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Role of Cytokines in Intervertebral Disc Degeneration: Pain and Disc-content

Makarand V. Risbud and Irving. M Shapiro

Department of Orthopedic Surgery, and Graduate Program in Cell and Developmental Biology, Jefferson Medical College, Philadelphia, PA

Abstract

Degeneration of the intervertebral disc is the major contributor to back/neck and radicular pain. It is characterized by an elevation in levels of the inflammatory cytokines tumor necrosis factor (TNF)- α , interleukin (IL)-1 α/β , IL-6 and IL-17 secreted by the disc cells themselves; these cytokines promote matrix degradation, chemokine production and changes in cell phenotype. The resulting imbalance between catabolic and anabolic responses leads to degeneration, as well as herniation and radicular pain. Release of chemokines from degenerating discs promote infiltration and activation of T and B cells, macrophages, neutrophils, and mast cells further amplifying the inflammatory cascade. Immunocyte migration into the disc is accompanied by the appearance of microvasculature and nerve fibers arising from the dorsal root ganglion (DRG). In this inflammatory milieu, neurogenic factors in particular nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) generated by disc and immune cells induce expression of pain associated cation channels in DRGs. Depolarization of these channels is likely to promote discogenic and radicular pain and reinforce the cytokine-mediated degenerative cascade. Taken together, the enhanced understanding of the contribution of cytokines and immune cells to catabolic and nociceptive processes provide new targets for treating symptomatic disc disease.

Introduction

For the past two hundred years, lower back pain has been associated with degeneration of the intervertebral disc and by inference linked to aging, excessive manual labor and more recently to genetic factors. It is estimated that as much as 84% of the population suffers from low back pain at some point in their lifetime¹, while 10% are chronically disabled. With approximately 30% of the American population suffering from lower back pain, there is a good chance that the reader has a painful back, hopefully not exacerbated by the time spent reading this review. Likewise, the lifetime incidence of neck-related pain is reported to be greater than 65% with up to 54% of population experiencing pain within the last 6 months². The socio-economic cost of the condition is enormous, estimated to be \$85 billion in 2008 (slightly more than the GDP of Oman, Ecuador, Croatia, Libya and Cuba)³; in the UK, in terms of lost productivity, disability benefits total more than £12 billion. As such, lower back pain is one of the most prevalent musculoskeletal conditions affecting Western society

Address Correspondence to: Makarand V. Risbud, Ph.D., Department of Orthopedic Surgery, Jefferson Medical College 1025 Walnut St., 511 College Bldg. Philadelphia, PA 19107, Tel: 215-955-1063, Fax: 215-955-9159, makarand.risbud@jefferson.edu.

and a huge drain on medical resources worldwide^{3,4}. A widely recognized contributor to back pain is degeneration of the intervertebral disc, the soft tissue between the vertebrae that absorbs and distributes applied loads and lends flexibility to the spine^{5–7}. As degeneration proceeds, there are elevated levels of inflammatory cytokines, enhanced aggrecan and collagen degradation, changes in disc cell phenotype⁸. The loss of hydrophilic matrix molecules leads to structural changes and spinal instability and is the main cause of herniation, sciatica and possibly stenosis⁸. However, although a vast majority of adults over the age of 30 have some form of structural degeneration of one or more discs this is not always accompanied by pain and may be a manifestation of ageing process^{9,10}. It is therefore likely that an event secondary to a structural deficit, such as injury or leakage of NP material through annular fissures results in recruitment of immune cells to the disc which then triggers pain generation. From this perspective, in the following discussion, the term disc degeneration is used in the context of symptomatic (painful) disease.

A number of diverse etiological factors are thought to serve as primary initiating events that lead to abnormal production of cytokines and catabolic molecules by the disc cells^{8–14}. These include genetic predisposition, smoking, infection, abnormal biomechanical loading, decreased nutrient transport across the endplate and ageing^{8–14}. While the relative importance of each of these factors is currently unknown they all lead to a common disease phenotype: loss of water signal on T2 weighted MRI referred to as a “black disc”, together with some degree of inflammation, and bulging or herniation of the NP (Figure 1). Degeneration is thought to be mediated by the abnormal production of pro-inflammatory molecules secreted by both the nucleus pulposus (NP) and annulus fibrosus (AF) (the fibrocartilaginous tissue that contains the NP) cells as well macrophages, T cells and neutrophils^{15–17}. These cytokines trigger a range of pathogenic responses by the disc cells that can promote autophagy, senescence and apoptosis^{8,18,19}. Secreted proinflammatory mediators include TNF- α , IL-1 α/β , IL-6, IL-17, IL-8, IL-2, IL-4, IL-10, IFN- γ , chemokines, prostaglandin (PGE)₂, of these TNF- α and IL-1 β are probably the most studied^{20–24}. TNF- α has been implicated in disc herniation and nerve irritation and ingrowth^{25,26}, while both TNF- α and IL-1 β induce upregulation of genes encoding matrix-degrading enzymes^{20,27,28}. It has been demonstrated that expression of IL-1 β and IL-1R are increased in degenerated disc tissue^{20,21}. In terms of promoting the degenerative response, significant increases in matrix metalloprotease (MMP)-1, -3, -7, -9 and -13 and A disintegrin-like and metalloprotease with thrombospondin type-1 motif (ADAMTS)-1, -4, -5, -9 and -15 have been noted in the NP, the gelatinous core of the disc, during degeneration and their presence has raised fundamental questions concerning the regulation of these proteolytic enzymes during the disease process^{29,30}.

