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Disc-overy of the Drivers of Inflammation Induced Chronic Low Back Pain: From Bacteria to Diabetes

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Abstract

The intervertebral disc is a unique avascular organ that supports axial skeleton flexion and rotation. The high proteoglycan content of the nucleus pulposus tissue, present at the center of the disc, is pivotal for its mechanical function, distribution of compressive loads. Chronic low back pain, a prevalent and costly condition, is strongly associated with disc degeneration. Degenerated discs exhibit high levels of inflammatory cytokines, matrix catabolizing enzymes, and an overall reduction in proteoglycan content. Although the cytokine profile of diseased discs has been widely studied, little is known of what initiates and drives inflammation and subsequent low back pain. Recent studies by Albert and colleagues have shown that anaerobic bacteria are present in a high percentage of painful, herniated discs and long-term treatment with antibiotics resolves symptoms associated with chronic low back pain. It is thought that these anaerobic bacteria in the disc may stimulate inflammation though toll-like receptors to further exacerbate disc degeneration. Despite the promise and novelty of this theory, there are other possible inflammatory mediators that need careful consideration. The metabolic environment associated with diabetes and atypical matrix degradation products also have the ability to activate many of the same inflammatory pathways as seen during microbial infection. It is therefore imperative that the research community must investigate the contribution of all possible drivers of inflammation to address the wide spread problem of discogenic chronic low back pain.

Introduction

Understanding the intervertebral disc (IVD) is necessary to address the serious global health problem of low back pain. Low back pain (LBP) is a profoundly debilitating and increasingly prevalent condition. It is currently the worldwide leading cause of disability. This condition is responsible for 58.2 million years lived with disability in 1990, 83 million in 2010, and an economic burden conservatively estimated at 85 billion dollars in 2005 alone (Buchbinder et al., 2013; Martin et al., 2008). Although LBP is a complex problem without one clear etiology, there is a strong association between LBP and disc degeneration. A study reviewing the MRIs of patients with persistent LBP showed disc degeneration in 87% of participants (Arnbak et al., 2015). Additionally, patients with severely degenerate discs are 3.2 times more likely to suffer from LBP (Livshits et al., 2011). Despite the strong link

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between disc degeneration and pain, degeneration is a normal consequence of aging that routinely does not cause discomfort, even with a corresponding reduction in mechanical function (Cheung et al., 2009; Dreischarf et al., 2014). Learning more about the healthy IVD and identifying the underlying pathology that distinguishes painful from non-painful degenerate discs will simultaneously generate new therapeutic targets for discogenic LBP and help continue to elucidate the mechanisms of disc degeneration. Developing effective treatments for painful disc degeneration will ameliorate the suffering of countless individuals.

Discs are compressible structures found sandwiched between each pair of adjacent vertebral bodies. This pattern of rigid bone separated by spongy disc enables the vertebral column both to distribute compressive loads and provide structural support to the axial skeleton, while still allowing for flexion and rotation. The IVD comprises three distinct tissues. The annulus fibrosus (AF), a concentric ring structure of organized lamellar collagen, laterally incases the proteoglycan rich inner nucleus pulposus (NP). The third component, two cartilaginous endplates, acts as the interface between the central portion of the disc and the neighboring vertebral bodies. Hydrophilic proteoglycan of the NP enables it to resist compressive loads through osmotic pressurization. The tensile strength of the AF prevents the gelatinous NP from extruding laterally from the disc space. The disc is the largest avascular tissue in the human body. While outer annulus has limited vascular supply, inner annulus and NP is completely devoid of any blood vessels. Consequently, metabolic support of much of the IVD is dependent on the endplates facilitating diffusion of nutrients and waste products between the disc and the adjacent vertebral body circulation. The central role of the NP in disc function makes its study pivotal to understanding disease pathogenesis, and thus much of the current research focuses on how the NP changes during development and degeneration.

