Inflammatory changes in ruptured canine cranial and human anterior cruciate ligaments

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Objective—To compare expression of tartrate-resistant acid phosphatase (TRAP) and cathepsin K and histologic changes in canine cranial cruciate ligaments (CCLs) and human anterior cruciate ligaments (ACLs).

Study Population—Sections of cruciate ligaments from 15 dogs with ruptured CCLs, 8 aged dogs with intact CCLs, 14 human beings with ruptured ACLs, and 11 aged human beings with intact ACLs.

Procedure—The CCLs and ACLs were evaluated histologically, and cells containing TRAP and cathepsin K were identified histochemically and immunohistochemically, respectively.

Results—The proportion of ruptured CCLs that contained TRAP+ cells was significantly higher than the proportion of intact ACLs that did but similar to proportions of intact CCLs and ruptured ACLs that did. The proportion of ruptured CCLs that contained cathepsin K+ cells was significantly increased, compared with all other groups. Numbers of TRAP+ and cathepsin K+ cells were significantly increased in ruptured CCLs, compared with intact ACLs. The presence of TRAP+ cells was correlated with inflammatory changes, which were most prominent in ruptured CCLs.

Conclusion and Clinical Relevance—Results suggest that synovial macrophage-like cells that produce TRAP are an important feature of the inflammatory associated with CCL rupture in dogs. Identification of TRAP and cathepsin K in intact CCLs and ACLs from aged dogs suggests that these enzymes have a functional role in cruciate ligament remodeling and repair. We hypothesize that recruitment and activation of TRAP+ macrophage-like cells into the stifle joint synovium and CCL epiligament are critical features of the inflammatory arthritis that promotes progressive degradation and eventual rupture of the CCL in dogs. (Am J Vet Res 2005;66:2073–2080)

There is a fundamental gap in the current understanding of the cellular and molecular events that lead to cranial cruciate ligament (CCL) rupture in dogs. There is often no history of trauma associated with the onset of lameness, young large-breed dogs are most commonly affected, and bilateral disease is common. This suggests that, in most instances, CCL rupture is not the result of an acute traumatic injury but represents the final stage of a progressive condition associated with an idiopathic, probably immune-mediated, inflammatory arthropathy. Loss of cellularity, decrease in ligament strength, degeneration of normal matrix architecture, and chondroid metaplasia all worsen with age in dogs that weigh > 15 kg, supporting the suggestion that this is a progressive condition. The suggestion that this condition is associated with an immune-mediated, inflammatory arthropathy is supported by the fact that ACL rupture in dogs is associated with generalized inflammation of the stifle joint synovium characterized by deposition of IgG and IgM in the synovial membrane, the presence of macrophage-like cells containing tartrate-resistant acid phosphatase (TRAP) and cathepsin K, and the presence of large numbers of major histocompatibility complex class II macrophages and dendritic cells. In contrast to the situation in dogs, rupture of the anterior cruciate ligament (ACL) in human beings is generally a result of an acute traumatic injury and occurs most often in younger patients during athletic activity. However, it is also possible that ACL rupture has a pathologic component in human patients, in that healing of partial ACL tears generally is poor, primary surgical repair of ruptured ACLs often does not result in a satisfactory outcome, and ACL rupture is more common in women. Histologic studies of intact and ruptured ACLs have revealed that there is an initial inflammatory response after ACL rupture, the synovium reforms around the frayed ends of the ligament remnants, and the regenerative response is coupled with neovascularization. Subsequently, there is a reduction in cellularity and remodeling of the retracted remnants. In intact ACLs, an area of chondroid metaplasia characterized by reduced vascularity and a fibrocartilaginous appearance can be found in the anterior portion of the ligament. The cells in this area are spheroid rather than elliptical or fusiform and occur in long, linear arrangements, similar to chondrocytes.

