FOR WHOM THE DISC TOLLS: INTERVERTEBRAL DISC DEGENERATION, BACK PAIN AND TOLL-LIKE RECEPTORS

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Abstract

Intervertebral disc (IVD) degeneration is characterised by catabolic and inflammatory processes that contribute largely to tissue degradation and chronic back pain. The disc cells are responsible for the pathological production of pro-inflammatory cytokines and catabolic enzymes leading to degeneration. However, this phenotypical change is poorly understood. Growing evidence in animal and human studies implicates Toll-like receptors (TLR) and their activation through danger-associated alarmins, found increasingly in degenerating IVDs. TLR signalling results in the release of pro-inflammatory cytokines and proteolytic enzymes that can directly cause IVD degeneration and back pain. This review aims to summarise the current literature on TLR activation in IVD degeneration and discuss potential treatment modalities to alleviate the inflammatory phenotype of disc cells in order to arrest IVD degeneration and back pain.

Keywords: Inflammation, proteases, catabolism, alarmins, pain, innervation.

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catabolic enzyme activity, the AF becomes prone to micro-fissuring and tears that weaken its structural integrity and increase the risk of NP bulging and herniation (Lotz and Ulrich, 2006). The catabolic state is driven by disc cells producing inflammatory and degenerative factors that, in addition to breaking down the tissue, promote leukocyte infiltration (Rajasekaran et al., 2019; Risbud and Shapiro, 2013). However, the phenotypic shift in NP and AF cells remains poorly understood. The search for much-needed disease-modifying drugs to stop and potentially reverse disc degeneration could be reliant on the knowledge of these molecular mechanisms. The present review summarises the recent advances implicating TLRs, a class of cytoplasmic type I receptors, in the inflammatory phenotype of degenerating IVDs.

Introduction

Disc degeneration

Chronic back pain is a leading cause of disability and one of the largest burdens on health-related quality of life worldwide (Balagué et al., 2012; Vos et al., 2013). Studies have shown a strong correlation between chronic back pain and IVD degeneration (Hartvigsen et al., 2018). IVD degeneration is strongly associated with ageing and is characterised by structural and biochemical changes to the central gel-like NP and the surrounding lamellar AF. Loss in load-bearing function of the disc is due to a generalised catabolism of the ECM and negatively charged proteoglycans in the NP, with consequent decrease in water content and compressibility of the disc. Through increased catabolic enzyme activity, the AF becomes prone to micro-fissuring and tears that weaken its structural integrity and increase the risk of NP bulging and herniation (Lotz and Ulrich, 2006). The catabolic state is driven by disc cells producing inflammatory and degenerative factors that, in addition to breaking down the tissue, promote leukocyte infiltration (Rajasekaran et al., 2019; Risbud and Shapiro, 2013). However, the phenotypic shift in NP and AF cells remains poorly understood. The search for much-needed disease-modifying drugs to stop and potentially reverse disc degeneration could be reliant on the knowledge of these molecular mechanisms. The present review summarises the recent advances implicating TLRs, a class of cytoplasmic type I receptors, in the inflammatory phenotype of degenerating IVDs.

Molecular pattern recognition is a crucial mechanism at the core of innate immunity and tissue repair. PRRs interact with a wide range of molecules from PAMPs to endogenous danger-associated molecules (alarmins or DAMPs) and induce an inflammatory response involved in tissue remodelling or host defence. RIG-1-like receptors, Nod-like receptors and most importantly the 10 mammalian members of the TLRs are the main actors that perform the PRR function in humans (Newton and Dixit, 2012). TLR1, 2, 4, 5, 6 and 10 are located mainly on the cell surface while TLR3, 7, 8 and 9 reside in endosomal vesicles (Takeda and Akira, 2015). TLR activation is induced in 3 steps (Fitzgerald and Kagan, 2020; Kumar et al., 2013) (Fig. 1).

**Fig. 1.** Schematic representation of TLR activation by TLR2/4 agonist and TLR4 agonist and downstream signalling pathways.
1. The agonist is trapped between the LRR-rich ectodomains of two TLR subunits. TLRs assemble in dimer pairs. In the present review, special attention is given to the TLR heterodimers TLR1/2 and TLR2/6 as well as the homodimer TLR4 due to their notable promiscuity in binding several alarmins and pathogens found in degenerating discs and their increased expression in degenerating IVD tissue. The proximity of two TLR subunits upon ligand binding induces the dimerisation their cytoplasmic TIR domain.

