Pro-inflammatory cytokine expression in synovium, synovial fluid, and articular cartilage of naturally osteoarthritic equine carpi

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Introduction:
Many cytokines including tumor necrosis factor alpha (TNFα) and interleukin one beta (IL-1β) are increased in osteoarthritic joints. They bind at different receptors but cause a similar increase in NFκB and JNK. These intermediates then increase the transcription of matrix metalloproteinases, cyclooxygenase-2, and inducible nitric oxide synthase. The end result is increased cartilage degradation and prostaglandin (PGE2) release leading to joint swelling and pain. Understanding cytokine changes during the onset and progression of osteoarthritis (OA) is a critical factor in the development of biomedical therapies.

The purpose of this study was to define pro-inflammatory cytokine alterations in synovium, synovial fluid, and cartilage during the progression from normal articulations to early and late stages of osteoarthritis.

Methods:
Equine carpi from horses age 2 to 11 years were used. Horses included those undergoing surgery for clinical osteochondral carpal fragmentation and various degrees of secondary arthritis, as well as horses being submitted to the pathology service for necropsy. Carpal joints were grouped as normal, or as mild, moderate, or severe OA according to gross and histologic examination.

Tissues were harvested at surgery or euthanasia, and RNA was isolated for qRT-PCR analysis. TNFα, IL-1β, aggrecanase 1 (ADAMTS-4), aggrecanase 2 (ADAMTS-5), matrix metalloproteinase 13 (MMP-13), interleukin 17 (IL-17), and collagen one alpha (Col-Iα) expression was determined in synovium. TNFα, IL-1β, ADAMTS-4, ADAMTS-5, MMP-13, IL-17, collagen two B (Col-IIb), and aggrecan expression was determined in cartilage. TNFα level in the synovial fluid was determined by enzyme-linked immunosorbent assay (ELISA).

Cytokine expression data and protein levels of the normal, mild OA, moderate OA, or severe OA groups were compared. A two-way analysis of variance (ANOVA) and Tukey’s and LSD post-hoc tests were performed on the qRT-PCR values.

Results:
Thirty-six horses with OA joints and 22 horses with normal joints were included in the synovium studies. Twenty-one horses with OA joints and 8 horses with normal joints were included in the cartilage studies. Thirty-one horses with OA joints and 22 horses with normal joints were included in the synovial fluid study.

Figure 1. Histology using H&E staining of synovium and cartilage from a severely affected osteoarthritic joint.

Significant increases in cytokine levels in OA synovium samples were seen for TNFα (p=0.01), ADAMTS-5 (0.0016) and MMP-13 (0.01). (Figures 3 and 4)

Significant increases in cytokine levels in OA cartilage samples were seen for TNFα (p=0.0007), IL-1β (p<0.001), ADAMTS-4 (p=0.0011), and MMP-13 (p<0.001). (Figures 1 and 5)

No expression of IL-17 was seen in either normal nor OA synovium and cartilage. In all cases where a significant difference in cytokine levels was noted on RT-PCR, the moderate OA mean value was the highest of all of the groups, and mean values decreased slightly from moderate to severe cases.

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