Orthopaedic Surgery
School of Medicine
University of Missouri Health

Thompson Laboratory for Regenerative Orthopaedics

Evaluation of Intervertebral Disc Metabolic Responses to Sustained IL-10 Stimulation using a Rat-Tail Whole Organ Explant Model

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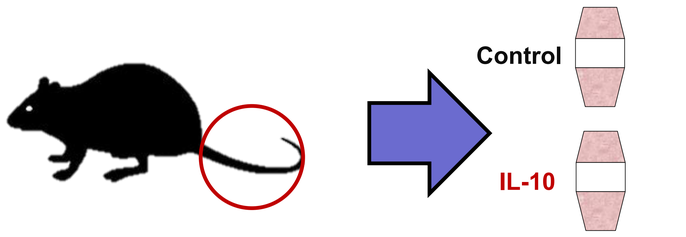
Introduction and Purpose

- Intervertebral disc (IVD) degeneration is commonly associated with debilitating low back pain and disability
- Previous studies have indicated degenerative IVD human tissues produce the anti-inflammatory cytokine IL-10
- This study was designed to assess the metabolic responses of the IVD to IL-10 stimulation using a rat tail whole organ IVD *ex vivo* culture model

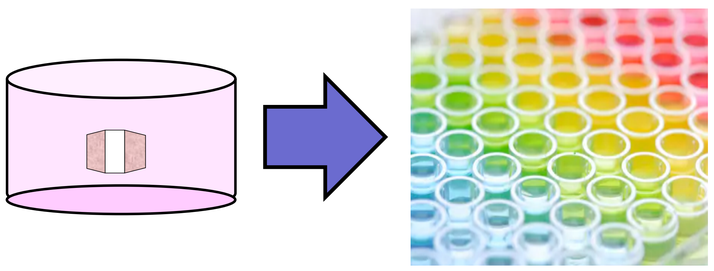
Hypothesis

- IVDs exposed to IL-10 stimulation will produce significantly lower levels of pro-inflammatory and degradative biomarkers compared to untreated controls

Methods



1) With IACUC approval, tails were harvested from skeletally mature Sprague Dawley rats (n=6), and whole organ IVD functional spinal units (n=24) were collected



2) IVDs were then randomly assigned and cultured with or without 10ng/ml IL-10 (n=12/group) in 2ml of DMEM for 12 days at 37˚C and 5% CO_2
3) Culture media were changed every 3 days, and stored at -20˚C for biomarker analyses
4) Media were analyzed for proteoglycan (GAG), PGE2, IL-6, IL-4, MMP Activity, GRO/KC, VEGF, TIMP-1, and NO using commercially available assays according to the manufacture's protocol.
5) Significant differences among groups at each time point were assessed using a Mann-Whitney Rank Sum test with significance set at $p<0.05$. Further, the data from all time points for each biomarker were summed, and significant differences between groups for cumulative production was assessed using Mann-Whitney Rank Sum test with significance set at $p<0.05$. Biomarker values were standardized to tissue wet weight.

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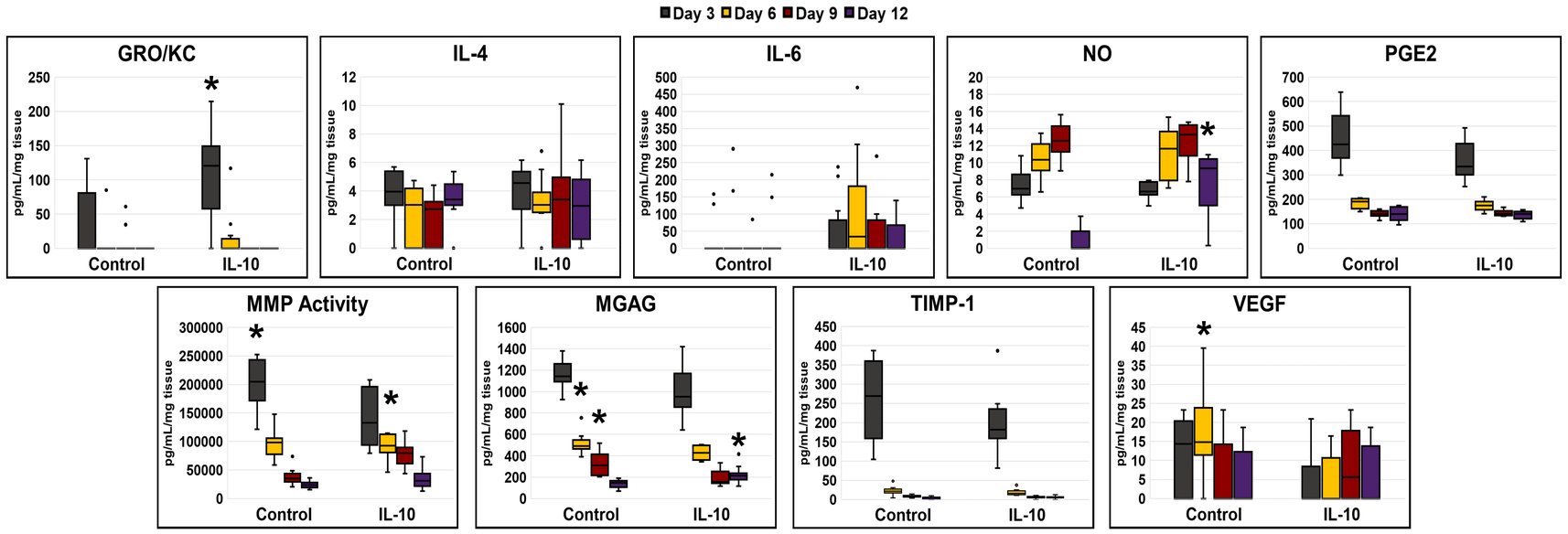
Results