2. The intracellular dimerised TIR domains recruit membrane bound TIR-domain-containing TIRAP or TRAM, which proceed to organise a SMOC around one of two key proteins: MyD88 or TRIF. Most TLR dimers signal through MyD88-dependent SMOC (myddosome). However, TLR4 homodimers can also activate the TRIF-dependent SMOC (triffosome).

3. In both myddosome and triffosome activation, TRAF6 induces IKK and MAPK phosphorylation. Ultimately, the transcription factors NF-κB and AP-1, which are translocated to the nucleus, are activated to induce transcription of pro-inflammatory genes. Recent research shows that the triffosome has the added ability to induce IFN1 signalling through IRF3.

These mechanisms are thought to induce an appropriate response according to the danger presented. For example, the bacterial cell wall component peptidoglycan, found mostly on gram-positive bacteria, can activate TLR2/6 to release pro-inflammatory and chemotactic signals to recruit cells specialised in phagocytosis. Alternatively, viral nucleic acids can activate the TLR3/TLR4 TRIF-dependent pathway for a more suitable IFN-mediated antiviral response. Dimer-agonist specificity, co-effector stimulation and signalling pathway cross-talks with cytokines and other alarmins enable the flexibility of downstream inflammatory responses observed (Lin et al., 2017). These inflammatory pathways are highly dependent on multiple positive feedback loops to amplify the inflammatory signal for quick and efficient response to danger. However, downsides of this efficient and flexible inflammatory response are the vicious inflammatory cycle TLR activation often creates. The study hypothesis was that these feedback loops are driving the inflammatory processes involved in disc degeneration (Fig. 2). The following sections will summarise the evidence supporting the presence of these feedback loops and their involvement in IVD degeneration and chronic back pain.

**TLR activation in IVDs**

**Disc infections or PAMPs**

Disc infections caused by low-virulence anaerobic bacteria such as *Cultibacterium acnes* (*C. acnes*, formerly...
Propionobacterium acnes) have been hypothesised as contributors to disc degeneration and Modic changes in the adjacent vertebral bone. Stirling et al. (2001) reported that 53% of degenerative disc samples contain live bacteria. Since then, multiple groups have performed similar experiments finding varying levels of live C. acnes in degenerative disc tissues: 0% (Li et al., 2016), 4.2% (Neto et al., 2019), 10% (Schmid et al., 2020), 15.6% (Ahmed-Yahia et al., 2019), 21% (Yuan et al., 2017), 32% (Tang et al., 2018), 35% (Salehpour et al., 2019), 37% (Javanshir et al., 2017). This discrepancy can be explained by the propensity for contamination of clinical samples by C. acnes, one of the most abundant bacteria found on human skin (Mollerup et al., 2016). The best evidence for this phenomenon, although contested by Brätten et al. (2019), is the double-blind randomised clinical control trial for the efficacy of antibiotic treatment to alleviate chronic low-back pain and Modic type I changes (Albert et al., 2013). Regardless of the prevalence of bacterial infiltration in degenerating discs, in vivo and in vitro animal investigations have revealed that C. acnes can survive in the disc environment and induce degeneration through the TLR pathway (Li et al., 2016; Lin et al., 2018). Human in vitro disc cell cultures respond to C. acnes contamination significantly increasing IL-6 mRNA production, which is decreased by Sparstolonin B (a TLR2/4 antagonist) treatment (Schmid et al., 2020). Interestingly, inoculation of rat caudal IVDs causes severe disc degeneration by triggering apoptosis of NP cells. Similar degeneration, with added Modic changes type I, is also found in inoculated rabbit IVDs (Chen et al., 2016). Furthermore, the time-dependent increase in Bax and cleaved caspase 3 following C. acnes infection is significantly reduced by knocking down TLR2 expression, indicating a central role for TLRs in bacterial infection of the IVD (Lin et al., 2018). Interestingly, new evidence suggests that the activation of the TLR2/MAPK pathway induces NP cells to phagocytose S. aureus. As an immune-deprived tissue, this mechanism might explain how discs attempt to control infection (Lin et al., 2019). Taken together, these studies suggest that the inflammatory response to infection produced by disc cells through TLRs can be detrimental to the disc tissue and enhance degeneration.