Although degeneration may begin with molecular changes within the NP and/or the AF, subsequent steps involve inflammatory changes in the discal and surrounding tissues^{23,31,32}. Structural deficits in the NP and AF and formation of tears and clefts and in some instances herniation allows immune cell activation and infiltration. This is supported by studies that showed that in herniated discs, along with the invading blood vessels, there is infiltration of CD68⁺ macrophages, neutrophils and T cells (CD4⁺, CD8⁺)^{23,31}. Noteworthy, the migration of immune cells into the disc is also accompanied by the appearance of nociceptive nerve fibers arising from the dorsal root ganglion (DRG)^{33–35}. At this time, NP tissue as well as

the infiltrating immune cells continue to release cytokines as well as neurogenic factors in particular NGF and BDNF. In DRG's these neurotrophins induce expression of the pain associated cation channels in particular the acid sensing ion channel (ASIC)3 and the transient receptor potential cation channel, subfamily V, member 1 (Trpv1)^{36–38}. Within this inflammatory milieu, channel depolarization would be expected to promote discogenic pain and reinforce cytokine mediated disc degeneration. Herein, we focus on the role of inflammatory cytokines secreted by both the resident disc cells (NP and AF) and invading immunocytes that are central to the pathogenesis of disc disease.

Contributions of TNF- α and IL-1 to the degenerative process

Human TNF is a secreted cytokine synthesized as a type II transmembrane protein that forms stable homotrimers. Processing by TNF- α -converting enzyme (TACE/ADAM17) results in release of the 51 kDa homotrimeric cytokine³⁹. Membrane bound and secreted proteins have overlapping as well as distinct biological actions. There are 2 cognate receptors, TNF-R1 (CD120a; p55/60) and TNF-R2 (CD120b; p75/80), unlike TNF-R1, TNF-R2 lacks death domain on its intracellular region⁴⁰. Proteolytic cleavage of the extracellular domains of both TNF-R1 and TNF-R2 generates their soluble forms which bind TNF with high affinity and serve as TNF antagonists. While both membrane bound and soluble TNF signal through ubiquitous TNF-R1, only membrane bound TNF signals via TNF-R2, a receptor that is restricted to hematopoietic and immune cells. Upon ligand binding, the homo trimeric TNF-R1 receptor complex (Complex I) undergoes a conformational change resulting in dissociation of a 60 kDa inhibitory protein, silencer of death domains (SODD) from the intracellular death domain of the receptor^{40,41}. This dissociation permits the recruitment of an adaptor, tumor necrosis factor receptor type 1-associated death domain protein (TRADD) that serves as a docking site for accessory proteins of the receptor complex including TNF receptor-associated factor2 (TRAF2) and RIP1^{40,41}. Downstream signaling is mediated by NF- κ B and MAPK pathways, a minor role of TNF-R1 in cell death pathway has also been observed mediated through formation of (Complex II or DISC) that occurs after internalization of Complex I^{40,41}.

Another cytokine that is closely linked to disc degeneration is IL-1^{20,21}. The IL-1 family consists of 11 cytokines, of which IL-1 α and IL-1 β are the most studied. Similar to TNF, all of the members of the IL-1 family, except IL-1Ra, are first synthesized as precursor proteins (31 kDa for IL-1 α and - β) which are subsequently processed to shorter 17 kDa active peptides. As there is no prototypic signal peptide required for processing, these proteins belong to the leaderless secretory protein group and thus secretion bypasses the ER and Golgi^{42,43}. Interestingly, while pro-IL-1 β is biologically inactive, does not engage membrane IL-1R1 and requires proteolytic processing by either intracellular cysteine protease, caspase-1 or extracellular neutrophilic proteases, the membrane-associated IL-1 α pro-peptide can signal through its cognate receptor to initiate cell-to-cell signaling^{42,43}. Moreover, unlike IL-1 β , IL-1 α is a dual function cytokine; its biological functions elicited through nuclear translocation of pro-IL-1 α or by the cleaved 16 kDa N-terminal pro-peptide^{42,43}. These activities are independent of binding to cell surface receptors (For details of IL-1 signaling see Figure 2). Both IL-1 and TNF- α are produced by cells of the intervertebral disc^{20–22} as well those of the immune system (macrophages, monocytes,

dendritic cells, B lymphocytes, NK cells). These cytokines have a number of common functions which include chemoattraction of neutrophils, induction of adhesion molecules on endothelial cells, stimulation of phagocytosis, and production of PGE2 by macrophages⁴²⁻⁴³.

