Detection of synovial macrophages in the joint capsule of dogs with naturally occurring rupture of the cranial cruciate ligament

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Objective—To test the hypotheses that the densities of macrophages in the synovial membranes and capsules of stifle joints in dogs with ruptured cranial cruciate ligaments are greater than those of normal joints and that those densities in affected joints are positively correlated with the chronicity and severity of the disease.

Animals—17 dogs with naturally occurring rupture of the cranial cruciate ligament and 5 healthy control dogs.

Procedure—All dogs underwent orthopedic and radiographic evaluations. In affected dogs, duration of clinical signs was used as an indicator of disease chronicity and the severity of osteoarthritis in the stifle joint was determined radiographically. Joint capsule specimens were evaluated histologically; macrophages, interleukin-6, and tumor necrosis factor-α were identified by use of immunocytochemical techniques.

Results—Compared with unaffected joints, macrophage density was increased in all affected joints. Duration of disease was significantly associated with radiographic severity of osteoarthritis and synovial macrophage density. Synovial macrophage density was significantly associated with severity of osteoarthritis and with the presence of interleukin-6 and tumor necrosis factor-α.

Conclusions and Clinical Relevance—Results suggest that synovial macrophages may be involved in the development of pathologic changes (including osteophyte formation) in the stifle joints of dogs with osteoarthritis secondary to rupture of the cruciate ligament. Determination of the importance of synovial macrophages in the development of changes in osteoarthritic joints may result in new treatment strategies that involve elimination of the deleterious effects of those cells. (*Am J Vet Res* 2005;66:493–499)

Osteoarthrosis is a common condition in multiple species[1-3] and is classified as a noninflammatory arthropathy with 2 forms: primary osteoarthrosis and secondary osteoarthrosis (also referred to as osteoarthritis or degenerative joint disease).[4] Although osteoarthrosis is classified as a noninflammatory joint disease, affected joints often have a focal or generalized synovitis; therefore, the disease is most commonly referred to as osteoarthritis.[5,6] No inherent predisposing cause is typically identified with primary osteoarthritis, whereas secondary osteoarthritis often develops in association with abnormalities that precede arthritic changes.[4,7] Secondary osteoarthritis can further be divided into 2 categories: osteoarthritis resulting from forces of abnormal intensity and distribution acting on normal joint structures or osteoarthritis resulting from normal forces acting on structures that are biomechanically inadequate.[5] In time, as with osteoarthritis secondary to rupture of the cranial cruciate ligament in dogs, morphologic abnormalities such as degradation and loss of articular cartilage, subchondral sclerosis, osteophytosis, and joint capsule thickening will develop.

Pathologic changes in joints associated with osteoarthritis include softening, fibrillation, ulceration, and loss of articular cartilage.[7] Biochemical alterations include matrix breakdown as a result of both enzymatic and mechanical factors.[5,6,8] Excessive production of metalloproteinases by chondrocytes is one of the major causes of the changes in matrix homeostasis and cartilage degradation.[5,6,8] In normal articular tissue, metalloproteinases are synthesized in low amounts.[5,8] In osteoarthritic cartilage, proinflammatory cytokines such as **tumor necrosis factor (TNF)-α**, **interleukin (IL)-1**, and IL-6 mediate the transcription of metalloproteinases.[5,6,8] It has been suggested that synovial lining cells are the primary source of these proinflammatory cytokines, and these cytokines have been demonstrated in the synovial membrane and synovial fluid of osteoarthritic joints.[9-11] However, it is not known which cells in the synovial membrane are responsible for the synthesis of these cytokines. Collectively, findings of these earlier studies have suggested that the synovial lining cells may play an important role in cytokine synthesis, increased metalloproteinase activity, and cartilage damage in osteoarthritic joints.

Osteophytes are considered one of the characteristic morphologic findings in joints with osteoarthritis. They develop as a reparative response to cartilage but may originate from the periosteum or synovium.[12] The trigger for the development of osteophytes is unknown. In osteophytes, several growth factors such as **transforming growth factor (TGF)-β**; bone morphogenic proteins 2, 3, 6, and 7; and cartilage-derived morphogenic proteins 1, 2, and 3 have been identified.[13-15] It has been demonstrated that blocking TGF-β inhibits osteophyte formation and that local adminis-

tration of TGF-β stimulates osteophyte formation.[13,16-18] The synovial lining of joints appears to provide the stimulus for osteophyte formation.[17,18] In an osteoarthritis model in mice, overexpression of TGF-β by synovial lining cells resulted in the development of chondro-osteophytes, whereas depletion of the synovial lining caused a dramatic decrease in osteophyte formation.[17] In another study[18] in mice, macrophage depletion before osteoarthritis induction almost completely eliminated osteophyte formation and further osteophyte formation following administration of TGF-β was almost completely inhibited in macrophage-depleted animals. The results of those studies suggest that under experimental conditions, TGF-β, the synovial membrane, and synovial macrophages contribute to osteophyte formation.