Alarmins
Sterile activation of TLRs in response to injury and stress is mediated through a class of ligands called DAMPs or alarmins. The term alarmin was introduced by Oppenheim in 2005 to classify endogenous molecules that have an immunostimulatory function once bioavailable (Oppenheim and Yang, 2005). For example, HMGB1 is a nuclear DNA-binding protein that can be actively secreted through non-canonical pathways by stressed and activated cells or passively released by leaky necrotic cells. Once released from the nucleus and the cell, the hydrophobic region of this protein can bind or interfere with multiple membrane receptors including TLR2 and TLR4 (Janko et al., 2014). Interestingly, a significant increase in HMGB1 expression and immunopositivity was found in advanced disc degeneration, with up to 8-fold increase in Thomson grade 5 discs (Gruber et al., 2015; Shah et al., 2018). Other intracellular alarmins, such as peroxiredoxin-5, are also increased or uniquely present in degenerating discs compared to healthy young and aged discs (Rajasekaran et al., 2019). The second alarmin-producing mechanism is through proteolysis of the ECM. With increased matrix-degrading enzyme activity during degeneration, the collagen network loses integrity and releases otherwise sequestered matrix proteins. The SLRPs' biglycan and decorin, for example, needs to be liberated from the matrix by proteases to become mobile and reach cell surface receptors (Roedig et al., 2019; Schaefer et al., 2005; Schaefer, 2014). Alarmins can also be generated by fragmenting core ECM components such as aggrecan, biglycan, fibronectin, fibromodulin and hyaluronan. In the case of aggrecan, the proteases MMP3, MMP13, ADAMTs-4 and ADAMTs-5 cleave the interglobular domain of the core protein to release a fragment named ACAN-32, a small peptide known to interact with TLR2 and cause inflammation (Lees et al., 2015; Miller et al., 2017). Additionally, various fragments of fibronectin have been shown to induce inflammatory responses such as a 30 kD, 40 kD, 45 kD and 75 kD fragment (Aota et al., 2005). Increased fragmentation of fibronectin and other ECM alarmins such as decorin, biglycan, fibromodulin and lumican are also found in degenerating IVDs (Brown et al., 2012; Melrose et al., 2001; Melrose et al., 2007; Singh et al., 2009). An exhaustive list of known alarmins is organised in Table 1.

TLR activation amplifies inflammation

Pro-inflammatory cytokines
The main outcome of TLR activation in any cell type is the production of pro-inflammatory cytokines (Takeda and Akira, 2015). The similarity in the intracellular domains of TLRs and IL-1 receptors explains the convergence of their pathways in ultimately activating the transcription factor NF-κB through MAPKs. Consequently, activation of most TLR dimers on disc cells will increase the production of pro-inflammatory cytokines. Studies in animal models and human samples have confirmed that IVD cells secrete IL-1β, IL-3, IL-5, IL-6, IL-8, IL-7, IL-13, IL-15, IFN-γ and TNF-α following TLR2 and/or TLR4 activation (Fang and Jiang, 2016; Krock et al., 2017; Quero et al., 2013; Rajan et al., 2013; Schmid et al., 2020). These cytokines are directly linked to the progression of IVD degeneration and their importance is highlighted by their wide influence on cellular responses, including chemokine production, synergistic effects on TLR activation and catabolic enzyme secretion (Johnson et al., 2015).
### Table 1. Alarmins activating TLRs found in IVDs.

<table>
<thead>
<tr>
<th>TLR</th>
<th>Alarmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>Fibronectin fragments (Oegema et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>HA acid fragments (Quero et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>Biglycan (Melrose et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>HMGB1 (Gruber et al., 2015; Shah et al., 2018)</td>
</tr>
<tr>
<td></td>
<td>HSP60, HSP70 (Klawitter et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>Versican (Sztrolovics et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Histones (Rajasekaran et al., 2019)</td>
</tr>
<tr>
<td></td>
<td>Fibromodulin (Melrose et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Aggrecan fragment (Sztrolovics et al., 1997)</td>
</tr>
<tr>
<td></td>
<td>Decorin (Zwambag et al., 2020)</td>
</tr>
</tbody>
</table>

| TLR4 | Fibronectin fragments (Oegema et al., 2000)                                                                                              |
|      | Thrombospondin-1 (Rajasekaran et al., 2019)                                                                                                |
|      | Resistin (Li et al., 2017; Liu et al., 2016)                                                                                              |
|      | Tenascin (Gruber et al., 2009)                                                                                                           |
|      | HSP60, HSP70 (Klawitter et al., 2014)                                                                                                   |
|      | Hyaluronic acid fragments (Quero et al., 2013)                                                                                           |
|      | HMGB1 (Gruber et al., 2015; Shah et al., 2018)                                                                                           |
|      | Biglycan (Melrose et al., 2007)                                                                                                          |
|      | Lumican (Melrose et al., 2007)                                                                                                           |
|      | S100A8/A9 (Grad et al., 2016)                                                                                                            |
|      | Decorin (Zwambag et al., 2020)                                                                                                           |

