9-21-2015

Disc in flames: Roles of TNF-α and IL-1β in intervertebral disc degeneration.

Z I Johnson
Department of Orthopaedic Surgery, Thomas Jefferson University

Z R Schoepflin
Department of Orthopaedic Surgery, Thomas Jefferson University

H Choi
Department of Orthopaedic Surgery, Thomas Jefferson University

I M Shapiro
Department of Orthopaedic Surgery, Thomas Jefferson University

M V Risbud
Department of Orthopaedic Surgery, Thomas Jefferson University

DISC IN FLAMES: ROLES OF TNF-α AND IL-1β IN INTERVERTEBRAL DISC DEGENERATION

Z.I. Johnson, Z.R. Schoepflin, H. Choi, I.M. Shapiro and M.V. Risbud*

Department of Orthopaedic Surgery, Sidney Kimmel Medical College and Graduate Program in Cell and Developmental Biology, Thomas Jefferson University, Philadelphia, PA, USA

Abstract

The intervertebral disc is an important mechanical structure that allows range of motion of the spinal column. Degeneration of the intervertebral disc – incited by aging, traumatic insult, genetic predisposition, or other factors – is often defined by functional and structural changes in the tissue, including excessive breakdown of the extracellular matrix, increased disc cell senescence and death, as well as compromised biomechanical function of the tissue. Intervertebral disc degeneration is strongly correlated with low back pain, which is a highly prevalent and costly condition, significantly contributing to loss in productivity and health care costs. Disc degeneration is a chronic, progressive condition, and current therapies are limited and often focused on symptomatic pain relief rather than curtailing the progression of the disease. Inflammatory processes exacerbated by cytokines tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are believed to be key mediators of disc degeneration and low back pain. In this review, we describe the contributions of TNF-α and IL-1β to changes seen during disc degeneration at both cellular and tissue level, as well as new evidence suggesting a link between infection of the spine and low back pain, and the emerging therapeutic modalities aimed at combating these processes.

Keywords: Intervertebral disc, nucleus pulposus, cytokines, extracellular matrix, tumour necrosis factor, interleukin-1, Toll-like receptor.

*Address for correspondence:
Makarand V. Risbud, PhD
1025 Walnut Street
Suite 511 College Building
Philadelphia, PA 19107, USA
Telephone Number: 1-215-955-1063
FAX Number: 1-215-955-9159
E-mail: makarand.risbud@jefferson.edu

Introduction

The intervertebral disc (IVD) comprises an outer circumferential annulus fibrosus (AF) and an inner cell-sparse, matrix-rich nucleus pulposus (NP), bordered superiorly and inferiorly by two cartilaginous endplates. The AF is a lamellar fibrocartilaginous structure rich in collagen I, derived from embryonic sclerotome, and is responsible for withstanding large hoop stress from the pressurised NP, and tensile and torsional stresses from motion of adjacent vertebrae (Guterl *et al*., 2013). The NP encompasses a physiologically hypoxic and hyperosmotic niche; cells residing within the NP are derived from the embryonic notochord (Risbud *et al*., 2010; Risbud and Shapiro, 2011; Johnson *et al*., 2014; Choi *et al*., 2015). NP cells are phenotypically defined in part by the distinct ratio of synthesised aggrecan and collagen II (Mwale *et al*., 2004; Risbud *et al*., 2015). The hydrated, aggrecan-rich extracellular matrix (ECM) resists large compressive loads from the trunk, allowing us to remain upright (Vergroesen *et al*., 2015). Degeneration of the NP, AF and IVD as a structure is often defined by functional and structural changes in the tissue, including excessive breakdown of ECM by proteases and increased cell senescence and death (Roughley, 2004; Adams and Roughley, 2006). IVD degeneration is strongly correlated with low back pain (LBP) (Chou *et al*., 2011; Livshits *et al*., 2011), which is a highly prevalent, costly, and crippling condition across the globe (Katz, 2006; Hoy *et al*., 2012). Inflammatory processes, exacerbated by cytokines tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are believed to be key events during disc degeneration and associated LBP (Risbud and Shapiro, 2014). In this review, we describe the specific contributions of TNF-α and IL-1β to cellular and tissue level changes seen during disc degeneration, discuss new evidence suggesting a link between infection of the spine and LBP, and the emerging therapeutic modalities aimed at combating these processes. Specifically, the effects of cytokines on breakdown of disc ECM and potential activation of Toll-like receptors, the immune response, disc cell homeostasis, and resolution of disc herniation are discussed, as well as potential triggering mechanisms for this cytokine activity.

Initiating events that may contribute to elevated cytokine production by disc cells and spinal tissues

It has been well established that inflammatory changes in the disc contribute to degeneration, and that trauma and genetic predisposition may contribute to some aspects of

this cascade. However, the question remains of the initiating event(s) that promotes production of these cytokines by NP and AF cells, especially in the absence of acute trauma or herniation. One possible initiator is chronic overload of the disc, which has been shown to cause degenerative changes in several models (Adams *et al*., 2000). Discs from rats subjected to *in vivo* dynamic compression overloading over 8 weeks showed increased matrix metalloproteinase (MMP)-associated aggrecan degradation products in both the AF and NP compared to sham discs (Iatridis *et al*., 2011). Some evidence exists, correlating bacterial infection with degenerative disc disease and LBP. In one study, 53 % of surgical microdiscectomy samples from patients with sciatica were positive for bacterial cultures (Stirling *et al*., 2001). In another recent study, herniated nuclear tissue removed during surgery was positive for microbial cultures in 46 % of patients, and significantly correlated with Modic changes in adjacent vertebral endplates, which are highly associated with LBP (Jensen *et al*., 2008; Albert *et al*., 2013a). Additional studies have also shown evidence of bacterial infection in some painful degenerate discs, even in the absence of overt clinical infection (Stirling *et al*., 2002; Fritzell *et al*., 2004; Agarwal *et al*., 2011; Arndt *et al*., 2012).

In 2008, Albert *et al.* (2008a) proposed the hypothesis that some cases of degenerative disc disease and low back pain have an infectious cause, supported by a pilot study investigating the efficacy of antibiotics for the treatment of patients with persistent LBP and Modic changes that did not respond to initial conservative therapy (Albert *et al*., 2008b). In this pilot study, there was a statistically and clinically significant improvement in patients receiving a course of amoxicillin-clavulanate antibiotics. A larger double-blind randomised clinical trial followed and similarly reported significant improvement in disability and pain in patients treated with antibiotics compared to placebo (Albert *et al*., 2013b). Importantly, some evidence suggests that clavulanate has anti-inflammatory and analgesic properties, which may confound interpretation of clinical findings by Albert *et al*. (Casellas *et al*., 1998; Hajhashemi and Dehdashti, 2014). In addition, some investigators have suggested that the presence of bacteria in disc samples arises from contamination of skin commensals (*e.g. Proprionibacterium acnes*) during non-sterile surgical and collection procedures (McLorinan *et al*., 2005; Ben-Galim *et al*., 2006; Carricajo *et al*., 2007; Wedderkopp *et al*., 2009); it may also arise from infected endplate and bony avulsions (Rajasekaran *et al*., 2013). In spite of the controversial nature, subclinical bacterial infection of endplate/vertebrae and/or discitis as a cause for disc degeneration and LBP warrants further consideration and careful investigation.

Bacterial infection of the disc may initiate inflammatory cascades through activation of components of the innate immune response. Toll-like receptors (TLRs) are plasma- and endolysosomal-bound pattern recognition receptors, best characterised as expressed on the surface of cells of the immune system (De Nardo, 2015). TLRs recognise pathogen-associated molecular patterns (PAMPs), and activate inflammatory signalling cascades (Kumar *et al*., 2009). Most relevant to bacterial infection are TLR2 and TLR4. TLR2 recognises a range of PAMPs including peptidoglycans expressed in the cell wall of Gram-positive bacteria (Zähringer *et al*., 2008), while TLR4 is activated by lipopolysaccharide (LPS) in the cell wall of Gram-negative bacteria (Poltorak *et al*., 1998). Activation both TLR2 and TLR4 signalling results in transcription of many pro-inflammatory cytokines through the MyD88 pathway (Kawai *et al*., 1999).

Interestingly, NP cells also express TLRs with a recent study identifying expression of TLR1/2/3/4/5/6/9/10 in isolated human IVD cells. Importantly, expression levels of TLR2 and TLR4 were raised with increasing grade of degeneration (Klawitter *et al*., 2014). Another study showed that treatment of IVD cells with LPS resulted in increased expression of TNF-α, IL-1β, and IL-6, and decreased expression of aggrecan and collagen II. Furthermore, injection of LPS into discs *in vivo* induced degenerative changes (Rajan *et al*., 2012). Ellman *et al*. (2012) demonstrated that inhibition of MyD88 attenuated the changes in catabolic gene expression incurred by LPS *in vitro* as well as in an *ex vivo* organ culture model. These studies suggest that not only can innate immunity initiate degenerative changes in the disc, but it can also perpetuate inflammatory cascades and promote progressive disease.

The TNF and IL-1 signalling pathways

TNF-α is synthesised by cells as a 26 kDa type II transmembrane protein termed mTNF. This membrane-bound form is processed to a 17 kDa soluble form, sTNF, by the metalloproteinase tumour necrosis factor-α-converting enzyme (TACE) also known as ADAM metallopeptidase domain 17 (ADAM17). The trimeric forms of both mTNF and sTNF are biologically active, although monomeric and dimeric forms also exist (Black *et al*., 1997). TNF-α interacts with either of two receptors of the tumour necrosis factor receptor (TNFR) superfamily, TNFR1 or TNFR2. mTNF can bind either receptor, while sTNF can bind TNFR1 only. TNFR1 activation by TNF-α leads to formation of two distinct TNF signalling complexes: Complex 1 has anti-apoptotic functions, while Complex II/death inducing signalling complex (DISC) induces apoptosis after receptor internalisation. TNFR2, on the other hand, lacks an intracellular death domain. Therefore, signalling through this complex is considered anti-apoptotic. Recent evidence, however, suggests that TNFR2 may induce degradation of TNF receptor-associated factor 2 (TRAF2), resulting in crosstalk between the TNFR1 and TNFR2 pathways. Signalling through Complex I activates the nuclear factor κB (NF-κB) and mitogen activated protein kinase (MAPK) pathways (Cabal-Hierro and Lazo, 2012) (See Fig. 1). Relevant to this review, high levels of TNF-α have been associated with disc degeneration (discussed in the following sections).

