Lymphocyte Proliferation to Collagen Type I in Dogs

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With 3 tables

Received for publication September 7, 2006

Summary

The objective of this study was to investigate if cellular reactivity to collagen type I exists in dogs with unilateral cranial cruciate ligament (CrCL) rupture and if it relates to disease progression. The patient group consisted of 10 dogs with unilateral CrCL rupture. The control dogs consisted of three healthy control dogs, and two healthy dogs with unilateral sham operations of the stifle joint. All dogs were assayed repeatedly every 6 months for 12–24 months. Peripheral blood mononuclear cells were isolated from whole blood and were cultured with human collagen type I at concentrations of 5, 20 and 40 \(\mu\)g/ml for 6 and 7 days. Lymphocyte reactivity to collagen type I occurred not only in dogs with CrCL rupture, but also in sham-operated dogs and healthy dogs. Five of the eight assays (63%) performed at the time of operation or at the time of diagnosis of CrCL rupture had a stimulation index (SI) \(\geq 3.0\). This was not significantly different compared to healthy control dogs, not to the sham-operated control dogs. The CrCL rupture was assessed intraoperatively in six cases. Three cases had partial rupture and three had complete rupture. Only one dog with partial rupture, and two dogs with complete rupture had a positive SI. An increase in proliferation to collagen type I was seen in dogs with CrCL rupture, whereas it either remained stable or decreased in the control dogs. No distinct pattern in lymphocyte reactivity to collagen type I could be established from the dogs that sustained a CrCL rupture in the contralateral stifle joint, although most dogs that did not sustain a CrCL rupture in the contralateral stifle joint remained negative during this study with exception of one dog. Further research is required to determine whether cellular reactivity to collagen type I may play an initiating role in cruciate degradation.

Introduction

Rupture of the cranial cruciate ligament (CrCL) is the most prevalent condition that affects the stifle joint in dogs. Partial or complete rupture of this ligament results in instability and inflammation of the stifle joint, and finally in osteoarthritis (OA). In the majority of the dogs, CrCL rupture occurs under normal daily loading, which normally should not damage this ligament. Despite excessive research, minimal progress has been made in understanding the changes that lead to the structural failure of the CrCL. Histopathological studies on synovial tissues from dogs with CrCL rupture reveal a chronic synovitis with infiltration of T lymphocytes, plasma cells and macrophages (Tirgari, 1977; Galloway and Lester, 1995; Lawrence et al., 1998; Hewicker-Trautwein et al., 1999), being suggestive for a cellular immune reaction. A recent histopathological study discovered a significant quantity of dendritic-like cells in the synovial tissue of dogs with natural occurring CrCL rupture (Lemburg et al., 2004). Dendritic cells are highly potent antigen presenting cells (APC) that take up, process and present antigen to naïve T cells. The antigen(s) presented by the dendritic cells in the synovium of stifle joints with CrCL rupture is (are) still unknown. However, one of the major components of joint tissue is collagen, and cellular reactivity to collagen has been reported in collagen-induced arthritis (CIA) in rodents and in rheumatoid arthritis (RA) in humans (Smolen et al., 1980; Kammer and Trentham, 1984). The cruciate ligaments, as well as the menisci, are mainly composed of collagen type I while the articular cartilage is mostly composed of collagen type II (Amiel et al., 1984; Morgan et al., 1987). It is, therefore, possible that collagen type I is a critical self-antigen in cruciate disease in dogs. An intriguing mystery in cruciate disease in dogs is the high occurrence of contralateral ruptures; one-third of the cruciate patients rupture their contralateral CrCL within 8 months after the first CrCL rupture (Pond and Campbell, 1972; Bennett et al., 1988; Doverspike et al., 1993; de Rooster, 2001). A possible explanation is that activated T cells against collagen type I enter the contralateral stifle joint and generate an inflammatory reaction that could ultimately result in degradation of the CrCL.

Proliferation assays performed with peripheral blood mononuclear cells (PBMC) are commonly used to investigate the reactivity of PBMC against a specific antigen (Barta and Barta, 1993). The objective of this study was to investigate the lymphocyte reactivity to collagen type I in dogs with CrCL rupture with a lymphocyte proliferation assay, and to discuss its possible role in the disease progression.