**TLR pathway sensitisation**

One of the strongest amplification mechanisms for inflammatory signals is through the self-regulation of the TLR/cytokine pathways. First, TLR activation leads to an increased expression of TLRs, as demonstrated by many studies (Table 2). TLR4 activation using LPS stimulation results in increased TLR2 and TLR4 expression in disc cells (Ellman et al., 2012; Rajan et al., 2013). Also, TLR2 activation using Pam2CSK4 or peptidoglycan increases TLR2 expression in disc cells (Krock et al., 2017). Separately, cytokines can increase TLR expression. Disc cells activated by IL-1β result in a significant increase in TLR2 mRNA expression, whereas TNF-α significantly upregulates TLR1, 2 and 4 (Klawitter et al., 2014; Qin et al., 2016). Gawri et al. (2014) demonstrated that also injurious loading increases TLR2 and TLR4 expression in IVD cells. Studies on other tissue types have found similar relationships between mechanical loading and induction of TLR expression, such as stretching alveolar epithelial cells (Kuhn et al., 2014), cardiomyocytes (Shyu et al., 2010) and chondrocytes (Wang et al., 2011).

Although the causality of TLR regulation through mechano-sensing is still being investigated, some studies have suggested that TLR upregulation is indirect and a consequence of alarmin production. Alarmins, such as PGE2 and HMGB1, are induced by mechanical stimulation and may, following TLR activation, induce their own expression (Wang et al., 2011; Wolfson et al., 2014). This causality still needs to be elucidated in the disc environment. However, to raise the sensitivity even more, some alarmins can be released directly from cells upon TLR activation, such as S100A8/A9 and HMGB1. Others are released indirectly after TLR activation through catabolic enzymes either liberating bound molecules, such as biglycan and decorin, from the matrix or by cleaving proteins into fragments, as in the case of aggregan (32-mer), fibronectin, fibromodulin and hyaluronan (Dziki et al., 2018). Interestingly, Klawitter et al. (2014) reported a proportional increase in TLR1, TLR2, TLR4 and TLR6 expression in degenerating discs, which could be the consequence of repeated exposure to alarmins and pro-inflammatory cytokines.

**Leukocyte recruitment**

Chemotaxis to the IVD may be beneficial in the case of reparative stem cells homing to the disc, attracted, for example, through CCL5 production by degenerative disc cells (Pattapa et al., 2014). However, an excessive chemotactic signal can worsen disc degeneration by recruiting leukocytes. Macrophages, neutrophils and T-cells have been shown to infiltrate herniated and degenerate discs following the release of a chemotactic factor from disc cells (Grönblad et al., 1994; Risbud and Shapiro, 2013). The harsh environment of the degenerating IVD may promote leukocytes to further enhance the catabolic milieu through the activity of both cytokine receptors and TLRs. In fact, in vivo studies have shown the beneficial effect on disc degeneration from blocking macrophage recruitment through CCR1 (Chou et al., 2020). CCL5 or RANTES, a well-documented chemokine attracting macrophages, monocytes and T-cells, is over-expressed in degenerating Thomson grade VI-V discs, likely through TNF-α stimulation (Gruber et al., 2014). Interestingly, alarmin-induced TLR activation controls also chemokine release from disc cells. Decorin stimulates rat AF cells to significantly increase production of the chemokines MCP-1, RANTES and MIP-2. The upregulation is reduced by the TLR4 antagonist TAK-242. Similarly, the alarmin resistin, produced during disc degeneration, induces...
Table 2. Summary of differential gene and protein expression studies downstream of TLR activation in IVDs. HS: Homo sapiens; BT: Bos taurus; RN: Rattus norvegicus; OC: Oryctolagus cuniculus; Chondrodystrophic canine.

