

## 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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### List of Abbreviations

ADAMTs	a disintegrin and metalloproteinase with thrombospondin motifs	IL	interleukin
AF	annulus fibrosus	iNOS	inducible nitric oxide synthase
AP-1	activator protein 1	IRAK1	IL-1 receptor-associated kinase 1
BAX	B-cell lymphoma 2-associated X protein	IRF	IFN regulatory factor 3
BDNF	brain-derived neurotrophic factor	IVD	intervertebral disc
CCL	chemokine ligand	JNK	c-Jun N-terminal kinases
CCR1	C-C chemokine receptor 1	LPS	lipopolysaccharide
COX	cyclooxygenase	LRR	leucine rich repeats
CXCL	C-X-C motif ligand 1	MAPK	mitogen-activated protein kinase
DAMPs	danger-associated molecular patterns	MCP-1	monocyte chemoattractant protein 1
ECM	extracellular matrix	MIP-2	macrophage inflammatory protein 2
G-CSF	granulocyte colony-stimulating factor	MMP	matrix metalloproteinase
HA	hyaluronic acid	NF-κB	nuclear factor kappa B
HMGB1	high-mobility group box 1	NGF	nerve growth factor
HSP	heat shock protein	NP	nucleus pulposus
HTRA	high temperature requirement	PAMPs	pathogen-associated molecular patterns
IFN	interferon	PGE2	prostaglandin E2
IKK	inhibitor of NF-κB kinase	PGES/PTGES2	prostaglandin E synthase-2
		PGN	peptidoglycan
		PRR	pattern recognition receptor
		RANTES	regulated on activation, normal T cell expressed and secreted
		RIG-I	retinoic-acid-inducible gene 1

SLRPs	small leucine-rich proteins
SMOC	supramolecular organising centre
SOCS1	suppressor of cytokine signalling 1
SPARC	secreted protein acidic and cysteine rich
SSL3	staphylococcal superantigen-like protein 3
TGF	transforming growth factor
TIR	Toll/IL-receptor
TIRAP	TIR-domain-containing adapter protein
TLR	Toll-like receptor
TNF	tumour necrosis factor
TRAF6	TNF receptor associated factor 6
TRAM	TIR-domain-containing adapter-inducing IFN- $\beta$ -related adapter molecule
TRIF	TNFR-associated factor
VEGF	vascular endothelial growth factor

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.

### TLRs

#### 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

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).

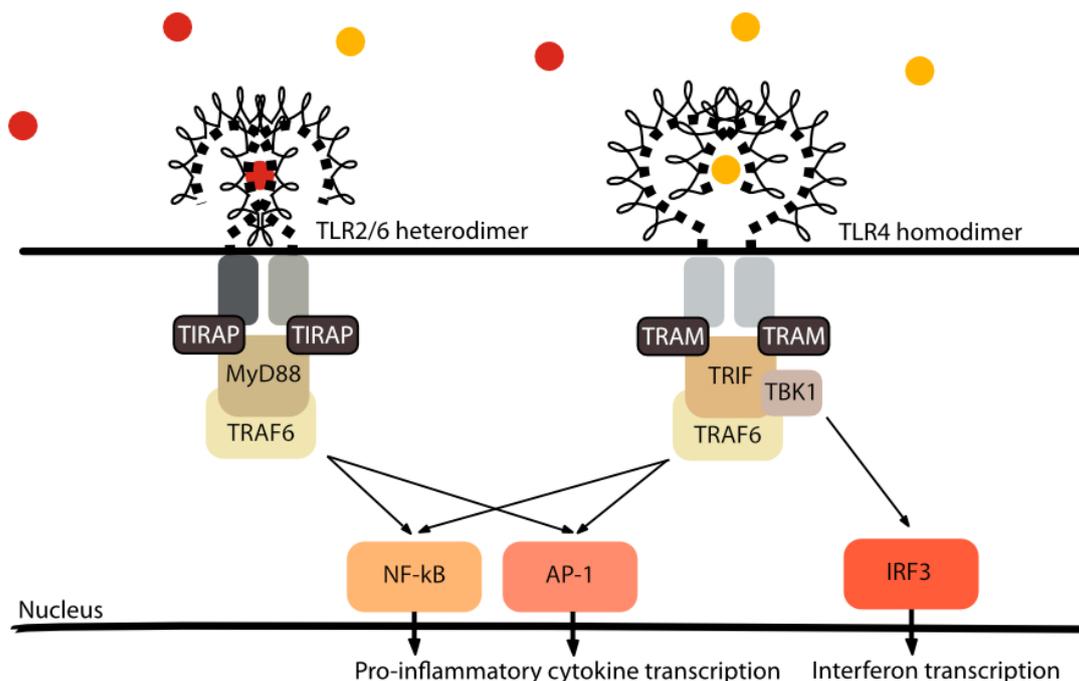


Fig. 1. Schematic representation of TLR activation by TLR2/4 agonist and TLR4 agonist and downstream signalling pathways.

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 (trifosome).
3. In both myddosome and trifosome activation, TRAF6 induces IKK and MAPK phosphorylation. Ultimately, the transcription factors NF- $\kappa$ B and AP-1, which are translocated to the nucleus, are activated to induce transcription of pro-inflammatory genes. Recent research shows that the trifosome 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