Increased levels of IL-1 and TNF- α along with other inflammatory mediators are present in degenerate and herniated intervertebral discs^{20,21} as well as in the epidural space²⁴. Le Maitre and Hoyland showed that disc cells from non-degenerate and degenerate tissues produced IL-1, IL-1Ra, IL-1R, and caspase-1 and that with the exception of IL-1Ra, expression increased with severity of disease²⁰. In a follow up study, these researchers demonstrated that degenerate and herniated discs exhibited raised expression of both TNF- α and IL-1 β with IL-1 β being higher than TNF- α ²¹. Likewise, there was a ten-fold elevation in IL-1R mRNA expression in degenerate discs and most cells displayed IL-1R immunopositivity²¹. However, no increase in TNF-RI gene or protein expression was observed in degenerate or herniated discs. The authors concluded that although both cytokines may be involved in the pathogenesis of disc disease, IL-1 may have a more prominent role than TNF- α , and thus would serve as a better target for therapeutic intervention²¹. Supporting these observations, Kepler *et al.* recently reported that human disc cells treated with IL-1 β or TNF- α unregulated the expression of substance P (SP); this neuropeptide also induces expression of IL-1 β , IL-6 and IL-8 in both NP and AF cells¹⁷. In a separate study, these investigators reported that IL-1 β and chemokine (C-C motif) ligand (CCL)5 were expressed at a significantly raised levels in painful discs as well as discs with increasing grades of degeneration⁴⁴. Recent work demonstrating the susceptibility of IL-1rn null mice to disc degeneration further validated the importance of IL-1 and IL-1ra in the pathogenesis of disc disease⁴⁵. However, in contrast to earlier work by Le Maitre²¹, Andrade and colleagues recently reported increased expression of both TNF-RI and TNF-RII in herniated NP tissue and showed a positive correlation between both TNF- α and TNF-RI protein levels and pain assessed by the visual analogue scale (VAS)⁴⁶. Thus, with respect to degeneration, herniation and discogenic pain, IL-1 β as well as TNF- α are the key inflammatory cytokines. Several recent organ culture models of disc degeneration supports this concept^{47,18}.

Cytokine production by disc cells was found to be responsive to various environmental stressors including smoking, abnormal mechanical loading, injury and infection⁴⁸⁻⁵¹. The cytokines upregulate a variety of catabolic mediators that include ADAMTS-4/5, MMP-1, -2, -3, -13, -14, and chemokines and suppress the expression of important matrix genes^{20,22,27,52}. Seguin and colleagues showed that TNF- α induced MMP-2 activity post translationally by controlling MMP-14 expression through the Extracellular signal-regulated kinases (ERK) pathway⁵³. Related to disc disease, syndecan-4 (SDC4), a key heparan sulfate proteoglycan concerned with activation of many growth factors and MMPs was seen to be required for ADAMTS-5 activation in NP cells in a TNF- α and IL-1 β dependent manner²⁷. It was noted that SDC4 promoted ADAMTS-5 processing and thus this HS proteoglycan indirectly regulated aggrecan turnover. Unlike chondrocytes⁵⁴, SDC4 dependent ADAMTS-5 processing in NP cells was independent of MMP-3 activity²⁷. In addition to post translational processing of ADAMTS-5²⁷, we and others have demonstrated

that expression of ADAMTS-4/5, is regulated by TNF- α and IL-1 β through mitogen activated protein kinase (MAPK) and NF- κ B signaling pathways^{53,55}. Concerning the role of these proteases in disc disease, Patel and colleagues⁵⁶ and Seki *et al.*⁵⁷ have demonstrated that while ADAMTS-4 protein levels increased with disease severity, ADAMTS-5 levels showed little change between early and late stages of the disease. Moreover, silencing of ADAMTS-5 alone was sufficient to block aggrecan degradation in rabbit discs⁵⁷. Lending support to these earlier reports, we found that silencing of either ADAMTS-4 or ADAMTS-5 in human NP cells resulted in inhibition of TNF- α induced aggrecan neoepitope generation⁵³. It is thus reasonable to assume that ADAMTS-4 and -5 are non-redundant and therapeutic blocking of one of these proteases would be expected to limit breakdown of the aggrecan-rich matrix and possibly mitigate structural changes.

As NF- κ B plays a critical role in mediating the effects of inflammatory cytokines, our group investigated if other regulatory molecules controlled the activity of this signaling pathway in NP cells. We noted that in the presence of inflammatory cytokines, prolyl hydroxylase (PHD)3, an otherwise important molecule in hypoxia inducible factor (HIF)-1 α signaling⁵⁸, formed a regulatory circuit with NF- κ B, serving as a transcriptional co-activator⁵⁹. PHD3 loss-of-function studies showed that there was a significant decrease in TNF- α /NF- κ B dependent induction of SDC4, ADAMTS5, MMP13 and COX2⁵⁹. Moreover, suppression of PHD3 expression and not its hydroxylase activity, prevented TNF- α mediated inhibition of aggrecan and collagen II, genes, critical for maintenance of the NP tissue matrix. Noteworthy, while cytokines also induced PHD3 expression through NF- κ B, HIF did not appear to be involved⁵⁹. These findings clearly indicate that PHD3 is a critical component of the cytokine transduction pathway in NP cells; as such, this molecule is likely to play a critical role in the pathogenesis of degenerative disc disease and represents a novel pharmacological target.