IL-1α and IL-1β, members of the IL-1 family of 11 cytokines, are first synthesised as precursor proteins and then activated through intracellular proteolytic cleavage by calpain and caspase-1, respectively. Secreted pro-IL-1β can also be activated extracellularly by neutrophil proteases. While pro-IL-1β requires this activation, membrane-bound

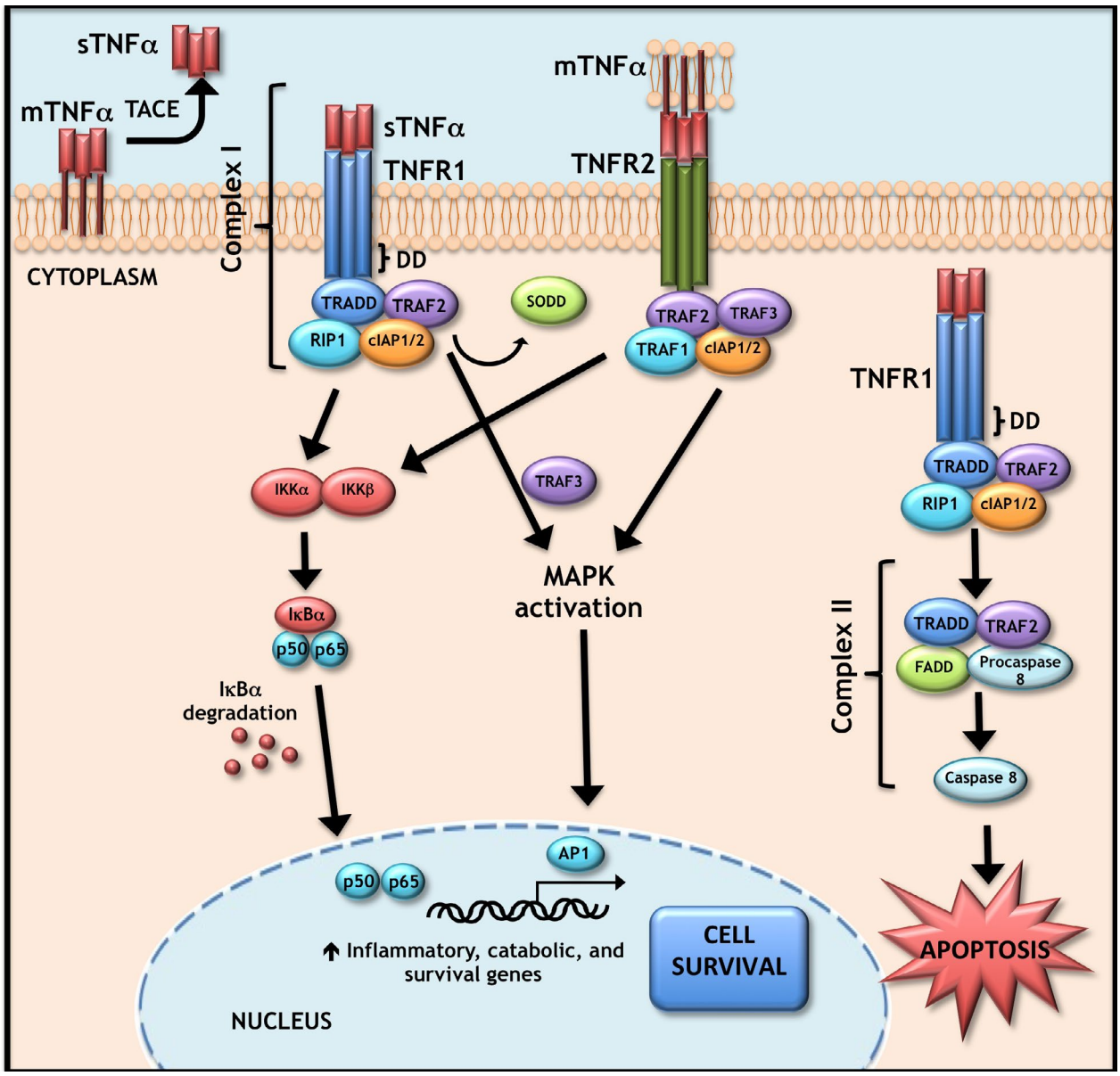

Fig. 1. TNF-α signalling pathway. Membrane-bound TNF (mTNF) is processed by the metalloproteinase TACE/ADAM-17 to the soluble form (sTNF). sTNF-α or mTNF-α may bind the transmembrane TNFR1 receptor, causing a conformational change that releases the inhibitory SODD protein. Binding results in the recruitment of several factors including TRADD, RIP1, TRAF2 and cIAP 1 and 2, resulting in formation of Complex I that signals through either the NF-κB or MAPK pathways to activate p65 or AP1, respectively. Complex I signalling results in transcription of inflammatory (chemokines, cytokines) and matrix catabolic genes (MMPs, ADAMTSs) as well as pro-survival genes (cIAP1 and 2, cFLIP, TRAF1, TRAF2). Alternatively, mTNF-α may activate the TNFR2 receptor to form a similar complex and downstream signalling cascade. In some instances, TNFR1 bound to sTNF-α may be internalised, initiating Complex II or DISC formation that leads to cleavage of procaspase 8 and finally cell apoptosis. Abbreviations: TNF-α, tumour necrosis factor α; TACE, TNF-α converting enzyme; ADAM-17, a disintegrin and metalloproteinase domain containing protein 17; TNFR1, TNF receptor 1; SODD, silencer of death domains; TNFR2, TNF receptor 2; TRADD, TNFR1-assoicated death domain protein; RIP1, receptor-interacting protein 1; TRAF2, TNF-receptor-associated factor 2; cIAP, baculoviral IAP repeat containing; DISC, death-inducing signalling complex; NF-κB, nuclear factor κB; MAPK, mitogen-activated protein kinase; DD, death domain.

pro-IL-1α can signal through interleukin 1 receptor type I (IL-1R1) on adjacent cells without proteolytic cleavage. In addition, the 16 kDa N-terminal propeptide cleavage product of pro-IL-1α (ppIL-1α) can translocate into the nucleus and is thought to function as a transcriptional modulator. Both IL-1α and IL-1β through binding IL-1R1, can stimulate NF-κB, JNK, and p38 MAPK signalling pathways, resulting in phosphorylation of various proteins involved in transcription of inflammatory and catabolic genes such as IL-6, IL-8, MCP-1, COX-2, IκBα, IL-1α, IL-1β and MKP-1 (Gabay *et al.*, 2010; Weber *et al.*, 2010).

TNF and IL-1 are closely associated with intervertebral disc degeneration

Associations between herniation, disc degeneration, and the inflammatory response have been well established. Herniated disc tissue exhibits a large inflammatory cell response with macrophages being predominant players and increased TNF-α, IL-1β and many other cytokines detected at the site of herniation (Grönblad *et al*., 1994; Takahashi *et al*., 1996). A 2012 study of NP samples from adolescent patients found an increased number of TNF-α-immunoreactive cells in painful herniated samples compared to non-painful scoliotic controls (Ohtori *et al*., 2012a). Importantly, during disc degeneration TNF-α and IL-1β are not only produced by leukocytes, but also by IVD cells themselves (Le Maitre *et al*., 2005; Le Maitre *et al*., 2007b). In addition, TNFR1, TNFR2, TACE, interleukin-1 receptor antagonist (IL-1ra) and IL-1R1 are expressed in human nucleus pulposus tissue, and expression of TNF-α and IL-1β increases with age and severity of degeneration in both humans and animal models (Oda *et al*., 2004; Le Maitre *et al*., 2005; Bachmeier *et al*., 2007; Le Maitre *et al*., 2007b; Wang *et al*., 2013b). Furthermore, another study confirmed expression of both TNFR1 and TNFR2 in NP tissue from patients with disc herniation, with levels of TNFR1 being positively correlated with degree of pain (Andrade *et al*., 2011). In addition, IL-1 gene family polymorphisms, IL-1RN G1812A, IL-1α C889T, and IL-1β C3954T, are associated with increased risk of LBP (Solovieva *et al*., 2004). Importantly, expression of these cytokines is not merely correlated with degeneration, but is believed to be causative of disease.

TNF and IL-1 contribute to disc disease through degradation of ECM

One meaningful characteristic of disc degeneration is disruption of the ECM. TNF-α and IL-1β have been shown in many contexts to induce degenerative changes to the ECM. Induction of catabolic enzymes a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and -5, and matrix metalloproteinases (MMPs) -1, -2, -3, -4, -13 and -14, and concurrent decrease in expression of anabolic ECM proteins aggrecan and collagen II is mediated by both IL-1β and TNF-α (Doita *et al*., 2001; Shen *et al*., 2003; Jimbo *et al*., 2005; Le Maitre *et al*., 2005; Séguin *et al*., 2005; Murata *et al*., 2006; Le Maitre *et al*., 2007a; Bachmeier *et al*., 2009; Pockert *et al*., 2009; Wang *et al*., 2011; Wang *et al*., 2014a). Séguin *et al*. (2008) showed that TNF-α-induced increase in MMP2 activity was specifically associated with upregulation of membrane type MT1-MMP. A recent study by Ye *et al*. (2015) showed that unlike in chondrocytes, NP cell expression of xylosyltransferase-1 (XT-1), a key enzyme in glycosaminoglycan synthesis, is unaffected by TNF-α and IL-1β treatment, suggesting that the effects of these cytokines on the ECM homeostasis are fairly cell-type-specific. Further studies have shown that TNF-mediated activation of ADAMTS4 occurs through ERK1, p38 and NF-κB pathways in NP cells (Tian *et al*., 2013). In organ culture models, treatment with TNF-α resulted in suppression of multiple collagen types, aggrecan, fibromodulin, increased expression of MMPs and pain-associated molecule nerve growth factor (NGF) (Ponnappan *et al*., 2011), and compromised disc biomechanics (Walter *et al*., 2015a). These results contrast with that of Hoyland *et al*. (2008), which showed that TNF-α treatment had little effect on matrix degradation as assessed by *in situ* zymography on gelatin, collagen II, and casein, although changes were seen after treatment with IL-1β. This differential response may be explained by differences in concentration, duration of TNF-α treatment, culture system, and/or species between the studies**.** Not surprisingly, the relative importance of each of these inflammatory mediators is still debated. Nonetheless, both of these cytokines have been shown under numerous conditions to control catabolic gene expression in disc tissues.