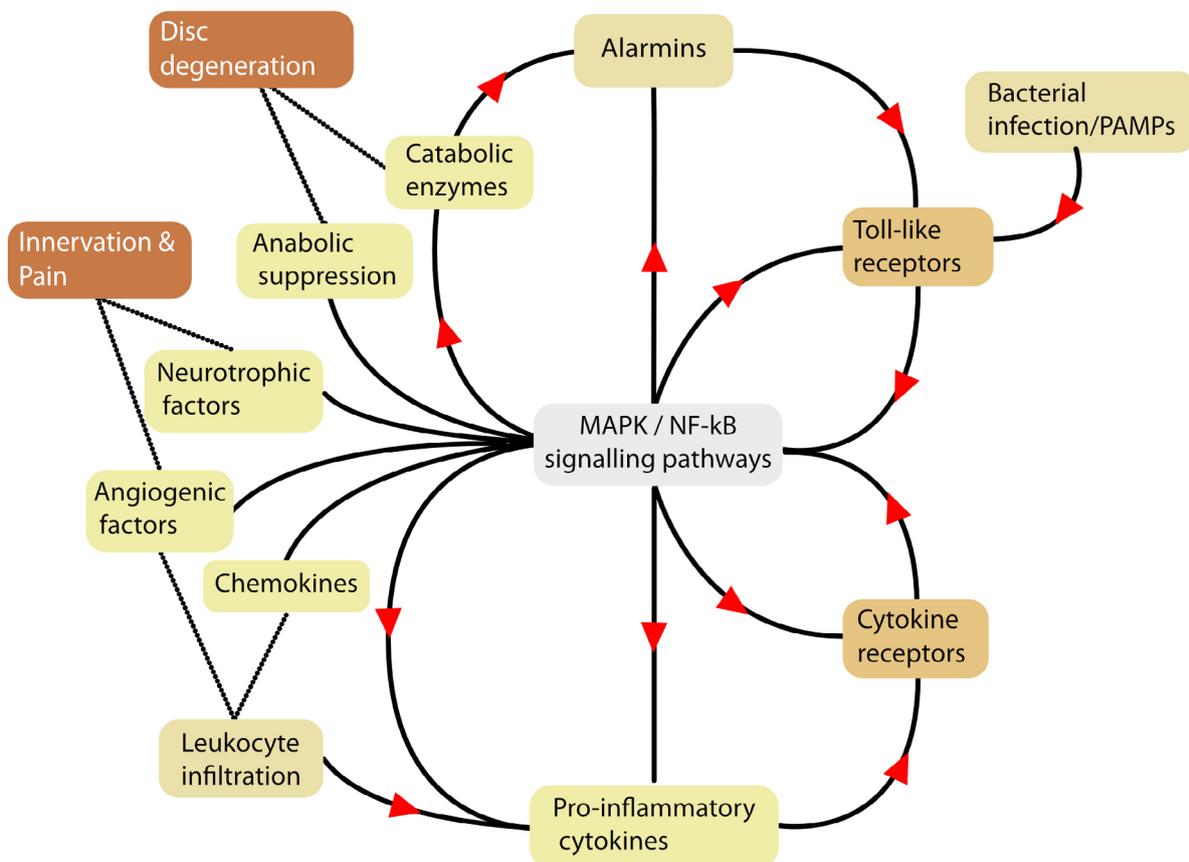


Fig. 2. TLR and cytokine receptor-centric positive feedback loops contributing to IVD degeneration and back pain.

*Propionibacterium 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 Bråten *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 vitro* human and *in vivo* 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 phagocytise *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 discs 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- $\kappa$ B 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-1 $\beta$ , IL-3, IL-5, IL-6, IL-8, IL-7, IL-13, IL-15, IFN- $\gamma$  and TNF- $\alpha$  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.**

TLR	Alarmin
TLR2	Fibronectin fragments (Oegema <i>et al.</i> , 2000) HA acid fragments (Quero <i>et al.</i> , 2013) Biglycan (Melrose <i>et al.</i> , 2007) HMGB1 (Gruber <i>et al.</i> , 2015; Shah <i>et al.</i> , 2018) HSP60, HSP70 (Klawitter <i>et al.</i> , 2014) Versican (Sztrolovics <i>et al.</i> , 2002) Histones (Rajasekaran <i>et al.</i> , 2019) Fibromodulin (Melrose <i>et al.</i> , 2007) Aggrecan fragment (Sztrolovics <i>et al.</i> , 1997) Decorin (Zwambag <i>et al.</i> , 2020)
TLR4	Fibronectin fragments (Oegema <i>et al.</i> , 2000) Thrombospondin-1 (Rajasekaran <i>et al.</i> , 2019) Resistin (Li <i>et al.</i> , 2017; Liu <i>et al.</i> , 2016) Tenascin (Gruber <i>et al.</i> , 2009) HSP60, HSP70 (Klawitter <i>et al.</i> , 2014) Hyaluronic acid fragments (Quero <i>et al.</i> , 2013) HMGB1 (Gruber <i>et al.</i> , 2015; Shah <i>et al.</i> , 2018) Biglycan (Melrose <i>et al.</i> , 2007) Lumican (Melrose <i>et al.</i> , 2007) S100A8/A9 (Grad <i>et al.</i> , 2016) Decorin (Zwambag <i>et al.</i> , 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-1 $\beta$  result in a significant increase in *TLR2* mRNA expression, whereas TNF- $\alpha$  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 aggrecan (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 chemoattractant 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- $\alpha$  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.*

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

the release of CCL4 through TLR4 and NF- $\kappa$ B. 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- $\alpha$  and LPS

stimulation of the NF- $\kappa$ B 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.

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

TLR target	Therapeutic agent
TLR2	Candesartan (Barakat <i>et al.</i> , 2014) OPN-305 (Reilly <i>et al.</i> , 2013) M2000 (Aletaha <i>et al.</i> , 2017) Sparstolonin B (Liang <i>et al.</i> , 2011) CU-CPT22 (Cheng <i>et al.</i> , 2012) SSL3 (Koymans <i>et al.</i> , 2018) C29/o-Vanillin (Mistry <i>et al.</i> , 2015) Imidaziquinolines (Kužnik <i>et al.</i> , 2011)
TLR4	Resatorvid/TAK242 (Krock <i>et al.</i> , 2018) Candesartan (Barakat <i>et al.</i> , 2014), Valsartan (Yang <i>et al.</i> , 2009) Fluvastatin (Földes <i>et al.</i> , 2008) Simvastatin (Methe <i>et al.</i> , 2005) Naloxone/Naltrexone (Lewis <i>et al.</i> , 2012) Curcumin (Youn <i>et al.</i> , 2006) Dioscin (Qi <i>et al.</i> , 2015) Eritoran/E5564 (Kim <i>et al.</i> , 2007) NI-0101 (Monnet <i>et al.</i> , 2017) M2000 (Aletaha <i>et al.</i> , 2017) FP7 (Perrin-Cocon <i>et al.</i> , 2017) Sparstolonin B (Liang <i>et al.</i> , 2011) 6-shoagol (Park <i>et al.</i> , 2009) EGb761 (Chen <i>et al.</i> , 2013) PSMa1-a3 (Chu <i>et al.</i> , 2018) Oleocanthol (Iacono <i>et al.</i> , 2010) Lactoferricin (Kim <i>et al.</i> , 2013) Boswellic acid (Wang <i>et al.</i> , 2014) Procyanidin B3 (Shang <i>et al.</i> , 2020) TAP2 (Park <i>et al.</i> , 2020)

**TLR activation promotes catabolism****Protease production**

Proteases involved in matrix degradation during IVD degeneration have been directly linked to NF- $\kappa$ B 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; Tian *et al.*, 2013). 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- $\kappa$ B (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).