Studies of the extracellular matrix of cruciate ligaments have not conclusively determined the role of enzymatic degradation in cruciate rupture. In rabbits, collagenase expression has been associated with cruciate ligament rupture. However, a study in humans found that whereas type I collagen mRNA is expressed in the ACL, neither collagenase, 72-kD gelatinase, nor tissue inhibitor of matrix metalloproteinase (TIMP)
mRNA is expressed at any time after ACL rupture. On the other hand, a more recent study suggests that matrix metalloproteinase-3 (MMP-3) and TIMP are expressed in ruptured ligaments. It is possible that other degradative enzymes are involved in ACL rupture or that the lack of expression of collagenolytic enzymes plays a role in the failure of ACLs to remodel and heal in humans.

A recent study of ruptured CCLs from dogs showed that localization of cathepsin K and TRAP is associated with cruciate ligament rupture. Cathepsin K is a cysteine proteinase important for cleavage of the N-terminal end of the collagen triple helix, allowing access for other proteolytic enzymes and MMPs. Cathepsin K is expressed in osteoclasts and macrophages but is also expressed by synovial and tendon fibroblasts, and this enzyme is thought to play a role in rheumatoid arthritis.

Tartrate-resistant acid phosphatase is a lysosomal enzyme expressed in dendritic cells, osteoclasts, and macrophages. Mice that lack TRAP have defects in endochondral ossification and macrophage function; thus, TRAP is thought to be involved in both bone resorption and innate immunity. In addition, cathepsin K and TRAP expression have been associated with degradation of matrix by macrophage-lineage cells, and TRAP is a marker for activated macrophages. As a result, expression of cathepsin K and TRAP in association with inflammatory changes in cruciate ligaments supports the concept that an unidentified trigger is promoting recruitment of macrophages in the stifle joint synovium. The development of synovitis in conjunction with expression of macrophage-derived matrix-degrading enzymes leads to degradation of the cruciate ligament’s structural properties. However, because the timing of the initial ligament injury in dogs is usually not known, it is difficult to correlate expression of these enzymes with the onset of CCL disruption.

We hypothesize that rupture of the CCL in most dogs represents the final stage of an immune-mediated inflammatory arthropathy in which collagenolytic enzyme products, such as cathepsin K, that are secreted by activated TRAP macrophages mediate irreversible destruction of the CCL. Because the mechanism of cruciate ligament rupture in dogs appears to be different from the mechanism in people, we further hypothesize that inflammatory changes and proteolytic enzyme expression would be more evident in ruptured CCLs from dogs than in ruptured ACLs from humans. The purpose of the study reported here, therefore, was to compare expression of TRAP and cathepsin K and histologic changes in ruptured and intact CCLs from dogs with findings for ruptured and intact ACLs from human beings.

Materials and Methods

CCLs—Ruptured CCLs from 15 dogs undergoing surgical treatment at the University of Wisconsin-Madison Veterinary Medical Teaching Hospital were used in the study. In all dogs, stifle joint instability had been identified by means of physical examination and CCL rupture was confirmed at surgery. Ruptured CCLs were collected during surgical debridement of the affected joints via a medial or lateral parapatellar arthrotomy.

Intact CCLs from 8 older dogs were also used in the study. These dogs had been euthanatized for reasons unrelated to the study; details for these dogs have been published previously. Information on age, weight, sex, and when applicable, duration of lameness was recorded for each dog.

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mouse IgG for 20 minutes at room temperature and rinsed with PBS-Tween (with 1% canine serum for canine tissue sections). Finally, slides were incubated with streptavidin–horseradish peroxidase conjugate for 20 minutes at room temperature, rinsed with PBS-Tween, and flooded with an insoluble 3.3′-diaminobenzidine tetrachloride–nickel-cobalt substrate. Slides were rinsed in water for 5 minutes, counterstained with nuclear fast red stain, and examined by means of light microscopy. The number of cathepsin K+ cells was counted on each slide. If > 200 cells were present on a slide, the number of cathepsin K+ cells was recorded as too numerous to count. Negative control slides omitting the primary or secondary antibody were included with each batch.

