Metabolic Response to Cytokine Stimulation by the Canine Infrapatellar Fat Pad

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Introduction

When evaluating the health and pathology of the knee it is essential to view the joint as a sum of its components and the interactions between these parts. In particular, when establishing the basis for a disease such as osteoarthritis (OA), it is important to know the role each component of the knee plays in the pathogenesis of this disease. Though it has been indicated as an important player in OA, the role of the infrapatellar fat pad (IPFP) has continued to remain unclear. In our study, we hope to evaluate the metabolic response of the IPFP to cytokine stimulation to better understand its role in the pathogenesis and pathobiology of OA.

Objective

To better understand the role of the IPFP in the release of inflammatory mediators into the joint and its potential role in the pathogenesis and pathobiology of osteoarthritis.

Hypotheses

1) Treatment with IL-1β, IL-6, IL-8 or MCP-1 will result in a significant increase in tissue inflammation and degradative enzyme production by the IPFP compared to negative control.
2) Combined cytokine culture will result in a significant increase in tissue inflammation compared to mono-culture.

Methods

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

Tissue Harvest: The IPFP, without synovial lining, was harvested from the knees of 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, 2) rcIL-6 (50ng/ml), 3) rcIL-8 (50ng/ml), 4) rcMCP-1 (50ng/ml), 5) rcIL-1β (10ng/ml), 6) rcMCP-1+rcIL-6 (25ng/ml each), 7) rcMCP-1+rcIL-8 (25ng/ml each), or 8) rcIL-6+rcIL-8 (25ng/ml each).

Tissue Culture: Explants were cultured in 1ml of media for 21 days. Media was changed every 3 days and collected for biomarker assessment. On day 21 of culture, tissue explants were collected and fixed in 10% formalin for histologic analysis.

Biomarker Analysis: Culture media from days 3, 6, and 9 were evaluated for the following markers: PGE_2; NO; MMP-1, 2, 3 and 13; total MMP activity; ADAMTS4 activity; and IL-6, IL-8, MCP-1, and KC using commercially available assays.

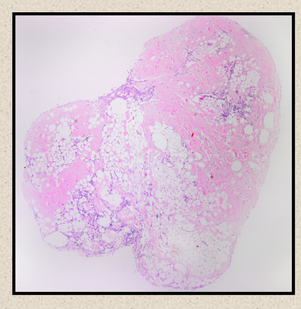
Statistical Analysis: Statistical significance was evaluated with SigmaPlot® using a one-way ANOVA for data between groups as well as biomarker levels over time with significance set at $p<0.05$.

Discussion

Previous studies have found that treatment of cartilage, as well as other tissues in the joint, with IL-1β stimulates an increased production of IL-6, IL-8, and MCP-1. However, there was no significant increase in inflammatory mediators or degradative enzyme production in explants treated with IL-6, IL-8 or MCP-1, alone or in combination, when compared to controls. This indicates that these cytokines do not induce inflammation at this concentration. It is possible that they are a part of a potential repair process by the tissue. Therefore, further studies will evaluate the role of these cytokines as potential contributors to the repair process of joint tissues during the development of osteoarthritis. These data also implicate the IPFP as a contributor to the inflammatory and degradative pathways associated with OA development and progression.

Conclusions

- IL-6, IL-8 and MCP-1 do not illicit an inflammatory or degradative response from the IPFP alone or in combination.
- The IPFP does contribute to the inflammatory and degradative pathways associated with OA as indicated by the response of the tissue to IL-1β stimulation.

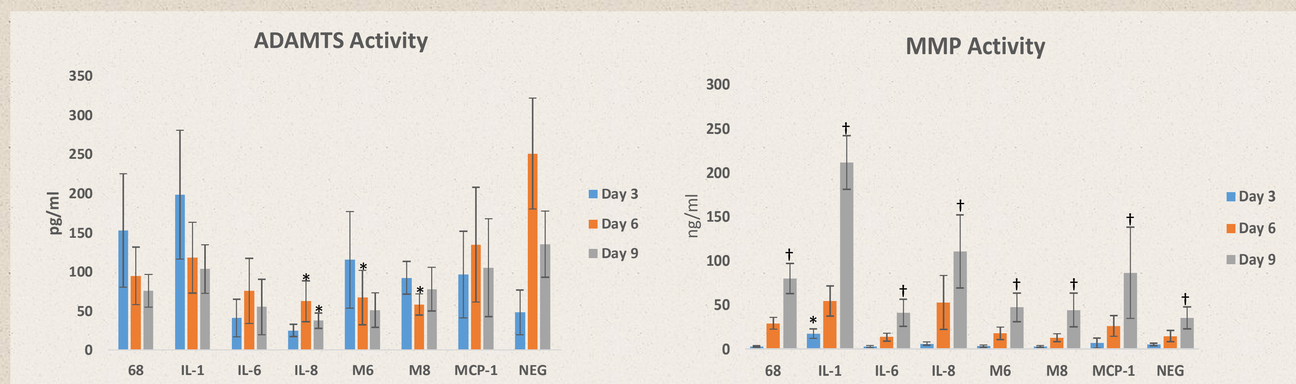


Representative histologic image of the canine infrapatellar fat pad. Histologic evaluation confirmed that synovial tissue was not harvested with the IPFP explants used for culture.