Aside from causing structural changes, inflammatory cytokines stimulate disc cells to produce chemotactic factors which promote recruitment of macrophages, neutrophils and T cells. Analysis of degenerate and herniated discs showed elevated levels of several chemokines including monocyte chemotactic protein (MCP)-1, CCL3, CCL4, CCL5, MCP-3, MCP-4, C-X-C motif chemokine 10 (CXCL10) as well as IL-8^{60-62,44}. Likewise, treatment of disc cells in culture with TNF- α and IL-1 β resulted in increased production of many of these chemokines^{17,44,61}. Recent studies demonstrated that NP cells expressed both CCL3 and CCL4, and their expression was regulated by TNF- α and IL-1 β . In concert with other cytokine responsive catabolic genes, CCL3 expression was controlled through MAPK and NF- κ B/p65 signaling pathways⁶¹. However, in contrast to p65, NF- κ B1/p50 was found to be inhibitory to CCL3 expression⁶¹. It was observed that by regulating CCL3 expression by NP cells, inflammatory cytokines promoted C-C chemokine receptor type 1 (CCR1) dependent macrophage migration. Analysis of human tissues indicated that the extent of CCL3 expression correlated positively with the grade of degeneration and that expression levels were higher in herniated tissue when compared with degenerate but contained discs⁴⁹. It is likely that if CCR1-CCL3 activity is blocked, then macrophage infiltration into the disc and the associated inflammatory and pain response would be limited. Similarly, when disc cells were cocultured with macrophages there was increased expression of IL-8, another

potent chemotactic factor, along with TNF- α , IL-6 and PGE-2⁶³. Further, co-neutralization of IL-8 and TNF- α significantly improved symptoms of mechanical hyperalgesia in both a disc autograft and a spinal nerve ligation model⁶³. These findings all point to the central role of inflammatory cytokines and chemotactic factors in the recruitment of immune cells to the disc and associated tissues, critical steps in the pain generation pathway.

In addition to the catabolic and pro-inflammatory effects discussed above, IL-1 β and TNF- α have been recently shown to influence disc cell senescence¹⁸, autophagy¹⁹ and expression of genes concerned with proliferation and renewal⁶⁴. Recent studies from our lab have shown that in NP cells, genes of the Notch pathway, concerned with regulation of cell proliferation⁶⁴, differentiation and fate determination, were up-regulated by these cytokines in NF- κ B and MAPK dependent fashion⁶⁵. In terms of clinical relevance, in human tissues, expression of Notch2 as well as IL-1 β and TNF- α was raised in histologically mid-grade degenerate discs⁶⁵. Similarly, a strong correlation between Notch2 and Notch1 and Notch target gene, Hey2 was also noted⁶⁵. These results provided a mechanistic link between the high levels of inflammatory cytokines and the dysregulated expression of genes of the Notch signaling pathway observed during disc disease⁶⁴ and suggest that the inflammatory milieu can alter disc cell renewal and differentiation, processes that are important for tissue repair and homeostasis.

Contribution of IL-6 to intervertebral disc degeneration

IL-6, a 26 kDa protein of 184 amino acids, that forms both monomers and dimers, signals through a type I cytokine receptor complex comprising the ligand-binding IL-6R α chain (CD126), and the signal-transducing component gp130 (CD130)⁶⁶. Several IL-6 family members including ciliary neurotrophic factor, leukemia inhibitory factor (LIF), IL-11, cardiotrophin-1 and oncostatin M signal through gp130. In contrast to the ubiquitously expressed gp130, IL-6R/CD126 expression is tissue restricted. A soluble form of IL-6R (sIL-6R) is generated either from an alternatively spliced IL-6R mRNA or more commonly by proteolytic cleavage of membrane bound IL-6R by ADAM-17. The complex of sIL-6R and IL-6 can bind gp130, induce dimerization and initiate signaling (aka trans-signaling) in cells that do not express trans-membrane IL-6R, thus serving as a paracrine factor⁶⁶. Binding of IL-6 to IL-6R/gp130 complex primarily signals through JAK/STAT, Ras and PI3K pathways¹⁶ and its function varies from growth and differentiation of B- and T- cells to acute-phase protein induction. Aside from T-cells and macrophages, IL-6 is secreted by intervertebral disc cells¹⁶ and raised expression levels were present in herniated discs⁶⁷. Studer *et al.* showed that IL-6 potentiates the catabolic actions of IL-1 and TNF- α on NP cells⁶⁸. When treated with a cocktail of cytokines, there was significant decrease in proteoglycan synthesis and a robust elevation in PGE-2 and MMP-13 levels⁵⁶. Aside from catabolic effects on NP cells, IL-6 induces TNF- α expression as well as apoptosis of neuronal cells in the DRG, events which may contribute to allodynia and hyperalgesia^{69,70}. A similar role for both IL-6 and TNF- α was noted in the generation of neuropathic pain following transection of L5 ventral root⁷¹. Further support to the contribution of IL-6 to sciatic pain was the observation that the IL-6 haplotype GGGA (based on 4 SNPs in exon 5) was more common in patients with symptomatic disc disease⁷². A later study by the same group using the additive inheritance model indicated that, the disease risk was significantly

elevated in young adults with an allele G of IL-6 SNPs rs1800795 and rs1800797. Moreover, haplotype analysis revealed an association between the GGG haplotype (SNPs rs1800797, rs1800796 and rs1800795) and disc disease, validating the importance of IL-6 in disease etiology⁷³, although the consequences of individual SNPs on IL-6 function in context of the disc remains to be elucidated. All these investigations indicate that targeting of IL-6 may have beneficial effects on both matrix degradation by NP cells, and importantly, nociception.