### 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-1 $\beta$  and TNF- $\alpha$  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-1 $\beta$  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-1 $\beta$  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-1 $\beta$  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- $\alpha$ , IL-1 $\beta$  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- $\alpha$ , IL-1 $\beta$  and IL-6 expression in a mouse model of disc degeneration. Furthermore, Sparstolonin B decreases TLR4, MyD88 and NF- $\kappa$ B 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.

#### References

- Ahmed-Yahia S, Decousser J-W, Flouzat-Lachaniette CH, Dervin G, Roubineau F, Audureau E, Hourdille A, Royer G, Eymard F, Chevalier X (2019) Is the discopathy associated with Modic changes an infectious process? Results from a prospective monocenter study. *Plos One* **14**: e0221030. DOI: 10.1371/journal.pone.0221030.
- Albert HB, Sorensen JS, Christensen BS, Manniche C (2013) Antibiotic treatment in patients with chronic low back pain and vertebral bone edema (Modic type 1 changes): a double-blind randomized clinical controlled trial of efficacy. *Eur Spine J* **22**: 697-707.
- Aletaha S, Haddad L, Roozbehkia M, Bigdeli R, Asgary V, Mahmoudi M, Mirshafiey A (2017) M2000 ( $\beta$ -D-mannuronic acid) as a novel antagonist for

blocking the TLR2 and TLR4 downstream signalling pathway. *Scand J Immunol* **85**: 122-129.

Anderson DG, Li X, Tannoury T, Beck G, Balian G (2003) A fibronectin fragment stimulates intervertebral disc degeneration *in vivo*. *Spine* **28**: 2338-2345.

Aoki Y, Rydevik B, Kikuchi S, Olmarker K (2002) Local application of disc-related cytokines on spinal nerve roots. *Spine* **27**: 1614-1617.

Aota Y, An HS, Homandberg G, Thonar EJM-A, Andersson GBJ, Pichika R, Masuda K (2005) Differential effects of fibronectin fragment on proteoglycan metabolism by intervertebral disc cells & colon; a comparison with articular chondrocytes. *Spine* **30**: 722-728.

Aota Y, An HS, Imai Y, Thonar EJ, Muehleman C, Masuda K (2006) Comparison of cellular response in bovine intervertebral disc cells and articular chondrocytes: effects of lipopolysaccharide on proteoglycan metabolism. *Cell Tissue Res* **326**: 787-793.

Balagué F, Mannion AF, Pellisé F, Cedraschi C (2012) Non-specific low back pain. *Lancet* **379**: 482-491.

Barakat W, Safwet N, El-Maraghy NN, Zakaria MNM (2014) Candesartan and glycyrrhizin ameliorate ischemic brain damage through downregulation of the TLR signaling cascade. *Eur J Pharmacol* **724**: 43-50.

Bråten LCH, Rolfsen MP, Espeland A, Wigemyr M, Aßmus J, Froholdt A, Haugen AJ, Marchand GH, Kristoffersen PM, Lutro O, Randen S, Wilhelmsen M, Winsvold BS, Kadar TI, Holmgard TE, Vigeland MD, Vetti N, Nygaard ØP, Lie BA, Hellum C, Anke A, Grotle M, Schistad EI, Skouen JS, Grøvlø L, Brox JI, Zwart J-A, Storheim K, group A study (2019) Efficacy of antibiotic treatment in patients with chronic low back pain and Modic changes (the AIM study): double blind, randomised, placebo controlled, multicentre trial. *BMJ* **367**: 15654. DOI: 10.1136/bmj.15654.

Brown S, Melrose J, Caterson B, Roughley P, Eisenstein SM, Roberts S (2012) A comparative evaluation of the small leucine-rich proteoglycans of pathological human intervertebral discs. *Eur Spine J* **21**: 154-159.

Campo GM, Avenoso A, Nastasi G, Micali A, Prestipino V, Vaccaro M, D'Ascola A, Calatroni A, Campo S (2011) Hyaluronan reduces inflammation in experimental arthritis by modulating TLR-2 and TLR-4 cartilage expression. *Biochim Biophys Acta* **1812**: 1170-1181.

Chen YJ, Tsai KS, Chiu CY, Yang TH, Lin TH, Fu WM, Chen CF, Yang RS, Liu SH (2013) EGb761 inhibits inflammatory responses in human chondrocytes and shows chondroprotection in osteoarthritic rat knee. *J Orthop Res* **31**: 1032-1038.

Chen Z, Zheng Y, Yuan Y, Jiao Y, Xiao J, Zhou Z, Cao P (2016) Modic Changes and disc degeneration caused by inoculation of propionibacterium acnes inside intervertebral discs of rabbits: a pilot study. *Biomed Res Int* **2016**: 1-7.

Cheng K, Wang X, Zhang S, Yin H (2012) Discovery of small-molecule inhibitors of the TLR1/TLR2 complex. *Angew Chem Int Ed Engl* **124**: 12412-12415.

Chou P-H, Chee A, Shi P, Lin C-L, Zhao Y, Zhang L, An HS (2020) Small molecule antagonist of C-C chemokine receptor 1 (CCR1) reduces disc inflammation in the rabbit model. *Spine J* **20**: 2025-2036.

Chu M, Zhou M, Jiang C, Chen X, Guo L, Zhang M, Chu Z, Wang Y (2018) *Staphylococcus aureus* phenol-soluble Modulins  $\alpha$ 1- $\alpha$ 3 act as novel toll-like receptor (TLR) 4 antagonists to inhibit HMGB1/TLR4/NF- $\kappa$ B signaling pathway. *Front Immunol* **9**: 862. DOI: 10.3389/fimmu.2018.00862.

Dziki JL, Hussey G, Badylak SF (2018) Alarmins of the extracellular space. *Semin Immunol* **38**: 33-39.

Dudli S, Liebenberg E, Magnitsky S, Lu B, Lauricella M, Lotz JC (2018) Modic type 1 change is an autoimmune response that requires a proinflammatory milieu provided by the 'Modic disc.' *Spine J* **18**: 831-844.

