Intervertebral disc degeneration correlates with neuroinflammation in a translational large animal model

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INTRODUCTION: Disc degeneration (DD) involves a progressive cascade of structural and biological changes that compromise disc mechanical function and height, often culminating in discogenic pain[1]. Inflammation plays a central role, with elevated cytokine production (IL6, TNFα, IL1β)[2] and immune cell infiltration (phagocytic macrophages)[3] driving matrix degradation and sensitizing local nociceptors. These inflammatory processes are often accompanied by aberrant neoinnervation of the normally aneural inner regions of the disc, facilitating pain signal transmission. We previously showed evidence of immune cell presence and innervation within a large animal DD model, supporting the use of such a model for the preclinical evaluation of novel therapeutics for disc regeneration[4]. However, while pain can be directly assessed in small animal models, and reliably correlates with DD severity, large animal models present a challenge, as no validated quantitative metrics of musculoskeletal pain currently exist for these models. To address this and establish surrogate measures of pain in our large animal model, we investigated neuroinflammation and pain pathways by quantifying glial fibrillary acidic protein (GFAP), ionized calcium-binding adaptor molecule 1 (Iba1), and substance P (SubP) in the spinal cord (SC) and dorsal root ganglia (DRG) adjacent to degenerated discs[5]. We hypothesized that increased discogenic inflammation, aberrant innervation, and immune cell infiltration in the intervertebral disc would correlate with neuroinflammation in this large animal model of DD.

METHODS: *Induction of Disc Degeneration:* Degeneration of the cervical C2-C3 or C4-C5 intervertebral discs of castrated male large frame goats (~3 years of age) was induced via intradiscal injection of 5U or 2U chABC in 200 µL of buffer (sterile PBS, 0.1% BSA), following IACUC approval. Intradiscal injection of chABC was performed percutaneously via an anterior approach using a 22G needle with 3D fluoroscopic guidance[6]. The C3-C4 level was utilized as an adjacent control. Additional cervical spines (C2-C5) were collected (n=3) from non-surgical goats to serve as level-matched healthy controls. *IVD MRI:* Sagittal MRI T2 maps (0.5 mm × 0.5 mm in-plane resolution; TEs = 13, 26, 39, 52, 71 ms) were obtained at 3T to quantify nucleus pulposus (NP) T2 relaxation times[7]. IVD *Histopathology:* Discs were fixed, decalcified and paraffin-embedded for histological grading[8]. *Immunohistochemical Analysis:* Bone-disc-bone segments of cervical IVDs, SCs and DRGs were prepared from explanted motion segments 12 weeks post-ChABC injection. Sections were fixed, decalcified, and paraffin embedded. Mid-sagittal spine sections were cut and used for immunohistochemical (IHC) analysis of inflammatory cytokines (IL6, TNFα), innervation (PGP9.5, 1:1 phosphorylated & non-phosphorylated NFH), and monocyte (Ly6C) and macrophage (CD68) cell markers. SC and DRG sections were used for IHC analysis of glial cell marker (GFAP), neural macrophage marker (Iba1) and neuropeptide (Sub P). Sections underwent antigen retrieval and overnight incubation with primary antibodies, followed by incubation with fluorescent secondary antibodies, and cover-slipped with mounting medium containing DAPI nuclear dye before imaging (Zeiss Axio Scan.Z1). *Protein expression analysis:* Immunofluorescence images were thresholded and target expression/localization was quantified as the percent fluorescent area within hand-drawn ROIs within the disc (NP, AF), SC (dorsal horn), and DRG. *Statistical analysis:* Relationships between inflammation, innervation, and immune cell infiltration in the IVD, glial cell activation, inflammation and pain in the SC and DRG were tested with Pearson correlations (GraphPad Prism), with significance set at $p < 0.05$.

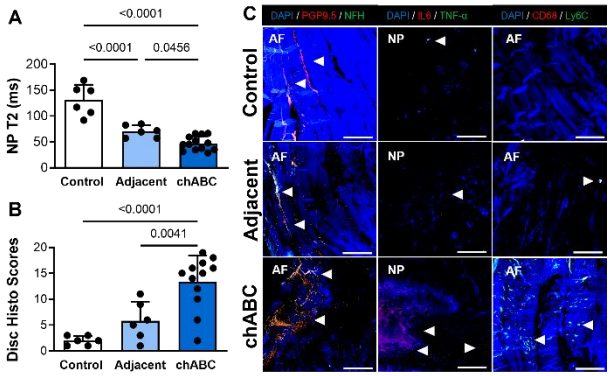

Fig 1: A) NP T2 relaxation times. B) IVD Histological scores. C) Inflammation, innervation and immune cell infiltration with chABC induced DD. Fluorescent images on chABC injected, adjacent and non-surgical control IVDs stained for inflammation, innervation and immune cell markers. Positive staining indicated by white arrows. Scale bar = 250µm