Role of IL-17 and IFN- γ in the pathogenesis of disc disease

IL-17A is the founder member of a group of 6 cytokines (IL17A-F) of the IL-17 family. It is a 35 kDa homodimeric, secreted glycoprotein of 155-amino acid monomers linked by a disulfide-bridge. Members of the IL-17 family share a similar protein structure, with four highly conserved cysteine residues required for their conformation. IL-17 interacts with type I cell surface receptor or IL-17R, a family that consists of five, broadly distributed receptors (IL-17RA-E) with individual ligand specificities⁷⁴. Signal transduction for IL-17A requires the presence of a heterodimeric complex consisting of both IL-17RA and IL-17RC. Signal transduction by these receptors is diverse and depends on the nature of the stimulus and tissue-type. Downstream of the IL-17/ IL-17R, AP-1, TNF-receptor associated factor (TRAF)6, NF- κ B, JNK, Erk1/2 and p38, have been implicated in signaling⁷⁴. IL-17 increases chemokine production and recruits monocytes and neutrophils to the site of inflammation. It is induced by IL-23 and is produced by T_H17, other lymphocytes and more recently by neutrophils, mast cells and even disc cells^{74–76}. In many instances, IL-17 acts synergistically with TNF- α and IL-1 to enhance the inflammatory response. Interferon-gamma (IFN- γ), a type II interferon, is another important molecule concerned with disc degeneration²³. It is secreted as a dimer that engages a heterodimeric receptor, IFN γ R1 and IFN γ R2⁷⁷. Similar to IL-6R, this receptor complex signals through the JAK-STAT pathway. IFN- γ has also been shown to interact with cell surface HS which inhibits its biological activity⁷⁸. Although, the physiological relevance of this interaction is not well understood, it is likely that binding to HS may protect it from proteolysis. IFN- γ is secreted by T_H1, T_C and NK cells as well as macrophages, myeloid cells and dendritic cells⁷⁷.

Due to its prominent role in inflammatory arthritis, considerable attention has been directed at IL-17⁷⁶. Kim *et al.* showed that IL-17 plays an important role in recruitment of T-cells and macrophages as well as glial and astrocytic activation during sciatic nerve injury and subsequent neuropathic pain⁷⁹. In disc cells, IL-17 and IFN- γ cause a synergistic increase in inflammatory mediator release, and an elevation in ICAM-1 expression⁸⁰. More recently, Shamji *et al.*²³ extended the earlier work by Park *et al.*⁸¹ and showed increased levels of IFN- γ , IL-12, IL-4, IL-6 as well as IL-17 in degenerated and to a greater extent in herniated discs. Similarly, evaluation of cytokine in disc lavage showed a significant elevation in IFN- γ of patients experiencing low back pain⁸². The presence of these cytokines imply that there is infiltration of immunocytes into the discal tissues. Supporting this idea, Shamji and colleagues found a significantly higher infiltration of CD68⁺ macrophage and a trend towards increased CD4⁺ T_H cells in herniated discs. Since IL-17 is secreted by T_H17 cells, it is likely that these cells may play an important role in the disc degeneration. However, the prominent IL-17 staining of non-degenerate control tissues²³, raised the possibility that other

cell types including resident disc cells may contribute to the production of this lymphokine. In a recent publication Gruber *et al.* confirmed this possibility by showing that disc cells produce IL-17 when stimulated by TNF- α and IL-1 β and that expression is raised in degenerate discs⁷⁵. Since Shamji *et al.*²³ showed that IL-12 and IFN- γ were both elevated in herniated tissues, a contribution from T_H1 cells is also likely. The presence of raised IL-4 also suggests involvement of T_H2 CD4⁺ cells in the degenerative process²³. Interestingly, these local inflammatory changes are reflected at the systemic level⁸³; in patients with lumbar disc herniation a significant increase in CD4⁺, CD8⁺, CD3⁺ and CD4⁺/CD8⁺ lymphocytes was seen in the peripheral circulation⁸³. A positive correlation was observed between the VAS pain scores and counts of CD4⁺ and CD4⁺/CD8⁺. The relationship between type of herniation, pain and T cell response in the peripheral circulation has also been investigated⁸⁴. It was noted that CD3⁺, CD4⁺, and CD4⁺/CD8⁺ cells in the ruptured disc herniation group were elevated. In addition, there was a positive correlation between CD4⁺, CD4⁺/CD8⁺ cells and pain as measured by positive straight leg raise (SLR) test⁸⁴.

Role of inflammatory cytokines in pain associated with disc degeneration

The onset of discogenic pain is characterized by nerve fiber ingrowth into an otherwise aneural tissue^{33–35}. The interplay between inflammatory cytokines and neurotrophins, produced by disc cells and infiltrating immunocytes as well as neurotrophin receptors and their modulators may guide this process. In the healthy state, nerve fiber ingrowth is prevented by the barrier function imparted by the high concentration of the chondroitin sulfate component of aggrecan and other matrix molecules which are inhibitory to nerve outgrowth⁸⁵. Noteworthy, during degeneration an increase in keratan sulfate: chondroitin sulfate ratio and proteolytic cleavage of aggrecan mediated by cytokine dependent ADAMTS4/5 activity may thus enhance nerve invasion^{27,52}. In addition, Sema3A, a member of class 3 semaphorin family, known to be inhibitory to axonal growth is highly expressed in the outer AF in the healthy state⁸⁶. In patients with discogenic pain, expression decreased with increasing grades of degeneration suggesting that Sema3A may inhibit nerve ingrowth.