TNF-α and IL-1β also suppress expression of matricellular protein connective tissue growth factor (CCN2/CTGF), an important regulator of cellular adhesion, proliferation, migration and ECM synthesis (Tran *et al*., 2010; Tran *et al*., 2014). Tran *et al*. (2014) demonstrated that the activity of these cytokines on CCN2 expression was NF-κB-dependent. In addition, CCN2 successfully suppressed IL-1β mediated induction of catabolic molecules MMP-3, ADAMTS5, syndecan-4 and prolyl hydroxylase 3 (PHD3), and this inhibition was through αvβ3 and α5β1 integrins, indicating a protective role of CCN2 in pathogenesis of disc disease.

Many of the effects of TNF-α and IL-1β on the ECM are mediated through the NF-κB signalling pathway and the heparan-sulphate proteoglycan syndecan-4. Fujita *et al.* (2012) showed that prolyl hydroxylase 3 (PHD3) acts as a coactivator of p65 to propagate matrix catabolism induced by TNF-α. TNF-α treatment of NP cells induces expression of PHD3, which then interacts with p65 to promote transcription of syndecan-4, ADAMTS5, MMP-13 and COX2. More recent work has confirmed that PHD2 plays a similar role in the NP in promoting p65-mediated transcription (Li *et al*., 2015). Syndecan-4 is required for TNF-α and IL-1β-dependent increase in expression and activity of ADAMTS5 and MMP-3 in rat NP cells (Wang *et al*., 2011; Wang *et al*., 2014a). Adding another dimension to cytokine action on disc cells, Ye *et al.* (2011) demonstrated a positive relationship between TNF-α and Wnt/β-catenin signalling in NP cells and showed that TNF-α activates the Wnt/β-catenin pathway, thereby increasing expression of MMP-13. Additionally, Wnt signalling induces TNF-α expression in NP cells, possibly leading to a pro-degenerative feed-forward loop between the two signalling pathways (Hiyama *et al*., 2013).

In vivo studies support the role of these endogenous cytokines in the degenerative process of the IVD. Mice lacking functional IL-1ra, an endogenous antagonist for IL-1R1, demonstrated loss of proteoglycans and increased expression of MMP-3, -7 and ADAMTS4 in their IVDs.

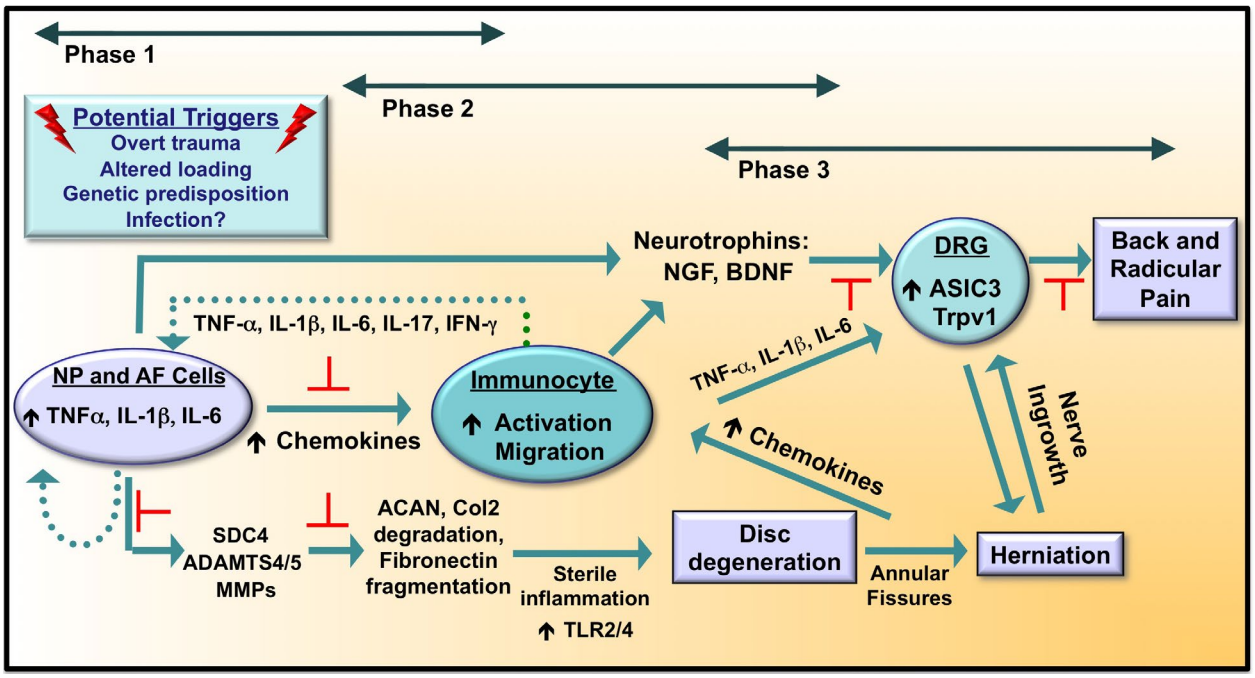

Fig. 2. Schematic showing the major phases of intervertebral disc degeneration and discogenic back pain. Potential triggering events include overt trauma to the disc, altered loading, genetic predisposition and spinal infection. These triggers result in NP and AF cell-mediated synthesis of cytokines such as TNF-α, IL-1β, and IL-6. These cytokines have at least three major effects: increased matrix breakdown through production of SDC4, ADAMTS4/5 and MMPs, activation and chemotaxis of immune cells to the disc, and induction of neurotrophins (NGF, BDNF) by disc cells and immunocytes. NGF and BDNF induce production of pain-associated channels ASIC3 and Trpv1 in the DRG. Presence of these factors in the inflammatory microenvironment of the degenerated disc is thought to promote DRG sensitisation and pain. Blocking of cytokine production in Phases 1 and 2 may have the most profound positive effect on disease progression and back pain. Red blocking symbols indicate possible areas for clinical intervention. Abbreviations: ADAMTS4/5, a disintegrin and metalloproteinase with thrombospondin motifs 4/5; ASIC3, acid-sensing ion channel 3; BDNF, brain-derived neurotrophic factor; β-NGF, β-nerve growth factor; DRG, dorsal root ganglion; IFN-γ, interferon-γ; MMPs, matrix metalloproteinases; SDC4, syndecan-4; TrpV1, transient receptor potential cation channel subfamily V member 1. (Adapted from Risbud and Shapiro, 2014)

Compared to wild type mice, discs of IL-1ra$^{-/-}$ mice had higher histological grade of degeneration, and disc cells exhibited diminished proliferative capacity (Phillips *et al.*, 2013). Similarly, Kang *et al.* (2015) showed that injection of TNF-α in a porcine model was sufficient to induce early-stage disc degeneration, characterised by matrix loss, annular fissure formation, and vascularisation.

Activation of TLRs by endogenous matrix ligands may exacerbate the disease progression through cytokine production

Additionally, matrix catabolism products may contribute to progression of disc disease by serving as endogenous ligands of TLRs and, thus, exacerbating the inflammatory state. Biglycan is a small, leucine-rich proteoglycan expressed in the IVD. Proteolytically-cleaved biglycan has been shown to activate proinflammatory cascades through binding to TLR2 and TLR4 in macrophages (Schaefer *et al.*, 2005). Relevant to this discussion, biglycan has been shown to be extensively fragmented in pathological human IVDs (Brown *et al.*, 2012). Similarly, fibronectin fragments (Fn-f) can act as endogenous ligands for TLR4 (Okamura *et al.*, 2001), fibronectin fragmentation increases in degenerated discs possibly due to increased ADAM8-mediated cleavage (Ruel *et al.*, 2014), and injection of 30 kDa N-terminal Fn-f in rabbit discs promotes degeneration (Anderson *et al.*, 2005). Similarly, versican, a large aggregating proteoglycan that is expressed in the disc (Sivan *et al.*, 2014), is shown to activate TLR2 and TLR6 (Kim *et al.*, 2009). While the *in vivo* evidence is limited, there are indeed studies showing activation of TLR by ECM components in NP, giving credence to the hypothesis. Fragments of hyaluronic acid (HA) increase expression of IL-1β, IL-6, IL-8, MMP-1, and MMP-13 by NP cells by binding to TLR2 (Quero *et al.*, 2013). Additionally, excessive mechanical loading of IVD cells, a contributor to disc disease, has also been shown to upregulate TLR2 and TLR4 expression (Gawri *et al.*, 2014). Thus, ECM proteins, particularly fragments generated during the degenerative process, binding to TLRs on NP cells can potentiate the

pathogenesis of disc degeneration (See schematic in Fig. 2 for various phases of disc degeneration and possible areas for intervention, as indicated by red blocking symbols).

