Interferon α/β Signaling Contributes to Local and Systemic Inflammation after Intervertebral Disc Injury

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INTRODUCTION: Low back pain is the leading cause of disability and is often associated with degeneration of the intervertebral disc (IVD), which is characterized by a local pro-inflammatory microenvironment[1,2]. IVD degeneration is also associated with systemic inflammation indicated by correlations between serum cytokine levels and disease severity in patients[3]. In pre-clinical models, it has been shown that intradiscal injury and inflammation can directly cause systemic inflammation, but the signaling pathways connecting these local and systemic inflammatory responses are unknown[4,5]. One pathway that contributes to IVD degeneration is TLR4-mediated signaling, which is most prominently known to activate NFκB. However, TLR4 can also activate interferon regulatory factors (IRFs) leading to Interferon α/β Signaling[6,7]. While Interferon α/β Signaling has not been widely studied in the context of the IVD, one study indicated IRF7 as a potential therapeutic target, with monocytes regarded as a primary driver of IRF7 expression[8,9]. Together, these findings motivate further investigation into Interferon α/β Signaling as a potential connection between local and systemic inflammation. The objective of this study was to evaluate Interferon α/β Signaling in the local and systemic inflammatory environments after IVD injury. The hypothesis was that Interferon α/β Signaling would increase both locally in the IVD and systemically in the blood after *in vivo* injury, and would be mediated, in part, by monocytes and macrophages.

METHODS: LPS-Injection Procedure: All animal procedures were performed with approval from the Institutional Animal Care and Use Committee (IACUC). Only male Sprague Dawley rats (n=36) were utilized to facilitate direct comparison with previous IVD injury models in the lab[4]. Rats were anesthetized with isoflurane, and using fluoroscopic guidance, 4 caudal (C) motion segments, C5-6 to C8-9, were injected percutaneously with 2.5 µl of saline (Injury) or 100 µg/mL LPS (Injury+LPS) using a 33G needle. An uninjured control group was also included. Annulus fibrosus (AF), nucleus pulposus (NP), and whole blood were collected at days 3, 7, 14, and 28 post-injury. **AF/NP & Blood RNA Isolation:** AF and NP were homogenized and cells were lysed with TRIzol prior to phase separation with chloroform. A high salt solution with isopropanol was used to precipitate RNA before purification with the RNeasy Mini Kit according to the manufacturer's protocol. RNA was isolated from blood using the RiboPure RNA Purification Kit according to the manufacturer's protocol. **Bulk RNA Sequencing:** RNA quality was validated using Tape Station with AF having RNA Integrity Numbers (RINs)>5.5 and blood having RINs>8.5. RNA sequencing was performed by the Genome Center using a STRPOLYA library prep and run on an AVITI instrument. Gene set enrichment analysis (GSEA) was used to determine enriched Reactome pathways between Injury+LPS and Injury with p-adjusted<0.05 considered significant[10]. **Peripheral Blood Mononuclear Cell (PBMC) Isolation & Sorting:** Whole blood was treated with red blood cell lysis buffer and PBMCs were isolated. Cells were resuspended in media, 40% fetal bovine serum (FBS), and 10% DMSO for storage in liquid nitrogen. PBMCs were thawed, stained, and subsets of immune cells were sorted using the FACSDiscover S8 Cell Sorter. Cells were sorted directly into cell lysis buffer and stored at -80°C (B Cells, T Cells, NK Cells, Neutrophils) or immediately processed for RNA using the RNeasy Micro Kit according to the manufacturer's protocol (Monocytes). **AF/NP & Monocyte Gene Expression:** RNA was converted to cDNA and expression of *Irf7*, *Rsad2*, and *Isg15* was measured using RT-qPCR. Significance in AF/NP was determined using two-way ANOVAs with Fisher's LSD post-hoc tests, and significance in monocytes was determined using one-way ANOVAs with Fisher's LSD post-hoc tests. **IVD Immunostaining:** Fixed samples were imbedded in paraffin, sectioned, and stained with primary antibodies (CD68, pIRF7) followed by secondary antibodies (AF488, AF594). DAPI and coverslips were applied before imaging.

RESULTS: In the comparison of LPS injection (Injury+LPS) versus saline injection (Injury), the Interferon α/β Signaling pathway was positively enriched in the AF at Day 3 and 28, and in the blood at Day 28 (Figure 1a). 12 of the core enriched genes were in common between comparisons, including *Irf7*, *Rsad2*, and *Isg15* (Figure 1b). Expression of these genes was further assessed in the AF and NP regions of the IVD over time after injury. Compared to uninjured controls, an early increase in AF expression of *Irf7* and *Isg15* was observed at Day 3 and/or Day 7, while a delayed increase in *Irf7*, *Rsad2*, and *Isg15* was observed in the NP, primarily at Day 14 and 28 (Figure 2a, b). Few significant differences in gene expression were observed between Injury+LPS and Injury in the AF or NP. Immunostaining of IVDs showed colocalization of the macrophage marker, CD68, and pIRF7, indicative of IRF7 activation. Similarly in the blood, sorted monocytes from the Injury+LPS group at Day 28 had increased expression of *Irf7* compared to Injury alone (Figure 3b).