Freemont and colleagues observed a direct relationship between invading nerve fibers and blood microvessels into painful discs, and suggested that NGF derived from endothelial cells is required for neuronal survival and ingrowth³⁵. Noteworthy, both NP and AF cells express low levels of NGF and BDNF and neurotrophin receptors TrkA, TrkB and p75^{NGFR}^{87,88}. Moreover, neurotrophin expression is increased in painful and degenerate discs and sensitive to IL-1 β and TNF- α .^{88–90} Accordingly, disc derived NGF may not only participates in nerve ingrowth, but directly act on DRGs and promote expression of ASIC3, a pH sensitive Na⁺ channel associated with ischemic and inflammatory pain^{36,87}. In concert, cytokines can also act on DRG, promote apoptosis^{67,68} as well as upregulate expression TrpV1, a nociceptive cation channel^{91,92}. Of note, nerve fibers that innervate disc tissue are categorized as nociceptive and thought to be derived from the DRG: they express, acetylcholinesterase, PGP 9.5, substance P (SP), BDNF, TrpV1, calcitonin gene related peptide (CGRP), neurofilament protein (NFP)^{92–96}. These relationships suggest a direct linkage between inflammatory cytokines, neurotrophins and nociception (Figure 4).

Human clinical studies

It is evident that inflammatory cytokines play a major role in all aspects of degenerative disc disease including pain generation. Table 1 lists a number of investigational anti-cytokine, and signaling pathway inhibitors for restoration of structural damage as well as mitigating neuropathic pain in either cell/organ culture or animal models. In contrast, human clinical trials are solely directed to alleviate symptoms of back or radicular pain associated with disc degeneration, herniation and spinal stenosis. To date, the results of these trials are mixed. In patients experiencing radicular pain due to lumbar spinal stenosis Ohtori and colleagues found that epidural application of TNF- α inhibitor etanercept⁹⁷, a TNF receptor-IgG fusion protein, or tocilizumab⁹⁸, an anti-IL-6R monoclonal antibody, to the spinal nerve produced pain relief. In a one month follow up, patients exhibited significant improvement in low back pain, leg pain, and leg numbness^{97,98}. In a randomized, double blinded, placebo-controlled trial Genevay *et al.* studied efficacy of two subcutaneous injections of adalimumab, a fully human antibody against TNF- α on severe acute sciatica^{99,100}. Both short term⁹⁹ and 3 year follow up¹⁰⁰ studies clearly showed a significant improvement in leg pain and reduction in the required number of back surgeries. Other pilot¹⁰¹ as well as randomized placebo-controlled^{102,103} clinical trials have also been performed to investigate the efficacy of TNF- α blockade on sciatic pain, using either subcutaneous¹⁰¹ or epidural injections of etanercept^{102,103} or intravenous infusions of infliximab¹⁰⁴, a mouse-human chimeric antibody. A pilot group control study of etanercept found that patients with severe sciatica exhibited marked improvement in visual analogue scale for leg pain (VASL) and low back pain (VASB), and two validated functional scores: the Oswestry disability index and the Roland Morris disability questionnaire. This improvement was apparent at 6 weeks after 3 subcutaneous injections when compared with patients who received standard care and i.v. methylprednisolone¹⁰¹. In a dose response study Cohen *et al.* demonstrated significant improvements in leg and back pain for the etanercept-treated patients¹⁰². However, in a recent study, etanercept was found to be less effective than steroids with respect to leg pain, back pain and functional capacity¹⁰⁵. Similarly, no significant differences in lumbar radicular pain were seen in the 1-year follow-up of the infliximab trial, although, a beneficial effect for patients with L3-L4 or L4-L5 herniation with a colocalized Modic change was noted¹⁰⁴. The discrepancy in outcomes between these studies may be due to technical issues such as problems with controlled delivery required to maintain the optimum therapeutic dose, inherent differences in the investigational molecules, limited cohort size, patient selection as well as study design rather than the actual importance of cytokines in the degenerative process. In addition to functional outcome measures, more sensitive, imaging technologies are necessary to detect tissue structural changes that may occur following the anti-cytokine therapy. These clinical studies are summarized in Table 2.

Conclusion

In conclusion, disc degeneration is characterized by three distinct but overlapping phases in which cytokines play a central role (Figure 4). In the first phase, an initiating event results in phenotypic changes and production of cytokines and chemokine by the both NP and AF cells. During the second phase there is further amplification of the inflammatory response by infiltrating immunocytes as well as neovascularization and nerve ingrowth into the

structurally deficient discal tissues. In the final stage, there is sensitization of nerve endings and the modulation of DRG pain channel activity by inflammatory mediators and neurotrophins resulting in pain. While cytokines play a critical role in each of the three defined stages, following injury cytokines may be critically important for the repair and the regeneration of peripheral nerves. In a recent study Nadeau *et al.*¹⁰⁶ showed that while mice lacking both IL-1 β and TNF, or both IL-1R1 and TNF-R1, exhibited decreased nociception, and recruitment of neutrophils and M1 macrophages following sciatic nerve injury, there was also impaired recovery of nerve function and axonal regeneration independent of neutrophil recruitment¹⁰⁶. These interesting results suggest that targeting specific IL-1 β /TNF-dependent responses, such as neutrophil and macrophage recruitment, that may be responsible for nociception could be a better therapeutic strategy for patients experiencing radicular pain associated with nerve root injury following disc herniation than complete blockage of cytokine production. Further, since cytokines form a complex regulatory network it is not unreasonable to assume that targeting actions of a single cytokine may have a limited clinical effect. What is now required is a therapy that simultaneously targets specific actions of multiple key cytokines (Figure 4). An enhanced understanding of the contribution of cytokines and immune cells to structural degeneration of the discal tissues, inflammation and pain could lead to the identification of novel targets for treating symptomatic disc disease without interfering with the tissue repair program.