Immune cells are activated by disc cells in response to TNF and IL-1

In addition to affecting matrix homeostasis, cytokines also induce NP cells to synthesise many cytokines and chemokines that can further enhance the inflammatory state by recruiting and activating immune cells. Gruber *et al*. (2013) showed that production of IL-17 is correlated with severity of degeneration and was induced by TNF-α and IL-1β. Human IVD cells, in response to IL-1β treatment, increase production of COX-2 and IL-6 as well as IL-1β itself, creating a positive feedback loop (Jimbo *et al*., 2005). When exposed to TNF-α, human IVD cells increased production of the tachykinin peptide Substance P, which subsequently induced expression of IL-1β, IL-6 and IL-8, a chemoattractant for neutrophils (Kepler *et al*., 2012). Similarly, IVD cells, following treatment with TNF-α and IL-1β, produce CCL5/RANTES, a chemoattractant for macrophages and eosinophils, and the expression is correlated with both discogenic pain and severity of degeneration (Kepler *et al*., 2013; Gruber *et al*., 2014a). When treated with IL-1β or TNF-α, both NP and AF cells produced monocyte chemoattractant protein-1 (MCP-1/CCL2) in a dose- and time-dependent manner. This was also demonstrated in a surgically-induced rabbit IVD herniation model (Yoshida *et al*., 2002; Yoshida *et al*., 2005). Human AF cells also induced expression of MCP-1/CCL2 in response to IL-1β treatment (Gruber *et al*., 2015). Other macrophage chemoattractants, such as CCL3/MIP-1α and CCL4/MIP-1β, are expressed by NP cells, and their expression is regulated by TNF-α or IL-1β through MAPK and NF-κB pathways. Interestingly, while p65 induces the expression of CCL3, p50 showed inhibitory effects on CCL3 expression. Importantly, expression of CCL3 has been correlated to severity of degeneration in human NP tissue (Wang *et al*., 2013b).

It is worth noting that IVD cells themselves express chemokine receptors, although their physiologic function and precise role in degeneration is not yet fully elucidated. IVD cells express CCL5 receptors CCR1, CCR3 and CCR5 (Gruber *et al*., 2014a). In a recent report, Phillips *et al*. (2015) showed expression of CCR1, CXCR1 and CXCR2 by human NP cells *in situ;* expression of CXCR2 significantly correlated with the grade of degeneration. Surprisingly, NP cells were also positive for CD4, a receptor exclusively expressed by immune cells, more prominently by the CD4+ T helper cells, an observation that requires further careful investigation. Even though human NP cells expressed these chemokine receptors, treatment of primary human NP cells with IL-16, CCL2, CCL3, CCL7 or CXCL8 did not induce inflammatory responses or ECM remodelling. Rather, IL-1β treatment was able to induce cytokines and chemokines as well as ECM remodelling, suggesting IL-1β as a key player in modulating inflammatory process in intervertebral disc (Phillips *et al*., 2015).

TNF and IL-1 affect homeostatic activities of IVD cells

In addition to the effects on the matrix, inflammatory cytokines can also directly affect disc cell survival and functionality. A study from Purmessur *et al*. (2013) showed in a bovine organ culture model that TNF-α induced cell senescence along with anti-anabolic and pro-catabolic activities of the resident cells. In a rat organ culture model of disc degeneration, tissues were cultured with TNF-α, IL-1β and serum limiting conditions, and showed differential expression of many genes involved in cell cycle, cell death, cellular growth and proliferation (Ponnappan *et al*., 2011). In addition, human AF cells exposed to both IL-1β and TNF-α significantly downregulated growth differentiation factor 5 (GDF5) (Gruber *et al*., 2014b), which has previously been demonstrated to be an important anabolic factor in disc (Li *et al*., 2004). Microarray analysis of human outer and inner AF tissue revealed higher GDF5 expression in herniated lumbar discs, compared to non-herniated lumbar discs (Gruber *et al*., 2014b). Another central mediator critical to disc cell proliferation is the Notch signalling pathway (Hiyama *et al*., 2011). TNF-α and IL-1β treatment of NP cells increased expression of NOTCH1 and NOTCH2 receptors along with their ligand Jagged-2, and downstream transcription factors HES1, HEY1 and HEY2. In addition, levels of NOTCH2 were increased in degenerative human NP tissues compared to non-degenerative samples, suggesting cytokine-mediated aberration in Notch signalling may affect cellular proliferation and differentiation in disc degeneration (Wang *et al*., 2013a).

There is emerging evidence that even transient exposure to cytokines may have lasting effects on the IVD. In a 2014 study, Maidhof *et al*. (2014) demonstrated that inflammatory stimuli have a permanent effect on IVD cell biophysical properties. After 24 h of treatment with TNF-α, hydraulic permeability and cell radius of NP cells were altered for 1 week. These authors also noted a decrease in the expression of water channel Aquaporin 1, which has been recently shown to be associated with severity of degenerative disc disease (Maidhof *et al*., 2014; Johnson *et al*., 2015). Similarly, in a bovine disc organ culture model, short-term TNF-α treatment has been shown to result in long-term catabolic effects without negatively affecting cell viability (Purmessur *et al*., 2013). Likewise, human AF cells treated with IL-1β displayed greater and sustained increase in intracellular calcium concentration in response to laminar fluid flow, indicating IL-1β "sensitises" the AF to mechanical loading and sheer stress (Elfervig *et al*., 2001).

TNF and IL-1 may play a critical role in resolution of disc herniation

While the high cytokines levels associated with chronic inflammation negatively affect the ECM and cell viability during disc degeneration, it is also important to note that TNF-α and IL-1β (Genevay *et al*., 2009**)** play an important role in natural resolution of disc herniation. It is well known that the majority of patients with disc herniation do not

develop symptoms of chronic LBP and radiculopathy due to neutrophil and macrophage-mediated timely resorption of the extruded NP tissue (Iwabuchi *et al*., 2008). One study has shown that TNF-α was required to induce MMP-3 in the herniated tissue, which acted as a chemoattractant for macrophages necessary for resorption (Haro *et al*., 2000). Immunoreactivity to TNF-α in the dorsal root ganglion (DRG) was increased following exposure to NP material in a rat model of disc herniation (Murata *et al*., 2004). Application of TNF-α inhibitor during surgery abrogated macrophage infiltration and VCAM-1 expression in the DRG. These results suggest that TNF-α plays a critical role in the coordination of macrophage infiltration following herniation of NP tissue (You *et al*., 2013). Likewise, inhibition of IL-1 by IL-1ra resulted in decreased active MMP-3 in human disc herniation samples, suggesting a contribution of IL-1 to this process (Genevay *et al*., 2009).

While these results demonstrate the importance of TNF-α and IL-1 in resolution of disc herniation, as discussed above, an unchecked inflammatory response is likely deleterious to the disc. It is therefore desirable to devise a strategy to carefully control timing and magnitude of the inflammatory response by using agents such as sTNFRII (Sinclair *et al*., 2011), IL-1ra (Le Maitre *et al*., 2006; Shamji *et al*., 2007; Gorth *et al*., 2012), and anti-chemotactic agents (Wang *et al*., 2013b).

New therapies and future directions for the treatment of disc disease

Several approaches have been studied to counteract the effects of TNF-α on disc degeneration. BMP-7 is able to counteract TNF-α-induced activation of NF-κB and subsequent induction of ADAMTS4 and ADAMTS5, thereby preventing TNF-induced matrix loss in human disc cells (Wang *et al*., 2014b). Platelet rich plasma, or PRP, has also been shown to reduce the TNF- and IL-1-mediated decreases in collagen II and aggrecan expression levels in human NP cells (Kim *et al*., 2014). Finally, LIM mineralisation protein-1 (LMP-1) has recently been shown to prevent TNF-α-mediated induction of MMP-3 and -13 in rat NP cells (Liu *et al*., 2015).

A recent study by Walter *et al*. (2015b) demonstrated that production of IL-1β, IL-6 and IL-8 by diseased human NP cells in response to TNF-α was at vastly different rates and magnitudes, suggesting different roles these cytokines may play in disc disease. Unlike reported by previous studies, blocking IL-6 or IL-1β did not affect the expression of other pro-inflammatory cytokines in response to TNF-α treatment. Anti-TNF-α therapy, given at the same time as TNF-α stimulation, was most effective at inhibiting expression of these cytokines (Walter *et al*., 2015b).

Other studies have suggested a possible role for anti-IL-1β therapy as a treatment modality. Co-treatment of agarose-encapsulated bovine NP cells with both IL-1β and IL-1ra abolished the catabolic effect caused by IL-1β (Smith *et al*., 2011). When IL-1ra was introduced to degenerate human IVD explants, matrix degradation as well as expression of MMPs and ADAMTSs were successfully blocked (Le Maitre *et al*., 2007a). Similarly, medium conditioned with IL-1ra encapsulated in PLGA microspheres was effective in attenuating the effect of IL-1β on bovine NP cells (Gorth *et al*., 2012). Shamji *et al*. (2007) used recombinant human elastin-like polypeptide (ELP)-IL-1ra fusion protein to promote prolonged release of IL-1ra, and were successful in decreasing TNF-α expression and ADAMTS4 and MMP-3 transcription in human IVD cells. In addition, Krupkova *et al*. (2014) demonstrated that degenerate human IVD cells treated *in vitro* with epigallocatechin 3-gallate (EPCG) significantly inhibited the expression of pro-inflammatory cytokines and MMPs in response to IL-1β treatment, and also showed *in vivo* reduction of radiculopathic pain in rats treated with EPCG.

A study by Gu *et al*. (2015) showed miR-146a has a protective effect on IL-1-induced IVD degeneration. When bovine NP cells transfected with a miR-146a mimic were treated with IL-1, induction of inflammatory cytokines and matrix protease gene expression was decreased. Additionally, IVDs from miR-146a$^{-/-}$ mice *ex vivo* showed greater degradation of proteoglycan and increased level of MMP-13 and ADAMTS-5 after treatment with IL-1, suggesting a new potential therapeutic approach in modulating IL-1 activity in disc disease (Gu *et al*., 2015). Importantly, in addition to these laboratory-based investigations, few clinical studies have tested efficacy of anti-TNF-α and anti-IL-1β therapies for treatment of back and radicular pain (see Table 1 for details). These clinical studies suggest that anti-cytokine therapies as well as, in a subset of patients, antibiotic regimens hold promise in treating low back and radicular pain.

