Inflammatory Cytokine Expression in a Goat Model of Intervertebral Disc Degeneration

Chenghao Zhang[1,2]
Sarah E. Gullbrand[1,2]
Thomas P. Schaer[1]
Dawn M. Elliott[3]
George R. Dodge[1,2]
Robert L. Mauck[1,2]
Neil R. Malhotra[1]
Lachlan J. Smith[1,2]

1. University of Pennsylvania, Philadelphia, PA
2. Corporal Michael J. Crescenz Philadelphia VA Medical Center, Philadelphia, PA
3. University of Delaware, Newark, DE

Introduction

Intervertebral disc (IVD) degeneration is strongly implicated as a leading cause of low back pain. Persistent, localized inflammation within the disc nucleus pulposus (NP) and annulus fibrosus (AF) is considered to be a key mediator of disc degeneration, and is associated with downstream catabolic enzyme activity and extracellular matrix destruction. Disc inflammation is characterized by expression of cytokines including IL-1β, IL-6 and TNF-α, amongst others, with expression levels positively correlated with severity of degeneration.[1]

There is currently a lack of validated preclinical animal models of disc degeneration that recapitulate clinically-relevant, persistent inflammatory cytokine expression. Our lab recently described a goat model of disc degeneration, in which different doses of chondroitinase ABC (ChABC) were used to reproducibly induce a spectrum of clinically-relevant structural, biomechanical and histological degenerative changes.[2]The objective of this study was to further enhance the clinical-relevance of this model by establishing whether these degenerative changes are associated with tissue-level expression of key inflammatory cytokines.

Methods

With IACUC approval, 9 goats underwent a surgical procedure to induce degeneration of the lumbar intervertebral discs. Using an open, lateral, retroperitoneal transpsoatic approach, L1-2, L2-3 and L3-4 lumbar discs were randomized to receive either subtotal nucleotomy (n=4) or injection of 200μL of either 0.1U, 1U or 5U ChABC via a 22G spinal needle (n=4 per dose). The L4-L5 (n=4) disc received a sham saline injection, and the T13-L1 or L5-L6 discs served as intact controls. Animals were euthanized 12 weeks after surgery, and lumbar spines harvested. Intervertebral discs with bony endplates intact were isolated, fixed in formalin, decalcified, and processed for paraffin histology. Mid-sagittal sections were double stained with either Alcian blue (glycosaminoglycans) and picrosirius red (collagen), or hematoxylin and eosin. Severity of degeneration was established via semi-quantitative histological grading.[2] Immunohistochemistry was performed to investigate relative expression of IL-1β, IL-6 and TNF-α in the NP and AF. Sections were counterstained with hematoxylin and imaged using bright field microscopy.Antibody reactivity and specificity was validated using goat cells pretreated with lipopolysaccharide. Expression levels were quantified by determining the percentage of positive vs. total cells in the NP and AF (average of 3 regions per disc). Differences in expression levels between intervention groups were established using Kruskal-Wallis tests with post-hoc Dunn's tests ($p<0.05$). Linear correlations between expression levels and histological grade were also evaluated.

Results

Multiple comparisons tests revealed significant effects of intervention type on expression levels of IL-1β, IL-6 and TNF-α in both the NP and AF (Figure 1). TNF-α expression was highest for 1U ChABC discs (both NP and AF), and significantly elevated compared to controls (Figures 1 and 2). IL-1β and IL-6 expression were both highest in the NP and AF of 5U ChABC discs, and significantly elevated compared to control, saline and nucleotomy. No cells (NP or AF) stained positive for any cytokines in any control discs. There were moderate, positive correlations between expression levels and overall histological grade for all three cytokines, which were significant with the exception of TNF-α in the AF (Figure 3).

