Comparison of the Response of Infrapatellar Fat Pad and Synovium to IL-1β and TNF-α *in vitro*

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Introduction

Osteoarthritis (OA) is a common, complex degenerative disease affecting humans and animals alike. Despite its prevalence, there is still much unknown regarding the pathogenesis and pathobiology of this disease. Two components of the knee that appear to be particularly important in maintaining the health and contributing to pathology of the joint are the synovium (SYN) and infrapatellar fat pad (IPFP). IL-1β and TNF-α are known to induce inflammation in many tissue types. In this study, we hope to compare the responses of SYN and IPFP to IL-1β and TNF-α stimulation to gain a better understanding of the role these two tissues play in the pathogenesis of OA.

Objective

To compare the specific responses of the IPFP and SYN as they respond to inflammation induced by IL-1β and TNF-α.

Hypothesis

IPFP and SYN will not have a significantly different response to IL-1β and TNF-α.

Methods

All procedures were approved by the IACUC and the animals used were euthanized for reasons unrelated to this study.

Tissue Harvest: IPFP and SYN were harvested from the knees of 3 dogs (n=6). Explants were created using a 4mm biopsy punch (n=8/dog), placed in M1 culture media, and incubated for 24 hours before being transferred to media containing an appropriate cytokine treatment.

Treatment Groups: Explants from each dog were placed into the following treatment groups: 1) NEG Control (M1 media), 2) IL-1β (2ng/ml), 3) TNF-α (2ng/ml), 4) IL-1β+TNF-α (2ng/ml).

Tissue Culture: Explants were cultured in 1ml of appropriately treated media for 6 days. Media was collected on days 3 and 6 for biomarker assessment. On day 6, wet weights of all tissues were established.

Biomarker Analysis: Culture media from days 3 and 6 was evaluated for the following markers: PGE_2; NO; MMP-1, 2, 3; total MMP activity; IL-6, IL-8, MCP-1, and KC using commercially available assays.

Statistical Analysis: Statistical significance was evaluated with SigmaPlot® using a one-way ANOVA for data between groups with significance set at $p<0.05$.

Discussion

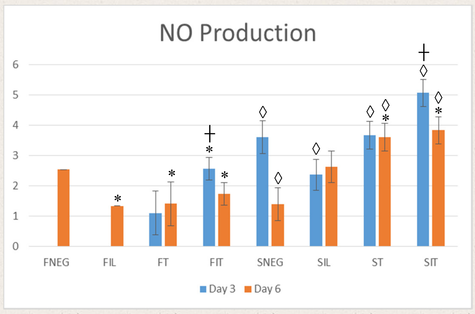
With minor exceptions, IPFP and SYN respond similarly to inflammatory stimulation by IL-1β and TNF-α. Compared to previous studies, more physiologic levels of cytokine stimulation were used in order to better understand the response of these tissues during OA pathogenesis and progression. The data from this study indicates that IPFP and synovium have a similar response to inflammatory stimulation, and may share a role in the pathogenesis of OA. TNF-α is a known contributor to inflammation in many tissue types. However, the response of the IPFP and SYN to TNF-α stimulation was significantly lower than the response to IL-1β for many of the biomarkers analyzed in this study. This indicates that compared to IL-1β, TNF-α may play a minor role in development and progression of OA for these tissues. Further exploration should compare inflammatory biomarker production of the IPFP and synovium to other tissues such as cartilage and menisci. A better understanding of the basic pathobiology of this complex disease lends the potential for development of early diagnosis and future treatment of OA.

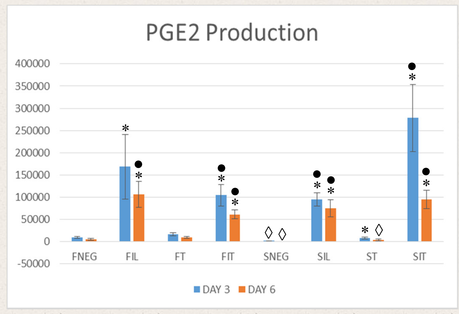
Conclusions

- IPFP and synovium have similar responses to stimulation by to IL-1β and TNF-α.
- Compared to IL-1β, TNF-α does not cause significant inflammation in IPFP and synovial tissues at a 2ng/ml concentration.