<table>
<thead>
<tr>
<th>Model</th>
<th>Organism</th>
<th>Agonist</th>
<th>Gene expression downstream</th>
<th>Protein expression downstream</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP cells</td>
<td>HS</td>
<td>HA fragment</td>
<td>Increase: IL-1β, IL-6, IL-8, COX-2, MMP1, MMP13</td>
<td>Increase: IL-6, MMP1</td>
<td>Quero et al., 2013</td>
</tr>
<tr>
<td>NP and AF cells</td>
<td>HS</td>
<td>PGN, LPS</td>
<td>Increase: IL-1β, IL-6, IL-8, COX-2, iNOS, PTGES2</td>
<td>Increase: NGF</td>
<td>Krock et al., 2016</td>
</tr>
<tr>
<td>NP cells</td>
<td>HS</td>
<td>C. acnes</td>
<td>Increase: IL-1β, IL-6, IL-8, COX-2, iNOS, PTGES2</td>
<td>Increase: IL-6, IL-8</td>
<td>Schmid et al., 2020</td>
</tr>
<tr>
<td>AF cells</td>
<td>HS</td>
<td>HMGB1</td>
<td>Increase: MMP13, ADAMTS-4, ADAMTS-5, TLR2, iNOS</td>
<td>Increase: prostaglandin E2, IL-6, IL-8, TNF-α</td>
<td>Fang and Jiang, 2016</td>
</tr>
<tr>
<td>NP cells</td>
<td>HS</td>
<td>LPS</td>
<td>Increase: MMP13, ADAMTS-4, ADAMTS-5, TLR2, iNOS</td>
<td>Increase: MMP13, ADAMTS-4, ADAMTS-5, TLR2, iNOS</td>
<td>Ellman et al., 2012</td>
</tr>
<tr>
<td>Whole disc</td>
<td>HS</td>
<td>Pam2CSK4, Fibronectin-fragment</td>
<td>Increase: NGF, IL-1β, TLR2</td>
<td>Increase: MMP3, MMP13, HTRA1, Cathepsin D, IL-3, IL-5, IL-6, IL-7, IL-10, IL-13, IL-15, IFN-γ, TGF-β, G-CSF, GRO, GM-CSF, CXCL1, CCL8, CCL7</td>
<td>Krock et al., 2017</td>
</tr>
<tr>
<td>Disc cells</td>
<td>BT</td>
<td>LPS</td>
<td>Increase: IL-1β, IL-6, TNF-α, TLR4</td>
<td>Increase: TNF-α, IL-1β, HMGB1 Decrease: collagen type II, aggrecan</td>
<td>Rajan et al., 2013</td>
</tr>
<tr>
<td>NP cells</td>
<td>RN</td>
<td>LPS</td>
<td>Increase: TLR4, TNF-α, IL-1β, IL-6</td>
<td>Increase: TNF-α, IL-1β, HMGB1</td>
<td>Qin et al., 2016</td>
</tr>
<tr>
<td>NP cells</td>
<td>HS</td>
<td>Resistin</td>
<td>Increase: CCL4</td>
<td>Increase: CCL4</td>
<td>Li et al., 2017</td>
</tr>
<tr>
<td>AF cells</td>
<td>RN</td>
<td>Decorin</td>
<td>Increase: MIP-2, RANTES, MCP-1, IL-6</td>
<td>Increase: CCL4</td>
<td>Zwambag et al., 2020</td>
</tr>
<tr>
<td>Whole disc</td>
<td>OC</td>
<td>Fibronectin-fragment</td>
<td>Decrease: aggrecan, collagen type II</td>
<td>Decrease: aggrecan, collagen type II</td>
<td>Anderson et al., 2003</td>
</tr>
<tr>
<td>NP cells</td>
<td>CC</td>
<td>LPS</td>
<td>Increase: TNF-α, IL-6, MMP3, MMP13, VEGF, PGES</td>
<td>Increase: TNF-α, IL-6, MMP3, MMP13, VEGF, PGES</td>
<td>Iwata et al., 2013</td>
</tr>
</tbody>
</table>

The release of CCL4 through TLR4 and NF-κB. The increase in CCL4 successfully attracts macrophages to the NP by binding to CCR1 (Li et al., 2017). In favour of leukocyte recruitment and infiltration, TLR activation results in neovascularisation through the production of angiogenic factors (Feng et al., 2018; Lee et al., 2011). An increase in potent factors, such as VEGF, was observed following TNF-α and LPS stimulation of the NF-κB pathway (Iwata et al., 2013; Ohba et al., 2009). Angiogenic factors attract new blood vessels to infiltrate AF fissures and encourage chemotaxis to the IVD (Haro et al., 2002). These results reveal another arm of the positive feedback loop potentially tying TLRs to disc degeneration through leukocyte recruitment.
TLR activation promotes catabolism

Protease production
Proteases involved in matrix degradation during IVD degeneration have been directly linked to NF-κB activity (Wuertz et al., 2012). Furthermore, TLR activation results in the production of MMP1, MMP3 and MMP13 (which are the most upregulated MMPs in degenerating discs) in IVDs (Quero et al., 2013). MMPs have the ability to cleave a wide range of ECM molecules, such as fibronectin, aggrecan, decorin, tenascin and collagen type I and II, which are the main structural components of cartilaginous tissues. ADAMTs-4 and -5, two proteases highly prevalent in cartilaginous tissue degradation, are also under the control of TLR activation in disc cells (Ellman et al., 2012). Although, proteases are involved in physiological tissue remodelling, their continuous activation will lead to tissue deterioration and cell death.