The osteoarthrosis-associated inflammatory changes in the joint capsule are believed to be in part secondary to cartilage wear particles and soluble cartilage-specific macromolecule degradation products.[7,19] Phagocytosis of cartilage wear particles by synovial macrophages induces chronic inflammation of the synovial membrane and joint capsule with subsequent synthesis of proteases and proinflammatory cytokines such as TNF-α, IL-1, and IL-6.[7] An important component of the inflammatory response is increased vascularization of the joint capsule. It has been reported that vascular endothelial growth factor immunoreactivity and endothelial cell proliferation are promoted with inflammation and that vascular endothelial growth factor is localized to synovial macrophages.[20] This would suggest that macrophages play an important role in the development and maintenance of synovial inflammation, as they do in osteophyte formation.

To date, data are available that indicate that synovial macrophages may play an important role in the pathogenesis of osteoarthritis, including involvement in osteophyte formation, cartilage destruction triggered by TNF-α and IL-1, and development and maintenance of synovial inflammation. A limitation of these earlier investigations of the role of synovial macrophages in the development of osteoarthritis is that almost all were conducted under experimental conditions and not in association with naturally occurring disease.

Therefore, we were interested in investigating whether macrophages may play a central role in naturally occurring osteoarthritis that develops secondary to rupture of the cranial cruciate ligament in dogs, similar to their role in experimentally induced osteoarthritis. The objectives of the prospective cohort study reported here were to test the hypotheses that the densities of macrophages in the synovial membranes and capsules of stifle joints in dogs with ruptured cranial cruciate ligaments are greater than those of clinically normal joints and that those densities in affected joints are positively correlated with the chronicity and severity of the disease. Disease chronicity was assessed on the basis of historical information, macrophages were identified by use of immunocytochemical techniques, and disease severity was determined via radiography of affected joints.

Materials and Methods

Seventeen dogs with ruptured cranial cruciate ligaments

that underwent surgical exploration of the affected stifle joint followed by surgical joint stabilization were included in the study. All were evaluated at the **Purdue University Veterinary Teaching Hospital (PUVTH)**. Dogs with a history of previous surgery, intra-articular injections, or arthrocentesis performed on the affected stifle joint were excluded. Normal joint capsule tissue for histologic and immunocytochemical evaluation was obtained from stifle joints of 5 healthy dogs that were euthanatized for reasons unrelated to the study.

The medical history of each affected dog, including information obtained from both the owner and the referring veterinarian, was documented. Data recorded included age, sex, weight, prior medications and response to treatment, and chronicity of disease (interval between onset of clinical signs and evaluation at the PUVTH). All dogs underwent a full orthopedic examination. The tentative diagnosis of rupture of the cranial cruciate ligament was made on the basis of results of a drawer or tibial compression test. For each dog, a CBC, serum biochemical profile, and urinalysis were performed.

A radiographic analysis of the affected stifle joints was performed. Radiographs obtained included mediolateral and caudocranial projections, and all radiographs were independently evaluated without knowledge of the dog's history, clinical signs, and histologic or immunocytochemical findings by 2 investigators (NWK and WRW [a board-certified veterinary radiologist]), according to previously established criteria.[21,22] Several radiographic features were scored subjectively, including loss of infrapatellar fat pad opacity (0, 1+, 2+, or 3+; 0 = normal appearance); synovial joint distention (0, 1+, 2+, or 3+; 0 = normal appearance); osteophyte production (0, 1+, 2+, or 3+; 0 = normal appearance) of the femur (both the medial and lateral condyles and medial and lateral trochlear ridges), patella (body and apex), fabellae (medial and lateral), and tibia (caudal, medial, lateral, and intercondylar eminence margins); enthesophyte production (0, 1+, 2+, or 3+; 0 = normal appearance of femur, tibia, and patella with a separate score for the cranial tibial plateau); subchondral sclerosis, irregularities, or cysts of femur, tibia, and patella (0 or 1+; 0 = normal appearance); subchondral erosion of femur, tibia, and patella (0 or 1+; 0 = normal appearance); subchondral bony remodeling (chronic shape change) of the femur, tibia, and patella (0 to 5+; 0 = normal appearance); and a soft tissue mineralization score with or without opacity in the intercondylar fossa area (0 or 1+; 0 = normal appearance). All affected dogs were assigned a total radiographic score (possible range, 0 to 80) that was calculated by adding all scores.

