Effect of IL-1β on Cytokine Production by Normal Canine Cartilage and Synovial Tissue in vitro

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Introduction
Osteoarthritis (OA) is a debilitating and progressive disease which results in the erosion and loss of articular cartilage. The development of OA is a complex process involving biomechanical, degradative, and inflammatory processes affecting multiple tissues within the joint. The cytokine IL-1β has been shown to play clinically important roles in the development and progression of OA. Treatment of cartilage explants and chondrocyte cell cultures with IL-1β has been reported to induce cell death, cartilage tissue proteoglycan loss, and increased release of PGE2, NO, and degradative enzymes in vitro. However, the majority of previously published work investigated only cartilage in culture, which does not address the complex interactions that occur among tissues in the joint during development and progression of OA. In an attempt to better mimic the in vivo joint environment in vitro, our laboratory has developed a cartilage and synovial tissue co-culture model. In this model, IL-1β is used to induce osteoarthritic changes in the co-cultured tissues. The present study focuses on understanding the roles of cartilage and synovial tissue in the pathogenesis of OA and identifying relevant biomarkers for disease mechanisms using this model. Our specific objectives were 1) to compare release of specific cytokines after IL-1β treatment of cartilage and synovial tissues cultured separately and together, and 2) to identify biomarkers for the screening of potential treatments of OA using the in vitro co-culture model.

Methods
Tissue Harvest and Culture: Full thickness articular cartilage (CART) and synovial (SYN) tissue was harvested from dogs (n=6) euthanized for reasons unrelated to this study, and 4mm CART and SYN tissue explants were created for culture. CART and SYN tissues were cultured alone as a mono-culture (C or S) or together as a co-culture (CO) in media supplemented with ITS serum replacement and with (P) or without (N) rhIL-1β (100ng/ml). Media were changed and collected every 3 days for biomarker analysis through 9 days of culture.

Media and Tissue Analysis: Media were tested for IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, GM-CSF, IP-10, IFNγ, KC, MCP-1, and TNFα by Luminex multiplex assay (Millipore). At the end of culture, tissues were papain digested and analyzed for DNA content using the PicoGreen Assay (Invitrogen). Cytokine data were standardized to the DNA content of the tissue analyzed for significance by one-way ANOVA with significance set at p<0.05.

Results
Undetected Analytes: The concentrations of IL-2, IL-4, IL-7, IL-10, IL-15, IL-18, IP-10, IFNγ, KC, MCP-1, and TNFα by Luminex multiplex assay (Millipore). At the end of culture, tissues were papain digested and analyzed for DNA content using the PicoGreen Assay (Invitrogen). Cytokine data were standardized to the DNA content of the tissue analyzed for significance by one-way ANOVA with significance set at p<0.05.

Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF): The production of GM-CSF was only consistently detected in the CO group on day 3, and after day 3 GM-CSF was only detected in two P-CO treated samples in the co-culture group. However, the P-CO group released significantly more GM-CSF to the media compared to the N-CO group on day 3.

Keratinocyte Chemotactic Factor (KC): KC production was highest on day 3 in all groups. Further, IL-1β treatment resulted in significantly higher KC production in the P-C group at all time points and in the P-CO group at days 6 and 9 compared to the corresponding control. There was no significant difference between the relevant C and S groups or the P-S and N-S groups at any time point, except the N-S group was significantly higher than the N-C group on day 9.

Interleukin (IL)-6 (Fig. 1-A): IL-6 was significantly higher in the CO group compared to the relevant C and S groups, and the P-CO group was significantly higher than the N-CO group at all time points. The concentration of IL-8 in the P-C group was significantly higher than the N-C group at all time points. There was no significant difference between the relevant C and S groups or the P-S and N-S groups at any time point, except the N-S group was significantly higher than the N-C group on day 9.

Interleukin (IL)-8 (Fig. 1-B): Of the cytokines tested, IL-8 had the highest media concentration. IL-8 was significantly higher in the CO groups compared to the relevant C and S groups, and the P-CO group was significantly higher than the N-CO group at all time points. The concentration of IL-8 in the P-C group was significantly higher than the N-C group at all time points. There was no significant difference between the relevant C and S groups or the P-S and N-S groups at any time point, except the N-S group was significantly higher than the N-C group on day 9.

Discussion
The data from this study indicate that the production of KC, MCP-1, IL-8, and IL-6 is consistently increased in response to IL-1β treatment in the co-culture model, identifying them as candidate biomarkers for the assessment of potential treatments using this model. These data also illustrate that culturing cartilage and synovial tissues together results in additive or synergistic increase in the basal and IL-1β stimulated production of these cytokines by the tissues in vitro. This effect was most pronounced for IL-8 and MCP-1, but was also evident for IL-6 and KC after IL-1β stimulation. These data also suggest that the increase in IL-8, KC, and MCP-1 observed in the synovial fluid of OA patients clinically may primarily be due to cartilage production, but factors released by the synovium may exacerbate this inflammatory response by the cartilage tissue in vivo. The low production of GM-CSF early in the model appears to implicate the synovial tissue as the primary source of this chemokine, but further study is required to determine if this is an accurate conclusion. The high production of KC on day 3 in all samples relative to subsequent days indicates that KC is responsive to the tissue damage resulting from tissue harvest and explant creation. Taken together, these data indicate that cartilage is more responsive than synovium in vitro to IL-1β for the cytokines and chemokines tested. Further, there is communication between the cartilage and synovial tissues that results in increased basal and IL-1β stimulated production of clinically relevant cytokines and chemokines tested in this study. Finally, co-culturing cartilage and synovial tissues in vitro may better mimic the in vivo response of both tissues to cytokine stimulation and OA development.