DETECTION OF A RANGE OF BACTERIAL SPECIES FROM THE KNEE JOINTS OF DOGS WITH ARTHRITIS/DEGENERATIVE ANTERIOR CRUCIATE LIGAMENT RUPTURE

INTRODUCTION

Bacteria are often found in joints affected with inflammatory arthritis. Mixtures of bacteria within joints have been implicated as a causative factor in the pathogenesis of arthritis. Polymicrobial populations of bacteria within synovial joints likely increase the risk that bacteria trigger immune system dysregulation and development of persistent synovitis and associated joint degeneration.

Inflammatory knee arthritis/degenerative ACL rupture is a common naturally occurring condition of the dog. In this disease, the knee synovium is infiltrated with a mixed population of inflammatory cells, which include B & T lymphocytes, macrophages, and dendritic cells [1]. Furthermore, expression of immune response genes is up-regulated in affected joints [2]. These findings suggest that antigen-specific immune responses are likely important in the pathogenesis of synovitis in this canine arthropathy. To further understand of the pathogenesis of synovitis in this canine inflammatory knee arthritis model, we determined whether bacteria could be detected in affected joints. We hypothesized that bacterial DNA would be detectable in dogs with inflammatory arthritis/degenerative ACL rupture, but not dogs with normal knees or experimentally-induced ACL rupture.

METHODS

Dogs. Experiment #1. Synovial membrane specimens were collected during surgical treatment of 43 dogs for ACL rupture and associated knee instability. In addition, specimens of synovium were collected from 12 normal dogs with intact ACL, and 16 Pond-Nuki dogs in which unilateral ACL rupture was induced experimentally in a normal knee for a period of 19 weeks. Experiment #2. Both synovial fluid and synovial membrane specimens were collected at surgery from a further 51 dogs with inflammatory knee arthritis/degenerative ACL rupture. Dogs with PCR-positive joints were treated with doxycycline at 5mg/kg bid orally for 10 weeks and follow-up synovial fluid specimens were then collected aseptically by percutaneous needle aspiration. Procedures were conducted with the approval from the Animal Care Committee of the University of Wisconsin-Madison.

Specimen collection and PCR for Bacterial DNA. Experiment #1. Synovial membrane specimens were collected aseptically during surgery. For the initial experiment, after extraction of DNA, a non-nested broad-range ‘panbacterial’ PCR method was used for detection of DNA from the bacterial 16S rRNA gene. The PCR system uses consensus primers that hybridize to a highly conserved region of the gene [3]. All PCR reactions were performed in a laminar flow hood and extensive precautions were taken to prevent contamination of the PCR system. PCR products were examined in UV light after electrophoresis on a 1.5% agarose gel and staining with ethidium bromide. PCR-positive specimens were cloned, and at least 4 clones were prepared from each synovial membrane specimen. Bacterial species were identified by a BLAST search of Genbank. In addition species-specific non-nested PCR methods were used to detect DNA from the Borrelia burgdorferi outer surface protein A (OspA) and 66-kDa protein (p66) genes. PCR-positive specimens were sequenced to confirm specificity. Experiment #2. Synovial fluid cells were centrifuged to isolate the cell pellet. The ‘panbacterial’ 16S rRNA and OspA PCR methods were used to test both synovial membrane and synovial fluid cell specimens for bacterial DNA sequences before and after doxycycline treatment. PCR products were examined in UV light after electrophoresis on a 1.5% agarose gel and staining with ethidium bromide.

Statistics. The Fisher’s Exact test was used to compare the proportion of dogs in each group with bacteria-positive knee tissues. Values of P were one-sided. Values of P < 0.05 were considered significant.

RESULTS

Dogs with bacterial DNA-positive knees. In Experiment #1, the presence of bacterial DNA in the knee synovium was significantly associated with the inflammatory knee arthritis/degenerative ACL rupture arthropathy. Synovial membrane specimens from 16 of 43 dogs with the ACL rupture arthropathy (37%) were PCR-positive. A 16S rRNA PCR product was found 14 dogs. Of these dogs, knee synovium from four dogs was also positive for Borrelia burgdorferi DNA. The remaining two dogs were positive for OspA only. None of the 12 specimens collected from dogs with normal knees and intact ACL were PCR-positive. One of the 16 synovial specimens collected from Pond-Nuki dogs was positive for OspA.

Response to doxycycline therapy. In Experiment #2, when PCR of both synovium and synovial fluid was performed in dogs with the ACL rupture arthropathy, 47% of knees were PCR-positive. Both 16S rRNA and OspA DNA were detectable with similar frequencies in synovium (16S rRNA – 33%; OspA – 5.9%) and synovial fluid (16S rRNA – 37%; OspA – 5.9%). Again, OspA DNA sequences were usually detectable together with 16S rRNA DNA sequences (4 of 6 dogs).

For 16S rRNA PCR-positive joints, doxycycline had a significant positive treatment effect (P < 0.05). In 6 dogs with 16S rRNA-positive joints before treatment, follow-up synovial fluid specimens collected after doxycycline treatment were PCR-negative, whereas one dog that was initially OspA PCR-positive remained PCR-positive.

Bacterial species identified in dogs with degenerative ACL rupture arthropathy. DNA from a wide range of bacterial species was identified in the dogs with the degenerative ACL rupture arthropathy. By sequencing of multiple clones, mixtures of bacteria were identified in 13 of 14 dogs. In addition to Borrelia burgdorferi (14% of dogs), other organisms commonly identified were uncultured Eubacterium species (21%), Stemmotrophomonas maltophilia (14%), Rhizobium radiobacter (9%), and Ralstonia solanacearum (7%).

DISCUSSION

DNA from a wide variety of bacterial species can often be found in the synovium of arthritic joints from human patients [3]. 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 degradation of synovial joints, the immune mechanisms involved are poorly understood and likely complex.

We found a significant association between the presence of bacterial DNA within synovium and the canine degenerative ACL rupture arthropathy. With analysis of both synovium and synovial fluid, the proportion of affected dogs with PCR-positive knees was 47%, a higher proportion compared with equivalent human studies [3]. In healthy dogs with normal knee joints, all joints were PCR-negative, suggesting that the normal canine knee is sterile. Our data from healthy dogs with induced ACL rupture suggest that translocation of mixtures of bacteria to the knee is not simply a consequence of joint instability. Borrelia burgdorferi was the only recognized joint pathogen identified. Borrelia burgdorferi was most often found together with mixtures of environmental bacteria, suggesting that mixtures of bacteria may be important for development of synovitis. Chronic Lyme arthritis develops preferentially in humans with specific MHC class II genotypes. Since the MHC class II gene complex of dogs models that of humans, a similar genetic susceptibility may exist in the dog.

OspA contamination is a possible explanation for identification of Borrelia burgdorferi in one Pond-Nuki dog and two degenerative ACL rupture arthropathy dogs that were ‘panbacterial’ PCR negative. Our PCR methods did not allow determination of organism viability. However, elimination of the 16S rRNA PCR product from joints after treatment with the bacteriostatic drug doxycycline suggests that mixtures of environmental bacteria within joints are viable. We hypothesize that the presence of mixtures of environmental bacteria and bacterial DNA within synovial joints is a key factor triggering persistent synovitis and progressive ACL rupture in this model; it is known that chronic synovitis degrades ACL structural properties [4].

REFERENCES


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