Dogs underwent surgical exploration of the affected stifle joint via a standard lateral parapatellar approach; immediately after opening the joint, a full-thickness section (approx 4 × 12 mm) of joint capsule including the synovial membrane was collected from the region adjacent to the incision. The specimen was divided into 2 parts; 1 part was placed into neutral-buffered 10% formalin for routine histologic evaluation, and the other was cryopreserved for immunocytochemical analysis. Tissue preserved in formalin was embedded in paraffin, sectioned at a thickness of 5 μm, and stained with H&E. Specimens collected for cryopreservation were washed with PBS solution and coated with an embedding medium.[a] Each coated specimen was then snap-frozen in 2-methylbutane and liquid nitrogen. Frozen specimens were stored at –80°C until cryosectioned (7-μm-thick sections).

Slides of the frozen sections were made in routine fashion. Each section was incubated with 100 μL of blocking serum in PBS solution (1:75 dilution) for 15 minutes in a humidity chamber at room temperature (approx 21°C). The slides were then incubated for 2 hours in a humidity chamber at room temperature with 100 μL/section of a 1:40 dilu-

tion of 1 of 2 cell adhesion antibodies to identify macrophages (CD11b and CD18),[b] a 1:100 dilution of purified anti-human IL-6 polyclonal antibody,[c] or a 1:100 dilution of an antibody to natural and recombinant human TNF-α antigen.[23,c] After incubation, sections were washed in PBS solution and incubated with 100 μL/section of a 1:200 dilution of anti-mouse IgG biotinylated antibody[d] in PBS solution for 30 minutes in a humidity chamber at room temperature. The slides were then washed in PBS solution, and incubated with 100 μL/section of a 1:111 dilution of avidin/biotinylated enzyme complex[e] in PBS solution for 1 hour in a humidity chamber at room temperature. A final wash in PBS solution was performed, and the slides were stained in a diaminobenzidine tetrahydrochloride substrate solution (0.125 g diaminobenzidine tetrahydrochloride in 200 mL tris-HCl buffer; 100 mL of 30% hydrogen peroxide was used to initiate the reaction). The sections were counterstained in Gill No. 2 hematoxylin, cleared in Scott tap water substitute, dehydrated in a graded series of alcohol solutions, cleared with xylene, and coverslipped. Additional slides were included as method (deletion of primary or secondary antibodies) and tissue (staining of lymph node) controls.

All slides were examined by 2 investigators (NWK and PWS [a board-certified pathologist]) without knowledge of patient's history, clinical signs, and radiographic or histological findings and were subjectively graded for synovial membrane thickness (1+, 2+, or 3+; 1+ = normal appearance); joint capsule vascularity (1+, 2+, or 3+; 1+ = normal appearance); and staining for CD11b, CD18, IL-6, and TNF-α (grade 1+ = little to no staining, 2+ = moderate uptake of stain, or 3+ = strong uptake of stain). Increased staining intensity with CD11b or CD18 was interpreted as an increase in the number of macrophages per unit area (macrophage density), and increased staining intensity with TNF-α or IL-6 was interpreted as increased tissue concentration.

Simple linear regression techniques were used to relate the variables of interest in the affected joint capsules. Because simultaneous presence of CD11b and CD18 is characteristic for macrophages and because of the high correlation (0.78; $P < 0.01$) between CD11b and CD18 staining intensity, the mean of the staining grades for these 2 markers was calculated to provide a combined grade that represented the domain that they share. Results for the staining grades for CD11b and CD18 are each reported separately, and the combined value is provided. The IL-6 and TNF-α staining grades were analyzed by use of the same statistical techniques. Results were summarized as correlations. Logarithms were analyzed for all the assumptions for simple linear regression. Statistical significance was assessed by use of 1-sided tests performed with significance set at 0.05 ($P \leq 0.05$). Regression diagnostics were used to verify model assumptions for the analysis. One dog with clinical signs of 2 days' duration was somewhat atypical for this variable; however, careful examination of plots indicated that the data for this dog were consistent with the overall pattern of the other

data and should be included in the analysis. Computer software[f] was used for all statistical analyses.