Review criteria

PubMed was searched for full-text, English-language original and review articles published between 1997 and 2013. The search terms used were: “intervertebral disc”, “intervertebral disc degeneration”, “back pain”, “sciatic pain”, “TNF- α ”, “IL-1”, “IL-17”, “IL-6”, “IFN- γ ”, “inflammatory cytokines”, “chemokines” and “neurotrophins”, either alone or in combination. The reference lists of identified articles were searched for further relevant papers.

Key Points

- Intervertebral disc degeneration is a widespread condition affecting a large percentage of the adult population with huge socio-economic costs.
- There is a strong association between disc disease and back/neck and radicular pain.
- Inflammatory cytokines play a major role in the pathogenesis of disc degeneration by promoting matrix breakdown and recruitment of immune cells to the discal tissues.
- Infiltration and activation of immune cells in the disc results in amplification of the inflammatory responses and release of neurotrophins.
- Inflammatory cytokines and neurotrophins promote pain generation through changes in nociceptive channel activity as well as apoptosis of cells in the DRG.

- An enhanced understanding of the contribution of cytokines and immune cells to disc degeneration, inflammation and nociception provides novel potential targets for treating symptomatic disc disease.

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Biographies

Dr. Makarand V. Risbud received his Ph.D. from the National Centre Cell Science, India and did post-doctoral studies at Harvard Medical School. He is currently a Professor of Orthopedic Surgery and member of the Cell and Developmental Biology Graduate Program at Thomas Jefferson University, Philadelphia. Dr. Risbud has published over 90 papers. His research is directed at understanding the role of microenvironmental niche factors including hypoxia, osmolarity and cytokines in regulating intervertebral disc function.

Dr Irving M. Shapiro holds a doctorate in Biochemistry and a degree in dental surgery from the University of London, UK. Formerly he was a Professor of Biochemistry at the University of Pennsylvania and currently is a Professor and Director of Orthopedic Research at Thomas Jefferson University. He has published nearly 300 papers. His research is primarily focused on the biology of skeletal tissues in health and disease.

B: MRI scan showing symptomatic herniation and disc disease

Figure 1. Relationship between key vertebral structures, a herniated cervical intervertebral disc and spinal nerves A) Schematic shows extrusion of the nucleus pulposus through annular fissures of a degenerating intervertebral disc. The herniated tissue impinges on a spinal nerve close to the dorsal root ganglion, causing an inflammatory response resulting in

radicular pain. B) T2 MRI image showing a lumbar disc herniation at L5-S1 in patient with symptomatic disc disease (arrow).

Figure 2.

IL-1 α and IL-1 β synthesis and signal transduction pathway. IL-1 α and - β are synthesized as precursor proteins (pro-IL-1, pro-IL-1 β) which then undergo proteolytic cleavage by either calpain or caspase-1 to produce the mature active forms, mIL-1 α and mIL-1 β respectively. While pro-IL-1 β is biologically inactive, myristoylated membrane associated pro-IL-1 α can signal through the IL-1R to initiate cell-to-cell signaling. In addition, nuclear translocation of pro-IL-1 α or the cleaved N-terminal pro-peptide that retains its nuclear localization sequence (NLS), elicits biological functions. Pro-IL-1 α , mIL-1 α and mIL-1 β all bind to IL-1RI, which recruits the IL-1 receptor accessory protein (IL-1RAcP) as a co-receptor. The receptor complex recruits two adaptor proteins, the myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor-activated protein kinase (IRAK). Sequential phosphorylation of IRAK4, IRAK1, IRAK2 and lastly TRAF6, an E3 ubiquitin ligase, results in polyubiquitination of signaling molecules, such as TGF- β -activated protein kinase (TAK-1) at K63. This modification allows for the association of TAK-1 with TRAF6 and subsequent activation of many signaling proteins: JNK, p38 and ERK1/2 as well as transcription factors: NF- κ B and AP-1 which control expression of a number of inflammatory and catabolic genes. Signaling through IL-1 receptor complex is modulated by inhibitory actions of IL-1RII, sIL-1RII, sIL-1RAcP as well as IL-1 family member, IL-1 receptor antagonist (IL-1Ra).

Figure 3.

Role of different classes of immune cells in amplifying the inflammatory response during intervertebral disc degeneration and the generation of back and radicular pain. Proinflammatory cytokines and soluble factors are secreted by both the NP and AF cells of the disc as well as immunocytes. Subtype of CD4⁺ T_H cells (except T_{REG}), CD8⁺ T_C, neutrophils, macrophages, B cells and mast cells infiltrate degenerate as well as herniated disc tissues. Major types of molecules that are secreted by the immune cells and their contribution to the disease process is listed. These proinflammatory factors promote expression of catabolic genes while at the same time suppressing expression of critical matrix genes by the NP and AF cells. The imbalance between synthesis and catabolism further accelerates degeneration, compromises tissue integrity and promotes pain generation.