Discussion

Inflammation is a defining feature of human disc degeneration and is a key mediator of tissue breakdown and painful innervation. Anti-inflammatory therapies such as catabolic cytokine inhibitors [3,4] have the potential to slow the degenerative cascade and provide a microenvironment more conducive to stem cell-based disc regeneration; however, the absence of a preclinical animal model that effectively recapitulates physiological inflammation represents an impediment to effective translation of such therapies. In this study, we provide evidence that an established goat model of disc degeneration is characterized by elevated expression of IL-1β, IL-6 and TNF-α, three cytokines that are well-established mediators of human disc degeneration. Specifically, we

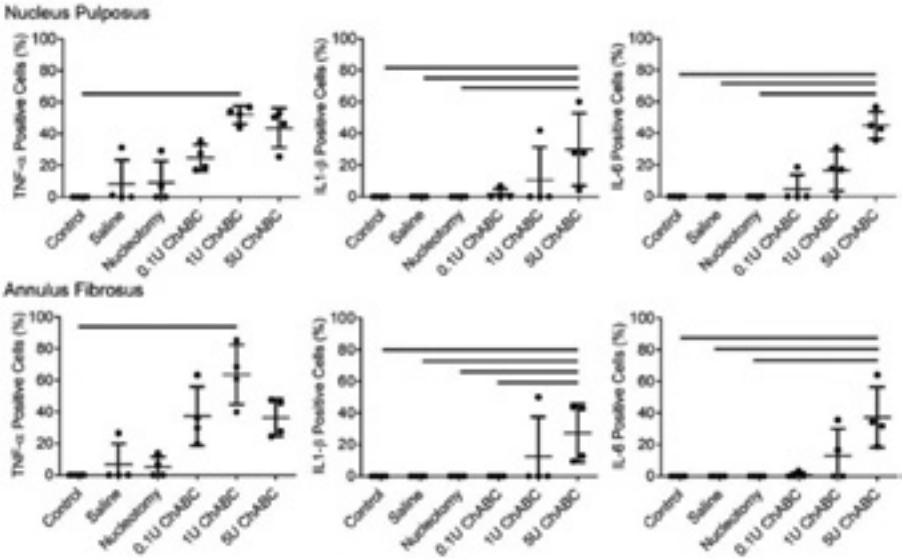
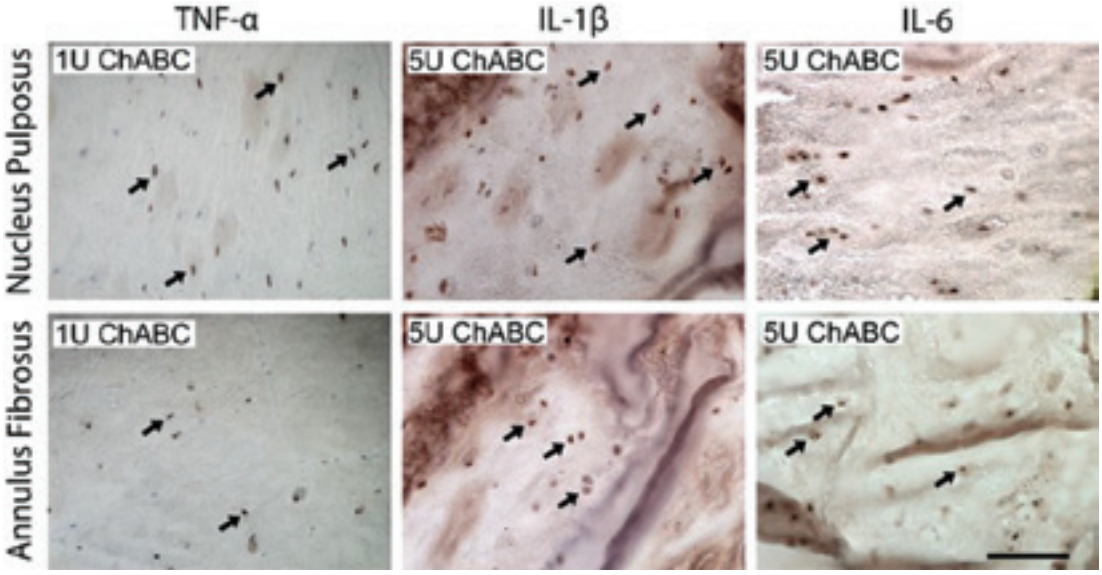


Figure 1. Quantification of expression levels of TNF-α, IL-1β and IL6 by cells in the NP and AF of goat discs 12 weeks following initiation of degeneration using different techniques. Bar indicate significant differences between groups ($p<0.05$). Mean ± SD.