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

Fang F, Jiang D (2016) IL-1 $\beta$ /HMGB1 signalling promotes the inflammatory cytokines release *via* TLR signalling in human intervertebral disc cells. *Bioscience Rep* **36**: e00379. DOI: 10.1042/BSR20160118.

Feng Y, Ke J, Cao P, Deng M, Li J, Cai H, Meng Q, Li Y, Long X (2018) HMGB1-induced angiogenesis in perforated disc cells of human temporomandibular joint. *J Cell Mol Med* **22**: 1283-1291.

Fitzgerald KA, Kagan JC (2020) Toll-like receptors and the control of immunity. *Cell* **180**: 1044-1066.

Földes G, Haehling S von, Okonko DO, Jankowska EA, Poole-Wilson PA, Anker SD (2008) Fluvastatin reduces increased blood monocyte Toll-like receptor 4 expression in whole blood from patients with chronic heart failure. *Int J Cardiol* **124**: 80-85.

Freemont AJ, Watkins A, Maitre CL, Baird P, Jeziorska M, Knight MTN, Ross ERS, O'Brien JP, Hoyland JA (2002) Nerve growth factor expression and innervation of the painful intervertebral disc. *J Pathology* **197**: 286-292.

Gawri R, Rosenzweig DH, Krock E, Ouellet JA, Stone LS, Quinn TM, Haglund L (2014) High mechanical strain of primary intervertebral disc cells promotes secretion of inflammatory factors associated with disc degeneration and pain. *Arthritis Res Ther* **16**: R21. DOI: 10.1186/ar4449.

Ge J, Chen L, Yang Y, Lu X, Xiang Z (2018) Sparstolonin B prevents lumbar intervertebral disc degeneration through Toll like receptor 4, NADPH oxidase activation and the protein kinase B signaling pathway. *Mol Med Rep* **17**: 1347-1353.

Grad S, Zhang Y, Rozhnova O, Schelkunova E, Mikhailovsky M, Sadovoy M, Haglund L, Ouellet J,

- Alini M (2016) Differential gene expression profiles of cells from normal, traumatic and idiopathic scoliotic discs identify molecular dysregulation in scoliosis. *Global Spine J* 6: s-0036-1582635-s-0036-1582635. DOI: 10.1055/s-0036-1582635.
- Grönblad M, Virri J, Tolonen J, Seitsalo S, Kääpä E, Kankare J, Myllynen P, Karaharju EO (1994) A controlled immunohistochemical study of inflammatory cells in disc herniation tissue. *Spine* 19: 2744-2751.
- Gruber H, Ingram J, Jr EH (2009) Tenascin in the human intervertebral disc: alterations with aging and disc degeneration. *Biotech Histochem* 77: 37-41.
- Gruber HE, Hoelscher GL, Bethea S, Ingram J, Cox M, Hanley EN (2015) High-mobility group box-1 gene, a potent proinflammatory mediator, is upregulated in more degenerated human discs *in vivo* and its receptor upregulated by TNF- $\alpha$  exposure *in vitro*. *Exp Mol Pathol* 98: 427-30.
- Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Norton HJ, Hanley EN (2014) Production and expression of RANTES (CCL5) by human disc cells and modulation by IL-1- $\beta$  and TNF- $\alpha$  in 3D culture. *Exp Mol Pathol* 96: 133-138.
- Haro H, Kato T, Komori H, Osada M, Shinomiya K (2002) Vascular endothelial growth factor (VEGF)-induced angiogenesis in herniated disc resorption. *J Orthopaed Res* 20: 409-415.
- Hartvigsen J, Hancock MJ, Kongsted A, Louw Q, Ferreira ML, Genevay S, Hoy D, Karpainen J, Pransky G, Sieper J, Smeets RJ, Underwood M, Group LLBPSW, Buchbinder R, Hartvigsen J, Cherkin D, Foster NE, Maher CG, Underwood M, Tulder M van, Anema JR, Chou R, Cohen SP, Costa LM, Croft P, Ferreira M, Ferreira PH, Fritz JM, Genevay S, Gross DP, Hancock MJ, Hoy D, Karpainen J, Koes BW, Kongsted A, Louw Q, Öberg B, Peul WC, Pransky G, Schoene M, Sieper J, Smeets RJ, Turner JA, Woolf A (2018) What low back pain is and why we need to pay attention. *Lancet* 391: 2356-2367.
- Iacono A, Gómez R, Sperry J, Conde J, Bianco G, Meli R, Gómez-Reino JJ, Smith AB, Gualillo O (2010) Effect of oleocanthal and its derivatives on inflammatory response induced by lipopolysaccharide in a murine chondrocyte cell line. *Arthritis Rheum* 62: 1675-1682.
- Iwata M, Ochi H, Asou Y, Haro H, Aikawa T, Harada Y, Nezu Y, Yogo T, Tagawa M, Hara Y (2013) Variations in gene and protein expression in canine chondrodystrophic nucleus pulposus cells following long-term three-dimensional culture. *Plos One* 8: e63120. DOI: 10.1371/journal.pone.0063120.
- Janko C, Filipović M, Munoz LE, Schorn C, Schett G, Ivanović-Burmazović I, Herrmann M (2014) Redox modulation of HMGB1-related signaling. *Antioxid Redox Signal* 20: 1075-1085. DOI: 10.1089/ars.2013.5179.
- Javanshir N, Salehpour F, Aghazadeh J, Mirzaei F, Alavi SAN (2017) The distribution of infection with *Propionibacterium acnes* is equal in patients with cervical and lumbar disc herniation. *Eur Spine J* 26: 3135-3140.
- Johnson Z, Schoepflin Z, Choi H, Shapiro I, Risbud M (2015) Disc in flames: roles of TNF- $\alpha$  and IL-1 $\beta$  in intervertebral disc degeneration. *Eur Cell Mater* 30: 104-117.
- Kim HM, Park BS, Kim J-I, Kim SE, Lee J, Oh SC, Enkhbayar P, Matsushima N, Lee H, Yoo OJ, Lee J-O (2007) Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. *Cell* 130: 906-917.
- Kim J-S, Ellman MB, Yan D, An HS, Kc R, Li X, Chen D, Xiao G, Cs-Szabo G, Hoskin DW, Buechter DD, Wijnen AJV, Im H-J (2013) Lactoferricin mediates anti-inflammatory and anti-catabolic effects *via* inhibition of IL-1 and LPS activity in the intervertebral disc. *J Cell Physiol* 228: 1884-1896.
- Klawitter M, Quero L, Klasen J, Liebscher T, Nerlich A, Boos N, Wuertz K (2012) Triptolide exhibits anti-inflammatory, anti-catabolic as well as anabolic effects and suppresses TLR expression and MAPK activity in IL-1 $\beta$  treated human intervertebral disc cells. *Eur Spine J* 21: 850-859.
- Klawitter M, Hakozaiki M, Kobayashi H, Krupkova O, Quero L, Ospelt C, Gay S, Hausmann O, Liebscher T, Meier U, Sekiguchi M, Konno S, Boos N, Ferguson SJ, Wuertz K (2014) Expression and regulation of Toll-like receptors (TLRs) in human intervertebral disc cells. *Eur Spine J* 23: 1878-1891.
- Klawitter M, Quero L, Klasen J, Gloess AN, Klopprogge B, Hausmann O, Boos N, Wuertz K (2012b) Curcuma DMSO extracts and curcumin exhibit an anti-inflammatory and anti-catabolic effect on human intervertebral disc cells, possibly by influencing TLR2 expression and JNK activity. *J Inflamm* 9: 29. DOI: 10.1186/1476-9255-9-29.
- Kokkola R, Li J, Sundberg E, Aveberger A-C, Palmblad K, Yang H, Tracey KJ, Andersson U, Harris HE (2003) Successful treatment of collagen-induced arthritis in mice and rats by targeting extracellular high mobility group box chromosomal protein 1 activity. *Arthritis Rheum* 48: 2052-2058.
- Koymans KJ, Feitsma LJ, Bisschop A, Huizinga EG, Strijp JAG van, Haas CJC de, McCarthy AJ (2018) Molecular basis determining species specificity for TLR2 inhibition by staphylococcal superantigen-like protein 3 (SSL3). *Vet Res* 49: 115. DOI: 10.1186/s13567-018-0609-8.
- Koymans KJ, Feitsma LJ, Brondijk THC, Aerts PC, Lukkien E, Lössl P, Kessel KPM van, Haas CJC de, Strijp JAG van, Huizinga EG (2015) Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3). *Proc Natl Acad Sci U S A* 112: 11018-11023.
- Krock E, Currie JB, Weber MH, Ouellet JA, Stone LS, Rosenzweig DH, Haglund L (2016a) Nerve growth factor is regulated by Toll-like receptor 2 in human intervertebral discs. *J Biol Chem* 291: 3541-3551.
- Krock E, Currie JB, Weber MH, Ouellet J, Stone LS, Rosenzweig DH, Haglund L (2016b) Toll-like receptor 2 regulates nerve growth factor synthesis *via* NF- $\kappa$ B signaling in human intervertebral disc cells.