Histologic examination—Sections that had undergone histochemical or immunohistochemical staining were examined for histologic evidence of epiligamentous proliferation (ie, increased cellularity of the epiligament), chondroid metaplasia (ie, cells having a more ovoid or round appearance than normal and a clonal appearance), matrix degradation (ie, areas with reduced numbers of collagen fibrils), and degree of regenerative response (ie, an increase in cellularity and vascularity with whorls of cells that appeared to be dividing). Degrees of epiligamentous proliferation, chondroid metaplasia, matrix degradation, increased cellularity, decreased cellularity, regenerative response, and increased vascularity were scored on a scale from 0 to 5, with 0 = no evidence; 1 = mild change in small area of the ligament; 2 = moderate change; 3 = moderate, diffuse changes; 4 = severe change; and 5 = severe, diffuse changes. Preliminary observation of the specimens revealed that areas with increased cellularity and other areas with decreased cellularity could be found within a single specimen; therefore, increased cellularity and decreased cellularity were included as separate variables. A single individual (JGB) blinded to specimen origin counted numbers of TRAP+ and cathepsin K+ cells and performed histologic examinations on all specimens.

Statistical analysis—The Kruskal-Wallis ANOVA by ranks and the Mann-Whitney U test were used to test for differences among the 4 groups (intact CCL, ruptured CCL, intact ACL, and ruptured ACL). Dependent variables included number of TRAP+ cells, number of cathepsin K+ cells, degree of epiligamentous proliferation, degree of chondroid metaplasia, degree of matrix degradation, degree of increased cellularity, degree of decreased cellularity, degree of regenerative response, and degree of increased vascularity. The Fisher exact test was used to compare proportions of ligaments that contained TRAP+ and cathepsin K+ cells among groups. The Spearman rank correlation method was used to examine possible associations between subject age or sex and the dependent variables. Values of $P < 0.05$ were considered significant.

Results

Median age of the 15 dogs from which ruptured CCLs were obtained was 4.3 years (range, 1.1 to 8.3 years), and median age for the 8 dogs from which intact CCLs were obtained was 11.4 years (range, 3 to 14 years). Median age of the 14 human patients from whom ruptured ACLs were obtained was 20 years (range, 15 to 45 years), and median age for the 11 human patients from whom intact ACLs were obtained was 72 years (range, 53 to 82 years).

Dogs from which ruptured CCLs were obtained consisted of 9 spayed females, 1 sexually intact female, 4 castrated males, and 1 sexually intact male. Dogs from which intact CCLs were obtained consisted of 5 spayed females and 3 castrated males. Nine of the human patients from whom ruptured ACLs were obtained were male, and 5 were female. Two of the human patients from whom intact ACLs were obtained were male, and 9 were female.

Intact CCLs—Chondroid metaplasia was common in the intact CCLs (median severity score, 3; Table 1), along with matrix degeneration and decreased cellularity. Median severity of decreased cellularity was significantly ($P = 0.04$) greater in intact CCLs than in ruptured CCLs, but median severity of regenerative responses was significantly ($P = 0.01$) lower in intact CCLs than in ruptured CCLs. Cells positive for TRAP were seen in only 4 of the 8 intact CCLs, with only low numbers of TRAP+ cells seen, and cathepsin K+ cells were seen in only 1 of the intact CCLs. However, median numbers of TRAP+ and cathepsin K+ cells in the intact CCLs were not significantly different from median numbers of TRAP+ and cathepsin K+ cells in ruptured CCLs ($P \geq 0.27$), ruptured ACLs ($P \geq 0.77$), or intact ACLs ($P \geq 0.97$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>CCL</th>
<th>ACL</th>
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<tr>
<td></td>
<td>Intact (8)</td>
<td>Ruptured (15)</td>
</tr>
<tr>
<td>Increased vascularity</td>
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<td>1</td>
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<td>2</td>
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<tr>
<td>Chondroid metaplasia*</td>
<td>3*</td>
<td>3*</td>
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<tr>
<td>Matrix degeneration*</td>
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<tr>
<td>Increased cellularity*</td>
<td>0*</td>
<td>3*</td>
</tr>
<tr>
<td>Regenerative response</td>
<td>0.5*</td>
<td>4*</td>
</tr>
<tr>
<td>Decreased cellularity*</td>
<td>2*</td>
<td>0*</td>
</tr>
<tr>
<td>TRAP+ cells*</td>
<td>10</td>
<td>200*</td>
</tr>
<tr>
<td>Cathepsin K+ cells*</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent numbers of ligaments. Histologic features were scored on a scale from 0 (no evidence) to 5 (severe and diffuse). Numbers of TRAP+ and cathepsin K+ cells were counted in a single longitudinal section from each ligament.

