EXPRESSION OF IMMUNE RESPONSE GENES IN CANINE INFLAMMATORY KNEE ARTHRITIS/DEGENERATIVE ANTERIOR CRUCIATE LIGAMENT RUPTURE

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INTRODUCTION

Chronic synovitis is an important factor promoting progressive degeneration of synovial joints over time. In the dog, persistent synovitis and development of inflammatory knee arthritis is likely an important factor promoting degenerative rupture of the anterior cruciate ligament (ACL), a naturally occurring condition of most breeds of dogs [1-2]. Synovial pathology in this canine condition is similar to human inflammatory arthritides, such as rheumatoid arthritis (RA). In RA, dysregulation of antigen-specific immune responses is a key feature promoting synovitis. Inflammatory changes in ruptured human ACL are less extensive [3].

To further understanding of the pathogenesis of joint inflammation in this canine knee arthritis model, we determined the pattern of immune response gene expression in dogs with inflammatory knee arthritis/degenerative ACL rupture. We hypothesized that immune-response gene expression would be up-regulated in dogs with inflammatory knee arthritis.

METHODS

**Blood and Joint Tissue Samples.** Peripheral blood and synovial fluid samples were collected from 32 dogs with inflammatory knee arthritis/ACL rupture during surgery, 8 healthy dogs with normal knees and intact ACL, and 9 dogs without ACL rupture and with degenerative osteoarthritis of a large synovial joint. Procedures were conducted with the approval of the Animal Care Committee of the University of Wisconsin-Madison. Peripheral blood mononuclear cells (PBMC) were isolated using commercial cell separation tubes (BD Vacutainer™ CPT™, Becton Dickinson, Franklin Lakes, NJ). Synovial fluid cells were isolated by centrifugation.

**Quantitative RT-PCR.** mRNA expression was quantified in PBMC and synovial fluid cells. Total RNA was isolated using standard RNAzol B methodology. Quantitative RT-PCR (qRT-PCR) was performed using a BioRad real-time thermocycler and commercially available SYBR green kits. Oligonucleotide primers for the genes of interest were designed for antigen-specific immune response genes (cathepsin S, tartrate-resistant acid phosphatase (TRAP), and invariant chain) and matrix turnover genes (cathepsin K, MMP-9, cathepsin S). PCR reactions were performed in duplicate.

**Data Analysis.** For each sample, the threshold cycle (Ct, values) obtained from the exponential region of the PCR amplification plot from the duplicate trials were averaged together. Relative gene expression for each of the genes-of-interest was then calculated using the ΔΔCt method [4]. PBMC gene expression was used as an internal control and the 18S rRNA gene was used as the housekeeping gene. Relative mRNA expression was calculated as 2^(-ΔΔCt). After log-transformation, a Student’s t test with a hypothesized mean equal to zero was used to determine whether synovial fluid gene expression was significantly different from PBMC (internal control). One-way ANOVA and a post-hoc t test were used to determine differences between groups. Differences were considered significant at \( P < 0.05 \).

RESULTS

Results are summarized in Figure 1A-E. Relative expression of the MMP-9 (196-fold), TRAP (35-fold), and invariant chain (14-fold) genes was significantly increased in the knee synovial fluid of dogs with ACL rupture, when compared with the knees of normal dogs \( (P \leq 0.05) \). In contrast, relative expression of all of the genes-of-interest in synovial fluid from joints affected with osteoarthritis was not significantly different, when compared with the knees of normal dogs. In the ACL rupture dogs, expression of cathepsin K (167-fold), MMP-9 (4053-fold), cathepsin S (60-fold), TRAP (51-fold), and invariant chain (15-fold) was significantly increased in knee synovial fluid, when compared with the internal PBMC control. In normal dogs, only cathepsin S (10-fold), cathepsin K (24-fold), and MMP-9 (21-fold) were significantly increased in knee synovial fluid, when compared with the internal PBMC control \( (P < 0.05) \). In dogs with osteoarthritis, only expression of cathepsin K (254-fold) and MMP-9 (208-fold) were increased in synovial fluid, when compared with the internal control \( (P < 0.05) \).

DISCUSSION

Although it is generally accepted that dysregulation of local immune responses within joints is a key factor in the development of persistent synovitis and progressive joint degradation, the immune mechanisms involved are poorly understood and likely complex. In normal dogs and dogs with osteoarthritis, matrix turnover genes were primarily up-regulated in joint tissues, when compared with PBMC internal controls, whereas both antigen-specific immune response genes and matrix turnover genes were up-regulated in dogs with inflammatory knee arthritis/degenerative ACL rupture. In particular, expression of TRAP and invariant chain was increased in dogs with the ACL rupture arthropathy, when compared with normal dogs. Levels of expression of these genes were also higher when compared with dogs with osteoarthritis \( (P < 0.05 \text{ and } P = 0.1, \text{ respectively}) \). Taken together, these data suggest that levels of expression of TRAP and invariant chain genes may be a useful biomarker for inflammatory arthritis in this canine model. Although, the key triggering antigens are not known, it is interesting to note that expression of TRAP in macrophages is thought to have a key role in immune clearance of bacteria [5]. These findings also suggest that antigen-specific immune responses are, at least in part, an important factor in the pathogenesis of synovitis in this model.

REFERENCES