Global Spine J 6: s-0036-1582634-s-0036-1582634. DOI: 10.1055/s-0036-1582634.

Krock E, Millecamps M, Currie JB, Stone LS, Haglund L (2018) Low back pain and disc degeneration are decreased following chronic toll-like receptor 4 inhibition in a mouse model. *Osteoarthritis Cartilage* **26**: 1236-1246.

Krock E, Rosenzweig DH, Currie JB, Bisson DG, Ouellet JA, Haglund L (2017) Toll-like receptor activation induces degeneration of human intervertebral discs. *Sci Rep* **7**: 17184. DOI: 10.1038/s41598-017-17472-1.

Kuhn H, Petzold K, Hammerschmidt S, Wirtz H (2014) Interaction of cyclic mechanical stretch and Toll-like receptor 4-mediated innate immunity in rat alveolar type II cells. *Respirology* **19**: 67-73.

Kumar H, Takeuchi O, Akira S (2013) Encyclopedia of Biological Chemistry (2nd Edition). Signaling. Editors: Lennarz W, Lane M. Academic Press pp: 396-401.

Kužnik A, Benčina M, Švajger U, Jeras M, Rozman B, Jerala R (2011) Mechanism of endosomal TLR inhibition by antimalarial drugs and Imidazoquinolines. *J Immunol* **186**: 4794-4804.

Lacagnina MJ, Watkins LR, Grace PM (2018) Toll-like receptors and their role in persistent pain. *Pharmacol Therapeut* **184**: 145-158.

Lee JM, Song JY, Baek M, Jung H, Kang H, Han IB, Kwon YD, Shin DE (2011) Interleukin-1 $\beta$  induces angiogenesis and innervation in human intervertebral disc degeneration. *J Orthop Res* **29**: 265-269.

Lees S, Golub SB, Last K, Zeng W, Jackson DC, Sutton P, Fosang AJ (2015) Bioactivity in an aggrecan 32-mer fragment is mediated *via* Toll-like receptor 2. *Arthritis Rheumatol* **67**: 1240-1249.

Lewis SS, Loram LC, Hutchinson MR, Li C-M, Zhang Y, Maier SF, Huang Y, Rice KC, Watkins LR (2012) (+)-Naloxone, an opioid-inactive Toll-like receptor 4 signaling inhibitor, reverses multiple models of chronic neuropathic pain in rats. *J Pain* **13**: 498-506.

Li B, Dong Z, Wu Y, Zeng J, Zheng Q, Xiao B, Cai X, Xiao Z (2016) Association between lumbar disc degeneration and propionibacterium acnes infection. *Spine* **41**: E764-E769.

Li Z, Wang X, Pan H, Yang H, Li X, Zhang K, Wang H, Zheng Z, Liu H, Wang J (2017) Resistin promotes CCL4 expression through Toll-like receptor-4 and activation of the p38-MAPK and NF- $\kappa$ B signaling pathways: implications for intervertebral disc degeneration. *Osteoarthritis Cartilage* **25**: 341-350.

Liang Q, Wu Q, Jiang J, Duan J, Wang C, Smith MD, Lu H, Wang Q, Nagarkatti P, Fan D (2011) Characterization of Sparstolonin B, a Chinese herb-derived compound, as a selective Toll-like receptor antagonist with potent anti-inflammatory properties. *J Biol Chem* **286**: 26470-26479.

Lin B, Dutta B, Fraser IDC (2017) Systematic investigation of multi-TLR sensing identifies regulators of sustained gene activation in

macrophages. *Cell Syst* **5**: 25-37.e3. DOI: 10.1016/j.cels.2017.06.014.