Materials and Methods

Control groups

Healthy control dogs

Three healthy experimental dogs were used as healthy control dogs. The dogs were kept at the Faculty of Veterinary Medicine and were housed, fed and cared for in accordance with institutional and ethical guidelines. They were examined before initiation of the study to exclude any musculoskeletal abnormalities. Radiographs of the shoulder, the elbow, the stifle and hip joints showed no signs of OA. Lymphocyte proliferation to collagen type I was seen in dogs with CrCL rupture in the study with exception of one dog.
proliferation assays to collagen type I were performed every 6 months for a total of three to four times.

**Sham-operated dogs**

A sham operation was performed on the left stifle joint of two healthy dogs. Both dogs were kept at the Faculty of Veterinary Medicine and were housed, fed and cared for in accordance with institutional and ethical guidelines. They were examined before the initiation of this study to exclude any musculoskeletal abnormalities. Radiographs of the shoulder, the elbow, the stifle and hip joints showed no signs of OA. The stifle joint was approached through a lateral arthrotomy. The patella was dislocated medially and a Gelpi retractor and Wallace retractor were used to open the stifle joint to inspect the intra-articular structures. There were no signs of OA, cruciate ligament or meniscal damage. The joints were routinely closed using single sutures of polyglactin 910 (Vicryl®; Johnson & Johnson, Dilbeek, Belgium). The dogs were allowed limited exercise for 6 weeks. Lymphocyte proliferation assays to collagen were performed only after the sham operation as the assay was not yet optimized at the time of operation. The assays were performed every 6 months, starting 6 months after the sham operation.

The entire study with the healthy control dogs and the sham-operated dogs was approved by the ethical commission of the Faculty of Veterinary Medicine, Ghent University, Belgium.

**Patient group**

Ten client-owned dogs admitted to the Ghent University, Veterinary School, Department of Diagnostic Imaging diagnosed with unilateral CrCL rupture were included in the present study. The owners gave written consent to present their dogs for re-examination every 6 months for 12–24 months. Diagnosis of CrCL rupture was based on hind limb lameness, palpable cranial drawer movement (with the exception of one dog), signs of OA and positive tibial compression test on standard mediolateral radiographs. Breed, age at time of initial presentation, body weight and sex were recorded. Data on the duration of lameness were available of all dogs. The affected stifle joints were stabilized using an extracapsular technique described (de Bruin et al., 2005). Briefly, diluted blood was supplemented with 10% (v/v) of a carbonyl iron/arabic gum mixture that was prepared as follows: a 10% (w/v) carbonyl iron suspension (Sigma, Bornem, Belgium) in 10 ml PBS supplemented with 1% penicillin (100 IU/mL) was washed four times with this solution (centrifugation for 10 min at 550 g and RT). Meanwhile, a 10% (w/v) arabic gum suspension (Sigma) in 10 ml PBS with 1% penicillin was prepared. Both were sterilized for a period of 15 min at 121°C. After cooling, equal amounts of the two suspensions were mixed.

The supplemented blood was incubated for 1 h at 38°C in a 5% CO₂ atmosphere, during which it was gently shaken every 10 min. After incubation, the 22 ml of blood was carefully layered onto 15 ml dense medium in a 50 ml falcon tube (thendense medium (density 1.079 g/cm³; osmolality 256 mOsm) is prepared by dissolving 7.1 g of Ficoll™ PM400 (Amersham Bioscience, Rosendaal, The Netherlands) and 9 g of sodium diatrizoate (Sigma) in 100 ml aqua dest.) and density gradient centrifugation (900 g for 30 min at room temperature (RT)) was performed. After centrifugation, cells from the interface were aspirated by pipette and diluted with an equal amount of Alsever’s solution (Gibco) (supplemented with 1% heat-inactivated homologous serum (HS) (Gibco), 1% penicillin (100 IU/mL) (Gibco) and streptomycin (100 μg/ml) (Gibco) and pelleted by centrifugation (550 g for 15 min at RT). When necessary, the remaining erythrocytes were lysed with ammonium chloride [0.8% (w/v)] for 10 min and the PBMC were again diluted with an equal amount of Alsever’s solution and pelleted by centrifugation (550 g for 10 min at RT). The PBMC were washed twice in incomplete RPMI [RPMI-1640 (Sigma) supplemented with 1% HS, penicillin (100 IU/ml) and streptomycin (100 μg/ml)] by centrifugation (400 g for 10 min at RT) and resuspension to remove platelets and to avoid clotting. The PBMC were adjusted to 3 x 10⁶ cells/ml in leucocyte medium [RPMI-1640 supplemented with 3% autologous serum, 2 mM l-glutamine (Gibco), 1 mM sodium pyruvate (Gibco), 1% non-essential amino acids (Gibco), 1% penicillin (100 IU/ml), 1% streptomycin (100 μg/ml) and 50 μM 2-mercaptoethanol (2-ME) (Gibco)].