Anabolic suppression
In addition to upregulation of matrix-degrading enzymes, TLR activation can also suppress matrix synthesis to further exacerbate catabolism. Both LPS and alarmin fibronectin-fragment have suppressive effects on proteoglycan expression in disc cells (Aota et al., 2006). Furthermore, TLR activation reduces the expression of other important structural proteins such as collagen type II and aggrecan, which are crucial for tissue organisation and function (Rajan et al., 2013). By promoting catabolic enzyme production and anabolic suppression, the TLR pathway seems to favour tissue catabolism once activated.

TLR activation and pain

TLR activation promotes innervation
Another pathology associated with TLR activation is discogenic and chronic back pain, in which the presence of neurites infiltrating the typically aneural IVDs are excited by factors released by degenerating IVDs (Freemont et al., 2002). Indeed, neurotrophic factors and NGF expression in human IVD cells are both regulated by TLR2 through NF-κB (Krock et al., 2016). Both NGF and BDNF promote neuron survival, maturation and growth. Additionally, in mature peripheral afferent fibres, NGF and BDNF can induce chronic neuronal sensitisation, resulting in the development of chronic pain (Pezet and McMahon, 2006).

Table 3. Potential therapeutic agents for TLR pathway suppression in disc degeneration.

<table>
<thead>
<tr>
<th>TLR target</th>
<th>Therapeutic agent</th>
</tr>
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<tbody>
<tr>
<td>TLR2</td>
<td>Candesartan (Barakat et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>OPN-305 (Reilly et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>M2000 (Aletaha et al., 2017)</td>
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<tr>
<td></td>
<td>Sparstolonin B (Liang et al., 2011)</td>
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<td></td>
<td>CU-CPT22 (Cheng et al., 2012)</td>
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<td></td>
<td>SSL3 (Koymans et al., 2018)</td>
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<td></td>
<td>C29/o-Vanillin (Mistry et al., 2015)</td>
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<tr>
<td></td>
<td>Imidaziquinollines (Kužnik et al., 2011)</td>
</tr>
<tr>
<td>TLR4</td>
<td>Resatorvid/TAK242 (Krock et al., 2018)</td>
</tr>
<tr>
<td></td>
<td>Candesartan (Barakat et al., 2014), Valsartan (Yang et al., 2009)</td>
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<td></td>
<td>Fluvastatin (Földes et al., 2008)</td>
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<td></td>
<td>Simvastatin (Methe et al., 2005)</td>
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<tr>
<td></td>
<td>Naloxone/Naltrexone (Lewis et al., 2012)</td>
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<td></td>
<td>Curcumin (Youn et al., 2006)</td>
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<td></td>
<td>Dioscin (Qi et al., 2015)</td>
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<td></td>
<td>Eriteron/E5564 (Kim et al., 2007)</td>
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<td></td>
<td>NI-0101 (Monnet et al., 2017)</td>
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<tr>
<td></td>
<td>M2000 (Aletaha et al., 2017)</td>
</tr>
<tr>
<td></td>
<td>FP7 (Ferrin-Cocon et al., 2017)</td>
</tr>
<tr>
<td></td>
<td>Sparstolonin B (Liang et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>6-shoagol (Park et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>EGB761 (Chen et al., 2013)</td>
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<td></td>
<td>PSMA1-a3 (Chu et al., 2018)</td>
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<td></td>
<td>Oleocanthal (Iacono et al., 2010)</td>
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<td></td>
<td>Lactoferricin (Kim et al., 2013)</td>
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<td></td>
<td>Boswellic acid (Wang et al., 2014)</td>
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<td></td>
<td>Procyanidin B3 (Shang et al., 2020)</td>
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<td></td>
<td>TAP2 (Park et al., 2020)</td>
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**TLR activation induces pain**