Lin Y, Cong H, Liu K, Jiao Y, Yuan Y, Tang G, Chen Y, Zheng Y, Xiao J, Li C, Chen Z, Cao P (2019) Microbicidal phagocytosis of nucleus pulposus cells against *Staphylococcus aureus* *via* the TLR2/MAPKs signaling pathway. *Front Immunol* **10**: 1132. DOI: 10.3389/fimmu.2019.01132.

Lin Y, Jiao Y, Yuan Y, Zhou Z, Zheng Y, Xiao J, Li C, Chen Z, Cao P (2018) *Propionibacterium acnes* induces intervertebral disc degeneration by promoting nucleus pulposus cell apoptosis *via* the TLR2/JNK/mitochondrial-mediated pathway. *Emerg Microbes Infect* **7**: 1. DOI: 10.1038/s41426-017-0002-0.

Liu C, Yang H, Gao F, Li X, An Y, Wang J, Jin A (2016) Resistin promotes intervertebral disc degeneration by upregulation of ADAMTS-5 through p38 MAPK signaling pathway. *Spine* **41**: 1414-1420.

Lotz JC, Ulrich JA (2006) Innervation, inflammation, and hypermobility may characterize pathologic disc degeneration: review of animal model data. *J Bone Jt Surg Am* **88** Suppl 2: 76-82.

Mai C-W, Kang YB, Hamzah AS, Pichika MR (2018) Comparative efficacy of vanilloids in inhibiting Toll-like receptor-4 (TLR-4)/myeloid differentiation factor (MD-2) homodimerisation. *Food Funct* **9**: 3344-3350.

Melrose J, Ghosh P, Taylor TKF (2001) A comparative analysis of the differential spatial and temporal distributions of the large (aggrecan, versican) and small (decorin, biglycan, fibromodulin) proteoglycans of the intervertebral disc. *J Anat* **198**: 3-15.

Melrose J, Smith SM, Fuller ES, Young AA, Roughley PJ, Dart A, Little CB (2007) Biglycan and fibromodulin fragmentation correlates with temporal and spatial annular remodelling in experimentally injured ovine intervertebral discs. *Eur Spine J* **16**: 2193-2205.

Methe H, Kim J-O, Kofler S, Nabauer M, Weis M (2005) Statins decrease Toll-like receptor 4 expression and downstream signaling in human CD14+ monocytes. *Arterioscler Thromb Vasc Biol* **25**: 1439-1445.

Miller RE, Belmadani A, Ishihara S, Tran PB, Ren D, Miller RJ, Malfait A-M (2015) Damage-associated molecular patterns generated in osteoarthritis directly excite murine nociceptive neurons through Toll-like receptor 4. *Arthritis Rheumatol* **67**: 2933-2943.

Miller RE, Miller RJ, Ishihara S, Tran PB, Golub SB, Last K, Fosang AJ, Malfait A-M (2017) An aggrecan fragment drives osteoarthritis pain through Toll-like receptor 2. *BioRxiv*: 160432. DOI: 10.1172/jci.insight.95704.

Mistry P, Laird MHW, Schwarz RS, Greene S, Dyson T, Snyder GA, Xiao TS, Chauhan J, Fletcher S, Toshchakov VY, MacKerell AD, Vogel SN (2015) Inhibition of TLR2 signaling by small molecule inhibitors targeting a pocket within the TLR2 TIR domain. *P Natl Acad Sci U S A* **112**: 5455-5460.