**Isolation of PBMC**

The method for isolating canine PBMC was previously described (de Bruin et al., 2005). Briefly, diluted blood was supplemented with 10% (v/v) of a carbonyl iron/arabic gum mixture that was prepared as follows: a 10% (w/v) carbonyl iron suspension (Sigma, Bornem, Belgium) in 10 ml PBS supplemented with 1% penicillin (100 IU/mL) was washed four times with this solution (centrifugation for 10 min at 550 g and RT). Meanwhile, a 10% (w/v) arabic gum suspension (Sigma) in 10 ml PBS with 1% penicillin was prepared. Both were sterilized for a period of 15 min at 121°C. After cooling, equal amounts of the two suspensions were mixed.

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**Blood sampling**

A volume of 20 ml blood was aseptically collected from the jugular vein into 50 ml syringes containing 20 ml PBS (Gibco) supplemented with 1% penicillin (100 IU/ml) (Gibco), streptomycin (100 μg/ml) (Gibco) and heparin 150 IU/ml (Heparine Rorer, Aventis, Belgium) for the isolation of PBMC. In addition to the 20 ml of blood for lymphocyte isolation, 4 ml of blood was aseptically collected for the isolation of the autologous serum.

**Collagen**

Highly purified human collagen type I (Southern Biotechnology Associates Inc., Birmingham, AL, USA) was dialysed against phosphate-buffered saline (PBS) solution (Gibco BRL; Life Technologies, Merelbeke, Belgium) for 24 h at 4°C. The collagen was aliquoted and stored at ~18°C until use. Thawing was performed by incubation at 4°C.

**PBMC proliferation assays with collagen**

Cultures were established in 96-well flat-bottomed microtitre plates (Cell star®, Greiner bio-one, Wemmel, Belgium). One hundred microlitres of cell suspensions were cultured in the presence of 100 μl of the three collagen solutions at final concentrations of 5, 20 and 40 μg/ml in leucocyte medium and in medium without collagen (unstimulated control) for 6 and 7 days at 38°C in a humidified atmosphere of 5% CO₂. Eighteen hours before harvesting, cultures were pulsed with [³H]-thymidine (1 μCi/well; Amersham ICN, Bucks, UK). Harvesting occurred onto glass fibre filters (Filter Mat; Wallac, Turku, Finland) with an automatic Combi Cell harvester (Skatron Instruments, model 11025, Lier, Norway). The radioactivity incorporated into the DNA was measured using...
a β-scintillation counter (Perkin Elmer, Brussels, Belgium). Stimulation was performed in duplicates or triplicates, depending on the amount of PBMC isolated. Positive control cultures consisted of PBMC with the mitogen concanavalin A (ConA) (10 μg/ml) (Sigma) and were cultured for 24 h after which they were pulsed with [3H]-thymidine for another 18 h.

**Calculation of the stimulation index**

Significance of the lymphocyte proliferation was expressed by using the stimulation index (SI), which is calculated as follows:

\[
SI = \frac{\Delta CPMs}{\Delta CPMns}
\]

where ΔCPMs is the mean counts per minute of collagen-containing cultures and ΔCPMns is the mean counts per minute of collagen-free cultures.

Lymphocyte proliferation was regarded as significant if the SI ≥ 3.0.

**Statistics**

All data were analysed using the statistical software package Statistix 4.1 (Tallahassee, FL, USA). The Mann–Whitney rank sum tests were used to compare data, such as age, body weight and duration of the lameness between dogs with partial and complete CrCL rupture.

As the various data sets were not normally distributed, results were expressed as medians (range). The Mann–Whitney rank sum tests were used to compare the SI values between the different groups. Because of the small sample sizes in the different groups, the Fisher's exact probability test was used to compare the number of dogs with positive SI in the patient group versus the different control dogs, and between the control dogs themselves. Tested hypotheses were accepted if the P-value was ≤ 0.05.