Silencing TLRs can reduce pain sensation caused by alarmins in *in vivo* models. Miller *et al.* (2015; 2017) found that alarmins, such as aggrecan 32-mer fragment and S100A8, stimulate TLR2 and TLR4, resulting in production of proalgesic chemokines in dorsal root ganglion nociceptive neurons and cause hyperalgesia when injected into a wild-type mouse knee. Furthermore, TLR-4-deficient mice, used as a model for neuropathic pain, are less sensitive to mechanical stimuli (Tanga *et al.*, 2005). Moreover, back pain behaviour is relieved when measured in response to mechanical stimuli and cold allodynia in SPARC-null mice. The antagonist used in the study was TAK-242, a TLR4-specific antagonist (Krock *et al.*, 2018). This was also seen in rats, where TAK-242 downregulates p65, IL-1β and TNF-α in the spinal cord dorsal horn, resulting in higher heat and mechanical pain thresholds (Zhao *et al.*, 2015). These results support evidence linking disc-secreted cytokines generating painful stimuli in neighbouring neurons (Aoki *et al.*, 2002). Studies on the effect of TLR activation upon discogenic pain are limited and need to be further explored for a more complete comprehension. For more information on the role of TLRs in persistent pain, a thorough review was published by Lacagnina *et al.* (2018).

To summarise, the multi-armed positive feedback loops centred around pro-inflammatory cytokines and TLRs are believed to play an important role in IVD degeneration. However, multiple *in vitro* and *ex vivo* experiments conducted in human, mouse, rat or bovine tissues show that these degenerative cascades can be triggered by TLR agonists as well as mechanical or inflammatory stimulation. Synthetic TLR agonists, alarmins or pathogen administration were all shown to induce disc degeneration (Krock *et al.*, 2017; Rajan *et al.*, 2013). This is supported by *in vivo* experiments on New Zealand white rabbits and rats where the injection of fibronectin-fragment, a known alarmin (Table 1), or LPS in the IVD causes a progressive inflammatory process similar to that seen in IVD degeneration (Anderson *et al.*, 2003; Dudli *et al.*, 2018). Therefore, it is imperative to find effective treatments to inhibit this vicious cycle and prevent disc degeneration.

**Therapeutic approaches**

Unfortunately, there is a lack of approved TLR-specific drugs to treat IVD degeneration. This section summarises different approaches that could be taken to reduce the degenerative environment induced by the TLR pathway in degenerating discs. The first approach is to neutralise molecules that will interfere with alarmin activity. The second is to suppress TLR expression in disc cells. The third is to modulate the downstream molecular cascade following TLR activation. For their similarity to disc cells, pathological changes and research interest, studies on TLR antagonists applied to chondrocytes or in osteoarthritis have been included. Further studies on TLR blocking agents in IVD cells are necessary to assess therapeutic efficiency and safety. A complete list of potential therapeutics can be found in Table 3.

**Interfering with TLR agonists**

Antibodies binding specific alarmins have had some experimental success in reducing arthritis in mice, including blocking synovial HMGB1 with a polyclonal antibody (Kokkola *et al.*, 2003). Pep-1, a HA-binding peptide, can prevent HA degradation into a lower molecular weight alarmin following IL-1β exposure and TLR activation (Campo *et al.*, 2011). The disadvantage of blocking specific alarmins is the risk of limited efficacy compared to blocking the receptor. Blocking the ligands also inhibits their beneficial effects independent of TLRs.

**Blocking TLR activation**

Many antagonists have been found to inhibit either ligand binding or dimerisation of TLRs to block downstream activation. Regarding TLR binding inhibitors, some compounds have a TLR dimer specificity, such as TAK242 (TLR4 homodimer) and CU-CPT22 (TLR1/2 heterodimer) (Cheng *et al.*, 2012; Takashima *et al.*, 2009). TAK242 has shown potential to attenuate the release of catabolic factors such as IL-6, IL-8, MMP1, MMP3 and MMP13 in human chondrocytes (Seny *et al.*, 2013). Exciting new research shows that systemic injection of TAK-242 in SPARC-null mice alleviates both IVD degeneration and back pain behaviour (Krock *et al.*, 2018). *In vitro* studies have shown the benefit of blocking the TLR pathway in mouse discs using lactoferricin (TLR4 antagonist) after LPS or IL-1β insult (Kim *et al.*, 2013). However, the translatability of TLR4 blocking in humans still needs evaluation. Other compounds, such as SSL3, have a wider blocking range on TLRs that seem to block TLR heterodimerisation (Koymans *et al.*, 2015). Taking efficacy and risk/reward into account, the most effective TLR inhibitor for treating disc degeneration should target TLR2 and TLR4 specifically. A list of potential TLR2 and TLR4 inhibitors can be found in Table 3.

