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Inflammation in intervertebral disc degeneration and regeneration

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Intervertebral disc (IVD) degeneration is one of the major causes of low back pain, a problem with a heavy economic burden, which has been increasing in prevalence as populations age. Deeper knowledge of the complex spatial and temporal orchestration of cellular interactions and extracellular matrix remodelling is critical to improve current IVD therapies, which have so far proved unsatisfactory. Inflammation has been correlated with degenerative disc disease but its role in discogenic pain and hernia regression remains controversial. The inflammatory response may be involved in the onset of disease, but it is also crucial in maintaining tissue homeostasis. Furthermore, if properly balanced it may contribute to tissue repair/regeneration as has already been demonstrated in other tissues. In this review, we focus on how inflammation has been associated with IVD degeneration by describing observational and *in vitro* studies as well as *in vivo* animal models. Finally, we provide an overview of IVD regenerative therapies that target key inflammatory players.

1. Introduction

Between 70 and 85% of all people have low back pain (LBP) at some time in their life. LBP can limit the activity in people younger than 45, causing a tremendous socio-economic impact [1]. The aetiology of LBP is unclear but in 40% of cases it is related to intervertebral disc (IVD) degeneration [2]. In 90% of sciatica cases, it is also associated with a herniated IVD that compresses a nerve root causing pain [3]. Novel strategies such as gene therapy, growth factor injection, cell-based therapies and tissue engineering approaches are being developed towards impairing degeneration or promoting regeneration of the IVD [4]. However, to achieve full IVD regeneration it is also necessary to recover the biomechanical properties of a native IVD and restore the biological behaviour of resident cells, including production of healthy extracellular matrix (ECM), while ensuring reduction of IVD-associated pain.

Traditionally, inflammation has mostly been seen as detrimental and correlated with disease progression, but it remains unclear whether it is a cause or consequence of IVD degeneration and herniation. A balanced inflammatory response may be required for restoring IVD function as recently suggested for other tissues [5,6]. In this review, we will discuss the inflammatory reaction in IVD, both in homeostasis and IVD degeneration, and comprehensively cover the strategies applied to inflammatory cells and factors targeted towards IVD regeneration.

2. The origin of inflammation in intervertebral disc

Inflammation has mostly been regarded as a response to infection or tissue injury, but the scientific community has been increasingly researching its physiological role in maintaining tissue homeostasis [7]. In general, the mechanisms of inflammation are dependent on the inducing agent and context, with the inflammatory response in the context of infection having been investigated the most. In the infection instigated inflammatory response, plasma and

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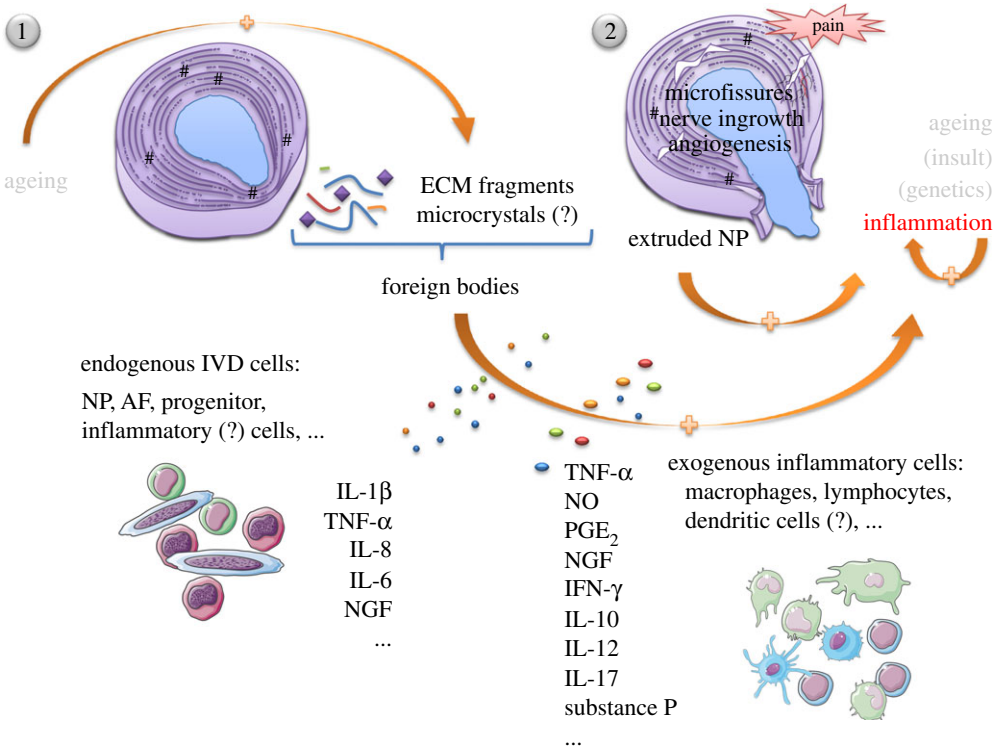