Like chondrocytes, NP cells inhabit a hypoxic niche, but equating NP cells to chondrocytes is analogous to calling dolphins fish; both animals have fins, but they have very different evolutionary origins. NP cells are a unique, dynamic, and diverse cell type. They are the only derivative from the embryonic notochord. They consist of two morphologically distinct populations on a continuum of differentiation, a group of larger vacuolated cells resembling notochordal cells and a second group of smaller cells resembling chondrocytes (Chen et al., 2005; Risbud and Shapiro, 2011; Risbud et al., 2015; Trout et al., 1982).

The origin, environment, and function of healthy NP cells are reflected in their gene expression profile: sonic hedgehog (shh), bradchyry, hypoxia-inducible factor-1 alpha (HIF-1α), glucose transporter-1 (GLUT-1), carbonic anhydrase 12 (CA12), aggrecan (agg) and collagen II (col II) at a ratio >20, keratin 18/19, and cluster of differentiation 24 (CD24) (Risbud et al., 2015). Shh and bradchyry are reflective of NP's notochordal origin (Mwale et al., 2004; Winkler et al., 2014). HIF-1α and GLUT-1 both represent an adaption of NP cells to survive in the metabolically demanding environment of the disc (Agrawal et al., 2007). Due to the hypoxic niche necessitating a reliance on glycolysis, CA12 maybe necessary for disc pH regulation (Power et al., 2011). Aggrecan, collagen 2, and keratin18/19 are structural components of the NP. Aggracan's predominance is necessary to create osmotic pressure to resist compression. The NP niche also puts unique osmotic requirements on its

cells, which respond by using Tonicity-Responsive Enhancer Binding Protein (TonEBP) to maintain viability and function (Johnson et al., 2014). As animals age, a dwindling population of vacuolated cells remains in the disc possibly due to their differentiation into morphologically distinct cells. The remaining vacuolated cells are thought to maintain overall cell viability and inhibit endothelial cell invasion (Cornejo et al., 2015; Erwin et al., 2011). Although this differentiation is a normal part of maturation, loading and injury can accelerate the process (Purmessur et al., 2013; Yang et al., 2009). Thus, understanding the function and phenotype of healthy NP can both help identify diseased discs and inform possible routes to treatment.

This review aims to synthesize current research describing pathology of degenerative disc disease, and discuss new theories concerning drivers of painful disc disease. We will in particular focus on the exciting yet controversial theory that subclinical bacterial infection is a driver of painful disc disease, while additionally exploring complementary and alternative theories of inflammation initiation.

Characteristics of the degenerating disc

Studying the inflammatory cytokine profile of degenerating discs has informed much of what we know about degenerative disc disease. During the degenerative process, NP and AF cells and then additionally invading immune cells secrete elevated levels of tumor necrosis factor alpha (TNFα), interleukin-1β (IL-1β), IL-6 and IL-17. These cytokines stimulate production of additional cytokines leading to matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) mediated extracellular matrix destruction, and altering synthetic and biophysical properties of NP cells (Maidhof et al., 2014). IL-1β and TNFα are the two most widely studied cytokines in the pathogenesis of disc degeneration. IL-1β is synthesized in an inactive pro-IL-1β form and activated by caspase-1 before secretion. IL-1β binds to IL-1β receptor that acts through myeloid differentiation primary response gene 88 (MYD88) to induce expression of matrix-degrading enzymes (Ellman et al., 2012; Risbud and Shapiro, 2014). IL-1β and its receptor expression increase corresponding to degradation severity, and the action of IL-1β interferes with aggrecan and collagen 2 transcription and translation (Le Maitre et al., 2005). On the other hand, TNFα is synthesized as a type II transmembrane protein. TNF-α-converting enzyme (TACE) cleaves the membrane bound portion to generate secreted TNFα. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) pathways are the two primary downstream targets of TNFα signaling in disc cells (Risbud and Shapiro, 2014). Importantly, in addition to transcriptional induction of several catabolic mediators, TNF-α promotes ADAMTS-5 processing and activation by elevating cell surface levels of SDC4, a heparan sulfate proteoglycan (Wang et al., 2011). A recent study has also shown the critical contribution of SDC4 in controlling TNF-α mediated transcriptional induction of MMP-3 (Wang et al., 2014). In addition to their well described catabolic functions, higher levels of inflammatory cytokines TNF-α and IL-6 have been shown to cause cell death in DRG neurons (Murata et al., 2011). Moreover, IL-6 and IL-8 are correlated with painful degenerate discs (Burke et al., 2002). Both IL-1β and TNFα also upregulate nerve growth factor (NGF), with IL-1β additionally inducing the expression of brain-derived neurotrophic factor (BDNF), and substance P (Abe et al., 2007;