Results

At initial evaluation at the PUVTH, the mean ± SD age of the 17 affected dogs was 69 ± 39 months (median, 74 months; range, 8 to 136 months). Twelve dogs were ovariohysterectomized females and 5 were castrated males. Breeds represented in the group included
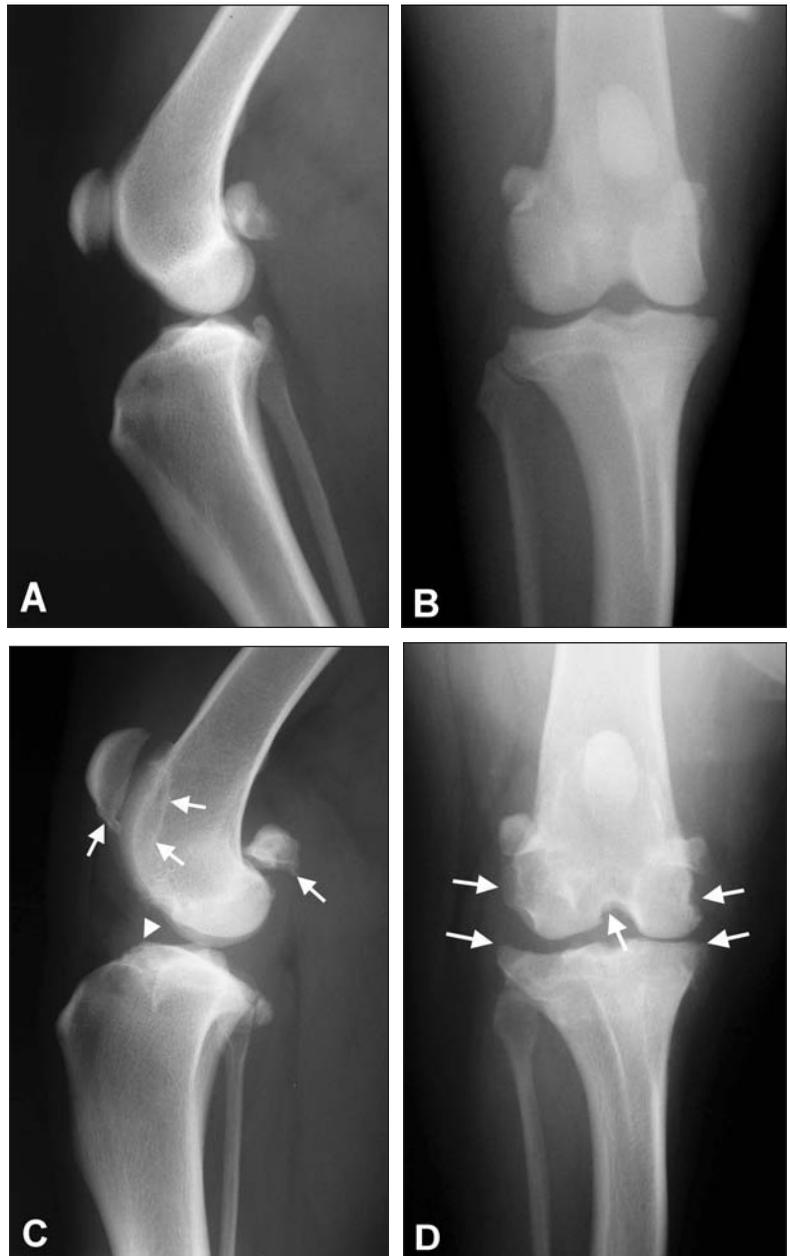