**Results**

**Clinical parameters**

The breeds included in the different groups are summarized in Table 1. Mean age, body weight, presence of a meniscal tear, and duration of lameness of the different groups are shown in Table 2.

The healthy control group consisted of three neutered females. The sham-operated dogs consisted of one female and one male.

At the initiation of the study, three dogs (30%) had complete rupture of the CrCL, of which two (66.6%) also had concomitant medial meniscus tear. The remaining seven dogs (70%) had partial CrCL rupture, of which three (43%) also had a medial meniscus tear. Eight dogs (80%) ruptured their CrCL without history of severe trauma. Eight dogs were female, two of them were neutered. Two dogs were male, only one was neutered. The dogs in the partial CrCL rupture group were significantly heavier than the dogs with complete CrCL rupture (P = 0.02).

No clinical signs of lameness were noticed in the healthy control group. The sham operation dogs were slightly lame for 1–2 months post-operatively. The mean duration of lameness in the patient group was 3.3 months (range 1–12 months). The mean duration of lameness was longer, although not significantly, in the dogs with partial rupture (mean 4.0 months) compared with the dogs with complete rupture (mean 1.7 months) (P = 0.17).

**Lymphocyte proliferation to collagen type I**

Table 3 shows the results of the collagen type I-induced lymphocyte proliferation for PBMC of dogs with cruciate disease and of the control dogs (healthy dogs and sham-operated dogs). In addition, the most relevant clinical data are presented. Lymphocyte proliferation to ConA was high in a majority of the dogs analysed but showed considerable variation (data not shown).

**Healthy control dogs**

A total of nine assays were available from these dogs (Table 3). Three of the nine assays (33.3%) had an SI ≥ 3.0. The median SI for all these assays was 2.4 (range 0.5–4.8). The SI of these dogs decreased in time.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Breeds</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>CrCL patients</td>
<td>Golden retriever</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>American Stafford terrier</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Labrador retriever</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Boxer</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bernese mountain dog</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>German shepherd</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mixed breed</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of dogs</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Number of dogs with MMT</th>
<th>Duration of lameness (mth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CrCL-R</td>
<td>10</td>
<td>4.6 (1.0–9.5)</td>
<td>33.4 (25–58)</td>
<td>5</td>
<td>3.3 (1–12)</td>
</tr>
<tr>
<td>cCrCL-R</td>
<td>3</td>
<td>4.5 (1.4–9.5)</td>
<td>25.8 (25–28)</td>
<td>2</td>
<td>1.7 (1–2)</td>
</tr>
<tr>
<td>pCrCL-R</td>
<td>7</td>
<td>4.6 (1.0–8.2)</td>
<td>36.7 (28–58)</td>
<td>3</td>
<td>4.0 (1.3–12)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>3</td>
<td>3.3 (2.7–3.8)</td>
<td>13.3 (13–14)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Sham-operated dogs</td>
<td>2</td>
<td>3.0 (2.2 + 3.5)</td>
<td>23.5 (18 + 29)</td>
<td>0</td>
<td>1.5 (1 + 2)</td>
</tr>
</tbody>
</table>

CrCL-R, cranial cruciate ligament rupture; cCrCL-R, complete CrCL-R; pCrCL-R, partial CrCL-R; MMT, medial meniscus tear; mth, months; nk, not known.
Sham-operated dogs

From the two sham-operated dogs, a total of six lymphocyte proliferation assays were available. Four of the six assays (66.7%) had an SI ≥ 3.0. This was not significantly different compared with the healthy control dogs (P = 0.31). The median SI of this group was 3.5 (range 0.7–24.2), which was not significantly different compared with the healthy control dogs (P = 0.59). One of the sham-operated dogs (sham dog 1), only had one positive assay (of the three assays in total), whereas the other dog remained positive (sham dog 2), twice with relatively high SI.