García-Cosamalón et al., 2010; Purmessur et al., 2008). Thus, not only could these neurotrophic factors cause pain by DRG sensitization and through retrograde signaling, but substance P has been shown to up regulate synthesis of inflammatory cytokines (IL-6, IL-8, and TNFα) further exacerbating to progression of disc degeneration (Kepler et al., 2015, 2012). While it is evident that inflammatory phenotype characterizes degenerate discs; little is known about how the inflammation is initiated and sustained to give rise to chronic LBP in this widespread disease.

Subclinical bacterial infection of the spinal motion segment as a novel initiator of LBP

There is growing interest and controversy in the notion that subclinical anaerobic bacterial infection could play a role in symptomatic disc degeneration. This subclinical infection is in contrast to LBP from overt discitis, where 80 percent of patients present with elevations in erythrocyte sedimentation rate, and 50 percent of patients present with a fever (Carragee et al., 1997; Sapico and Montgomerie, 1979). Changes in endplate radiological appearance may lead to clues to identifying patients who are suffering from bacteria mediated LBP. Modic et al. used MRI to detect changes in vertebral bone marrow associated with degenerative disc disease; Type 1 changes were described as decreased signal intensity on T1-weighted images with increased signal intensity on T2-weighted images, and Type 2 changers were described as increased signal intensity on T1-weighted images with slightly increased intensity on T2-weighted images (Modic et al., 1988). Fat appears bright and increases the signal intensity in T1 images, whereas water increases in the signal intensity on T2 images. Consequently the painful Type 1 changes are associated with edema in the vertebral bodies. More recently, Jensen et al. showed that these same Modic Type 1 changes are strongly correlated with LBP (Jensen et al., 2008). In 2013, Albert et al. proposed that anaerobic bacteria, predominantly *Propionibacterium acnes (p. acnes)*, might be responsible for these radiologic changes associated with LBP. The discs in their study infected with anerobic bacteria were significantly more likely to have Modic Type 1 changes than both discs infected with aerobic bacteria and discs with no detectable infection (Hanne B. Albert et al., 2013). Any time a bacterial culture is taken from a surgical sample, there is a chance of environmental contamination, which could have affected Albert et al.'s results. However, they found a difference between the aerobic and anaerobic bacteria groups. Moreover, other studies have also found anaerobic bacteria in nucleus material, thus adding strength to the notion that the hypoxic conditions in the inner AF and NP may be preferable for survival and colonization of anerobic microorganisms (Agarwal et al., 2010; Fritzell et al., 2004).

The hypothesis that subclinical bacterial infection can cause LBP is further supported by the ability of antibiotic treatment to resolve back pain in patients with Type 1 Modic changes. In a double-blind randomized clinical controlled trial, Albert et al. found that a 100 day course of amoxicillin-clavulanate resulted in significant improvement in both disability and pain of patients suffering from LBP (Hanne B Albert et al., 2013). This is one of the most strikingly successful experimental treatments for LBP to date, but along with the possible shortcomings of their earlier discussed study, there are possible confounding effects of amoxicillin-clavulanate. There is some evidence suggesting that clavulanate has anti-inflammatory action in the treatment of ulcerative colitis, and analgesic properties during morphine withdrawal in mice, which may lead to a misinterpretation of this clinical study

(Casellas et al., 1998; Hajhashemi and Dehdashti, 2014). It is important to note that treating back pain with long term antibiotics also raises global health concerns by exacerbating the already serious problem of antibiotic resistance (Roca et al., 2015). Despite these limitations, subclinical bacterial infection is a promising new theory of LBP. Additional investigation into the underlying mechanisms linking anaerobic bacterial infection to LBP could enhance our collective understanding the disease and identify treatments directly targeting the pathways contributing to painful disc degeneration.