**Suppressing TLR expression**

As TLRs are overexpressed in degenerating IVDs, suppressing their expression in these tissues could be a viable therapeutic pathway to fend off aberrant activation. Some compounds such as curcumin, o-vanillin and triptolide have been found to decrease TLR2 and TLR4 mRNA expression after IL-1β pre-stimulation in human IVD cells (Klawitter *et al.*, 2012). These effects were concurrent with lowered pro-inflammatory cytokine and protease expression, suggesting that the reduced TLR expression is a secondary effect to decreased inflammation. Another interesting option for suppressing TLR expression in diseased tissues is the use of gene-based therapies. MicroRNAs are important regulators of...
TLR expression. MiR-155 represses the negative regulator SOCS1 and MiR-146a downregulates TLR downstream components TRAF6 and IRAK1 (O’Neill et al., 2011). Similarly, Zhang et al. (2018) showed that overexpression of MiR-150-5p in disc cells inhibits increased TLR4 expression induced by LPS. This approach also reduces the LPS-mediated secretion of TNF-α, IL-1β and IL-6, all while reversing the downregulation of aggrecan and collagen type II (Zhang et al., 2018). Therefore, interfering with microRNAs using expression vectors or antisense oligonucleotides might be an interesting therapy to block TLR pathways in IVDs. However, more studies are needed to solve delivery and off-target issues and improve safety and efficacy in vivo.

Blocking downstream effectors of TLR activation

The use of some compounds, such as vanillin, shown to interfere with MyD88 recruitment has resulted in recent advances in relieving pro-inflammatory and catabolic states of IVD cells (Mai et al., 2018; Mistry et al., 2015). The Chinese herb Sparstolonin B, interfering with MyD88 recruitment, downregulates TNF-α, IL-1β and IL-6 expression in a mouse model of disc degeneration. Furthermore, Sparstolonin B decreases TLR4, MyD88 and NF-κB protein expression, suggesting that TLR pathway downregulation results in de-sensitisation (Ge et al., 2018). Studer et al. (2007) showed some efficacy in blocking MAPKs, such as JNK or p38, for suppressing inflammation. However, their usefulness is limited due to their ubiquity and having important functions in many other pathways. This probably explains why toxicity and side effects are commonly reported. A development of isoform-specific inhibitors may help in reducing side effects without affecting the response of targeted MAPK inhibition.

Limitations

There are two main limitations to this review. First, the evidence presented in the literature is still limited. Indeed, only two in vivo studies have described the inflammatory cascade inducing IVD degeneration through TLR activation. However, the abundance of research showing the consistent effects of TLR activation ex vivo and in vitro in other cell types solidify the IVD-specific literature covered in the present review. The second limitation is the lack of knowledge on the applicability of TLR-based therapeutics for IVD degeneration. As such, most of the therapeutic options listed in Table 3 were tested in other cell types. Although the TLR inhibition studies referenced have shown beneficial effects on disc homeostasis and inflammatory state, it is currently not known whether TLR inhibition has the potential to reverse IVD degeneration. Future studies are needed to confirm this hypothesis.

Perspectives

The question of the relative importance of the TLR pathway for tissue inflammation degradation and pain related to IVD degeneration remains largely unanswered. However, strong evidence is accumulating implicating TLRs in degenerating cartilaginous tissues, including IVDs. Further advances should aim to observe if the inflammatory and catabolic phenotype of disc cells can be reduced or reversed by TLR pathway interference in order to limit IVD degeneration and pain.

Conclusion

This review highlights the implication of TLR pathways in the phenotypic shift of NP and AF cells contributing to IVD degeneration and back pain. Although well controlled TLR activation may be important in tissue remodelling, uncontrolled activation of this pathway may lead to aberrant inflammation and tissue breakdown. Therefore, blocking TLRs may offer a potential disease-modifying therapy for IVD degradation. This is important since there is currently an absence of disease-modifying drugs for IVD degeneration and back pain, which remains one of the most prevalent and debilitating diseases worldwide.

Search method

The search for literature was structured around different keywords, such as “Toll-like receptors”, “infections”, “alarmins” and “pain” in conjunction with the keyword “intervertebral disc” using the boolean operator AND. These keywords were searched in 3 main databases: Google Scholar, NCBI (PubMed) and the McGill Library catalogue.

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