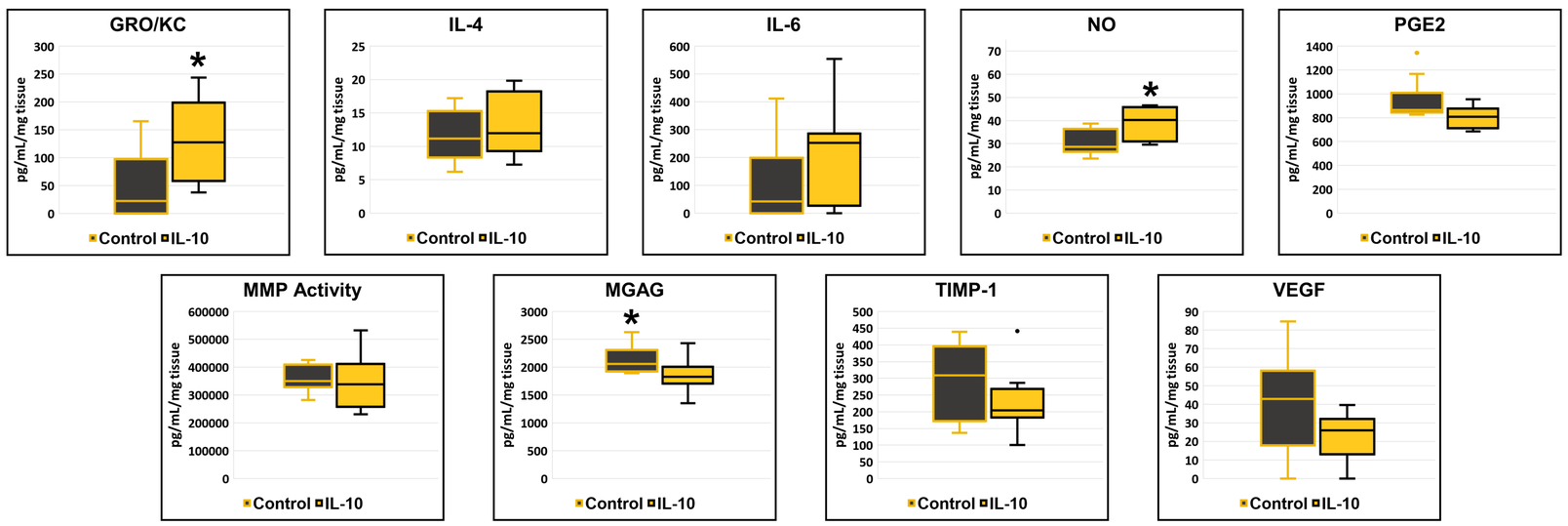
Metabolic Responses by Day



(*) Significantly higher production at p = 0.05

- Release of GAG to the media was significantly lower in the IL-10 group compared to the NEG control group on day 6 and 9, and higher in the IL-10 group compared to the NEG control on day 12.
- Level of MMP activity was significantly lower on day 3 of culture, and higher on day 9 of culture, in the IL-10 group compared to the NEG control.
- The IL-10 group produced significantly higher GRO/KC on day 3, lower VEGF on day 6, and higher NO on day 12 compared to the NEG control

Cumulative Biomarker Release



- Cumulative release levels of GRO/KC and NO by IL-10 stimulated discs were significantly higher compared to control discs.
- Cumulative release levels of GAG to culture media by control discs was significantly lower compared to IL-10 stimulated discs

Conclusions

- Stimulation with IL-10 did not have a consistent effect on the pro-inflammatory or degradative metabolic responses of the IVD during culture over time
- Stimulation with IL-10 appears to contribute to an increase in the cumulative pro-inflammatory metabolic responses by the IVD based on GRO/KC and NO production during culture
- Stimulation with IL-10 appears to decrease tissue ECM degradation over time based on the lower cumulative release of GAG to the media during culture
- Further study is required to determine how these metabolic responses to IL-10 stimulation by the IVD relates to the development and progression of symptomatic clinical IVD degeneration