DISCUSSION: Injury caused by percutaneous injection caused structural changes by Day 14 compared to uninjured controls (Not Shown). However, local inflammatory stimulation in the Injury+LPS group also exacerbated inflammation systemically relative to Injury alone. Specifically, RNA sequencing showed enrichment of the Interferon α/β Signaling pathway in both the AF and blood, with 12 core enriched genes commonly upregulated, including *Irf7* and interferon stimulated genes (ISGs), *Rsad2* and *Isg15* (Figure 1). Recent results in the lab have also indicated a dysregulation of Interferon α/β Signaling in the blood of patients with LBP and spine pathology compared to healthy controls, supporting clinical relevance of this pathway (Not Shown). Locally, the AF and NP showed temporally distinct responses to Injury in Interferon α/β Signaling genes, where AF expression increased early after injury, followed by NP (Figure 2). Additionally, local expression was mediated primarily by injury from injection, with few additional impacts of LPS inflammatory stimulation. IRF7 inhibition has previously been shown to have protective effects on IVD structure after injury, supporting the role of IRF7 in IVD degeneration[9]. As monocytes and macrophages have been identified as primary drivers of Interferon α/β Signaling, specifically through IRF7, cell type specific expression was also investigated. Immunostaining of the IVD demonstrated colocalization of the macrophage marker, CD68, with activated pIRF7 (Figure 3a). Similarly, a sorted population of monocytes from blood in the Injury+LPS group had increased expression of *Irf7* compared to Injury alone (Figure 3b). Together, these results establish Interferon α/β Signaling as a novel pathway connecting the local and systemic inflammatory responses, in part, through monocyte and macrophage-mediated expression.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the signaling pathways and cell types contributing to local intradiscal and systemic inflammatory responses could inform therapeutic targets for inflammation-driven IVD degeneration.

REFERENCES: [1] Buchbinder, R. et al., 2013, Best Pract Res Clin Rheumatol. [2] Silva, J. et al., 2019, Front Immunol. [3] Jacobsen, H. et al., 2020, Osteoarthr Cartil. [4] Lisiewski, L. et al., 2024, FASEB J. [5] Burt K. et al., 2024, Sci Adv. [6] Rajan, N. et al., 2013, Spine (Phila Pa 1976). [7] Lin, Q. et al., 2011, Int Immunopharmacol. [8] Wang L. et al., 2024, Exp Dermatol. [9] Xu, P. et al., 2024, Front Immunol. [10] Love, M. et al., 2014, Genome Biol.

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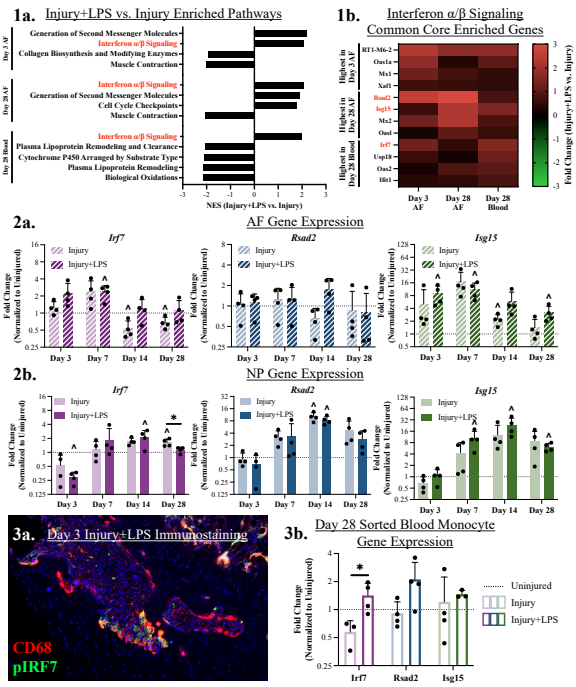

Figure 1: a) Enriched pathways and **b)** common Interferon α/β Signaling core enriched genes between Injury+LPS and Injury in Day 3 AF, Day 28 AF, and Day 28 Blood. **Figure 2: a)** AF and **b)** NP gene expression of *Irf7*, *Rsad2*, and *Isg15* over time after injury. **Figure 3: a)** Representative image of CD68+ macrophages co-stained with pIRF7 on Day 3 after Injury+LPS injection. **b)** Gene expression of *Irf7*, *Rsad2*, and *Isg15* in monocytes flow-sorted from PBMCs at Day 28 after injection.