RESULTS: chABC treatment induced DD, evidenced by increased T2 relaxation times and elevated histology scores at 12 weeks (**Fig1A, B**). Significant degeneration was also detected at the C3/4 levels adjacent to ChABC-injected discs compared to non-surgical controls (**Fig1A, B**). Within the disc, chABC increased innervation, inflammation, and immune cell infiltration, with greater PGP9.5 expression in the AF of injected discs and elevated NFH expression in adjacent discs (**Fig 1C**). chABC-treated discs also showed increased Ly6C in the AF (**Fig 1C**). For DRG and SC analyses, chABC and adjacent discs were pooled. In the SC, chABC animals exhibited significant increases in Iba1 and substance P, with trending elevations of GFAP and Iba1 in the DRG compared to controls without spinal pathology (**Fig 2C, D, F, G**). Correlation analyses revealed significant positive associations between DRG neuroinflammation and disc innervation/inflammation. Notably, PGP9.5, NFH, and IL-6 in the disc correlated with substance P expression in the DRG **(Fig 2I)**, indicating that disc inflammation and innervation are linked to neuroinflammation and pain signaling in the DRG.

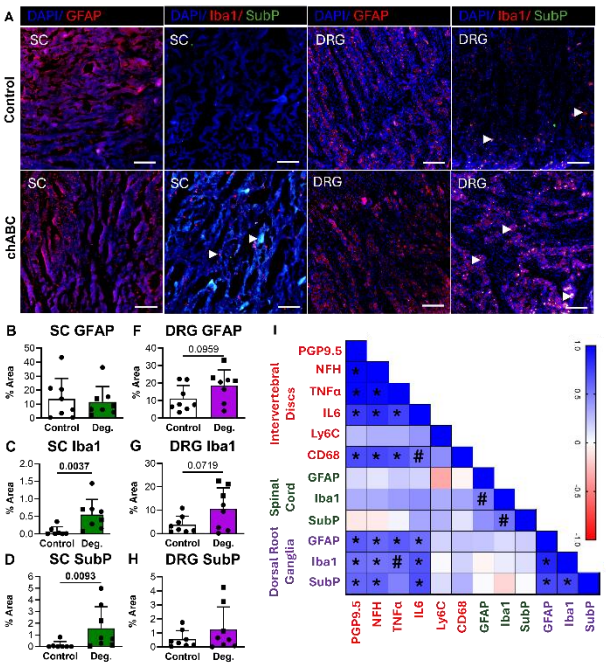

Fig 2: A) Glial cell activation, immune cell and pain markers with chABC induced DD in SC and DRGs. Scale bar = 50µm. **Expression of markers in SC: B)** GFAP, **C)** Iba1, and **D)** Substance P. **Expression of markers in DRGs: F)** GFAP, **G)** Iba1, and **H)** Substance P. **I) IVD, SC and DRG inflammation, innervation and immune cell infiltration correlation matrix.** Correlation matrix across study outcomes using Pearson r statistical analysis. Significance was observed in correlation data by * = $p<0.05$, # = $0.10 > p > 0.05$.

DISCUSSION: The purpose of this study was to determine whether discogenic inflammation, innervation, and immune cell infiltration correlated with neuroinflammation in a large animal model of DD. These findings demonstrate that enzymatically induced DD not only disrupts local disc integrity but also provokes adjacent segment degeneration[9], underscoring that degeneration at one level can propagate to neighboring segments. The association between degeneration, innervation, and immune cell infiltration highlights the interplay between inflammation and nociceptive nerve growth in disc pathology[10]. Moreover, the link between disc-derived inflammatory and innervation markers and substance P in the DRG suggests that peripheral disc changes can sensitize central pain pathways, providing mechanistic insight into how disc degeneration may drive pain. Compared to rodent models[5], this work highlights that similar neuroinflammatory processes occur in a translationally relevant large animal model, strengthening its utility for preclinical therapeutic studies. A key limitation is that neural pathways remain undefined, particularly with concurrent facet degeneration in this model. Future retrograde tracing studies will be needed to separate disc- versus facet-driven contributions across spinal levels. Overall, these findings identify disc-innervation–immune cell crosstalk as a driver of pain.

SIGNIFICANCE: This study defines nociceptive and inflammatory responses in a large-animal disc degeneration model and introduces a quantitative pain readout, strengthening future translational studies of novel therapeutics for disc regeneration.

REFERENCES: [1] Gopal + Adv Exp Med Biol., 2012 [2] Li + J Inflamm Res., 2022 [3] Nakazawa + Spine J., 2018 [4] Burt + ORS 2024., 1005 [5] Lai + Int J Mol Sci., 2024 [6] Gullbrand + Ecm., 2024 [7] Jaumard + J Biomech Eng., 2011 [8] Lee + JOR Spine., 2021 [9] Diwan + JOR Spine., 2022 [10] Cai + Orthop Surg., 2020.

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