected cartilage at different stages of the disease process in conjunction with the roles of various cytokines in the development of OC to delineate the complex etiopathogenesis of OC.

Although results of the present study do not definitively answer questions regarding the etiopathogenesis of OC, they do provide important information regarding the potential for the clinical use of OC-affected cartilage for autologous grafting of articular cartilage defects. Owing to the limited healing potential of articular cartilage, replacement of abnormal cartilage with variable biological materials has been attempted.[53] Autologous chondrocyte transplantation has been studied because of multiple problems associated with the use of other biological tissues.[54,55] An autologous chondrocyte graft derived from resected OC-affected cartilage could be an advantageous method for resurfacing the original OC-induced articular cartilage defect, provided the reimplanted cells are capable of appropriate tissue repair. However, the altered viability of and ECM molecule production by cultured chondrocytes from OC-affected cartilage that we detected in the present study suggest that further evaluation of the use of autologous chondrocyte grafts may not be justified.

[a]Gibco BRL, Grand Island, NY.
[b]Fisher Scientific, Fair Lawn, NJ.
[c]Chemicon International Inc, Temecula, Calif.
[d]Provided by Dr. Gary Gibson, The Henry Ford Hospital, Detroit, Mich.
[e]Vectastain, Vector Laboratories Inc, Burlingame, Calif.
[f]Zymed, San Francisco, Calif.
[g]Sigma Chemical Co, St Louis, Mo.
[h]SigmaStat 2.03, Jandel Scientific, San Rafael, Calif.

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