Figure 4.

Schematic of major interdependent phases leading to disc degeneration and pain. Following initial insult/s, disc cells upregulate expression of inflammatory cytokines and chemokines that include TNF- α , IL- β , IL-6 as well as IL-17 and CCLs. In this inflammatory environment, disc cells express several catabolic molecules like SDC4, ADAMTS-4/5, MMPs. These enzymes promote degradation of predominant extracellular matrix molecules such as aggrecan (ACAN) and collagen II (Col2). Continuous structural breakdown of matrix molecules of the NP and AF results in mechanical instability, annular tears and in many instances herniation. In the second phase of the disease, release of chemokines and cytokines from the degenerated disc enhances activation and infiltration of immunocytes into tissues further amplifying the inflammatory response. Infiltration of immune cells is accompanied by the appearance of microvasculature and nociceptive nerve fibers that arise from the dorsal root ganglion (DRG). In the third phase of the disease, neurogenic factors in particular NGF and BDNF produced by the herniated disc as well as the immunocytes induce expression of the pain associated cation channels like ASIC3 and Trpv1 in the DRGs. In this inflammatory milieu, activation of these channels is likely to promote discogenic pain and reinforce cytokine mediated disc degeneration. Possible sites of pharmacological intervention are indicated.

Table 1

Effects of some anti-cytokine and pathway inhibitor molecules on disc degeneration and radicular pain

Molecules	Model	Effects
IL1ra	NP tissue explants and tissue engineered NP constructs Nerve root compression with chromic gut sutures	Inhibition of matrix degradation and other catabolic effects of IL-1 β , restoration of GAG matrix and biomechanical properties ^{28,107,108} Partial reversal of allodynia, and diminished astrocytic reactivity ^{109,110}
Lactoferricin (LfcinB)	In vitro cell and disc organ culture models	Inhibition of IL-1 β and LPS-mediated induction in MMP-1, -3, -13 and ADAMTS-4/5 gene expression. Restored proteoglycan production ¹¹¹
Triptolide	In vitro human NP cell culture	Inhibition of IL-1 β mediated induction in IL-6/-8, PGE2, MMP1/2/3/13 gene expression and restored expression of aggrecan and collagen II ¹¹²
MyD88 peptide inhibitor	Disc organ culture model	Blocked LPS- or IL-1 β -mediated induction in MMP-1/13, ADAMTS-4, -5, iNOS and TLR-2 gene expression. Prevented IL-1-mediated proteoglycan depletion ¹¹³ .
anti-TNF- α antibody	L5 nerve root ligation and disc autograft model	In combination with anti-IL-8 antibody improved symptoms of mechanical hyperalgesia ⁶³
sTNF-RI	Nerve root compression with chromic gut sutures	Partial reversal of mechanical allodynia, reduced astrocytic reactivity ^{109,110,114}
sTNF-RII	In vitro NP cell culture DRG exposure and nerve constriction or ligation, in rats	Suppression of NO and PGE2 ¹¹⁵ Restoration of normal gait, Partial reversal of allodynia ¹¹⁶⁻¹¹⁸
Nemo Binding Domain (8K-NBD) peptide	Ercc1 deficient accelerated aging mouse model	Restored disc proteoglycan synthesis and maintenance of disc cellularity ¹¹⁹
NF-kB decoy	L5 DRG compression and NP placement in rats	Restoration of molecular changes in DRG, some reversal of thermal hyperalgesia and mechanical allodynia ¹²⁰

Table 2

Clinical studies of anti-cytokine therapy for back and radicular pain

Targeted Molecules	Drug/Compound	Clinical Study	Outcome
TNF- α	Etanercept (TNF receptor-IgG fusion protein)	Prospective randomized trial of patients experiencing radicular pain due to lumbar spinal stenosis	Significant improvement in low back pain, leg pain, and leg numbness ⁹⁷
		Randomized, placebo-controlled trial of Sciatica patients receiving epidural injections	Improvements in back and leg pain ^{102,103}
		Multicenter, 3-group, randomized, placebo-controlled trial of Sciatica patients	Found to be less effective than steroids with respect to back pain, leg pain, and functional capacity ¹⁰⁵
		Pilot Study of patients experiencing acute, severe Sciatica	Patients showed reduced back pain and leg pain at 6 week follow up, but no difference at 10 day ¹⁰¹
	Adalimumab (fully human antibody against TNF- α)	Randomized, Double Blind, placebo-controlled trial of patients experiencing severe and acute Sciatica	Significant improvement in leg pain and reduction in required back surgeries at short term and 3 year follow ups ^{99,100}
	Infliximab (mouse-human chimeric antibody)	Randomized, placebo-controlled trial of patients experiencing disc-herniation-induced Sciatica	No significant difference in lumbar radicular pain seen in 1 year follow up but a noted beneficial effect for patients with L3-L4 or L4-L5 herniation with a colocalized Modic change ¹⁰⁴
IL-6	Tocilizumab (anti-IL-6R monoclonal antibody)	Randomized study of patients experiencing radicular pain due to lumbar spinal stenosis	Significant improvement in low back pain, leg pain, and leg numbness ⁹⁸