Figure 1. Inflammation in the IVD. It is unclear whether inflammation is the cause or consequence of disc degeneration and herniation, and what may trigger activation and recruitment of different immune cells. The normal ageing process allied to some genetic pre-disposition causes the IVD to degenerate giving rise to profound changes in the ECM—loss of proteoglycan content, dehydration, malnutrition, decrease of native cell population, matrix breakdown and calcifications. In this scenario, the natural response to mechanical loading is compromised and the IVD becomes prone to microfissures and consequent ingrowth of blood and nerve vessels. (1) Disc herniation may also occur when the AF is no longer able to sustain the NP. ECM fragments and microcrystals may internally elicit an inflammatory response, stimulating endogenous IVD cells to produce pro-inflammatory mediators, that will further feed the cascade of tissue degeneration—IL-1β, IL-8, IL-6. (2) NP is recognized as non-self by the immune system. Hence, its exposure (both in microfissures and herniation) may propagate an immunologic response, with recruitment of macrophages, lymphocytes and other possible inflammatory cells, in order to eliminate the foreign body. Discogenic pain has been many times attributed to TNF-α, PGE2, NO and IFN-γ secretion by macrophages, concomitantly with NGF and substance P production, accompanying the processes of nerve ingrowth and angiogenesis inwards the degenerated IVD. Activated B and T lymphocytes are also recruited to the site, contributing to the positive pro-inflammatory feedback loop established. It is not well understood how endogenous IVD cells interact with exogenous inflammatory cells and whether they positively contribute to tissue resorption and regeneration or not. Spontaneous disc regression is currently believed to be a consequence of macrophage activity.

leucocytes are recruited to the site of infection and soluble mediators that lead to recruitment and activation of other cell types are secreted. A complex cascade of events is triggered that eventually leads to clearing infection from the tissue and resolution of inflammation. In response to tissue injury, there also exists a vascular response and an orchestrated recruitment and activation of various cell types. However, the IVD is an avascular tissue and therefore it is unsurprising that the inflammatory response is different in this context.

The process of IVD degeneration implies a cascade of structurally disrupting events, normally starting with declining nutrition of cells within the central IVD, followed by accumulation of cell waste products and degraded matrix molecules. This creates an increasingly acidic environment which further compromises cell viability [8]. Various causes have been hypothesized to play a role in the pathogenesis of degenerative disc disease (DDD), such as endplate calcification, leading to an impairment of normal nutrition routes, excessive mechanical loading, genetic pre-disposition, unhealthy habits, ageing and spine infection [9–16]. Regardless of the cause, inflammation is an omnipresent player, and its association to LBP is clear [17]. Yet, it remains uncertain what may trigger the recruitment of immune cells to IVD and the associated inflammatory response (figure 1).

