Toll-like receptor expression in synovium from three different canine osteoarthritis models
+1Kuroki, K; 1Stoker, AM; ... The synovium was covered by a thin layer of synoviocytes. Nuclei were counter stained with DAPI (blue fluorescence).

INTRODUCTION

The Toll-like receptors (TLRs) are membrane proteins that are homologous to the Toll host defense system first identified in Drosophila and stimulate innate immune responses via recognition of a variety of microbe-derived molecules. In addition to recognizing microbes through microbe-associated molecular patterns (MAMPs), TLRs also recognize endogenous ligands called damage-associated molecular patterns (DAMPs). DAMP molecules, such as heat-shock proteins, high-mobility group box 1 protein, hyaluronan, fibronectin, and fibrinogen, are recognized by TLR-2 and/or TLR-4. Activation of TLR pathways can induce transcription factors, resulting in inflammatory responses characterized by gene transcription of cytokines, chemokines, inducible nitric oxide synthase and proteinases, and may provoke autoimmune responses and tissue injuries. Recently, increased attention has been given the roles of TLRs in various inflammatory conditions, while there has been little focus on TLRs in osteoarthritis (OA). Further study is needed to confirm involvement of TLRs in OA and delineate their roles in the disease mechanism. The present study was designed to be a first step in this process by examining synovial tissues from canine stifles (knees) with or without OA for the presence of TLR-2 and TLR-4 expression. The study hypothesis was that TLR-2 and TLR-4 gene expression levels would be significantly higher in synovial tissues obtained from OA joints than normal joints.

METHODS

All procedures were approved by the animal care and use committee (ACUC). Twenty-one purpose-bred female hounds dogs were used for surgical induction of OA. All dogs underwent one of fourarthroscopic procedures on one knee joint: transection of anterior cruciate ligament (ACLt) (n=5), creation of full-thickness grooves in the medial femoral condyle cartilage using a 3 mm diameter ring curette (GR) (n=6), meniscal release by transection at the caudal horn junction with the caudal meniscotibial ligament (MR) (n=5) or probe manipulation of all joint landmarks without insult (SHAM) (n=5). The non-operated, contralateral knee joints served as an internal control for each procedure group. Twelve weeks after surgery, dogs were euthanatized and synovial tissues were collected from each knee for real-time reverse transcription-polymerase chain reaction (PCR) for TLR-2 and TLR-4. Gene expression levels were determined by comparison to the house keeping gene Glyceraldehydes-3-phosphate dehydrogenase. Data from each procedure group were combined and mean ± standard errors (SEM) were determined. A one-way ANOVA was performed to determine differences among procedure groups with respect to TLR-2 and TLR-4 expression levels, respectively. When significant differences among groups were obtained, Tukey’s pairwise comparison test was performed to determine which groups were different from each other. Significance was set at $p < 0.05$.

RESULTS

Meaningful RNA was successfully yielded from both operated and non-operated knee joints of 18 dogs (4 CrCLt, 6 GR, 3 MR, and 5 SHAM). TLR-4 gene expression levels in synovial tissues obtained from ACLt operated knee joints were significantly higher than the contralateral non-operated knee control or any other operated and non-operated groups ($p < 0.01$) (Figure 1). In contrast, there were no significant differences in TLR-2 gene expression levels among the procedure groups and control groups (Figure 1). In order to confirm TLR-4 expression at the protein level, immunofluorescence with the use of a TLR-4 antibody was conducted. TLR-4 immunofluorescence expression was observed in the synovial cells lining the synovium obtained from all the three dogs with knee OA secondary to CrCL insufficiency (Figure 2). TLR-4 immunofluorescent expression was rarely observed in the synovial tissues obtained from the dog with non-osteoarthritic apparently healthy knee joints (Figure 3).

DISCUSSION

The present study clearly demonstrated that gene expression levels of TLR-4 are significantly elevated in synovial tissues in canine osteoarthritic joints secondary to the transection of the ACL. The dogs used for the gene expression analyses were purpose-bred research dogs and the possibility of microbial joint infection which may activate TLRs in response to MAMP molecules is unlikely. In addition, we did not see elevated expression of TLR-4 in the purpose-bred research dogs with other insults to the joints or their contralateral joints. Moreover, there was no difference in the expression level of TLR-2 gene among all procedure groups and non-operated control groups. This suggests that something unique to ACL insufficiency, whether it be exposed ligament, marked joint instability, and/or severity of the OA, incites the TLR-4 pathway as part of the mechanism of disease. This is further verified by TLR-4 expression as determined by immunofluorescence in synovial tissues obtained from client-owned dogs with OA secondary to ACL insufficiency. Microscopic changes in synovial tissues in knee OA can vary from little discernible change to synovial hyperplasia with lymphoplasmacytic inflammation. What determines the apparent involvement of the inflammatory responses in at least a subset of OA pathology is an important question to be answered. Activation of TLR pathways may be a key innate immune response that initiates and perpetuates the adaptive immune responses with persistent inflammation in a portion of OA patients. It is warranted to elucidate the specific roles of TLR pathways in the disease mechanisms of OA.