Figures 2. Representative images showing positive expression of TNF-α, IL-1β and IL-6 by NP and AF cells (arrows = examples) in degenerate goat intervertebral discs. Scale = 100μm.

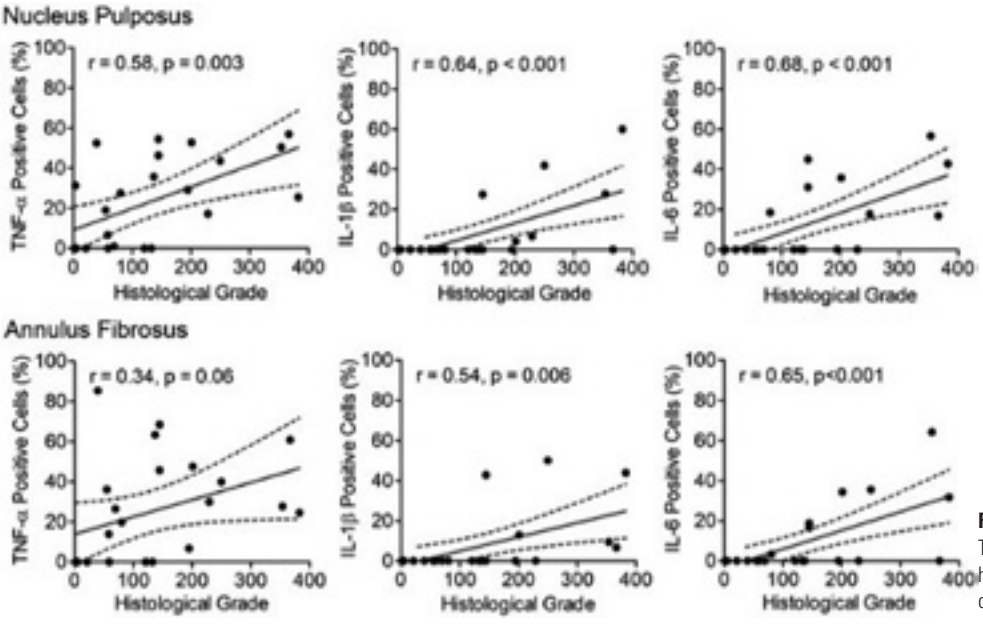


Figure 3. Linear correlations between expression of TNF-α, IL-1β and IL-6 by NP and AF cells, and the overall histological grade of goat discs 12 weeks following initiation of degeneration. Dotted lines = 95% confidence intervals.

found that cytokine expression was significantly elevated in moderately and severely degenerate discs (1U and 5U ChABC), suggesting that these discs may provide the most suitable models for evaluating anti-inflammatory therapies. Ongoing studies will seek to establish corresponding expression of downstream catabolic enzymes and pain mediators, and our long term goal is to leverage this model to evaluate anti-inflammatory therapies for disc degeneration.

Significance

Anti-inflammatory therapies represent a promising treatment strategy for painful disc degeneration. The animal model described here provides a platform for preclinical evaluation of such therapies and progressing them towards clinical use.

References

1. Risbud MV and Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol* 2014; 10(1): 44-56.

2. Gullbrand SE, Malhotra NR, Schaer TP, *et al.* A large animal model that recapitulates the spectrum of human intervertebral disc degeneration. *Osteoarthritis Cartilage* 2017; 25(1): 146-56.

3. Gorth DJ, Mauck RL, Chiaro JA, *et al.* IL-1ra delivered from poly(lactic-co-glycolic acid) microspheres attenuates IL-1ß-mediated degradation of nucleaus pulposus in vitro. *Arthritis Res Ther* 2012; 14(4): R179.

4. Sainoh T, Orita S, Miyagi M, *et al.* Single intradiscal administration of the tumor necrosis factor-alpha inhibitor, Etanercept, for patients with discogenic low back pain. *Pain Med* 2016; 17(1): 40-5.