- Mollerup S, Friis-Nielsen J, Vinner L, Hansen TA, Richter SR, Fridholm H, Herrera JAR, Lund O, Brunak S, Izarzugaza JMG, Mourier T, Nielsen LP, Hansen AJ (2016) *Propionibacterium acnes*: disease-causing agent or common contaminant? Detection in diverse patient samples by next-generation sequencing. *J Clin Microbiol* **54**: 980-987.
- Monnet E, Lapeyre G, Poelgeest E van, Jacqmin P, Graaf K de, Reijers J, Moerland M, Burggraaf J, Min C de (2017) Evidence of NI-0101 pharmacological activity, an anti-TLR4 antibody, in a randomized phase I dose escalation study in healthy volunteers receiving LPS. *Clin Pharmacol Ther* **101**: 200-208.
- Neto NA, Wajchenberg M, Kanas M, Gotfryd AO, Lenza M, Martins DE (2019) Subclinical *Propionibacterium acnes* infection estimation in the intervertebral disc (SPInE-ID): protocol for a prospective cohort. *BMJ Open* **7**: e017930. DOI: 10.1136/bmjopen-2017-017930.
- Newton K, Dixit VM (2012) Signaling in innate immunity and inflammation. *Csh Perspect Biol* **4**: a006049. DOI: 10.1101/cshperspect.a006049.
- Oegema TR, Johnson SL, Aguiar DJ, Ogilvie JW (2000) Fibronectin and its fragments increase with degeneration in the human intervertebral disc. *Spine* **25**: 2742-2747.
- Ohba T, Haro H, Ando T, Wako M, Suenaga F, Aso Y, Koyama K, Hamada Y, Nakao A (2009) TNF- $\alpha$ -induced NF- $\kappa$ B signaling reverses age-related declines in VEGF induction and angiogenic activity in intervertebral disc tissues. *J Orthop Res* **27**: 229-235.
- O'Neill LA, Sheedy FJ, McCoy CE (2011) MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol* **11**: 163-175.
- Oppenheim JJ, Yang D (2005) Alarmins: chemotactic activators of immune responses. *Curr Opin Immunol* **17**: 359-365.
- Pattappa G, Peroglio M, Sakai D, Mochida J, Benneker L, Alini M, Grad S (2014) CCL5/RANTES is a key chemoattractant released by degenerative intervertebral discs in organ culture. *Eur Cell Mater* **27**: 124-136.
- Park H, Hong J, Yin Y, Joo Y, Kim Y, Shin J, Kwon HH, Shin N, Shin HJ, Beom J, Kim DW, Kim J (2020) TAP2, a peptide antagonist of Toll-like receptor 4, attenuates pain and cartilage degradation in a monoiodoacetate-induced arthritis rat model. *Sci Rep* **10**: 17451. DOI: 10.1038/s41598-020-74544-5.
- Park S-J, Lee M-Y, Son B-S, Youn H-S (2009) TBK1-targeted suppression of TRIF-dependent signaling pathway of Toll-like receptors by 6-Shogaol, an active component of ginger. *Biosci Biotechnology Biochem* **73**: 1474-1478.
- Perrin-Cocon L, Aublin-Gex A, Sestito SE, Shirey KA, Patel MC, André P, Blanco JC, Vogel SN, Peri F, Lotteau V (2017) TLR4 antagonist FP7 inhibits LPS-induced cytokine production and glycolytic reprogramming in dendritic cells, and protects mice from lethal influenza infection. *Sci Rep* **7**: 40791. DOI: 10.1038/srep40791.
- Pezet S, McMahon SB (2006) Neurotrophins: mediators and modulators of pain. *Annu Rev Neurosci* **29**: 507-538.
- Qi M, Zheng L, Qi Y, Han X, Xu Y, Xu L, Yin L, Wang C, Zhao Y, Sun H, Liu K, Peng J (2015) Dioscin attenuates renal ischemia/reperfusion injury by inhibiting the TLR4/MyD88 signaling pathway *via* up-regulation of HSP70. *Pharmacol Res* **100**: 341-352.
- Qin C, Zhang B, Zhang L, Zhang Z, Wang L, Tang L, Li S, Yang Y, Yang F, Zhang P, Yang B (2016) MyD88-dependent Toll-like receptor 4 signal pathway in intervertebral disc degeneration. *Exp Ther Med* **12**: 611-618.
- Quero L, Klawitter M, Schmaus A, Rothley M, Sleeman J, Tiaden AN, Klasen J, Boos N, Hottiger MO, Wuertz K, Richards PJ (2013) Hyaluronic acid fragments enhance the inflammatory and catabolic response in human intervertebral disc cells through modulation of Toll-like receptor 2 signalling pathways. *Arthritis Res Ther* **15**: R94. DOI: 10.1186/ar4274.
- Rajan NE, Bloom O, Maidhof R, Stetson N, Sherry B, Levine M, Chahine NO (2013) Toll-like receptor 4 (TLR4) expression and stimulation in a model of intervertebral disc inflammation and degeneration. *Spine* **38**: 1343-1351.
- Rajasekaran S, Tangavel C, Anand SV, Raja DC, Nayagam SM, Matchado MS, Raveendran M, Shetty AP, Kanna RM, Dharmalingam K (2019) Inflammaging determines health and disease in lumbar discs - evidence from differing proteomic signatures of healthy, ageing and degenerating discs. *Spine J* **20**: 48-59.
- Reilly M, Miller RM, Thomson MH, Patris V, Ryle P, McLoughlin L, Mutch P, Gilboy P, Miller C, Broekema M, Keogh B, McCormack W, Rooij JW de (2013) Randomized, double-blind, placebo-controlled, dose-escalating phase I, healthy subjects study of intravenous OPN-305, a humanized anti-TLR2 antibody. *Clin Pharmacol Ther* **94**: 593-600.
- Risbud MV, Shapiro IM (2013) Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatology* **10**: 44-56.
- Roedig H, Nastase MV, Wygrecka M, Schaefer L (2019) Breaking down chronic inflammatory diseases: the role of biglycan in promoting a switch between inflammation and autophagy. *FEBS J* **286**: 2965-2979.
- Salehpour F, Aghazadeh J, Mirzaei F, Ziaei E, Alavi SAN (2019) *Propionibacterium acnes* infection in disc material and different antibiotic susceptibility in patients with lumbar disc herniation. *Int J Spine Surg* **13**: 146-152.
- Schaefer L (2014) Complexity of danger: the diverse nature of damage-associated molecular patterns. *J Biol Chem* **289**: 35237-35245.
- Schaefer L, Babelova A, Kiss E, Hausser H-J, Baliova M, Krzyzankova M, Marsche G, Young MF, Mihalik D, Götte M, Malle E, Schaefer RM, Gröne H-J (2005) The matrix component biglycan is proinflammatory and signals through Toll-like

receptors 4 and 2 in macrophages. *J Clin Invest* **115**: 2223-2233.

Schmid B, Hausmann O, Hitzl W, Achermann Y, Wuertz-Kozak K (2020) The role of *Cutibacterium acnes* in intervertebral disc inflammation. *Biomedicines* **8**: 186. DOI: 10.3390/biomedicines8070186.

Seny D de, Cobraiville G, Charlier E, Neuville S, Esser N, Malaise D, Malaise O, Calvo FQ, Relic B, Malaise MG (2013) Acute-phase serum amyloid A in osteoarthritis: regulatory mechanism and proinflammatory properties. *Plos One* **8**: e66769. DOI: 10.1371/journal.pone.0066769.

Shah BS, Burt KG, Jacobsen T, Fernandes TD, Alipui DO, Weber KT, Levine M, Chavan SS, Yang H, Tracey KJ, Chahine NO (2018) High mobility group box-1 induces pro-inflammatory signaling in human nucleus pulposus cells *via* Toll-like receptor 4-dependent pathway. *J Orthop Res* **37**: 220-231.

Shang P, Tang Q, Hu Z, Huang S, Hu Y, Zhu J, Liu H (2020) Procyanidin B3 alleviates intervertebral disc degeneration *via* interaction with the TLR4/MD-2 complex. *J Cell Mol Med* **24**: 3701-3711.

Shyu K-G, Wang B-W, Lin C-M, Chang H (2010) Cyclic stretch enhances the expression of Toll-like receptor 4 gene in cultured cardiomyocytes *via* p38 MAP kinase and NF- $\kappa$ B pathway. *J Biomed Sci* **17**: 15. DOI: 10.1186/1423-0127-17-15.

Singh K, Masuda K, Thonar EJ-MA, An HS, Cs-Szabo G (2009) Age-related changes in the extracellular matrix of nucleus pulposus and anulus fibrosus of human intervertebral disc. *Spine* **34**: 10-16.

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

Studer RK, Aboka AM, Gilbertson LG, Georgescu H, Sowa G, Vo N, Kang JD (2007) p38 MAPK inhibition in nucleus pulposus cells. *Spine* **32**: 2827-2833.

Sztrolovics R, Alini M, Roughley PJ, Mort JS (1997) Aggrecan degradation in human intervertebral disc and articular cartilage. *Biochem J* **326**: 235-241.