One hypothesis for the cause of the IVD inflammatory response relies on endogenous factors, such as crystals and ECM breakdown products, which could induce the inflammatory response [7]. Crystal deposits of calcium pyrophosphate dihydrate (CPPD), cuboid microstructures (characterized as magnesium whitlockite) and hydroxyapatite (HA) have been observed in degenerated IVD specimens [15,18–20]. In articular cartilage, regions with crystals showed altered amounts of collagen, calcium-binding proteins, decorin and large proteoglycan content, as well as abnormal pericellular matrix deposition [21,22]. Phagocytosis of crystals present in joints and pericellular tissues can trigger activation of the NOD-like receptor family pyrin domain containing 3 (NALP3) inflammasome. This cytoplasmic multimolecular protein complex regulates activity of caspase-1 and maturation and release of interleukin (IL)-1β [7,23], the latter being commonly found in degenerated IVD [24].

ECM breakdown products generated during tissue dysfunction or damage may also promote an inflammatory response as has been shown in various models [25,26]. The IVD is mostly composed of ECM molecules, including collagens, proteoglycans and other matrix proteins (see table 1 for more information), which are continuously synthesized and degraded by local existing proteases to maintain

Table 1. Main extracellular matrix components of a young and healthy IVD.

name	distribution/localization	putative/possible function
collagens		
fibril-forming collagens		
type I	AF and NP	confers tensile stiffness allowing torsion and flexion [27–30]
type II	AF and NP	confines PG within the matrix to retain more water to allow larger deformations and withstand greater compressive loads [31,32]
type III	NP and outer AF	organizes pericellular environment; allows extensibility of tissue [33–35]
type V	AF and NP (increased in AF cells when compared to NP cells)	regulates fibril diameter (smaller if this collagen is more abundant) influencing mechanical properties [35,36]
type XI [37]	all over, mostly NP	regulates fibril diameter (smaller if this collagen is more abundant) influencing mechanical properties [35,38]
beaded-filament forming collagens		
type VI	all over, mostly NP	helps cell fixation to the matrix and facilitates collagen bundles' sliding and lubrication [39,40]
FACIT collagens		
type IX	NP	maintains matrix integrity [41,42]
type XII	AF	might regulate fibrillogenesis [29,43]
type XIV	AF	might regulate fibrillogenesis [29,43]
proteoglycans		
aggregating PGs		
aggrecan	AF and NP	maintains IVD's osmotic pressure; may act as an anti-angiogenic factor due to its inhibition of endothelial cell migration [40,44,45]
versican	all over, mostly AF	favours the attachment of adjacent lamellae, contributes to resistance to compressive forces and facilitates cell migration, since it is an anti-adhesive molecule [35,40]
non-aggregating PGs		
small leucine-rich proteoglycans (SLRPs)		
decorin	outer AF and fibrillar NP	regulates collagen fibril diameter and spacing, maintaining uniform patterning; GFs' reservoir (TGF-β), modulating ECM metabolism [46–48]
biglycan	outer AF and NP (fibrillar and pericellular region)	GFs' reservoir (TGF-β), modulating ECM metabolism [46,47]
asporin	outer and inner AF, rarely NP	GFs' reservoir (TGF-β), modulating ECM metabolism; may play a major role in modulating chondrocyte matrix homeostasis [49,50]
fibromodulin	AF and NP	regulates collagen fibril diameter and spacing, maintaining uniform patterning; GFs' reservoir (TGF-β), modulating ECM metabolism [44,47]
lumican	AF and NP	regulates collagen fibril diameter and spacing, maintaining uniform patterning [44,51]
prolargin (encoded by PRELP*)	all over, mostly AF	anchors basement membranes to the underlying connective tissue [44,52]
chondroadherin	AF and NP	binds integrin ad collagen; regulates cell metabolism and ECM structure, promoting matrix homeostasis [44,53,54]
osteoglycin/ mimecan	AF and NP	unknown [44]
other matrix proteins		
other PGs		
perlecan	AF and NP	has a role in cell proliferation and differentiation by acting as co-receptor for FGFs; matrix organization and stabilization; role in FGF signalling [40,55]

(*Continued*.)