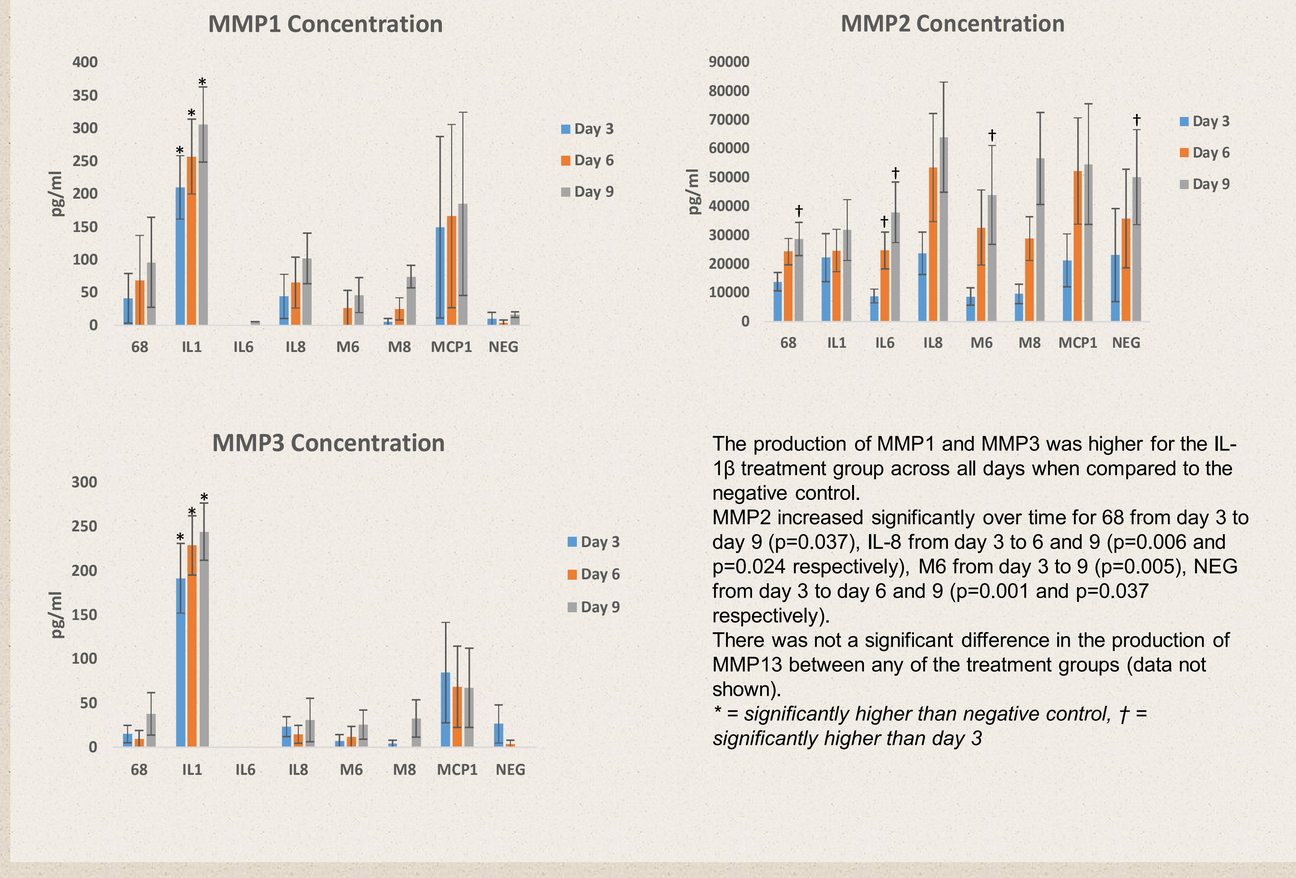
There were no apparent significant differences in histologic appearance of the infrapatellar fat pad between treatment groups.