TLR signaling as one of the important links between disc inflammation and LBP

Relevant to the discussion above, activation of toll-like receptor signaling (TLR) may possibly link subclinical bacterial infection with inflammation induced LBP. Toll-like receptors are an integral part of the innate immune system. They recognize common microbial components and induce inflammatory cytokine production. These cytokines initiate the innate immunological response while also instructing the development of acquired antigen-specific immunity (Takeda and Akira, 2005). TLRs 1/2/4/6 are expressed by disc cells in a degeneration dependent manner making this signaling cascade relevant to disease pathogenesis (Klawitter et al., 2014; Rajan et al., 2012). Similar to IL-1 signaling, both TLR 2 and TLR 4 utilize the MyD88 adaptor protein to initiate downstream signaling (Kawai et al., 1999). Upon ligand engagement, MyD88 recruits members of the interleukin-1 receptor-associated kinase (IRAK) family that activate TNF receptor-associated factor 6 (TRAF6) (Kawagoe et al., 2008) which in turn signals through tumor growth factor beta activated kinase 1 and then through NF-κB and MAPK signaling (Adhikari et al., 2007).

In the IVD, cytokines and TLR constitute a feedforward loop whereby inflammatory cytokines stimulate expression of TLRs, and TLR signaling in turn triggers the production of inflammatory cytokines promoting degradation. Lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls, engages TRL4 triggering the expression of IL-1β, TNFα, IL-6 as well as nitric oxide (NO) (Rajan et al., 2012). Inhibition of MyD88 attenuates this LPS induced inflammatory gene expression (Ellman et al., 2012). It is interesting to note that *p. acnes* is a gram positive bacteria and is not expected to stimulate LPS sensitive TLR4, but instead been shown to signal through pathogen-associated molecular patters (PAMPs) - TLR2 axis (Zähringer et al., 2008). However, it is important to note that direct stimulation of TLR4 is not the only way to amplify TLR4 dependent inflammatory response; in other human tissues, inoculation with *p. acnes* has been shown to increase TLR4 expression and to sensitize TLR4 through lymphocyte antigen 96 (MD-2) upregulation (Jugeau et al., 2005; Romics et al., 2004). The inflammatory environment in degenerated discs potentiates TRL mediated responses; stimulation with IL-1β or TNFα increases the expression of TLR1/4 mRNA, and significantly increased the transcriptional and translational expression of TLR2 (Klawitter et al., 2014). In addition to TLR signaling, subclinical bacterial infection may stimulate caspase-1 mediated IL-1β activation though NACHT, LRR, and PYD domainscontaining protein 3 (NLRP3). Expression levels of NLRP3, caspase-1, and IL-1β were all found to be higher in degenerate discs (Chen et al., 2015). Although the possible link between subclinical bacterial infection to persistent inflammation and painful disc degeneration warrants further investigation, bacteria are only one of possible ways to

activate inflammation in the disc. Even something as seemingly straightforward as mechanical loading can have complex signal mediated actions on disc disease. Along with other effects, mechanical loading upregulates TNFα. IL-6, TLR2 and TLR4 expression (Gawri et al., 2014). It is important to recognize the complexity IVD degeneration to adequately treat this debilitating condition. The influence of extracellular metabolic stress and matrix breakdown products on disc inflammation cannot be neglected.