Current treatment modalities for disc degeneration and LBP are limited and lack efficacy (Martin *et al*., 2008; Williams *et al*., 2014). Therefore, these new, targeted therapeutic strategies hold much promise. Careful consideration must be taken, however, when using anti-cytokine treatment. As mentioned above, TNF-α and IL-1β play necessary positive roles in natural resolution of NP herniation. Additionally, also mentioned above, even transient exposure to LPS or TNF-α results in lasting biophysical changes in NP cells (Maidhof *et al*., 2014). In light of this evidence, the timing of targeted anti-inflammatory treatment modalities is likely to have a critical impact on overall efficacy and success of therapy. Although a definitive "cure" for disc degeneration is far in the future, current research is providing hope for researchers and clinicians for improving patient outcomes for degenerative disc disease and LBP.

Acknowledgements

Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Numbers R01AR055655, R01AR064733, R01AR050087, T32AR052273 (ZIJ) and F30AR066506 (ZRS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We wish to confirm that there are no known conflicts of interest associated with this publication

Table 1. Clinical trials of different therapeutic modalities for low back and radicular pain.

Drug	Study design	Outcome
Etanercept (TNF-α decoy receptor)	Multicentre, double-blind, RCT for treating symptomatic lumbar disc herniation	Two transforaminal injections of etanercept resulted in clinically and statistically significant reduction in worst back pain and mean daily worst leg pain at 4 weeks post-treatment (Freeman *et al*., 2013)
	Prospective, randomized trial for treating radicular pain in patients with lumbar spinal stenosis	Epidural injection of etanercept was more effective than dexamethasone for improving low back pain, leg pain, and leg numbness (Ohtori *et al*., 2012)
	Multicentre, 3-group, RCT for treating pain due to lumbosacral radiculopathy	No improvement when compared to placebo, and had worse efficacy than epidural steroid injection in improving pain and functionality (Cohen *et al*., 2012)
	Triple-blind RCT for treating acute sciatica secondary to lumbar disc herniation	The ODI and VAS improved at 6 weeks and at a 3-month follow-up (Okoro *et al*., 2010)
	Double-blind, controlled study for treating patients with subacute lumbosacral radiculopathy	Significant improvements in leg and back pain at 6 months (Cohen *et al*., 2009)
	Open cohort, historical group controlled study for treating patients with severe sciatica	Improvement of leg pain, low back pain at 6 weeks, as well as Roland Morris disability questionnaire and the ODI (Genevay *et al*., 2004)
Adalimumab (Anti-TNF-α antibody)	Multicentre, double-blind, RCT for treating acute and severe sciatica and imaging-confirmed lumbar disc herniation	Small but significant improvement in sciatica, and fewer surgical procedures in short term, and markedly reduced back surgery in a 3-year follow-up (Genevay *et al*., 2010; Genevay *et al*., 2012)
Infliximab (Anti-TNF-α antibody)	RCT for treating patients with acute/ subacute sciatica due to herniated disc	No difference compared to placebo group in improving pain at 1 year, but shortened symptom duration and less straight leg raising restriction in cases of L4-L5/L3-L4 herniation with Modic change (Korhonen *et al*., 2006)
IL-1Ra-enriched Authologous Conditioned Serum (ACS; Orthokine)	Prospective, double-blind, reference-controlled, single centre, investigator-initiated study for treatment of lumbar back pain	Significant clinical improvements and VAS score, and reduction in pain and disability, ACS showed statistically significant superiority over triamcinolone at week 22 (Becker *et al*., 2007)
Bioclavid (Amoxicillin-clavulanate)	Double-blind, RCT for treatment of chronic low back pain and Modic type 1 changes	Statistically significant improvement on lumbar pain, leg pain, and disease-specific disability in antibiotic-treated group at 1 year (Albert *et al*., 2013)
	Pilot prospective uncontrolled study for treating patients with low back pain and Modic type 1 changes	Clinically and statistically significant improvement in low back pain intensity, number of days with pain, disease-specific and patient-specific function, and global perceived effect at long-term follow up (mean 10.8 months) (Albert *et al*., 2008)

Abbreviations: RCT, randomised controlled trial; VAS, visual analogue scale; ODI, Oswestry disability index.

and there has been no significant financial support for this work that could have influenced its outcome.

References

Adams MA, Roughley PJ (2006) What is intervertebral disc degeneration, and what causes it? Spine **31:** 2151-2161.

Adams MA, Freeman BJ, Morrison HP, Nelson IW, Dolan P (2000) Mechanical initiation of intervertebral disc degeneration. Spine **25:** 1625-1636.

Agarwal V, Golish SR, Alamin TF (2011) Bacteriologic culture of excised intervertebral disc from immunocompetent patients undergoing single level primary lumbar microdiscectomy. J Spinal Disord Tech **24:** 397-400.

Albert HB, Kjaer P, Jensen TS, Sorensen JS, Bendix T, Manniche C (2008a) Modic changes, possible causes and relation to low back pain. Med Hypotheses **70:** 361-368.

Albert HB, Manniche C, Sorensen JS, Deleuran BW (2008b) Antibiotic treatment in patients with low-back pain associated with Modic changes Type 1 (bone oedema): a pilot study. Br J Sports Med **42:** 969-973.

Albert HB, Lambert P, Rollason J, Sorensen JS, Worthington T, Pedersen MB, Norgaard HS, Vernallis A, Busch F, Manniche C, Elliott T (2013a) Does nuclear tissue infected with bacteria following disc herniations lead to Modic changes in the adjacent vertebrae? Eur Spine J **22:** 690-696.

Albert HB, Sorensen JS, Christensen BS, Manniche C (2013b) Antibiotic treatment in patients with chronic low back pain and vertebral bone edema (Modic type 1 changes): A double-blind randomized clinical controlled trial of efficacy. Eur Spine J **22:** 697-707.

Anderson DG, Li X, Balian G (2005) A fibronectin fragment alters the metabolism by rabbit intervertebral disc cells *in vitro*. Spine **30:** 1242-1246.

Andrade P, Visser-Vandewalle V, Philippens M, Daemen MA, Steinbusch HWM, Buurman WA, Hoogland G (2011) Tumor necrosis factor-α levels correlate with postoperative pain severity in lumbar disc hernia patients: Opposite clinical effects between tumor necrosis factor receptor 1 and 2. Pain **152:** 2645-2652.

Arndt J, Charles YP, Koebel C, Bogorin I, Steib J-P (2012) Bacteriology of degenerated lumbar intervertebral disks. J Spinal Disord Tech **25:** E211-E216.

Bachmeier BE, Nerlich AG, Weiler C, Paesold G, Jochum M, Boos N (2007) Analysis of tissue distribution of TNF-α, TNF-α-receptors, and the activating TNF-α-converting enzyme suggests activation of the TNF-α system in the aging intervertebral disc. Ann NY Acad Sci **1096:** 44-54.

Bachmeier BE, Nerlich A, Mittermaier N, Weiler C, Lumenta C, Wuertz K, Boos N (2009) Matrix metalloproteinase expression levels suggest distinct enzyme roles during lumbar disc herniation and degeneration. Eur Spine J **18:** 1573-1586.

Becker C, Heidersdorf S, Drewlo S, de Rodriguez SZ, Krämer J, Willburger RE (2007) Efficacy of epidural perineural injections with autologous conditioned serum for lumbar radicular compression: an investigator-initiated, prospective, double-blind, reference-controlled study. Spine **32:** 1803-1808.

Ben-Galim P, Rand N, Giladi M, Schwartz D, Ashkenazi E, Millgram M, Dekel S, Floman Y (2006) Association between sciatica and microbial infection: true infection or culture contamination? Spine **31:** 2507-2509.

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP (1997) A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature **385:** 729-733.

Brown S, Melrose J, Caterson B, Roughley P, Eisenstein SM, Roberts S (2012) A comparative evaluation of the small leucine-rich proteoglycans of pathological human intervertebral discs. Eur Spine J **21:** S154-159.

Cabal-Hierro L, Lazo PS (2012) Signal transduction by tumor necrosis factor receptors. Cell Signal **24:** 1297-1305.

Carricajo A, Nuti C, Aubert E, Hatem O, Fonsale N, Mallaval FO, Vautrin AC, Brunon J, Aubert G (2007) Propionibacterium acnes contamination in lumbar disc surgery. J Hosp Infect **66:** 275-277.

Casellas F, Borruel N, Papo M, Guarner F, Antolín M, Videla S, Malagelada JR (1998) Antiinflammatory effects of enterically coated amoxicillin-clavulanic acid in active ulcerative colitis. Inflamm Bowel Dis **4:** 1-5.

Choi H, Johnson ZI, Risbud MV (2015) Understanding nucleus pulposus cell phenotype: A prerequisite for stem cell based therapies to treat intervertebral disc degeneration. Curr Stem Cell Res Ther **10:** 307-316.

Chou D, Samartzis D, Bellabarba C, Patel A, Luk KDK, Kisser JMS, Skelly AC (2011) Degenerative magnetic resonance imaging changes in patients with chronic low back pain: a systematic review. Spine **36:** S43-53.

Cohen SP, Bogduk N, Dragovich A, Buckenmaier CC, Griffith S, Kurihara C, Raymond J, Richter PJ, Williams N, Yaksh TL (2009) Randomized, double-blind, placebo-controlled, dose-response, and preclinical safety study of transforaminal epidural etanercept for the treatment of sciatica. Anesthesiology **110:** 1116-1126.

Cohen SP, White RL, Kurihara C, Larkin TM, Chang A, Griffith SR, Gilligan C, Larkin R, Morlando B, Pasquina PF, Yaksh TL, Nguyen C (2012) Epidural steroids, etanercept, or saline in subacute sciatica: a multicenter, randomized trial. Ann Intern Med **156:** 551-559.

De Nardo D (2015) Toll-like receptors: Activation, signalling and transcriptional modulation. Cytokine **74:** 181-189.

Doita M, Kanatani T, Ozaki T, Matsui N, Kurosaka M, Yoshiya S (2001) Influence of macrophage infiltration of herniated disc tissue on the production of matrix metalloproteinases leading to disc resorption. Spine **26:** 1522-1527.

Elfervig MK, Minchew JT, Francke E, Tsuzaki M, Banes AJ (2001) IL-1β sensitizes intervertebral disc annulus cells to fluid-induced shear stress. J Cell Biochem **82:** 290-298.