Results

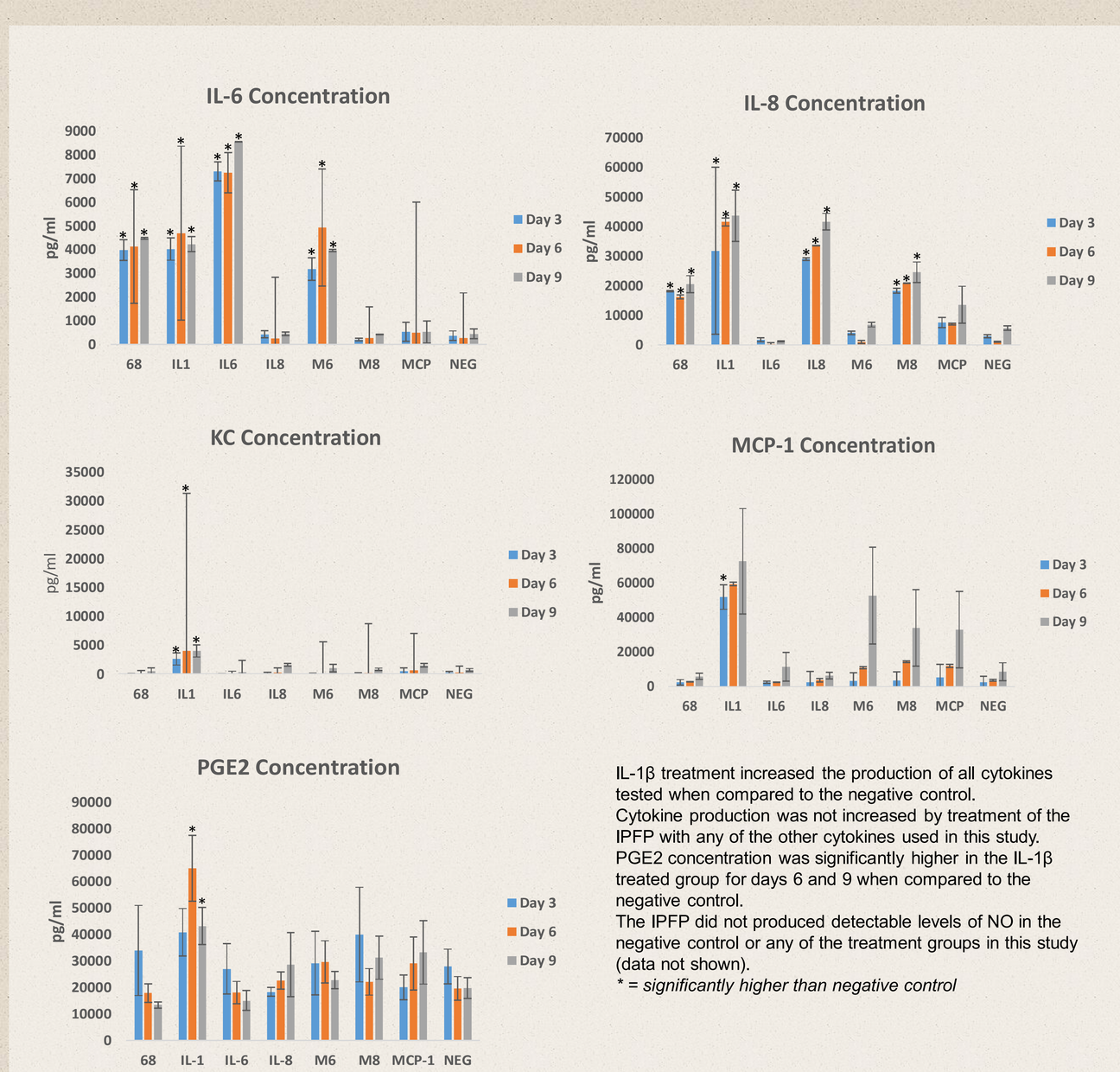
Treatment Groups: 68 (IL-6 + IL-8); IL-1 (IL-1β); IL-6; IL-8; M6 (MCP-1 + IL-6), M8 (MCP-1 + IL-8); MCP-1; NEG (Negative Control)



ADAMTS4 activity was significantly lower on day 6 for IL-8 (p=0.032), M6 (p=0.042), M8 (p=0.023) as well as on day 9 for IL-8 (p=0.047) when compared to negative control.
MMP activity increased significantly from day 3 to day 9 for all treatment groups. Additionally, day 3 IL-1β was significantly higher than the negative control (p=0.041).
** = significantly higher than negative control, † = significantly higher than day 3*



The production of MMP1 and MMP3 was higher for the IL-1β treatment group across all days when compared to the negative control.
MMP2 increased significantly over time for 68 from day 3 to day 9 (p=0.037), IL-8 from day 3 to 6 and 9 (p=0.006 and p=0.024 respectively), M6 from day 3 to 9 (p=0.005), NEG from day 3 to day 6 and 9 (p=0.001 and p=0.037 respectively).
There was not a significant difference in the production of MMP13 between any of the treatment groups (data not shown).
** = significantly higher than negative control, † = significantly higher than day 3*



IL-1β treatment increased the production of all cytokines tested when compared to the negative control.
Cytokine production was not increased by treatment of the IPFP with any of the other cytokines used in this study.
PGE2 concentration was significantly higher in the IL-1β treated group for days 6 and 9 when compared to the negative control.
The IPFP did not produced detectable levels of NO in the negative control or any of the treatment groups in this study (data not shown).
** = significantly higher than negative control*

Acknowledgments: *Funded by The Comparative Orthopaedic Laboratory and a gift from Zoetis Animal Health.*