Damage-associated molecular patters (DAMPs) as novel mediators of chronic inflammation and LBP

In addition to the drivers of inflammation described above, some endogenous molecules and their atypical cleavage products have the ability to stimulate sterile inflammation in the disc. This family of molecules, collectively called damage-associated molecular patters (DAMPs), include fragments of commonly found extracellular matrix components such as hyaluronan and fibronectin as well as high-mobility group protein B1 (HMGB1), a nuclear factor for chromatin assembly. Hyaluronic acid fragments (12 to 24 mer) have been shown to enhance pro-inflammatory cytokine production (IL-1β, IL-6, and IL-8) and matrix degrading enzymes (MMP-1, MMP-3, and MMP-13) leading to an inflammatory environment and matrix breakdown. These effects are mediated by TLR2 signaling and can be inhibited by high molecular weight hyaluronan (Quero et al., 2013). The TLR2 signaling cascade activated by hyaluronan fragments is the same MyD88-IRAK-TRAF6 dependent cascade activated by both LPS and CpG sequence DNA binding (Kawagoe et al., 2008; Scheibner et al., 2006). It is interesting to note that by binding TLR2 instead of TRL4 or CD44, hyaluronan fragments stimulate NP cells in a manner that more closely resembles macrophages than chondrocytes, hinting at the unique nature and perhaps origin of these cells (Campo et al., 2012, 2010; Scheibner et al., 2006).

HMGB1 is another DAMP that is receiving attention in the disc field. HMGB1 is both passively released from necrotic cells and secreted by monocytes. It likely exerts its inflammatory function by binding to RAGE receptors (Scaffidi et al., 2002; Voll et al., 2008). Delivery of anti-HMGB1 reduces pain and TNF expression after application of NP tissue to DRG in rat study (Otoshi et al., 2011). Fibronectin fragments are another possible inflammatory mediator in disc degeneration. Fibronectin fragments are absent in infant cadaveric human IVDs, but increase in concentration with aging and severity of disc degeneration (Ruel et al., 2014). Delivery of 30 kDa fibronectin fragments into rabbit lumbar discs induced a degenerative phenotype similar to spontaneous human disc degeneration. Comparing their results to a PBS-injected sham, fibronectin fragment injection caused a significant decrease in disc height and a significant reduction in proteoglycan synthesis (Liu et al., 2013). These effects may be due to increase in MMP-9 and MMP-13 along with the simultaneous decrease in collagen 2 and aggracan expression observed in rabbit NP cells *in vitro* (Anderson et al., 2005). In contrast to signaling through RAGE and TLR, fibronectin fragments exert their catabolic effects through integrin $\alpha_5\beta_1$ (Xia and Zhu, 2011). The enhanced catabolism caused by fibronectin fragments could also be a result of their out competing CCN2 for $\alpha_5\beta_1$ binding. Noteworthy, CCN2 causes context dependent effects in the IVD, promoting aggrecan expression and inhibiting the catabolic effects of IL-1β when bound to $\alpha_5\beta_1$ and otherwise, possibly through HSPGs, promoting catabolism (Tran et al.,

2014). Thus, atypical matrix cleavage products may interfere with disc homeostasis pushing the homeostatic balance more towards catabolism.

Systemic metabolic syndrome as a driver of disc inflammation and LBP

Obesity and diabetes are two epidemics in Western society that increase the risk of developing painful disc degeneration. Beyond the greater forces that the spinal motion segments of an obese individual experience, diabetes creates a niche environment that can drive disc degeneration. Advanced glycation end products (AGEs) associated with diabetes can accelerate disc degeneration by inducing catabolism and promoting inflammation. Although diabetes and obesity are linked, recent research has attempted to isolate the effects of diabetes by comparing both obese Sprague-Dawley rats to diabetic obese UCD-T2DM rats while using lean rats as an additional control. The diabetic rats alone had an elevated AGE concentration, which was associated with diminished glycosaminoglycan and water content of discs. These compositional changes resulted an experimentally consistent decrease in disc mechanics (Fields et al., 2015). In a separate study, Tsai and colleagues showed that treatment with AGEs of both rat and human NP cells caused a dose dependent increase in MMP-2 and extracellular signal-related kinases (ERK) activity, and elevation in advanced AGE-specific receptor (RAGE) mRNA expression, which all could lead to enhanced NF-κB activation (Tsai et al., 2014). Both the anti-inflammatory pentosan-polysulfate and AGE-inhibitor pyridoxamine, FDA approved compounds, are able to reduce the deleterious effects streptozotocin induced diabetes on disc disease in mice (Illien-Junger et al., 2013). Independent of the aberrant reactions caused by hyperglycemia leading to AGE production, elevated blood glucose concentration, common to diabetes, has been shown to inhibit NP cell proliferation *in vitro,* which could further alter cellular homeostasis in the disc (Johnson et al., 2008). Taken together these studies suggest that diabetes, obesity and their metabolic consequences may encourage disc degeneration.

