

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

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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-1 β (IL-1 β) 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-1 β 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.

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-1 β (IL-1 β) 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-1 β 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.

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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 microdisectomy 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.* *Propriionibacterium 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-1 β , 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 I 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 κ B (NF- κ B) 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-1 α and IL-1 β , 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-1 β can also be activated extracellularly by neutrophil proteases. While pro-IL-1 β requires this activation, membrane-bound

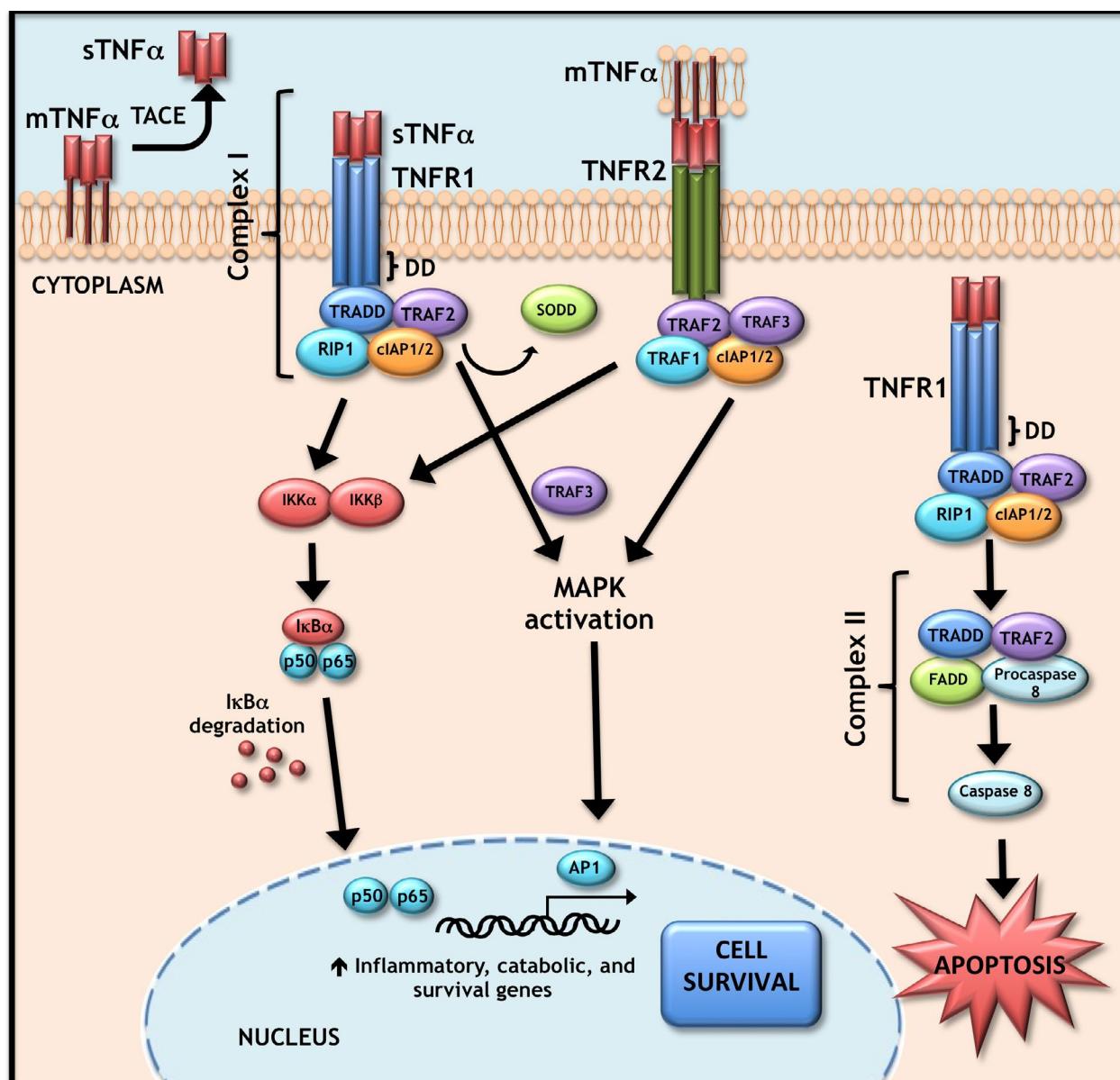


Fig. 1. TNF- α signalling pathway. Membrane-bound TNF (mTNF) is processed by the metalloproteinase TACE/ADAM-17 to the soluble form (sTNF). sTNF- α or mTNF- α may bind the transmembrane TNFR1 receptor, causing a conformational change that releases the inhibitory SODD protein. Binding results in the recruitment of several factors including TRADD, RIP1, TRAF2 and cIAP 1 and 2, resulting in formation of Complex I that signals through either the NF- κ B or MAPK pathways to activate p65 or AP1, respectively. Complex I signalling results in transcription of inflammatory (chemokines, cytokines) and matrix catabolic genes (MMPs, ADAMTs) 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-associated 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- κ B, nuclear factor κ B; MAPK, mitogen-activated protein kinase; DD, death domain.

pro-IL-1 α 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-1 α (ppIL-1 α) can translocate into the nucleus and is thought to function as a transcriptional modulator. Both IL-1 α and IL-1 β through binding IL-1R1,

can stimulate NF- κ B, 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, I κ B α , IL-1 α , IL-1 β 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-1 β 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-1 β 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-1 β 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-1 α C889T, and IL-1 β 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-1 β 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-1 β 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-1 β 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- κ B 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-1 β . 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-1 β 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- κ B-dependent. In addition, CCN2 successfully suppressed IL-1 β mediated induction of catabolic molecules MMP-3, ADAMTS5, syndecan-4 and prolyl hydroxylase 3 (PHD3), and this inhibition was through $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins, indicating a protective role of CCN2 in pathogenesis of disc disease.