Ellman MB, Kim JS, An HS, Chen D, Kc R, An J, Dittakavi T, van Wijnen AJ, Cs-Szabo G, Li X, Xiao G, An S, Kim SG, Im HJ (2012) Toll-like receptor adaptor signaling molecule MyD88 on intervertebral disk homeostasis: *In vitro*, *ex vivo* studies. Gene **505:** 283-290.

Freeman BJC, Ludbrook GL, Hall S, Cousins M, Mitchell B, Jaros M, Wyand M, Gorman JR (2013) Randomized, double-blind, placebo-controlled, trial of transforaminal epidural etanercept for the treatment of symptomatic lumbar disc herniation. Spine **38:** 1986-1994.

Fritzell P, Bergström T, Welinder-Olsson C (2004) Detection of bacterial DNA in painful degenerated spinal discs in patients without signs of clinical infection. Eur Spine J **13:** 702-706.

Fujita N, Gogate SS, Chiba K, Toyama Y, Shapiro IM, Risbud MV (2012) Prolyl hydroxylase 3 (PHD3) modulates catabolic effects of tumor necrosis factor-α (TNF-α) on cells of the nucleus pulposus through co-activation of nuclear factor κB (NF-κB)/p65 signaling. J Biol Chem **287:** 39942-39953.

Gabay C, Lamacchia C, Palmer G (2010) IL-1 pathways in inflammation and human diseases. Nat Rev Rheumatol **6:** 232-241.

Gawri R, Rosenzweig DH, Krock E, Ouellet JA, Stone LS, Quinn TM, Haglund L (2014) High mechanical strain of primary intervertebral disc cells promotes secretion of inflammatory factors associated with disc degeneration and pain. Arthritis Res Ther **16:** R21.

Genevay S, Stingelin S, Gabay C (2004) Efficacy of etanercept in the treatment of acute, severe sciatica: a pilot study. Ann Rheum Dis **63:** 1120-1123.

Genevay S, Finckh A, Mezin F, Tessitore E, Guerne PA (2009) Influence of cytokine inhibitors on concentration and activity of MMP-1 and MMP-3 in disc herniation. Arthritis Res Ther **11:** R169.

Genevay S, Viatte S, Finckh A, Zufferey P, Balagué F, Gabay C (2010) Adalimumab in severe and acute sciatica: a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum **62:** 2339-2346.

Genevay S, Finckh A, Zufferey P, Viatte S, Balagué F, Gabay C (2012) Adalimumab in acute sciatica reduces the long-term need for surgery: a 3-year follow-up of a randomised double-blind placebo-controlled trial. Ann Rheum Dis **71:** 560-562.

Gorth DJ, Mauck RL, Chiaro JA, Mohanraj B, Hebela NM, Dodge GR, Elliott DM, Smith LJ (2012) IL-1Ra delivered from poly(lactic-co-glycolic acid) microspheres attenuates IL-1β mediated degradation of nucleus pulposus *in vitro*. Arthritis Res Ther **14:** R179.

Grönblad M, Virri J, Tolonen J, Seitsalo S, Kääpä E, Kankare J, Myllynen P, Karaharju EO (1994) A controlled immunohistochemical study of inflammatory cells in disc herniation tissue. Spine **19:** 2744-2751.

Gruber HE, Hoelscher GL, Ingram JA, Norton HJ, Hanley EN (2013) Increased IL-17 expression in degenerated human discs and increased production in cultured annulus cells exposed to IL-1β and TNF-α. Biotech Histochem **88:** 302-310.

Gruber HE, Hoelscher GL, Ingram JA., Bethea S, Norton HJ, Hanley EN (2014a) Production and expression of RANTES (CCL5) by human disc cells and modulation by IL-1β and TNF-α in 3D culture. Exp Mol Pathol **96:** 133-138.

Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Hanley EN (2014b) Growth and differentiation factor-5 (GDF-5) in the human intervertebral annulus cells and its modulation by IL-1β and TNF-α *in vitro*. Exp Mol Pathol **96:** 225-229.

Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Cox M, Hanley EN (2015) Proinflammatory cytokines modulate the chemokine CCL2 (MCP-1) in human annulus cells *in vitro*: CCL2 expression and production. Exp Mol Pathol **98:** 102-105.

Gu S-X, Li X, Hamilton JL, Chee A, Kc R, Chen D, An HS, Kim J-S, Oh C, Ma Y-Z, van Wijnen AJ, Im H-J (2015) MicroRNA-146a reduces IL-1 dependent inflammatory responses in the intervertebral disc. Gene **555:** 80-87.

Guterl CC, See EY, Blanquer SBG, Pandit A, Ferguson SJ, Benneker LM, Grijpma DW, Sakai D, Eglin D, Alini M, Iatridis JC, Grad S (2013) Challenges and strategies in the repair of ruptured annulus fibrosus. Eur Cell Mater **25:** 1-21.

Hajhashemi V, Dehdashti KH (2014) Antinociceptive effect of clavulanic acid and its preventive activity against development of morphine tolerance and dependence in animal models. Res Pharm Sci **9:** 315-321.

Haro H, Crawford HC, Fingleton B, Shinomiya K, Spengler DM, Matrisian LM (2000) Matrix metalloproteinase-7-dependent release of tumor necrosis factor-α in a model of herniated disc resorption. J Clin Invest **105:** 143-150.

Hiyama A, Skubutyte R, Markova D, Anderson DG, Yadla S, Sakai D, Mochida J, Albert TJ, Shapiro IM, Risbud MV (2011) Hypoxia activates the notch signaling pathway in cells of the intervertebral disc: Implications in degenerative disc disease. Arthritis Rheum **63:** 1355-1364.

Hiyama A, Yokoyama K, Nukaga T, Sakai D, Mochida J (2013) A complex interaction between Wnt signaling and TNF-α in nucleus pulposus cells. Arthritis Res Ther **15:** R189.

Hoy D, Bain C, Williams G, March L, Brooks P, Blyth F, Woolf A, Vos T, Buchbinder R (2012) A systematic review of the global prevalence of low back pain. Arthritis Rheum **64:** 2028-2037.

Hoyland JA, Le Maitre CL, Freemont AJ (2008) Investigation of the role of IL-1 and TNF in matrix degradation in the intervertebral disc. Rheumatology **47:** 809-814.

Iatridis JC, Godburn K, Wuertz K, Alini M, Roughley PJ (2011) Region-dependent aggrecan degradation patterns in the rat intervertebral disc are affected by mechanical loading *in vivo*. Spine **36:** 203-209.

Iwabuchi S, Ito M, Chikanishi T, Azuma Y, Haro H (2008) Role of the tumor necrosis factor-alpha, cyclooxygenase-2, prostaglandin E2, and effect of low-intensity pulsed ultrasound in an *in vitro* herniated disc resorption model. J Orthop Res **26:** 1274-1278.

Jensen TS, Karppinen J, Sorensen JS, Niinimäki J, Leboeuf-Yde C (2008) Vertebral endplate signal changes (Modic change): A systematic literature review of prevalence and association with non-specific low back pain. Eur Spine J **17:** 1407-1422.

Jimbo K, Park JS, Yokosuka K, Sato K, Nagata K (2005) Positive feedback loop of interleukin-1β upregulating production of inflammatory mediators in human intervertebral disc cells *in vitro*. J Neurosurg Spine **2:** 589-595.

Johnson ZI, Shapiro IM, Risbud MV (2014) Extracellular osmolarity regulates matrix homeostasis in the intervertebral disc and articular cartilage: Evolving role of TonEBP. Matrix Biol **40:** 10-16.

Johnson ZI, Gogate SS, Day R, Binch A, Markova DZ, Chiverton N, Cole A, Conner M, Shapiro IM, Le Maitre CL, Risbud MV (2015) Aquaporin 1 and 5 expression decreases during human intervertebral disc degeneration: novel HIF-1-mediated regulation of aquaporins in NP cells. Oncotarget **6:** 11945-11958.

Kang R, Li H, Rickers K, Ringgaard S, Xie L, Bünger C (2015) Intervertebral disc degenerative changes after intradiscal injection of TNF-α in a porcine model. Eur Spine J **24:** 2010-2016.

Katz JN (2006) Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. J Bone Joint Surg Am **88 Suppl 2:** 21-24.

Kawai T, Adachi O, Ogawa T, Takeda K, Akira S (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity **11:** 115-122.

Kepler CK, Markova D, Hilibrand AS, Vaccaro AR, Risbud MV, Albert TJ, Anderson DG (2012) Substance P stimulates production of inflammatory cytokines in human disc cells. Spine J **12:** S109.

Kepler CK, Markova DZ, Dibra F, Yadla S, Vaccaro AR, Risbud MV, Albert TJ, Anderson DG (2013) Expression and relationship of proinflammatory chemokine RANTES/CCL5 and cytokine IL-1β in painful human intervertebral discs. Spine **38:** 873-880.

Kim S, Takahashi H, Lin W-W, Descargues P, Grivennikov S, Kim Y, Luo J-L, Karin M (2009) Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. Nature **457:** 102-106.

Kim H-J, Yeom JS, Koh Y-G, Yeo J-E, Kang K-T, Kang Y-M, Chang B-S, Lee C-K (2014) Anti-inflammatory effect of platelet-rich plasma on nucleus pulposus cells with response of TNF-α and IL-1. J Orthop Res **32:** 551-556.

Klawitter M, Hakozaki M, Kobayashi H, Krupkova O, Quero L, Ospelt C, Gay S, Hausmann O, Liebscher T, Meier U, Sekiguchi M, Konno S-I, Boos N, Ferguson SJ, Wuertz K (2014) Expression and regulation of toll-like receptors (TLRs) in human intervertebral disc cells. Eur Spine J **30:** 1878-1891.

Korhonen T, Karppinen J, Paimela L, Malmivaara A, Lindgren K-A, Bowman C, Hammond A, Kirkham B, Järvinen S, Niinimäki J, Veeger N, Haapea M, Torkki M, Tervonen O, Seitsalo S, Hurri H (2006) The treatment of disc-herniation-induced sciatica with infliximab: one-year follow-up results of FIRST II, a randomized controlled trial. Spine **31:** 2759-2766.