Conclusion

The clinical success of antibiotic use for treating low back pain is exciting yet controversial. Recent data suggests that anaerobic *p. acnes* could induce its inflammatory effects on the intervertebral disc through TLR signaling. Despite this clinical success, focusing only on bacteria as a driver of inflammation would miss the effects of aberrant mechanical loading, metabolic syndromes such as diabetes, and endogenous atypical cleavage products that initiate many of the same downstream effects as bacteria. An effective treatment for painful disc degeneration is likely to be comprehensive. Understanding the contribution and interplay between all inflammatory drivers will lead to a responsible and effective treatment for low back pain.

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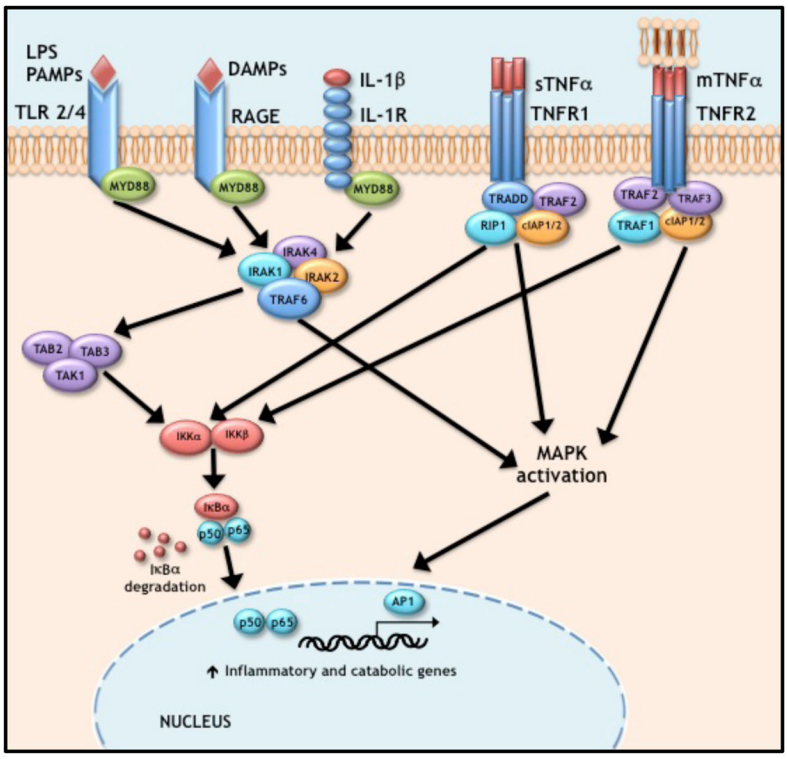

Figure 1. Signaling pathways driving inflammation

TRL, RAGE, and IL-1 all initiate their inflammatory effects though MYD88 with subsequent action though members of the IRAK family that activate TRAF6. TNF-α can simulate the cell in both its soluble and membrane bound form by activating TNFR1 and TNFR2 respectively. TNFR1 ligand binding results in a conformational change leading to the recruitment of TRADD, RIP1, TRAF2, and CIAP1/2. Ligand binding to TNFR2 results in the recruitment of similar down stream factors. All five of these receptor pathways converge on NF-κβ and MAPK activation to induce their inflammatory and catabolic effects.