*Value for all CCLs (ruptured and intact) was significantly ($P < 0.05$) different from value for all ACLs (ruptured and intact).

CCL = Cranial cruciate ligament, ACL = Anterior cruciate ligament.

*In each row, values with different superscript letters were significantly ($P < 0.05$) different.
Intact ACLs—Moderate matrix degeneration, chondroid metaplasia, and decreased cellularity were frequently seen in areas of the core of intact ACLs (Figure 1), and many intact ACLs had regions of chondroid metaplasia and matrix degeneration interspersed through hypocellular areas of organized matrix. In many intact ACLs, however, there was no increase in vascularity or cellularity and no evidence of epiligamentous proliferation. Low numbers of TRAP+ cells were seen in 2 of the 11 intact ACLs, and none of the intact ACLs had cathepsin K+ cells. Median numbers of TRAP+ and cathepsin K+ cells in intact ACLs were significantly lower than median numbers of cells in ruptured CCLs (P = 0.005 and 0.04, respectively; Table 1).

Ruptured CCLs—Most ruptured CCLs had moderate or severe regenerative responses with moderate chondroid metaplasia, increased cellularity, and increased vascularity. Many ruptured CCLs had focal areas of severe regenerative response interspersed throughout a generally poorly organized matrix with mild increases in cellularity (Figure 2). Median severity of regenerative response and increased cellularity were significantly higher in ruptured CCLs than in intact ACLs or intact CCLs (Table 1). Additionally, median severity of chondroid metaplasia in ruptured CCLs was significantly (P = 0.006) higher than median severity in ruptured ACLs.

Eleven of the 15 ruptured CCLs contained TRAP+ cells, and most of these had > 200 TRAP+ cells. Ten of the ruptured CCLs contained cathepsin K+ cells, and 5 of these had > 200 cathepsin K+ cells. These TRAP+ and cathepsin K+ cells were often seen in the epiligamentous region of the ruptured CCLs but were also seen in the core region (Figure 2). The proportion of ruptured CCLs that contained TRAP+ cells (11/15) was significantly (P < 0.01) higher than the proportion of intact ACLs that did (2/11) but was not significantly different from proportions of intact CCLs (4/8) and ruptured ACLs (10/14) that had TRAP+ cells. The proportion of ruptured CCLs that contained cathepsin K+ cells (10/15) was significantly (P < 0.05) higher than the proportions of intact CCLs (1/8), ruptured.
ACLs (3/14), and intact ACLs (0/11) that did. Median numbers of TRAP+ and cathepsin K+ cells in ruptured ACLs were significantly higher than median numbers of these cells in intact ACLs (P = 0.005 and 0.04, respectively).

Ruptured ACLs—Most ruptured ACLs had mild regenerative responses with mild increases in vascularity and epiligamentous proliferation. There was little chondroid metaplasia and no evidence of matrix degeneration in ruptured ACLs. However, there were some areas where ligament fibroblasts had a slightly more ovoid than fusiform appearance in association with a pronounced regenerative response (Figure 1). In some areas of ruptured ACLs, there was normal-appearing cellularity and crimp pattern. Ten of the 14 ruptured ACLs contained TRAP+ cells, and 3 contained cathepsin K+ cells within the epiligamentous region. However, only low numbers of these cells were seen, and only 1 ruptured ACL had > 200 TRAP+ and cathepsin K+ cells (Figure 2). The 9 ruptured ACLs from male patients had a maximum of 3 TRAP+ cells/slide, and none had any cathepsin K+ cells. The proportion of ruptured ACLs that contained TRAP+ cells (10/14) was significantly (P = 0.01) higher than the proportion of intact ACLs that did (2/11), but the proportion of ruptured ACLs that contained cathepsin K+ cells (3/14) was not significantly (P = 0.02) different from the proportion of intact ACLs (0/11) that did. Median severities of matrix degeneration and chondroid metaplasia in ruptured ACLs were significantly (P < 0.001) lower than median severities in intact or ruptured CCLs (Table 1). Areas of regeneration with mild chondroid metaplasia and epiligamentous proliferation and other areas of histologically normal matrix and cellularity were typically seen. This was in contrast to the intact ACLs, which, in areas of decreased cellularity, more frequently had severe chondroid metaplasia of the remaining cells and matrix degeneration, and the ruptured and intact CCLs, which had expansive areas in their cores with enlarged chondroid cells with cloning and matrix degeneration (Figure 1).