Figure 1—Radiographic views of 2 canine stifle joints that were or were not affected with osteoarthritis. A and B—Lateral and caudocranial radiographic views, respectively, of a nonosteoarthritic stifle joint. By use of a scoring system in which several radiographic features of joints were scored subjectively, this joint would be assigned a cumulative score of 0 (maximum score possible, 80). C and D—Lateral and caudocranial radiographic views, respectively, of a stifle joint affected with osteoarthritis secondary to rupture of the cranial cruciate ligament. Note loss of the infrapatellar fat pad opacity; synovial joint distention; osteophytosis of the femur, sesamoid bones, and tibia (arrows); and enthesophyte production on the femur and cranial tibial plateau (arrowhead). This joint was assigned a cumulative score of 29.
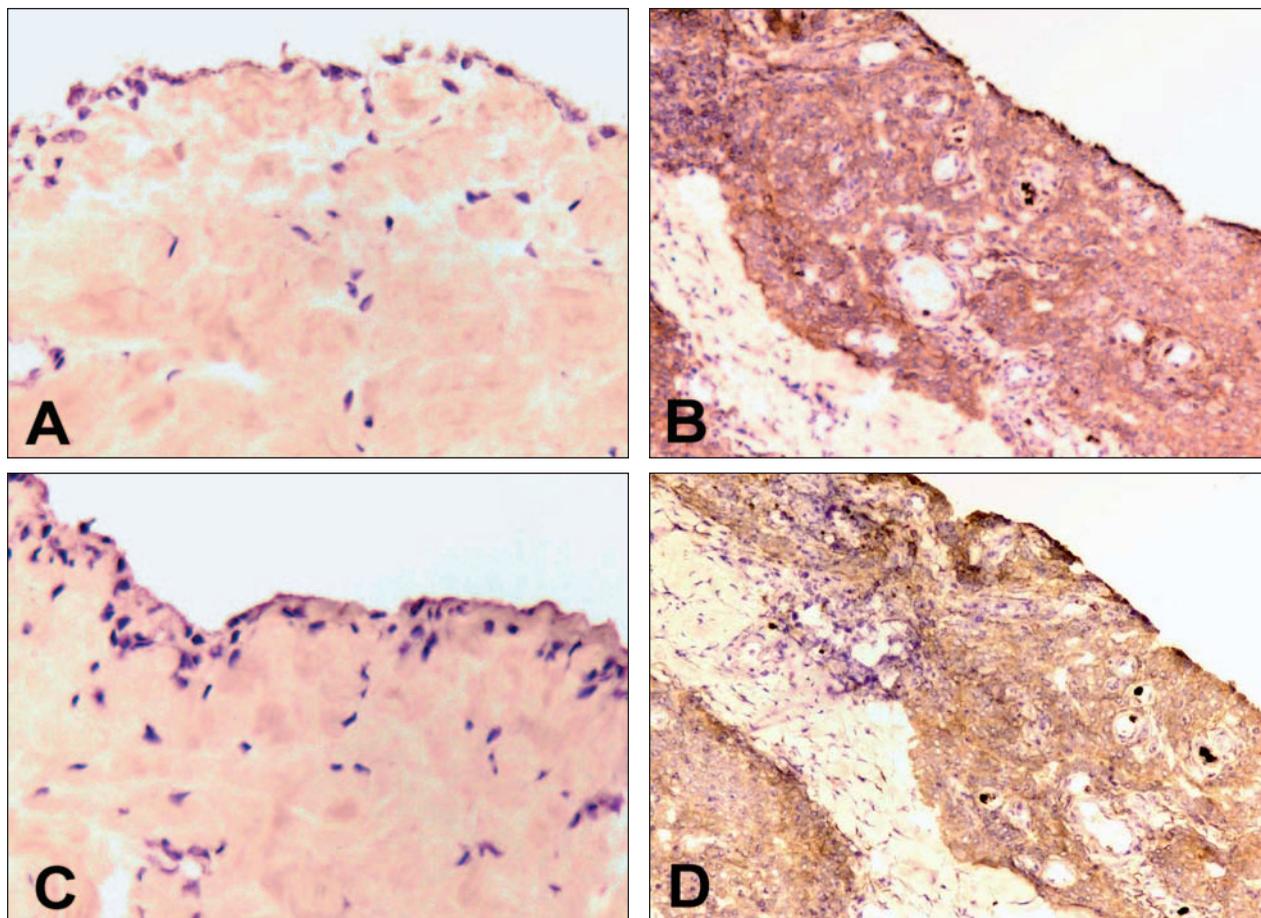
Figure 2—Results of immunohistochemical staining with anti-CD11b or anti-CD18 monoclonal antibodies in specimens of synovial membrane obtained from canine stifle joints that were or were not affected with osteoarthritis. A—Anti-CD18 antibody staining of synovium obtained from a normal nonosteoarthritic stifle joint. B—Anti-CD18 antibody staining of synovium obtained from an osteoarthritic stifle joint. C—Anti-CD11b antibody staining of synovium obtained from a normal nonosteoarthritic stifle joint. D—Anti-CD11b antibody staining of synovium obtained from an osteoarthritic stifle joint. In panels A and C, notice the 1 cell layer thickness of the synovial membrane and that the subsynovial tissue has minimal cellularity and vascularity. There is no immunostaining in these sections. In panels B and D, notice the marked thickening and hypertrophy of the synovial membrane, extensive vascularity of the subsynovial tissue, and prominent staining throughout the sample.