Dogs with CrCL rupture

A total of eight lymphocyte proliferation assays to collagen type I were performed with seven dogs at the time of surgery or diagnosis of rupture (one dog was counted twice for the time of rupture as it was assayed at the time of surgery of the initially affected stifle and at diagnosis of rupture of the contralateral stifle joint). Five of these eight assays (62.5%) had an SI ≥ 3.0 after stimulation with collagen type I. This was not significantly different compared with the healthy control dogs (P = 0.15), or to the sham-operated dogs (P = 1.00). The median SI of all dogs sampled at operation time or diagnosis of rupture was 4.5 (range 1.1–12.1), which tended to be higher than the SI of the healthy control dogs (2.4; range 0.5–4.6). However, this was not significant (P = 0.14). The status of the CrCL rupture (partial or complete) was assessed intraoperatively in six of the eight cases. There were three cases with partial CrCL rupture and three cases with complete CrCL rupture. Only one of the three cases with partial CrCL rupture (33.3%) had an SI ≥ 3.0, whereas two of the three with complete rupture (66.7%) had an SI ≥ 3.0 (P = 1.00). The median SI of the cases with partial CrCL rupture was 2.5 (range 1.1–4.6) and of the cases with complete CrCL rupture was 7.2 (range 1.1–12.1), which was not significantly different (P = 0.42).

All dogs with CrCL rupture were tested repeatedly for lymphocyte proliferation against collagen type I at different time intervals. An increase in SI was seen in seven of the 10 dogs (dogs 1, 2, 3, 5, 7, 8, 10). Five of the 10 dogs sustained a CrCL rupture in the contralateral stifle joint during this study. One dog remained negative during the entire study (dog 1), one dog (dog 5) showed a slight increase in SI until rupture, one dog (dog 4) was positive before and at rupture of the contralateral CrCL. The other two dogs (dogs 2 and 3) only had a positive SI at the time of CrCL rupture of the contralateral stifle joint. The five dogs that did not sustain a CrCL rupture in the contralateral stifle joint during this study remained negative with exception to one dog which had a positive SI once after rupture (dog 10) that turned negative again.

Optimal stimulating conditions with collagen

No single dose of collagen was optimal for stimulating a lymphocyte proliferation in all dogs, although significantly more dogs responded with 20 μg/ml (P = 0.008). In addition, dogs with cruciate pathology had significantly more lymphocyte reactivity to 20 μg/ml than the control dogs (P = 0.011). Among the dogs with CrCL rupture, eight dogs responded optimally to 20 μg/ml and none to 5 and 40 μg/ml. Of the control dogs (healthy and shams), one responded optimally to 5 μg/ml, 2–20 μg/ml and 2-40 μg/ml. Optimal response was seen after 6 or 7 days culture.

Discussion

To the best of our knowledge, this is the first study investigating lymphocyte responsiveness to collagen in dogs with CrCL rupture. Our results demonstrate that sensitization to collagen type I not only occurs in dogs with cruciate disease, but also in sham-operated dogs and healthy dogs. This suggests that sensitivity to collagen type I in dogs is not joint

Table 3. Evaluation of lymphocyte proliferation to collagen type I in dogs with CrCL rupture, sham-operated dogs and healthy control dogs.