Comparison of ACLs and CCLs—When all CCLs (ruptured and intact) were compared with all ACLs (ruptured and intact), numbers of TRAP+ and cathepsin K+ cells were significantly higher in CCLs than in ACLs.
Additionally, median severity of matrix degeneration, chondroid metaplasia, and increased cellularity were significantly higher in CCLs than in ACLs (Table 1).

Focal areas of chondroid metaplasia locally surrounded by matrix degeneration were seen in intact ACLs but not in intact CCLs. In contrast, chondroid metaplasia was distributed throughout the core region of intact CCLs, with no areas of hypocellular matrix.

Correlations among variables—When intact and ruptured ACLs were considered as a group, severity of matrix degeneration, chondroid metaplasia, and decreased cellularity were positively correlated with age (Table 2). The number of TRAP+ cells was positively correlated with number of cathepsin K+ cells, severity of epiligamentous proliferation, severity of regenerative response, and severity of increased vascularity. When intact and ruptured CCLs were considered as a group, number of TRAP+ and cathepsin K+ cells were positively correlated. In addition, the proportion of ruptured CCLs that contained cathepsin K+ cells (10/15) was significantly higher than the proportions of intact CCLs (1/8), ruptured ACLs (3/14), and intact ACLs (0/11) that did, and the proportion of ruptured CCLs that contained TRAP+ cells (11/15) was significantly higher than the proportion of intact ACLs that did (2/11). In addition, ruptured CCLs had more severe regenerative responses and increases in cellularity than did intact CCLs. These changes were not associated with age of the dogs and therefore might be part of an inflammatory process that promotes rupture of the CCL. Intact CCLs from older dogs in the present study had evidence of chondroid metaplasia, but in these dogs, it was associated with matrix degeneration and decreased cellularity without any increase in vascularity. Thus, it was more similar to the chondroid metaplasia seen in intact ACLs from human patients.

Chondroid differentiation in the anterior portion of the ACL in humans has been described.20 Further, it

Table 2—Spearman rank correlations between various histologic features in ruptured (n = 14) and intact (11) ACLs from human patients.

<table>
<thead>
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<th>Variable</th>
<th>Epiligamentous proliferation</th>
<th>Chondroid metaplasia</th>
<th>Matrix degeneration</th>
<th>Decreased cellularity</th>
<th>Increased cellularity</th>
<th>Regenerative response</th>
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<th>TRAP+ cells</th>
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NS = No significant correlation. + = Significant (P < 0.05) positive correlation. – = Significant (P < 0.05) negative correlation.

Table 3—Spearman rank correlations between various histologic features in ruptured (n = 15) and intact (8) CCLs from dogs.

<table>
<thead>
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<th>Variable</th>
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See Table 2 for key.

Discussion

Results of the present study suggest that there are species-specific differences in inflammatory changes in ruptured cruciate ligaments from dogs and people and that synovial macrophage-like cells that produce TRAP and cathepsin K are an important feature of the inflammation associated with CCL rupture in dogs. Recent studies4,9 have suggested that CCL rupture in dogs is associated with joint inflammation and upregulation of collagenolytic enzymes, such as cathepsin K and TRAP, that participate in macrophage-mediated joint destruction. For both the canine and human cruciate ligaments in the present study, numbers of TRAP+ and cathepsin K+ cells were positively correlated. In addition, the proportion of ruptured CCLs that contained cathepsin K+ cells (10/15) was significantly higher than the proportions of intact CCLs (1/8), ruptured ACLs (3/14), and intact ACLs (0/11) that did, and the proportion of ruptured CCLs that contained TRAP+ cells (11/15) was significantly higher than the proportion of intact ACLs that did (2/11). In addition, ruptured CCLs had more severe regenerative responses and increases in cellularity than did intact CCLs. These changes were not associated with age of the dogs and therefore might be part of an inflammatory process that promotes rupture of the ACL. Intact CCLs from older dogs in the present study had evidence of chondroid metaplasia, but in these dogs, it was associated with matrix degeneration and decreased cellularity without any increase in vascularity. Thus, it was more similar to the chondroid metaplasia seen in intact ACLs from human patients.