mixed (n = 7), Labrador Retriever (3), Rottweiler (3), Golden Retriever (1), Cocker Spaniel (1), Akita (1), and Bernese Mountain dog (1). Mean weight at the time of initial evaluation was 36.2 ± 9.4 kg (median, 36.5 kg; range, 15 to 55 kg). Mean duration of clinical signs prior to initial evaluation was 230 ± 297 days (median, 150 days; range, 2 to 1,095 days). Thirteen of the 17 dogs had received $\geq$ 1 medical treatments prior to evaluation at the PUVTH, including carprofen (n = 8), etodolac (2), deracoxib (1), aspirin (1), orally administered chondroprotective agents (7), and cortisone (1). Results of CBCs were within reference limits in all but 1 dog that had slight leukopenia. Six of the 17 affected dogs had high serum lipase concentration, and 1 had high serum liver enzyme concentrations; urinalyses revealed that 3 dogs had crystalluria and 1 had bacteria observed in the urine.

Radiographic findings included joint distention, osteophyte and enthesophyte formation, subchondral sclerosis, erosion, other signs of bony remodeling, and soft tissue mineralization (**Figure 1**). These changes varied in severity among dogs. In dogs with a long duration of clinical signs, osteophytosis was most com-

monly noted, whereas in dogs with a short duration of clinical signs, joint distention was more frequently observed. The mean total radiographic score was 15.1 $\pm$ 9.0 (median, 13; range, 1 to 34).

Routine histologic examination of formalin-fixed and cryopreserved samples of tissue obtained from affected joints revealed synovitis that was characterized by thickening, villous hypertrophy, and increased vascularization of the synovial membrane. Affected joint capsules also appeared to have an increased number of macrophages, lymphocytes plasma cells, and fibroblasts, compared with the number of those cells in joint capsules from unaffected dogs. Qualitatively, macrophage density (as indicated by staining intensity with CD11 and CD18 antibodies) was increased in all dogs, compared with control tissues.

On further examination of specimens, the mean synovial membrane thickness grade was 2.06 (median grade, 2; range, 1 to 3), and the mean vascularization grade was 2.24 (median grade, 2; range, 1 to 3). The mean membrane thickness and mean vascularization grades were both 1 in the control samples.

The presence of macrophages was determined via staining for the cell adhesion molecule markers CD11b

and CD18. The mean CD11b staining grade was 2.24 (median grade, 2; range, 1 to 3), and the mean CD18 staining grade was 2.47 (median grade, 3; range, 1 to 3). Control samples were classified as grade 1 for both markers (**Figure 2**). The mean staining grades for the macrophage-produced cytokines IL-6 and TNF-α were 2.29 (median grade, 2; range, 1 to 3) and 2.06 (median grade, 2; range, 1 to 3), respectively. Control specimens were classified as grade 1 for the cytokine markers.

Regression analyses were performed (**Table 1**). No significant correlations were found between osteoarthritis severity, age, and sex. However, significant relationships were detected (at the 0.05 level or better). Duration of clinical signs was related to the total radiographic score and to macrophage density represented by the combined CD11b and CD18 staining grades and the CD18 staining grade alone. The combined cell adhesion molecule staining grades were associated with the total radiographic score, the combined TNF-α and IL-6 staining grades, TNF-α staining grade alone, IL-6 staining grade alone, and the grade of synovial membrane thickness. Similarly, the staining grade for CD11b was associated with the combined TNF-α and IL-6 staining grades, TNF-α staining grade alone, IL-6 staining grade alone, and grade of synovial membrane thickness; the staining grade for CD18 was associated with the total radiographic score, combined TNF-α and IL-6 staining grades, TNF-α staining grade alone, and IL-6 staining grade alone. It is important to note that although some relationships had positive

Table 1—Relationships among duration of clinical signs of disease and radiographic scores for pathologic changes in 17 canine stifle joints affected with osteoarthritis and grades of immunohistochemical staining for cell adhesion molecule markers CD11b and CD18 (alone and combined) and tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 (alone and combined), joint capsule vascularity, and synovial membrane thickness in specimens of joint capsules and synovial membranes obtained from those joints.