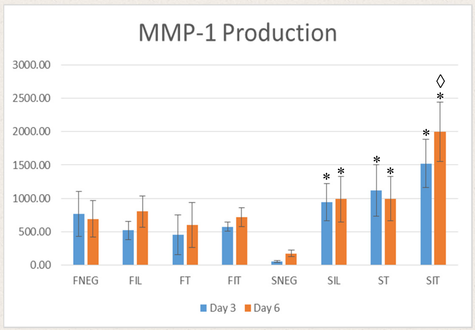
Results

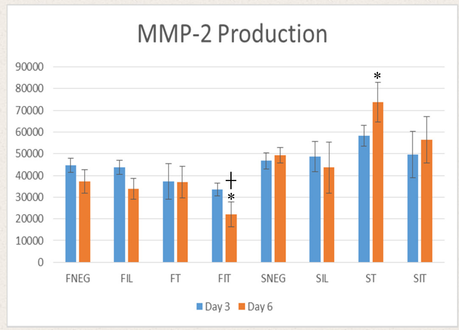
Treatment Groups: FNEG (IPFP in M1), FIL (IPFP in IL-1β), FT (IPFP in TNF-α), FIT (IPFP in IL-1β and TNF-α). SNEG (SYN in M1), SIL (SYN in IL-1β), ST (SYN in TNF-α), SIT (SYN in IL-1β and TNF-α)

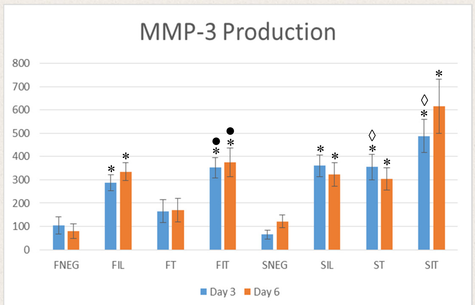


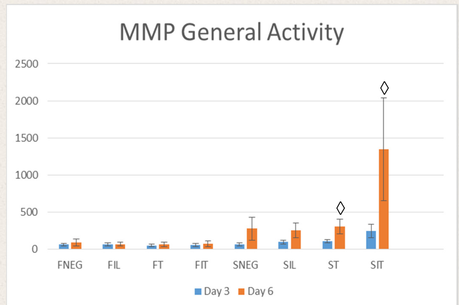


NO production was significantly different between SIL and FIL on day 3 and all other treatments on days 3 and 6. PGE2 production in the FT group was significantly less than that of the FIL group on day 6 and the FIT group on days 3 and 6. ST was significantly less than SIL and SIT treatment groups on all days.
**=Significantly different from NEG, ◊=Significantly different from IPFP equivalent, ┼ = Significantly different from SIL or FIL, ●=Significantly different from FT or ST.*









MMP-1 production was significantly different in the synovial treatment groups from the negative control while this was not the case for the IPFP. FT MMP-3 was not significantly different from negative control but was significantly less than FIT. No major significant differences found in MMP-2 production or MMP general activity.
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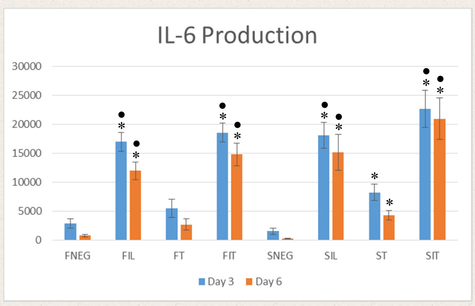

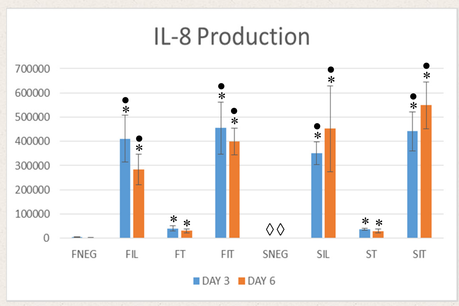

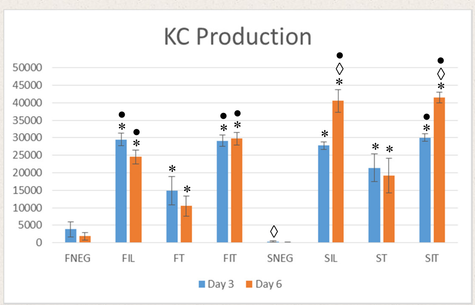

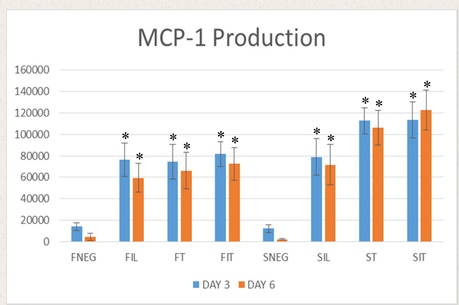

FT production was significantly lower than IL and IT treatment groups for both tissues for IL-6, IL-8 and KC. KC SIL and SIT production were significantly different from their IPFP equivalent on day 6. MCP-1 production was significantly different from NEG on all days in both IPFP and SYN.
**=Significantly different from NEG, ◊=Significantly different from IPFP equivalent, ┼ = Significantly different from SIL or FIL, ●=Significantly different from FT or ST.*

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