Table 1. (*Continued.*)

name	distribution/localization	putative/possible function
fibronectin	all over the disc	preserves structural integrity of the ECM; involved in cell adhesion through interaction with integrins [56–58]
elastin	all over the disc	preserves structural integrity of the ECM; helps to regain disc height and shape after deformation [59–61]
COMP	all over, mostly AF	preserves structural integrity of the ECM; binds other matrix proteins and catalyses polymerization of type II collagen fibrils; prevents vascularization of cartilage [62,63]
thrombospondin	AF	preserves structural integrity of the ECM; mediates cell adhesion, matrix–matrix interactions, cell migration and proliferation in other tissues; might prevent vascularization of the tissue; activates TGF-β complex [64,65]

Table 2. Main IVD proteinases.

name	distribution/localization	putative/possible function
aggrecanases		
ADAMTS1, 4, 5, 9 and 15	*ADAMTS1:* NP and AF *ADAMTS4:* low levels NP and AF *ADAMTS5:* low levels NP and AF *ADAMTS9:* NP and AF *ADAMTS15:* low levels NP and AF	degrades aggrecan [66–69], as well as versican, biglycan, fibromodulin, COMP, TSP1, TSP2, nidogen, among other substrates [70]
collagenases		
MMP1, 8 and 13	*MMP1:* low levels, mostly inner AF and NP *MMP8:* low levels *MMP13:* low levels, mostly NP	cleaves fibrillar collagen [66–68]
gelatinases		
MMP2 and 9	*MMP2:* low levels, mostly inner AF and NP *MMP9:* low levels AF and NP	degrades denatured collagen and basement membrane collagen [68]
stromelysin		
MMP3 and 10	*MMP3:* low levels, mostly in the adult NP *MMP10:* only checked in the NP	digests non-collagenous matrix proteins and denatured collagen [66,68,71]
matrilysin		
MMP7	NP and inner AF	degrades aggrecan and collagen type II [72]
other MMPs		
MMP19	AF and NP	cleaves aggrecan, COMP, types I and IV collagen, and fibronectin and acts on tenascin; can interfere with stabilization of capillary-like structures, possibly playing a role in the avascular status of the disc; regulates IGF-mediated proliferation in other tissues by proteolysis of IGFBP3 [73]

homeostasis (table 2) [66,74–76]. However, when an imbalance occurs, degradation products might trigger inflammation. For instance, fibronectin fragments alleviate metalloproteinase (MMP) inhibition and so promote monocyte migration *in vitro* [77]. This certainly facilitates their recruitment into the inflamed region. Fragments of laminin, collagen type XIV and fibrin can also modulate inflammatory cell infiltration and proliferation in other systems [26]. In cartilage explant cultures, fibromodulin fragments are produced following IL-1 stimulation [78]. In different *in vitro* settings, fragments originated from elastin, laminins, collagen (type I and IV), fibronectin, ectactin/nidogen, thrombospondin and hyaluronan also induce protease and cytokine production, independent of their chemotactic activity [79]. Some of the

aforementioned studies were performed in highly vascularized model systems (i.e. cardiovascular, lung or renal tissues). While these systems are very different from healthy adult IVDs, which are largely avascular, their findings might still be of relevance when studying phenomena associated with disc herniation or sequestration, in which blood vessels are much more abundant [80].

It appears that fragment release initiates and propagates the inflammatory response locally. Many of these fragments (e.g. originated from biglycan, fibronectin, hyaluronan) signal through toll-like receptor-2 (TLR2) and/or TLR4 in other model systems [81,82]. TLR4, in particular, is a well-known pattern recognition receptor involved in innate immune responses that has been implicated in inflammatory degeneration [83]. In human IVD cells, hyaluronic acid fragments (fHA) lead to increased mRNA expression levels of inflammatory and catabolic genes IL-1β, IL-6, IL-8, cyclooxygenase (COX)-2, metaloprotease-1 and -13, and IL-6 [84]. However, while IL-6 production is dependent on TLR2 it is independent of TLR4. It should be noted that low and high molecular weight molecules can have different effects, even through the same pathways. For example, high molecular weight hyaluronan protects epithelial cells against pro-apoptotic stimuli through NF-κB activation, in a TLR dependent way. Low molecular weight degradation products can induce inflammation, promoting macrophage mediated production of IL-1β and tumour necrosis factor alpha (TNF-α), through activation of the NF-κB/IκBα complex [82]. It is difficult to assess the overall role of ECM proteins within an immune setting because of their dual roles and because many proteases and a variety of fragments are released simultaneously. This difficulty is exacerbated by the scarcity of *in vivo* data, owing to limitations in the techniques used to detect fragments and immune cells, which are present at low concentrations and are short lived [85].

