**In Vivo Lypopolysaccharide (LPS) Injection Provokes Secretion of Multiple Pro-Inflammatory Mediators of Intervertebral Disc Degeneration**

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**Introduction:** Human intervertebral disc (IVD) degeneration is accompanied by elevated levels of pro-inflammatory cytokines, particularly IL-1β and TNF-α [1-3]. Cytokine secretion by disc cells increases catabolic breakdown of the tissue, resulting in a positive feedback of disc integrity loss and further inflammation [4-6]. Animal models of disc degeneration using stab or laceration of the disc generally reproduce morphological changes of IVD degeneration. Using these models, transient increases in the expression or secretion of pro-inflammatory cytokines has been confirmed [7-9]. Several studies have shown that gene expression of TNF and IL-1 are upregulated for 2 to 3-weeks post annular laceration [7, 8]. Additionally, disc stab injuries have been shown to induce acute (4 day) increases in IL-1β secretion, while TNF-α or IL-6 are unchanged up to 12 weeks post stab [9].

In this study, we propose an alternate approach for inducing inflammation that is not initiated by physical destruction of the disc integrity. We propose to initiate inflammation by injection of rat caudal discs with lipopolysaccharide (LPS), an endotoxin widely used in preclinical models of inflammation. We hypothesize that injection of LPS into rat caudal discs will result in increased levels of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor (TNF-α) as well as high mobility group protein (HMGB-1) and macrophage migration inhibitory factor (MIF). In this study, LPS is administered at sublethal dosages with micro needles (<10% disc height) in order to limit the potential disruption of the disc by needle injection.

**Methods:** Male Sprague Dawley Rats were used (n=8; 300-350 g). Rats were anesthetized and a 2” incision was made exposing caudal motion segments (Co 3-4, Co 4-5, Co 5-6, Co 6-7). LPS Injection: A 33G needle was inserted 4mm into the center of the disc with clamp guidance. A microliter syringe was then used to inject 2.5µl of LPS (1µg/ml) into the NP disc space, yielding a dosage of 2.5 ng/disc. PBS (2.5µl) was injected into the adjacent disc to serve as a sham control. Animals were allowed unrestricted activity for either 1or 7 days and then sacrificed for biochemical analysis of the treated discs.

**Protein extraction:** Individual discs were dissected and homogenized in an extraction buffer containing 20mM Tris (pH 7.6), 120mM NaCl, 10mM EDTA, 10% Glycerol, 1% Nonidet P40, and protease inhibitor (Roche Complete) for 48 hours at 4°C with agitation. Lysates were then centrifuged serially at 3000 rpm and then at 55,000 rpm for 30 minutes at 4°C. Protein content was measured via Bradford Assay (Pierce Biotechnology, Rockford, IL). ELISA: Disc lysates were analyzed by ELISA for TNF-α and IL-1β (R&D Systems, Minneapolis, MN), against standard curves (Spectramax Plus, Molecular Devices). Cytokines levels were normalized to sample protein content, and analyzed using ANOVA with LSD post-hoc test, with p<0.05 considered statistically significant (STATISTICA). Immunoblotting: Protein lysate (25 µg) was analyzed by quantitative western blotting to detect the presence of HMGB1 and MIF. Samples were run by SDS-PAGE using the Invitrogen NuPAGE system and transferred to PVDF membrane. β-actin was used as a loading control and recombinant HMGB1 (lane 1) and MIF (lane 2) were also loaded as a positive control of these novel cytokines. Primary antibodies used to detect proteins were: mouse monoclonal anti-Actin (Millipore), Rabbit polyclonal anti-HMG-B1 (AbCam) and rabbit polyclonal anti-MIF (Courtesy of C. Metz). Secondary antibodies were conjugated to infrared dyes (LI-COR Biosciences) and used to visualize proteins were: mouse monoclonal anti-Actin (Millipore), Rabbit polyclonal anti-HMG-B1 (AbCam) and rabbit polyclonal anti-MIF (Courtesy of C. Metz). Secondary antibodies were conjugated to infrared dyes (LI-COR Biosciences) and used to visualize.

**Results:** Injection of LPS into the NP disc space resulted in increased levels of pro-inflammatory cytokinesTNF-α and IL-1β as compared to discs that were treated with PBS (Figures 1-2). In discs injected with PBS, the concentration of IL-1β was relatively low 1 day post injection (0.1±0.04 ng/mg protein). At this time point, LPS injected discs had significantly higher levels of IL-1β vs. PBS sham (0.3±0.02 ng/mg protein; p<0.05; Figure 1). Elevated levels of IL-1β persisted 7 days post injection with LPS (3.6±1.2 ng/mg protein), and was significantly higher than PBS sham discs (p<0.05; Figure 1). Overall, there was ~10X increase in IL-1β overt time in both treatment groups (day 1 vs. 7).

**Discussion:** The concentration of TNF-α was also significantly greater for LPS vs. PBS groups 1 day post injection (0.23±0.003 ng/mg protein; p<0.05, Figure 2). While TNF-α concentration 7 days post LPS injection was higher than PBS, the difference was not statistically significant (Figure 2). Additionally, no significant temporal increase in TNF-α in either sham or LPS treated discs was seen between day 1 and day 7 discs.

HMGB1 and MIF were detected in discs treated with LPS at 1 and 7 days post disc injection (Figures 3 & 4). While basal levels of HMGB1 were present in PBS sham group at both time points, higher levels of HMGB1 were detected in LPS treated discs (Figures 3 & 4). MIF was not detectable in sham discs, whereas increased detection was seen in discs injected with LPS both at 1 and 7 days post treatment.

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**References:**


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