Chondroid differentiation in the anterior portion of the ACL in humans has been described.20 Further, it
has been suggested that formation of fibrocartilage within the ACL is associated with stresses from contact with the intercondylar fossa during extension.\textsuperscript{30} Our data show that severity of chondroid metaplasia, matrix degeneration, and decreased cellularity in the ACL correlate with age of the patient. Similar changes were seen in CCLs from dogs but were not correlated with age of the dogs. Furthermore, there was no significant difference in severity of chondroid metaplasia between ruptured and intact CCLs.

This raises the question as to whether chondroid metaplasia is a normal age-related change of the CCL in dogs or is an important factor influencing the risk of CCL rupture. It is hard to separate changes seen in intact CCLs from older dogs from changes seen in ruptured canine CCLs with regard to this histologic feature because it is probable that a low level of cruciate degradation is present in intact ligaments from older dogs.\textsuperscript{3} If CCL rupture develops as a consequence of an inflammatory arthropathy, no true negative control can be identified until a breed or type of dog that does not experience progressive CCL rupture is identified.

One limitation of the present study was that we were only able to collect ruptured ACLs from younger patients and intact ACLs from older patients. Because rupture of the ACL occurs most often in younger patients, it would have been better to compare ruptured ACLs with intact ACLs from younger individuals. However, such specimens were not available. We were also unable, in the present study, to collect information regarding the use of nonsteroidal anti-inflammatory drugs in study subjects prior to collection of the cruciate ligaments, and there may be differences between dogs and humans in regard to their use. Results of the present study could have been more definitive if higher numbers of ligaments had been used. However, we did find significant differences among groups. We did not know how long after rupture the ACLs were collected from the human patients; however, an in-depth study\textsuperscript{99} of temporal histologic changes has been published.

In the present study, ruptured ACLs from human patients were less similar to ruptured CCLs from dogs than intact ACLs were to intact CCLs. In particular, ruptured ACLs did not have as much chondroid metaplasia or matrix degeneration as did ruptured CCLs. When all ACLs (ruptured and intact) were compared with all CCLs (ruptured and intact), the ACLs had significantly less severe chondroid metaplasia, matrix degeneration, and increased cellularity than did the CCLs and lower numbers of TRAP and cathepsin K\textsuperscript{+} cells. Our data support the idea that TRAP\textsuperscript{+} cells have a functional role in cruciate ligament remodeling and repair, and our finding that cathepsin K\textsuperscript{+} and TRAP\textsuperscript{+} cells could be seen in ruptured ACLs is intriguing, given that ACL rupture is most often a result of acute trauma in human beings. It is possible that in people, expression of TRAP and cathepsin K following ACL rupture is associated with remodeling of the ligament remnants. Although it is possible that these enzymes are not expressed in the CCL in dogs prior to rupture, we found that numbers of TRAP and cathepsin K\textsuperscript{+} cells were significantly higher in CCLs than in ACLs, suggesting that rupture of the CCL is associated with more pronounced joint inflammation. Examination of synovial membrane from human knees with ACL rupture would help to clarify this point.

If data from the present study are considered in light of the fact that CCL rupture in dogs is not usually associated with a traumatic event and typically is bilateral, then it seems likely that CCL rupture in dogs is a progressive process. These data also suggest that naturally occurring CCL rupture in dogs is not a good model for ACL rupture in humans because naturally occurring CCL ruptures are associated with much greater inflammatory and reparative responses. We currently hypothesize that CCL rupture in most dogs represents the final stage of an immune-mediated inflammatory arthropathy in which expression of macrophage products, such as TRAP and cathepsin K, is part of the rupture mechanism.\textsuperscript{99,100,101}

References