Explanatory variable	Response variable	Correlation	*P* value
CD11b	CD18	0.780	< 0.01
Duration	CD11b + CD18	0.525	0.02
Duration	CD11b	0.408	0.05
Duration	CD18	0.578	< 0.01
CD11b + CD18	Total radiographic score	0.413	0.05
CD11b + CD18	TNF-α + IL-6	0.668	< 0.01
CD11b + CD18	TNF-α + IL-6	0.668	< 0.01
CD11b + CD18	TNF-α	0.663	< 0.01
CD11b + CD18	IL-6	0.587	< 0.01
CD11b + CD18	Vascularity	0.311	0.11
CD11b + CD18	Thickness	0.467	0.03
CD11b	Total radiographic score	0.342	0.09
CD11b	TNF-α + IL-6	0.615	< 0.01
CD11b	TNF-α	0.673	< 0.01
CD11b	IL-6	0.493	0.02
CD11b	Vascularity	0.361	0.08
CD11b	Thickness	0.521	0.02
CD18	Total radiographic score	0.433	0.04
CD18	TNF-α + IL-6	0.644	< 0.01
CD18	TNF-α	0.579	< 0.01
CD18	IL-6	0.612	< 0.01
CD18	Vascularity	0.229	0.19
CD18	Thickness	0.356	0.08

associations with one another, those associations failed to achieve significance at the 0.05 level. With a larger sample size, correlations of this magnitude would be significant.

Discussion

In the present study, macrophages and the cytokines TNF-α and IL-6 were detected in the synovial membranes and joint capsules of all 17 dogs with ruptured cranial cruciate ligaments. There was a positive relationship between the duration of clinical signs (chronicity) and macrophage density and between the duration of clinical signs and the radiographically assessed severity of the joint changes. The presence of macrophages was also associated with the immunocytochemical staining for TNF-α and IL-6. Thus, these findings supported the hypotheses of the present study and suggest that the synovial membrane and synovial macrophages may play an important role in the development of osteoarthritic changes in stifle joints of dogs with rupture of the cranial cruciate ligament.

The findings in our study are consistent with those of previous reports[1,24-27] of the histopathologic changes and cell types in stifle joint capsules of dogs with ruptured cranial cruciate ligaments. As reported by other investigators, inflammatory changes in the joint capsules of dogs with osteoarthritis secondary to naturally occurring rupture of the cranial cruciate ligament were identified in our study. In addition, all affected joint capsules examined in the present study contained macrophages, and the macrophage density (determined via immunostaining intensity) increased with the chronicity of the osteoarthritis.

In the specimens obtained from dogs with naturally occurring rupture of the cranial cruciate ligament, the presence of TNF-α and IL-6 in the synovial membrane was consistent with findings of other experiments involving models of osteoarthritis in animals[5,8] and with results of examination of biopsy specimens obtained from humans with naturally occurring osteoarthritis.[9] The cytokines produced in the synovial lining of synovial joints reach articular cartilage via the synovial fluid.[5] Indeed, the concentrations of TNF-α and IL-6 in the synovial fluid from stifle joints of dogs with transected cranial cruciate ligaments are significantly increased, compared with concentrations in the stifle joints of their unoperated normal contralateral limbs.[10] It is not known which cell type in the synovial membrane synthesizes these cytokines. However, the significant correlations between the staining intensity of the macrophage markers and TNF-α and IL-6 markers in the present study suggest that synovial macrophages may be the source. Our findings also indicate that in naturally occurring osteoarthritis, as in experimentally induced osteoarthritis, articular cartilage damage may be caused by cytokine synthesis in the synovial cell lining with subsequent upregulation of metalloproteinase synthesis by chondrocytes.[5]

Other synovial changes in stifle joints of dogs with ruptured cranial cruciate ligaments that were subjectively graded in our study were synovial membrane thickness and vascularity; in agreement with findings of a previous study,[20] there was no significant correla-

tion between the radiographic score and synovial membrane thickness or vascularity. The combined staining grade for the macrophage markers CD11b and CD18 was positively correlated with synovial membrane thickness but not with synovial membrane vascularity. Thus, our findings did not support those of a previous study[20] in humans, which suggested that macrophages are associated with angiogenesis. This difference between our findings and those of the previous investigations may be a consequence of differences in patient selection. In our study, dogs had a relatively acute stifle joint injury; in the studies[20,28] in humans, patients had end-stage osteoarthritis (biopsy samples were obtained during total joint replacement) in which pathologic changes often mimic those associated with rheumatoid arthritis.