Krupkova O, Sekiguchi M, Klasen J, Hausmann O, Konno S, Ferguson SJ, Wuertz-Kozak K (2014) Epigallocatechin 3-gallate suppresses interleukin-1β-induced inflammatory responses in intervertebral disc cells *in vitro* and reduces radiculopathic pain in rats. Eur Cell Mater **28:** 372-386.

Kumar H, Kawai T, Akira S (2009) Toll-like receptors and innate immunity. Biochem Biophys Res Commun **388:** 621-625.

Le Maitre CL, Freemont AJ, Hoyland JA (2005) The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. Arthritis Res Ther **7:** R732-R745.

Le Maitre CL, Freemont AJ, Hoyland JA (2006) A preliminary *in vitro* study into the use of IL-1Ra gene therapy for the inhibition of intervertebral disc degeneration. Int J Exp Pathol **87:** 17-28.

Le Maitre CL, Hoyland JA, Freemont AJ (2007a) Interleukin-1 receptor antagonist delivered directly and by gene therapy inhibits matrix degradation in the intact degenerate human intervertebral disc: an *in situ* zymographic and gene therapy study. Arthritis Res Ther **9:** R83.

Le Maitre CL, Hoyland JA, Freemont AJ (2007b) Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1beta and TNFalpha expression profile. Arthritis Res Ther **9:** R77.

Li X, Leo BM, Beck G, Balian G, Anderson GD (2004) Collagen and proteoglycan abnormalities in the GDF-5-deficient mice and molecular changes when treating disk cells with recombinant growth factor. Spine **29:** 2229-2234.

Li J, Yuan W, Jiang S, Ye W, Yang H, Shapiro IM, Risbud MV (2015) Prolyl-4-hydroxylase domain protein 2 controls NF-κB/p65 transactivation and enhances the catabolic effects of inflammatory cytokines on cells of the nucleus pulposus. J Biol Chem **290:** 7195-7207.

Liu H, Pan H, Yang H, Wang J, Zhang K, Li X, Wang H, Ding W, Li B, Zheng Z (2015) LIM mineralization protein-1 suppresses TNF-α induced intervertebral disc degeneration by maintaining nucleus pulposus extracellular matrix production and inhibiting matrix metalloproteinases expression. J Orthop Res **33:** 294-303.

Livshits G, Popham M, Malkin I, Sambrook PN, Macgregor AJ, Spector T, Williams FMK (2011) Lumbar disc degeneration and genetic factors are the main risk factors for low back pain in women: the UK Twin Spine Study. Ann Rheum Dis **70:** 1740-1745.

Maidhof R, Jacobsen T, Papatheodorou A, Chahine NO (2014) Inflammation induces irreversible biophysical changes in isolated nucleus pulposus cells. PLoS One **9:** e99621.

Martin BI, Deyo RA, Mirza SK, Turner JA, Comstock BA, Hollingworth W, Sullivan SD (2008) Expenditures and health status among adults with back and neck problems. JAMA **299:** 656-664.

McLorinan GC, Glenn J V, McMullan MG, Patrick S (2005) Propionibacterium acnes wound contamination at the time of spinal surgery. Clin Orthop Relat Res **437:** 67-73.

Murata Y, Onda A, Rydevik B, Takahashi K, Olmarker K (2004) Distribution and appearance of tumor necrosis factor-alpha in the dorsal root ganglion exposed to experimental disc herniation in rats. Spine **29:** 2235-2241.

Murata Y, Onda A, Rydevik B, Takahashi I, Takahashi K, Olmarker K (2006) Changes in pain behavior and histologic changes caused by application of tumor necrosis factor-alpha to the dorsal root ganglion in rats. Spine **31:** 530-535.

Mwale F, Roughley P, Antoniou J (2004) Distinction between the extracellular matrix of the nucleus pulposus and hyaline cartilage: a requisite for tissue engineering of intervertebral disc. Eur Cell Mater **8:** 58-63.

Oda H, Matsuzaki H, Tokuhashi Y, Wakabayashi K, Uematsu Y, Iwahashi M (2004) Degeneration of intervertebral discs due to smoking: Experimental assessment in a rat-smoking model. J Orthop Sci **9:** 135-141.

Ohtori S, Inoue G, Eguchi Y, Orita S, Takaso M, Ochiai N, Kishida S, Kuniyoshi K, Aoki Y, Nakamura J, Ishikawa T, Arai G, Miyagi M, Kamoda H, Suzuki M, Sakuma Y, Oikawa Y, Kubota G, Inage K, Sainoh T, Toyone T, Yamauchi K, Kotani T, Akazawa T, Minami S, Takahashi K (2012a) Tumor necrosis factor-α-immunoreactive cells in nucleus pulposus in adolescent patients with lumbar disc herniation. Spine **38:** 459-462.

Ohtori S, Miyagi M, Eguchi Y, Inoue G, Orita S, Ochiai N, Kishida S, Kuniyoshi K, Nakamura J, Aoki Y, Ishikawa T, Arai G, Kamoda H, Suzuki M, Takaso M, Furuya T, Toyone T, Takahashi K (2012b) Epidural administration of spinal nerves with the tumor necrosis factor-alpha inhibitor, etanercept, compared with dexamethasone for treatment of sciatica in patients with lumbar spinal stenosis: a prospective randomized study. Spine **37:** 439-444.

Okamura Y, Watari M, Jerud ES, Young DW, Ishizaka ST, Rose J, Chow JC, Strauss JF (2001) The extra domain A of fibronectin activates Toll-like receptor 4. J Biol Chem **276:** 10229-10233.

Okoro T, Tafazal SI, Longworth S, Sell PJ (2010) Tumor necrosis alpha-blocking agent (etanercept): a triple blind randomized controlled trial of its use in treatment of sciatica. J Spinal Disord Tech **23:** 74-77.

Phillips KLE, Jordan-Mahy N, Nicklin MJH, Le Maitre CL (2013) Interleukin-1 receptor antagonist deficient mice provide insights into pathogenesis of human intervertebral disc degeneration. Ann Rheum Dis **72:** 1860-1867.

Phillips KLE, Cullen K, Chiverton N, Michael ALR, Cole AA, Breakwell LM, Haddock G, Bunning RAD, Cross AK, Le Maitre CL (2015) Potential roles of cytokines and chemokines in human intervertebral disc degeneration: interleukin-1 is a master regulator of catabolic processes. Osteoarthritis Cartilage **23:** 1165-1177.

Pockert AJ, Richardson SM, Le Maitre CL, Lyon M, Deakin JA, Buttle DJ, Freemont AJ, Hoyland JA (2009)

Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration. Arthritis Rheum **60:** 482-491.

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science **282:** 2085-2088.

Ponnappan RK, Markova DZ, Antonio PJ, Murray HB, Vaccaro AR, Shapiro IM, Anderson DG, Albert TJ, Risbud MV (2011) An organ culture system to model early degenerative changes of the intervertebral disc. Arthritis Res Ther **13:** R171.

Purmessur D, Walter BA, Roughley PJ, Laudier DM, Hecht AC, Iatridis J (2013) A role for TNFα in intervertebral disc degeneration: A non-recoverable catabolic shift. Biochem Biophys Res Commun **433:** 151-156.

Quero L, Klawitter M, Schmaus A, Rothley M, Sleeman J, Tiaden AN, Klasen J, Boos N, Hottiger MO, Wuertz K, Richards PJ (2013) Hyaluronic acid fragments enhance the inflammatory and catabolic response in human intervertebral disc cells through modulation of toll-like receptor 2 signalling pathways. Arthritis Res Ther **15:** R94.

Rajan N, Bloom O, Maidhof R, Stetson N, Sherry B, Levine M, Chahine NO (2012) Toll-like receptor 4 (TLR4) expression and stimulation in a model of intervertebral disc inflammation and degeneration. Spine **38:** 1343-1351.

Rajasekaran S, Bajaj N, Tubaki V, Kanna RM, Shetty AP (2013) The anatomy of failure in lumbar disc herniation: an *in vivo*, multimodal, prospective study of 181 subjects. Spine **38:** 1491-500.

Risbud MV, Shapiro IM (2011) Notochordal cells in the adult intervertebral disc: new perspective on an old question. Crit Rev Eukaryot Gene Expr **21:** 29-41.

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

Risbud MV, Schipani E, Shapiro IM (2010) Hypoxic regulation of nucleus pulposus cell survival: from niche to notch. Am J Pathol **176:** 1577-1583.

Risbud MV, Schoepflin ZR, Mwale F, Kandel RA, Grad S, Iatridis JC, Sakai D, Hoyland JA (2015) Defining the phenotype of young healthy nucleus pulposus cells: Recommendations of the Spine Research Interest Group at the 2014 annual ORS meeting. J Orthop Res **33:** 283-293.

Roughley PJ (2004) Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. Spine **29:** 2691-2699.

Ruel N, Markova DZ, Adams SL, Scanzello C, Cs-Szabo G, Gerard D, Shi P, Anderson DG, Zack M, An HS, Chen D, Zhang Y (2014) Fibronectin fragments and the cleaving enzyme ADAM-8 in the degenerative human intervertebral disc. Spine **39:** 1274-1279.

Schaefer L, Babelova A, Kiss E, Hausser HJ, Baliova M, Krzyzankova M, Marsche G, Young MF, Mihalik D, Götte M, Malle E, Schaefer RM, Gröne HJ (2005) The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. J Clin Invest **115:** 2223-2233.

Séguin CA, Pilliar RM, Roughley PJ, Kandel RA (2005) Tumor necrosis factor-alpha modulates matrix production and catabolism in nucleus pulposus tissue. Spine **30:** 1940-1948.

Séguin CA, Pilliar RM, Madri JA, Kandel RA (2008) TNF-alpha induces MMP2 gelatinase activity and MT1-MMP expression in an *in vitro* model of nucleus pulposus tissue degeneration. Spine **33:** 356-365.