Numerous studies suggest that the IVD might endogenously include inflammatory-like cells [86,87]. In particular, it has been shown *in vitro* that a population of IVD cells can phagocytize beads and apoptotic bodies [86]. In turn, human surgical non-herniated nucleus pulposus (NP) samples presented a high number of resident CD68+ cells [87]. Furthermore, a recent robust analysis of cytokine/chemokine expression profile of human NP cells has presented clear evidence that NP cells, or at least some of them, are producers of specific inflammation-associated molecules, even in basal conditions (non-degenerated NP) [88]. In addition, infiltrated leukocytes (CD11b-positive cells) were found even in prolapsed IVDs, where NP is supposedly intact and isolated from any vascular source of immune cells [88]. The question of whether these cells could be resident macrophages or macrophage-like cells remains. Although pleiotropic, cytokines and chemokines have three modes of action: (i) stimulating the production of other inflammatory mediators and MMPs, (ii) enhancing matrix degradation, and (iii) recruiting inflammatory cells and activating phagocytosis [89–94]. Together, these effects can contribute to disease progression in the IVD.

It should be stressed that extreme mechanical loading has also been shown to alter ECM properties (through proteinase activation) and promote inflammation, contributing to IVD degeneration [95]. Apart from *in vitro* studies, organ cultures of bovine caudal IVDs have shown that compression induces apoptosis, produces inflammatory mediators and alters matrix integrity, leading to development of the disease [96].

In a more advanced degenerative stage, the well contained and apparently 'sealed' NP (immune privileged) becomes exposed to immune cells, which, responding to an inflammatory stimulus, may arise from newly formed blood vessels that invade pathological clefts and tears found in the annulus fibrosus (AF). Nociceptive nerve fibre ingrowth also accompanies angiogenesis and is believed to be the origin of discogenic pain that actively contributes to LBP [97–100]. Indeed, while the probable sites for focal damage and inflammation are vertebral endplates and AF (the only sites where the IVD is vascularized) [101], the NP is capable of attracting leukocytes and increasing vascular permeability when implanted subcutaneously [100]. An increase in expression of some cytokines and MMPs in herniated IVD may occur when molecules seen as 'non-self' by immune cells become exposed. This may also be linked to the phenomenon of spontaneous regression or disappearance of extruded IVD fragments, which has been attributed to matrix degradation and phagocytosis by recruited/infiltrated macrophages [102–106]. In cases with transligamentous extrusion, which can occur when the NP is potentially more exposed to immune cells, regression occurs [107]. Furthermore, the survival rate of subcutaneously transplanted rat NP cells is higher in immunocompromized NOD mice, and both rat macrophages and NK cells lyse autologous NP cells *in vitro*, indicating that immune cell populations respond to NP tissues [108].

In the next sections, we will review observational, *in vitro* and *in vivo* studies of the inflammatory milieu in IVD.

3. Inflammatory key players in intervertebral disc

3.1. Observational studies

A range of cytokines have been found in human IVDs in varying amounts, depending on whether the IVD is healthy, degenerated or herniated. Table 3 groups by methodology (observational, *in vitro* or pathway analysis) some of the most important studies that have clarified which inflammatory factors are expressed during homeostasis and with degeneration. Importantly, the identity of the cells producing these mediators (i.e. NP cells, AF cells, native IVD cells only, native cells plus infiltrating inflammatory cells) is highlighted.