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Clinical diagnosis</th>
<th>Disease duration (mth) at day 0</th>
<th>Disease course</th>
<th>CrCL-R initial/contralateral stifle</th>
<th>Day 0</th>
<th>6 mth</th>
<th>12 mth</th>
<th>18 mth</th>
<th>24 mth</th>
<th>30 mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>CrCL-R</td>
<td>12</td>
<td>R-contra</td>
<td>12 mth</td>
<td>Partial/partial</td>
<td>1.9</td>
<td>1.8</td>
<td>2.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dog 2</td>
<td>CrCL-R</td>
<td>3</td>
<td>R-contra</td>
<td>12 mth</td>
<td>Partial/complete</td>
<td>2.5</td>
<td>12.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dog 3</td>
<td>CrCL-R</td>
<td>1</td>
<td>R-contra</td>
<td>12 mth</td>
<td>Complete/complete</td>
<td>1.5</td>
<td>6.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dog 4</td>
<td>CrCL-R</td>
<td>2</td>
<td>R-contra</td>
<td>18 mth</td>
<td>Partial/unknown</td>
<td>4.5</td>
<td>3.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dog 5</td>
<td>CrCL-R</td>
<td>0.5</td>
<td>R-contra</td>
<td>12 mth</td>
<td>Partial/unknown</td>
<td>1.1</td>
<td>2.6</td>
<td>3.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dog 6</td>
<td>CrCL-R</td>
<td>2</td>
<td>Stable &gt; 8 mth</td>
<td>18 mth</td>
<td>Complete/stable</td>
<td>2.6</td>
<td>0.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Dog 7</td>
<td>CrCL-R</td>
<td>2</td>
<td>Stable &gt; 18 mth</td>
<td>18 mth</td>
<td>Partial/stable</td>
<td>–</td>
<td>–</td>
<td>1.2</td>
<td>2.5</td>
<td>–</td>
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<tr>
<td>Dog 8</td>
<td>CrCL-R</td>
<td>2</td>
<td>Stable &gt; 18 mth</td>
<td>18 mth</td>
<td>Complete/stable</td>
<td>–</td>
<td>–</td>
<td>1.8</td>
<td>2.1</td>
<td>–</td>
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<tr>
<td>Dog 9</td>
<td>CrCL-R</td>
<td>4</td>
<td>Stable &gt; 18 mth</td>
<td>18 mth</td>
<td>Partial/stable</td>
<td>4.6</td>
<td>1.4</td>
<td>UR</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Dog 10</td>
<td>CrCL-R</td>
<td>4</td>
<td>Stable &gt; 30 mth</td>
<td>18 mth</td>
<td>Partial/stable</td>
<td>–</td>
<td>0.7</td>
<td>0.25</td>
<td>0.89</td>
<td>5.7</td>
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<td>Sham dog 1</td>
<td>Sham</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.9</td>
<td>UR</td>
<td>1.3</td>
<td>–</td>
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<tr>
<td>Sham dog 2</td>
<td>Sham</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11.2</td>
<td>3.1</td>
<td>24.2</td>
<td>–</td>
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<tr>
<td>Control dog 1</td>
<td>Healthy</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.1</td>
<td>3.7</td>
<td>1.3</td>
<td>0.5</td>
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<tr>
<td>Control dog 2</td>
<td>Healthy</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.8</td>
<td>1.9</td>
<td>1.8</td>
<td>–</td>
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<td>Control dog 3</td>
<td>Healthy</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.4</td>
<td>2.1</td>
<td>–</td>
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</tbody>
</table>

CrCL, cranial cruciate ligament; CrCL-R, CrCL rupture; mth, months; R-contra, rupture of the contralateral CrCL; †Died; UR, unreliable; –, not tested at this time-point.
disease specific. The same has been suggested in humans, as PBMC isolated from patients with different arthropathies, and healthy controls, also proliferate when cultured with different types of collagens (Stuart et al., 1976; Smolen et al., 1980; Golds et al., 1983; Elkayam et al., 1991; Snowden et al., 1997; Kim et al., 1999).

An increase in proliferation to collagen type I was seen at the time of CrCL rupture in three of the five dogs, meaning that the number of lymphocytes with reactivity to collagen type I amplified over time in these patients, whereas a decrease was seen in the healthy control dogs and one of the sham-operated dogs. This difference in proliferation between these groups might be due to a pre-clinical CrCL rupture in the contralateral stifle joint in the cruciate disease group. Yet, no difference in lymphocyte reactivity to collagen type I could be found between dogs that sustained a CrCL rupture in their contralateral stifle joint and dogs that did not during this study. An explanation might be found in recent studies from human patients with RA studying paired PBMC and synovial fluid mononuclear cells (SFMC) samples cultured with collagen type II. These studies demonstrated that the T-lymphocyte responses were more frequent and vigorous in SFMC than in PBMC (Kim et al., 1999; Park et al., 2001; Kim and Kim, 2005) which might suggest that these collagen-specific T-lymphocytes home to joints. We have not investigated SFMC reactivity to collagen type I in this study, however, it would be worthwhile to test this concept in the future as it could result in a clearer differentiation of the immune response in dogs that will sustain a CrCL rupture in their contralateral stifle joint and dogs that will not.

In conclusion, our study demonstrates that lymphocyte reactivity to collagen type I does occur in dogs with CrCL rupture especially at the time of symptomatic rupture. However, some of the sham-operated dogs and the healthy dogs tested positive as well. From our results it is difficult to determine whether the presence of a cellular immune response to collagen type I plays an initiating role in cruciate degradation or whether it evolves after ligament damage. Future studies should focus on examining reactive lymphocytes isolated from synovial fluid.

References


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