Sztrolovics R, Grover J, Cs-Szabo G, Shi S, Zhang Y, Mort JS, Roughley PJ (2002) The characterization of versican and its message in human articular cartilage and intervertebral disc. *J Orthop Res* **20**: 257-266.

Takashima K, Matsunaga N, Yoshimatsu M, Hazeki K, Kaisho T, Uekata M, Hazeki O, Akira S, Iizawa Y, Ii M (2009) Analysis of binding site for the novel small-molecule TLR4 signal transduction inhibitor TAK-242 and its therapeutic effect on mouse sepsis model. *Brit J Pharmacol* **157**: 1250-1262.

Takeda K, Akira S (2015) Toll-like receptors. *Curr Protoc Immunol* **109**: 14.12.1-14.12.10. DOI: 10.1002/0471142735.im1412s109.

Tang G, Wang Z, Chen J, Zhang Z, Qian H, Chen Y (2018) Latent infection of low-virulence anaerobic bacteria in degenerated lumbar intervertebral discs. *BMC Musculoskelet Disord* **19**: 445. DOI: 10.1186/s12891-018-2373-3.

Tanga FY, Nutile-McMenemy N, DeLeo JA (2005) The CNS role of Toll-like receptor 4 in innate

neuroimmunity and painful neuropathy. *Proc Natl Acad Sci U S A* **102**: 5856-5861.

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

Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, AlMazroa MA, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basáñez M-G, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabé E, Bhalla K, Bhandari B, Bikbov B, Abdulhak AB, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT-A, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, Vaccaro KC de, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, Leo DD, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Jarlais DCD, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fèvre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FGR, Franklin R, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gosselin R, Grainger R, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo J-P, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Ma J, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Memish ZA, Mensah GA, Merriman TR, Meyer A-C, Miglioli V, Miller M, Miller TR, Mitchell PB, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh

M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KV, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N, Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA, Popova S, Porrini E, Pourmalek F, Prince M, Pullan RL, Ramaiahk D, Ranganathan D, Razavi H, Regan M, Rehm JT, Rein DB, Remuzzi G, Richardson K, Rivara FP, Roberts T, Robinson C, Leòn FRD, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S, Sampson U, Sanchez-Riera L, Sanman E, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz S, Shepard DS, Shin H, Shivakoti R, Silberberg D, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA, Sliwa K, Smith E, Smith JL, Stapelberg NJ, Steer A, Steiner T, Stolk WA, Stovner LJ, Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsen T, Tsilimbaris MK, Ubeda C, Undurraga EA, Werf MJ van der, Os J van, Vavilala MS, Venketasubramanian N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG, Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC, Williams SR, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh P-H, Zaidi AK, Zheng Z-J, Zonies D, Lopez AD, Murray CJ (2013) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**: 2163-2196.

Wang P, Zhu F, Tong Z, Konstantopoulos K (2011) Response of chondrocytes to shear stress: antagonistic effects of the binding partners Toll-like receptor 4 and caveolin-1. *FASEB J* **25**: 3401-3415.

Wang Q, Pan X, Wong HH, Wagner CA, Lahey LJ, Robinson WH, Sokolove J (2014) Oral and topical boswellic acid attenuates mouse osteoarthritis. *Osteoarthritis Cartilage* **22**: 128-132.

Wolfson RK, Mapes B, Garcia JGN (2014) Excessive mechanical stress increases HMGB1 expression in human lung microvascular endothelial cells *via* STAT3. *Microvasc Res* **92**: 50-55.

Wuertz K, Vo N, Kleitsas D, Boos N (2012) Inflammatory and catabolic signalling in intervertebral discs: The roles of NF- $\kappa$ B and MAP kinases. *Eur Cell Mater* **23**: 102-120.

Yang J, Jiang H, Yang J, Ding J-W, Chen L-H, Li S, Zhang X-D (2009) Valsartan preconditioning protects against myocardial ischemia-reperfusion injury through TLR4/NF- $\kappa$ B signaling pathway. *Mol Cell Biochem* **330**: 39-46.

Youn HS, Saitoh SI, Miyake K, Hwang DH (2006) Inhibition of homodimerization of Toll-like receptor 4 by curcumin. *Biochem Pharmacol* **72**: 62-69.

Yuan Y, Zhou Z, Jiao Y, Li C, Zheng Y, Lin Y, Xiao J, Chen Z, Cao P (2017) Histological identification of *Propionibacterium acnes* in nonpyogenic degenerated intervertebral discs. *Biomed Res Int* **2017**: 1-7.

Zhang Q, Weng Y, Jiang Y, Zhao S, Zhou D, Xu N (2018) Overexpression of miR-140-5p inhibits lipopolysaccharide-induced human intervertebral disc inflammation and degeneration by downregulating Toll-like receptor 4. *Oncol Rep* **40**: 793-802.

Zhao Y, Xin Y, Gao J, Teng R-Y, Chu H-C (2015) Analgesic effect of TAK-242 on neuropathic pain in rats. *Int J Clin Exp Med* **8**: 11202-11207.

Zwambag DP, Molladavoodi S, Guerreiro MJ, DeWitte-Orr SJ, Gregory DE (2020) Immunostimulatory capacity of decorin in the rat tail intervertebral disc and the mechanical consequence of resultant inflammation. *Eur Spine J* **29**: 1641-1648.

## Discussion with Reviewer

**Zhen Li:** Do you think blocking of TLRs could be used in the future as a treatment therapy for IVD disease?

**Authors:** We believe so. Since the TLRs are an integral part of the cytokine-mediated inflammatory process seen at the core of IVD degeneration and that alarmins are increasingly found in degenerating discs, the literature supports the fact that inhibiting this pathway leads to a dampening of the catabolism and inflammation found in IVDs. However, it remains to be seen whether this dampening is enough to bring balance to tissue homeostasis to arrest the vicious cycle of degeneration.

**Zhen Li:** Blocking TLRs seems to offer a promising therapeutic treatment for management of inflammatory and degenerative disc disease. Is there any side effect anticipated if TLR blockers are applied systematically or locally for treatment of disc disease?

**Authors:** TLRs are important as the first line of defence at the core of innate immunity. Therefore, a systemic inhibitory approach could bring adverse immunosuppressive effects. Ideally, treatments should be applied locally with slow release or short-term exposure at a higher dose.

**Editor's note:** The Guest Editor responsible for this paper was Andrea Vernengo.