Shamji MF, Betre H, Kraus VB, Chen J, Chilkoti A, Pichika R, Masuda K, Setton LA (2007) Development and characterization of a fusion protein between thermally responsive elastin-like polypeptide and interleukin-1 receptor antagonist: Sustained release of a local antiinflammatory therapeutic. Arthritis Rheum **56:** 3650-3661.

Shen B, Melrose J, Ghosh P, Taylor F (2003) Induction of matrix metalloproteinase-2 and -3 activity in ovine nucleus pulposus cells grown in three-dimensional agarose gel culture by interleukin-1beta: a potential pathway of disc degeneration. Eur Spine J **12:** 66-75.

Sinclair SM, Shamji MF, Chen J, Jing L, Richardson WJ, Brown CR, Fitch RD, Setton LA (2011) Attenuation of inflammatory events in human intervertebral disc cells with a tumor necrosis factor antagonist. Spine **36:** 1190-1196.

Sivan SS, Hayes AJ, Wachtel E, Caterson B, Merkher Y, Maroudas A, Brown S, Roberts S (2014) Biochemical composition and turnover of the extracellular matrix of the normal and degenerate intervertebral disc. Eur Spine J. **23:** S344-353.

Smith LJ, Chiaro JA, Nerurkar NL, Cortes DH, Horava SD, Hebela NM, Mauck RL, Dodge GR, Elliott DM (2011) Nucleus pulposus cells synthesize a functional extracellular matrix and respond to inflammatory cytokine challenge following long-term agarose culture. Eur Cell Mater **22:** 291-301.

Solovieva S, Leino-Arjas P, Saarela J, Luoma K, Raininko R, Riihimäki H (2004) Possible association of interleukin 1 gene locus polymorphisms with low back pain. Pain **109:** 8-19.

Stirling A, Worthington T, Rafiq M, Lambert PA, Elliott TSJ (2001) Association between sciatica and *Propionibacterium* acnes. Lancet **357:** 2024-2025.

Stirling AJ, Rafiq M, Mathur K, Elliott TSJ, Worthington T, Lambert PA (2002) Association between sciatica and skin commensals. J Bone Joint Surg Br **84B:** 147.

Takahashi H, Suguro T, Okazima Y, Motegi M, Okada Y, Kakiuchi T (1996) Inflammatory cytokines in the herniated disc of the lumbar spine. Spine **21:** 218-224.

Tian Y, Yuan W, Fujita N, Wang J, Wang H, Shapiro IM, Risbud MV (2013) Inflammatory cytokines associated with degenerative disc disease control aggrecanase-1 (ADAMTS-4) expression in nucleus pulposus cells through MAPK and NF-κB. Am J Pathol **182:** 2310-2321.

Tran CM, Markova D, Smith HE, Susarla B, Ponnappan RK, Anderson DG, Symes A, Shapiro IM, Risbud MV (2010) Regulation of CCN2/connective tissue growth factor expression in the nucleus pulposus of the

intervertebral disc: role of Smad and activator protein 1 signaling. Arthritis Rheum **62:** 1983-1692.

Tran CM, Schoepflin ZR, Markova DZ, Kepler CK, Anderson DG, Shapiro IM, Risbud MV (2014) CCN2 suppresses catabolic effects of interleukin-1β through α5β1 and αvβ3 integrins in nucleus pulposus cells: Implications in intervertebral disc degeneration. J Biol Chem **289:** 7374-7387.

Vergroesen P-PA, Kingma I, Emanuel KS, Hoogendoorn RJW, Welting TJ, van Royen BJ, van Dieën JH, Smit TH (2015) Mechanics and biology in intervertebral disc degeneration: a vicious circle. Osteoarthritis Cartilage **23:** 1057-1070.

Walter BA, Likhitpanichkul M, Illien-Junger S, Roughley PJ, Hecht AC, Iatridis JC (2015a) TNFα transport induced by dynamic loading alters biomechanics of intact intervertebral discs. PLoS One **10:** e0118358.

Walter BA, Purmessur D, Likhitpanichkul M, Weinberg A, Cho SK, Qureshi SA, Hecht AC, Iatridis JC (2015b) Inflammatory kinetics and efficacy of anti-inflammatory treatments on human nucleus pulposus cells. Spine doi: 10.1097/BRS.0000000000000932.

Wang J, Markova D, Anderson DG, Zheng Z, Shapiro IM, Risbud MV (2011) TNF-α and IL-1β promote a disintegrin-like and metalloprotease with thrombospondin type I motif-5-mediated aggrecan degradation through syndecan-4 in intervertebral disc. J Biol Chem **286:** 39738-39749.

Wang H, Tian Y, Wang J, Phillips KLE, Binch ALA, Dunn S, Cross A, Chiverton N, Zheng Z, Shapiro IM, Le Maitre CL, Risbud MV (2013a) Inflammatory cytokines induce NOTCH signaling in nucleus pulposus cells: Implications in intervertebral disc degeneration. J Biol Chem **288:** 16761-16774.

Wang J, Tian Y, Phillips KLE, Chiverton N, Haddock G, Bunning RA, Cross AK, Shapiro IM, Le Maitre CL, Risbud MV (2013b) Tumor necrosis factor α- and interleukin-1β-dependent induction of CCL3 expression by nucleus pulposus cells promotes macrophage migration through CCR1. Arthritis Rheum **65:** 832-842.

Wang X, Wang H, Yang H, Li J, Cai Q, Shapiro IM, Risbud MV (2014a) Tumor necrosis factor-α- and interleukin-1β-dependent matrix metalloproteinase-3 expression in nucleus pulposus cells requires cooperative signaling *via* syndecan 4 and mitogen-activated protein kinase-nuclear factor κB axis: Implications in inflammatory disc disease. Am J Pathol **184:** 2560-2572.

Wang Z, Hutton WC, Yoon ST (2014b) Bone morphogenetic protein-7 antagonizes tumor necrosis factor-α-induced activation of nuclear factor κB and up-regulation of the ADAMTS, leading to decreased degradation of disc matrix macromolecules aggrecan and collagen II. Spine J **14:** 505-512.

Weber A, Wasiliew P, Kracht M (2010) Interleukin-1 (IL-1) pathway. Sci Signal **3:** cm1.

Wedderkopp N, Thomsen K, Manniche C, Kolmos HJ, Secher Jensen T, Leboeuf Yde C (2009) No evidence for presence of bacteria in modic type I changes. Acta Radiol **50:** 65-70.

Williams CM, Maher CG, Latimer J, McLachlan AJ, Hancock MJ, Day RO, Lin C-WC (2014) Efficacy of paracetamol for acute low-back pain: A double-blind, randomised controlled trial. Lancet **384:** 1586-1596.

Ye S, Wang J, Yang S, Xu W, Xie M, Han K, Zhang B, Wu Z (2011) Specific inhibitory protein Dkk-1 blocking Wnt/β-catenin signaling pathway improves protective effect on the extracellular matrix. J Huazhong Univ Sci Technol Med Sci **31:** 657-662.

Ye W, Zhou J, Markova DZ, Tian Y, Li J, Anderson DG, Shapiro IM, Risbud MV (2015) Xylosyltransferase-1 expression is refractory to inhibition by the inflammatory cytokines tumor necrosis factor α and IL-1β in nucleus pulposus cells. Am J Pathol **185:** 485-495.

Yoshida M, Nakamura T, Kikuchi T, Takagi K, Matsukawa A (2002) Expression of monocyte chemoattractant protein-1 in primary cultures of rabbit intervertebral disc cells. J Orthop Res **20:** 1298-1304.

Yoshida M, Nakamura T, Sei A, Kikuchi T, Takagi K, Matsukawa A (2005) Intervertebral disc cells produce tumor necrosis factor alpha, interleukin-1beta, and monocyte chemoattractant protein-1 immediately after herniation: an experimental study using a new hernia model. Spine **30:** 55-61.

You C, Zhu K, Liu X, Xi C, Zhang Z, Xu G, Yan J (2013) Tumor necrosis factor-α-dependent infiltration of macrophages into the dorsal root ganglion in a rat disc herniation model. Spine **38:** 2003-2007.

Zähringer U, Lindner B, Inamura S, Heine H, Alexander C (2008) TLR2 - promiscuous or specific? A critical re-evaluation of a receptor expressing apparent broad specificity. Immunobiology **213:** 205-224.

Discussion with Reviewers

Reviewer I: In the concluding paragraph the importance of the appropriate timing of anti-inflammatory treatment is highlighted. In view of the phases described in Fig. 2, at which stage(s) would anti-inflammatory therapy be most appropriate?

Authors: Because of the chronic and progressive nature of disc disease, anti-inflammatory treatments are best started earlier in the disease process. We are therefore of the opinion that Phases I and II would be most appropriate for anti-inflammatory therapy. The caveat, however, is that often times the disease is not clinically manifested until much later in its course, which likely would limit the maximal benefits of such treatments.

Reviewer II: The study by Albert *et al*. (2013b) has raised major interest amongst clinicians. How much relevance do the authors assign to bacterial infection during degenerative disc disease and which approach would they apply to further investigate this aspect?

Authors: The nucleus pulposus is an avascular and confined tissue, making bacterial colonisation difficult when the continuity of the tissue is maintained, thus making primary infection of the nucleus pulposus an unlikely initiating cause of disc disease. The Albert study does suggest, however, that bacterial infection in a certain subset of patients can at least promote symptomatic disc disease and chronic LBP. It is important to note that all

of the patients in the Albert study had previous nuclear herniations, where extruded NP material would be exposed to vasculature and surrounding connective tissue, and Type I Modic changes. It would therefore be interesting to explore whether a correlation exists between persistent and refractory LBP following herniation, and increased likelihood of bacteraemia (*e.g.* prolonged catheter use, recent oral surgery, history of infective endocarditis, *etc.*), when disc material could potentially be colonised. One could then investigate whether bacterial infection post-herniation alters hernia resolution and/or promotes persistent inflammatory and nociceptive cascades with involvement of immune cells. A more comprehensive study that does not exclude chronic LBP patients without Modic changes would further provide understanding of the potential contribution of bacterial infection to progression of disc disease in the general population.

Editor's Note: Scientific Editor in charge of the paper: Stephen Ferguson.