3.1.1. *Post-mortem* samples

Separating NP from AF tissue upon discectomy is a very challenging task, particularly when dealing with degenerated human IVD tissue. In cases of disc herniation (e.g. extruded, sequestered), the IVD is invaded by other cell types, confounding analysis of molecules released by regions of the IVD. Samples taken *post-mortem*, which are not contaminated by infiltrating inflammatory cells (or at least not to the same extent as herniated discs), are therefore superior when investigating IVD homeostasis. For instance, TNF-α was substantially expressed in autopsy material in fetal/infantile and older adult NP, whereas it was sparsely expressed in adolescent and young adult NP. It was not found in the AF of young adults (below 25 years), but significantly increased in older individuals [24]. Also, calcium-dependent phospholipase A2 (PLA2), a regulator of prostaglandin E2 (PGE_2) production, has been found in both cadaveric and surgical samples, and IVDs of middle-aged cases had higher PLA2

Table 3. Inflammatory mediators found in the human IVD.

mediator	condition	producing cells	references
inflammatory factors that are expressed during homeostasis			
observational studies			
TNF-α	*post-mortem* and non-degenerate samples	IVD cells	[24,71,109]
IL-1β		IVD cells	[71,109–112]
IL-1α, IL-1Ra, IL-1RI, and ICE		NP and AF cells	[112]
IL-6, IL-8, RANTES		AF and NP cells	[110]
NGF		NP and AF cells in monolayer and alginate bead culture	[71,113]
NGF receptor (trkA)		NP and AF cells in monolayer and alginate bead culture	[113]
substance P			[71]
PLA2		NP and AF cells	[114]
CCL3 and CCL4		IVD cells	[115]
NOTCH		IVD cells	[109]
MMPs		IVD cells	[71,92]
inflammatory factors that are expressed with degeneration			
observational studies			
TNF-α	herniations (including subligamentous and transligamentous), protrusion, extrusion, sequestration, spondylosis, scoliosis, degenerated or discogenic pain	IVD cells and infiltrating cells	[24,71,116–121]
IL-1β			[71,111,117,118,120,121]
IL-1α			[117–119]
IL1-Ra, NO			[118]
IL-6			[17,117,118,122]
IL-8			[17,119]
IL-12, IL-17, IFN-γ			[122]
IL-20 (and its receptors)			[90]
IL-10, TGF-β, RANTES			[119]
IL-16, CCL2, CCL7, CXCL8			[88]
substance P			[71,118]
PGE_2			[17,118]
COX-2			[121]
PLA2			[114,121]
NGF			[71]
VEGF, bFGF			[116]
GM-CSF			[117]
CD20, CD45RO, CD68			[107]
MMPs			[71,107,116,118]
in vitro studies			
TNF-α	degenerate, sciatica, discogenic pain, extrusion, sequestration	IVD cells and infiltrating cells treated with different inflammatory stimulus (including IL-1β, TNF-α, substance P, IL-17, IL-20, IFN-γ, LPS) in monolayer or three-dimensional cell culture, or co-cultured with macrophage-like cells, or exposed to high mechanical strain	[95,121,123–125]

(*Continued*.)

Table 3. (*Continued.*)