In the study of this report, macrophages were identified immunocytochemically by use of anti-CD11b and anti-CD18 monoclonal antibodies. The CD18 antigen is a leukointegrin that consists of a β-2 chain that is unique to leukocytes.[29-32] The CD11b antigen is present on the cell surface of granulocytes and monocytes and is 1 of 3 β chains that are noncovalently bound to the β-2 chain.[29-31] The CD11b antigen is expressed by macrophages in the synovial membrane and joint capsule of humans and dogs.[29-32] By use of immunocytochemical techniques to detect both the CD18 and CD11b chains, macrophages in joint capsules can be reliably visualized and their density in the tissues graded; the antibodies used in those techniques do not distinguish resting macrophages from activated macrophages. However, the presence of TNF-α and IL-6 in the synovium and their strong correlation with the combined staining grades of the CD11b and CD18 markers in joint capsule specimens obtained from dogs with naturally occurring rupture of the cranial cruciate ligament suggested that the synovial macrophages detected in those specimens were activated.

In the present study, we identified a positive relationship between synovial macrophage density and the total radiographic score of stifle joints in dogs with naturally occurring rupture of the cranial cruciate ligament. The method used in our study to evaluate osteoarthritis radiographically placed emphasis on osteophyte formation. From results of research conducted in different species, several investigators have suggested that macrophages may play a crucial role in the development of osteophytes through the production of TGF-β and other substances.[13,16-18] Our findings that macrophage density, duration of disease, and radiographic evidence of osteoarthritis are strongly correlated in stifle joints of dogs with naturally occurring rupture of the cranial cruciate ligament certainly support this notion. By producing various cytokines and growth factors, macrophages may be responsible not only for the initiation of osteophyte formation but also for the perpetuation of osteophyte formation and growth.

Some of the limitations of our study are the number of animals included, the subjectivity of our definition of chronicity of osteoarthritis, and the subjective histologic grading of the joint capsule specimens. To the authors' knowledge, no similar studies have been reported previously and it was difficult to determine a priori what the sample size of the present study should be. The sample size and analytic procedures used in our study required a correlation to be 0.41 or larger to be significant at the 5% level. The additional power provided by a larger sample size would be very likely to reveal additional significant associations. In the present study, the assessment of chronicity (duration of clinical signs) was based on the observation of lameness by the owner. Because cranial cruciate rupture in dogs may be insidious in onset and not associated with trauma, the initial lameness may have not been noticed by the owner and not documented in the records of the referring veterinarians. Indeed, the reported duration of clinical signs in the dogs of our study may have been underestimated, but we do not believe that such an underestimation affected the overall results of the study. Finally, we opted for less time-consuming, subjective histologic grading of tissue specimens; more objective grading and quantification would have been preferable, particularly from a statistical viewpoint.

The results of the present study have indicated that the densities of macrophages in the synovial membrane and joint capsule of osteoarthritic stifle joints in dogs are greater than those of clinically normal joints; furthermore, the macrophage densities in the synovial membrane and joint capsule may be related to the chronicity and severity of osteoarthritis in dogs. Our data provide evidence that in naturally occurring osteoarthritis secondary to cranial cruciate ligament rupture in stifle joints of dogs, synovial macrophages may play an important role in the development of pathologic changes in articular cartilage and joint capsule as well as in the development of osteophytes. Identification of synovial macrophages as central players in the development of cartilage lesions, osteophytes, and joint capsule inflammation may open the way for new treatment strategies that are based on elimination of the deleterious effects of synovial macrophages. For humans with rheumatoid arthritis, such an approach has resulted in the development of a new, successful class of therapeutic agents. The mechanisms of action of these agents include neutralization of cytokines, cytokine receptor blockade, and folate-mediated drug delivery to macrophages.[33,34] It may be that similar approaches may result in complete arrest or inhibition of rapidly progressive osteoarthritis and, as such, provide an important addition to the options for treatment of osteoarthritis in dogs.

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