Responses of Articular Cartilage and Synovial Tissue to Impact Injury and Inflammation

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Introduction:
Osteoarthritis (OA) is a debilitating disease associated with progressive loss of articular cartilage. Tissue injury from supraphysiological loads and joint inflammation are two factors known to play key roles in the development and progression of OA. Both of these factors are also associated with increased degradative enzyme activity that directly depletes the extracellular matrix of articular cartilage. While loss of cartilage is considered the hallmark of OA, all articular tissues contribute to the development and progression of OA, and perhaps most importantly, to the pain and dysfunction experienced by OA patients. However, the relationships among inflammation, supraphysiological load and the production and regulation of degradative enzymes by different articular tissues are poorly understood. Therefore, this study was designed to assess the effects of cytokine stimulation and impact injury on cartilage and synovial tissues with respect to matrix metalloproteinases (MMP) production, MMP activity, and ADAMTS4 activity in vitro.

Methods:
Tissue collection: Articular cartilage tissue was obtained from the humeral head and synovial tissue was obtained from the knee of 13 dogs that were euthanatized for reasons unrelated to this study. Explants of these tissues were created using a 6mm (cartilage) or 4mm (synovium) dermal punch and cultured alone (CART or SYN) or together as co-culture (CO).

Tissue culture: For the inflammation model, explants were treated with 50ng/ml IL-1ß, and to simulate a traumatic injury to the joint, a single impact load was applied to the cartilage explant as described below. The explants from each dog were randomly assigned to the following groups: Inflammation (1) CART 50 (2) SYN 50 (3) CO 50; Injury (4) CART IM (5) CO IM; or Both (6) CART IMC (7) CO IMC. Explants cultured without cytokine treatment or impact injury served as negative controls (8) CART N, (9) SYN N, and (10) CO N. Culture media were collected and changed every 3-4 days for biomarker analysis. Tissues were cultured up to 21 days in 2ml of DMEM at 37°C.

Impact Injury: An Instron 8821S servo-hydraulic testing machine was used to apply a single impact load to the tissue. The cartilage explant was placed in a stainless steel well (6mm diameter by 2.54mm deep) and a 3.9mm diameter flat punch attached to the ram was used to measure the thickness of the explant. The thickness measurement was used to calculate the parameters to apply a 75% strain impact at 100mm/sec.

Media Biomarker Analysis: Media were analyzed for the concentration of MMP-1, MMP-2, and MMP-3 using a multiplex assay.

Data Analysis: All statistical analyses were performed using a computer software program (Sigma Plot®, San Rafael, CA). A Student’s t-test was performed to determine differences between groups with significance set at p<0.05.

Results:
MMP Concentration: Under basal and cytokine stimulated conditions, the synovial tissue released significantly higher concentrations of MMP-1, MMP-2, and MMP-3 to the media compared to the cartilage tissue. Further, when the cartilage and synovial tissues were co-cultured, there was not a consistent significant difference between the CO groups and SYN groups for MMP production. Cytokine stimulation significantly increased the production of MMP-3 in CART 50, SYN 50, and CO 50 compared to their respective controls. Cytokine stimulation also significantly increased the production of MMP-1 in the CART 50 and CO 50 group compared to their respective controls, but not in the SYN 50 group. Impact injury to the cartilage tissue did not result in a significant increase in any of the MMPs tested compared to CART N group. Additionally, combining impact and cytokine stimulation did not result in a significant increase in MMP production compared to the respective cytokine-only treatment groups.

MMP Activity: Under basal conditions, the SYN N group had significantly higher levels of MMP activity compared to the CART N group. Additionally, the SYN N group had significantly higher levels of MMP activity compared to the CO N group. MMP activity increased significantly in all cytokine-treated groups compared to their respective controls. Impact injury did not significantly affect MMP activity in cartilage tissue, and the combination of impact injury and cytokine stimulation did not result in a significant increase in MMP activity compared to cytokine-only treatment.

ADAMTS4 Activity: Under basal conditions, the SYN N group had significantly higher levels of ADAMTS4 activity compared to the CART N group. Additionally, the SYN N group had significantly higher levels of ADAMTS4 activity compared to the CO N group. Cytokine stimulation did not stimulate a significant increase in ADAMTS4 activity in the SYN 50 and CART 50 groups compared to
their respective controls, but there was a significant increase in ADAMTS4 activity in the CO 50 and CO IMC groups compared to the CO N group. Impact injury alone did not stimulate an increase in ADAMTS4 activity in the CART IM and CO IM groups compared to their respective controls.

**Discussion:** These data indicate that synovial tissue is a primary source of degradative enzymes (MMPs and ADAMTS4) within the joint. Synovial explants produced higher levels of MMPs, and MMP and ADAMTS4 activities were higher in synovial cultures compared to cartilage cultures. Intriguingly, when cartilage and synovial tissues were co-cultured, there were not significant decreases in production of MMPs, but there was a significant decrease in MMP and ADAMTS4 activities. These findings suggest that articular cartilage produces higher levels of factors that can suppress the activity of degradative enzymes present in the joint, such as tissue inhibitors of matrix metalloproteinases (TIMPs), in order to regulate extracellular matrix turnover in an attempt to protect itself from degradation. As expected, cytokine treatment significantly increased production and activity of MMPs in this model. However, it was surprising to find that ADAMTS4 activity was significantly increased in the cytokine-treated co-culture groups, but not the mono-culture groups. Further study is required to elucidate this important finding and determine why the loss of ADAMTS4 regulation occurs in co-culture. It was also surprising that impact injury did not result in significant increases in degradative enzyme production or activity in this model. It is possible that the level of strain used was so destructive to the tissue that lack of cell viability influenced the relative production of degradative enzymes. Taken together, these data begin to characterize the relative production and regulation of degradative enzymes by cartilage and synovium in response to inflammation and injury. Ongoing research is aimed at further characterizing articular tissues’ responses to insults that result in OA and their interactions in vitro and in vivo.

**Significance:** These data continue to characterize the relative production and regulation of degradative enzymes by cartilage and synovium in response to inflammation and injury.

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**References:**

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