mediator	condition	producing cells	references
IL-1β	discogenic pain and *post-mortem*		[110,112,124–126]
IL-1α, IL-1Ra, IL-1RI, and ICE	degenerate and *post-mortem*		[112]
IL-6	discogenic pain, scoliosis, sciatica, extrusion, sequestration, degenerate, myelopathy or radiculopathy and *post-mortem*		[89,90,95,110,124,126–129]
IL-8	discogenic pain, scoliosis, sciatica, extrusion, sequestration, degenerate, myelopathy or radiculopathy and *post-mortem*		[90,95,110,124,126–128]
IL-17A	protrusion, extrusion and scoliosis		[123]
IL-15, IFN-γ, CXCL9, TLR-2, TLR-4, MCP-3	*post-mortem*		[95]
RANTES	discogenic pain and *post-mortem*		[110]
MCP-1	discogenic pain, scoliosis, sciatica, extrusion, sequestration and *post-mortem*		[90,95,127]
TFGF-β1	discogenic pain, scoliosis, sciatica and *post-mortem*		[95,127]
substance P	myelopathy or radiculopathy		[126]
bFGF	scoliosis, sciatica and discogenic pain		[127]
PGE_2	degenerate, scoliosis, sciatica and discogenic pain		[89,121,128–130]
COX-2	extruded and sequestrated IVD tissue		[121]
NGF	*post-mortem*		[95,113]
NGF receptor (trkA)	*post-mortem*		[113]
PGF2α	degenerate		[128]
NO	degenerate, scoliosis		[89,124,129,130]
ICAM-1 (CD54)	degenerate and scoliosis		[89]
MMPs	degenerate, non-degenerate, extrusion and sequestration		[90,92,112,130]
NOTCH	protrusion		[109]
pathway analysis			
NF-κB, MAPK and C/EBP$\beta\rightarrow$ CCL3	discectomy	IVD cells and infiltrating cells	[115]
NF-κB$\rightarrow$ADAMTS-4 and -5	DDD and myelopathy	NP and infiltrating cells treated with IL-1β and TNF-α	[131]
NF-κB$\rightarrow$Sox9 and collagen type II	spine trauma	IVD cells treated with IL-1	[132]
NF-κB and MAPK$\rightarrow$NOTCH pathway	protrusion	NP cells	[109]

activity than those of younger and older subjects, indicating an important physiological role in maintaining homeostasis [114]. Like TNF-α or PLA2, many other inflammatory key players have been localized in non-degenerated human IVD tissue (table 3). Importantly, IVD native enzyme activity has also been studied. It was shown in the intact IVD that IL-1 is a key cytokine mediating IVD matrix degradation, by measuring enzyme activity (*in situ* zymography (ISZ)) against gelatin, collagen II and casein matrices [92]. Also, MMP-10 expression (at mRNA and protein levels) was increased in the symptomatic degenerate IVD, when compared to non-symptomatic one—possibly contributing to matrix degradation and initiation of nociception [71]. An additional perspective is given by studies that identified factors not naturally produced by native IVD cells: immunoreactivity for IL-4, IL-6, IL-12 and interferon (IFN)-γ was modest in surgical IVD tissue, being higher in herniated IVD samples and virtually non-existent in the control

samples taken from *post-mortem* non-degenerated IVDs [122]. The majority of these reports used *post-mortem* IVD samples as healthy controls, exposing the role of IVD native cells in IVD homeostasis. This knowledge is of potential interest for the development of endogenous therapeutic routes to restore homeostasis in DDD.

3.1.2. Degenerated samples

Regarding human degenerated IVD samples, early studies detected the presence of IL-1, intracellular adhesion molecule-1 (ICAM-1), lymphocyte function-associated antigen (LFA) and fibroblast growth factor (FGF) [133]. Immunoreactivity for some cytokines (IL-4, IL-6, IL-12, IFN-γ, TNF-α) seemed modest but evident in herniated and degenerated discs, with substantial macrophage infiltration [116,122]. Also, pathologic discs highly expressed IL-17, suggesting the involvement of Th17 lymphocytes in disc herniation [123]. Others have shown a higher expression of TNF-α, IL-1β, IL-6, IL-8, IL-20, PGE_2 and nitric oxide (NO) in herniated discs [17,90,117]. Some inflammatory key players have also been associated with pain in human IVD: RANTES and IL-1β expression was significantly higher in painful versus painless discs, contrarily to IL-6 and IL-8 [110]. A strong difference was observed in the levels of nerve growth factor (NGF), neurofilament-68, growth-associated protein (GAP)-43, and substance P in invading nerve fibers, in and around the outer layer of uncontained herniated versus spondylotic IVDs [116]. Another study that evaluated 91 cytokine- and chemokine-associated genes in human NP cells showed that NP cells are a source of IL-16, CCL2, CCL7 and CXCL8 [88]. Some of the pro-inflammatory cytokines usually present at increased levels in human degenerated discs, such as IL-1β and TNF-α, may mediate catabolic effects, decreasing proteoglycan production and enhancing MMP expression [71,111,134,135].