Many of the effects of TNF- α and IL-1 β on the ECM are mediated through the NF- κ B 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-1 β -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.

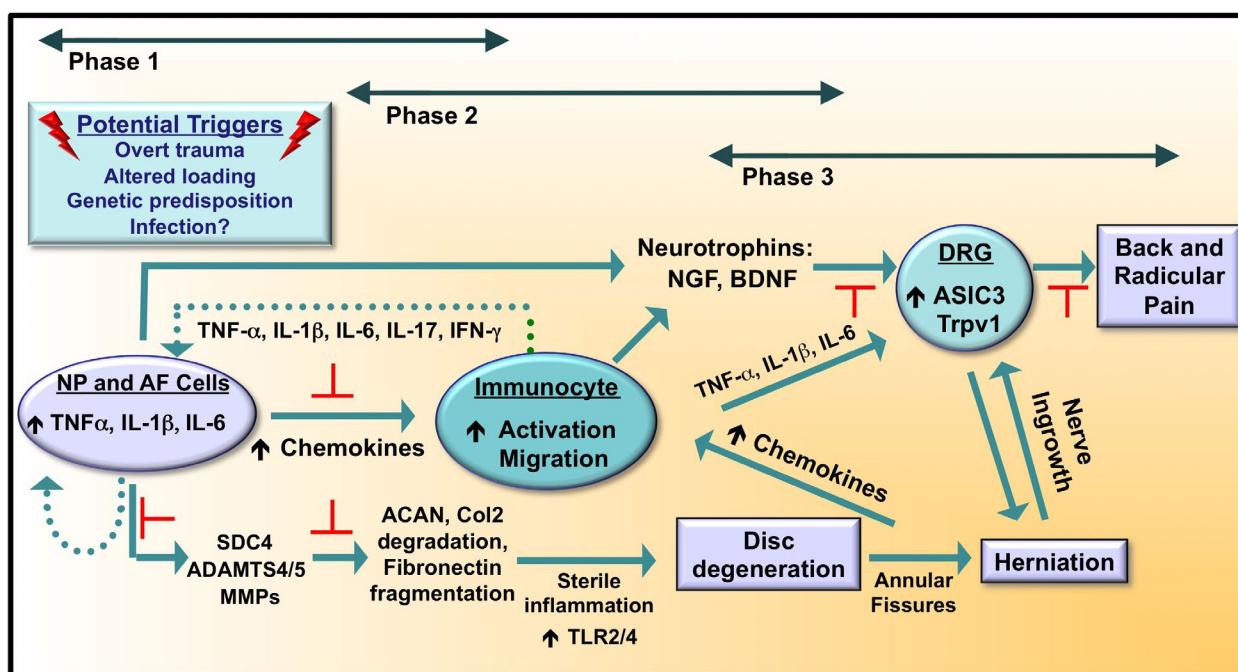


Fig. 2. Schematic showing the major phases of intervertebral disc degeneration and discogenic back pain. Potential triggering events include overt trauma to the disc, altered loading, genetic predisposition and spinal infection. These triggers result in NP and AF cell-mediated synthesis of cytokines such as TNF- α , IL-1 β , 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-1 β , 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-1 β . Human IVD cells, in response to IL-1 β treatment, increase production of COX-2 and IL-6 as well as IL-1 β 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-1 β , IL-6 and IL-8, a chemoattractant for neutrophils (Kepler *et al.*, 2012). Similarly, IVD cells, following treatment with TNF- α and IL-1 β , 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-1 β 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-1 β treatment (Gruber *et al.*, 2015). Other macrophage chemoattractants, such as CCL3/MIP-1 α and CCL4/MIP-1 β , are expressed by NP cells, and their expression is regulated by TNF- α or IL-1 β through MAPK and NF- κ B 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-1 β treatment was able to induce cytokines and chemokines as well as ECM remodelling, suggesting IL-1 β 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-1 β 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-1 β 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-1 β 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-1 β displayed greater and sustained increase in intracellular calcium concentration in response to laminar fluid flow, indicating IL-1 β "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-1 β (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- κ B 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-1 β , 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-1 β 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-1 β therapy as a treatment modality. Co-treatment of agarose-encapsulated bovine NP cells with both IL-1 β and IL-1ra abolished the catabolic effect caused by IL-1 β (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-1 β 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-1 β 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-1 β 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-1 β 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.

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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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 2010)
	Double-blind, controlled study for treating patients with subacute lumbosacral radiculopathy	Significant improvements in leg and back pain at 6 months (Cohen <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 2010; Genevay <i>et al.</i> , 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 <i>et al.</i> , 2006)
IL-1Ra-enriched Autologous 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 2008)

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

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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.