3.2. *In vitro* studies

Different *in vitro* studies have focused on studying the sources and role of some inflammatory mediators associated with herniated and degenerated IVD. An increase in IL-6, IL-8 and PGE_2 was observed in control and degenerated human IVD tissues upon lipopolysaccharide (LPS) stimulation [127]. Furthermore, it was shown that substance P, expressed by IVD cells, upregulates IL-1β, IL-6 and IL-8 in both NP and AF, and RANTES and TNF-α in AF only [126]. Also, NP cells were shown to express the CCL3 ligand (also known as macrophage inflammatory protein (MIP)-1α), which is well known for its chemotactic and pro-inflammatory effects, through activation of the MAPK, NF-κB and C/EBP signalling pathways after treatment with IL-1β or TNF-α [115]. These studies suggest a contribution of native IVD cells to the inflammatory milieu. However, others have defended the hypothesis that immune cells, such as macrophages, neutrophils and T cells, can be recruited to degenerated IVD [135]. This hypothesis is supported by evidence that Th17 cells expressing CCR6 are recruited to degenerated IVD by CCL20 secretion from NP [123], and that macrophages can migrate after stimulation with conditioned medium from rat NP cells treated with IL-1β or TNF-α [115].

TNF-α, which is one of the most studied pro-inflammatory molecules, is known to promote aggrecan degradation, disc catabolism and expression of pro-inflammatory cytokines and NGF, without any recovery [136]. TNF-α is an adipokine that has been associated with higher numbers of bovine IVD senescent cells and is therefore implicated with the inability of degenerated IVD to repopulate by itself [137]. Curiously, although many studies have focused on the role of TNF-α in IVD degeneration [136], Hoyland and co-authors suggest instead that IL-1β is the key regulator of matrix degradation in degenerated IVD: IL-1 has greater expression in the IVDs clinically associated with chronic LPB and treatments against IL-1β were shown to inhibit matrix degradation [92]. IL-1 is upregulated in degenerated human discs, inducing MMP7, MMP13 and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs)—suggesting a deregulation of the normal IVD homoeostasis [138]. TNF-α blockers had no effect on matrix-degrading activity, suggesting that its upregulation in DDD is not associated with matrix degradation [92] but instead with neighbouring nerve root irritation, which is confirmed by other studies [134,139]. Hence, TNF-α might be contributing to discogenic pain in cases where nerve ingrowth into IVD degenerative fissures occurs [140,141].

3.3. *In vivo* studies

Although some IVD degeneration related inflammatory mediators identified *in vitro* have not yet been studied *in vivo*, recent evidence has shed light on the role of several molecules. The majority of studies have been conducted in rat, rabbit and porcine animal models.

In a rat animal model of IVD herniation, NP exposure led to increased IL-6, TNF-α and IFN-γ levels. Other cytokines (IL-1β, IL-10, IL-1α and IL-2), already increased by the surgical procedure, were not altered [142]. In another study, TNF-α was identified in rat herniated IVDs and associated with radicular pain [139]. Also, a rat model of caudal annular incision demonstrated a transient peak in IL-1β 4 days following injury. This model was characterized by NP size decrease, annular collagen layer disorganization, and cellular metaplasia of annular fibroblasts to chondrocyte-like cells. However, no significant changes in TNF-α or IL-6 were seen [143]. In a lumbar rabbit annular incision model, no alteration in IL-1α or TNF genes was observed in whole IVDs at either one or three weeks after injury [144]. In the same model, IL-1β, transforming growth factor (TGF)-β1 and iNOS (inducible nitric oxide synthase) gene expression increased after three weeks, but decreased between weeks 6 and 12, having a second peak at 24 weeks—possibly showing a long-term pro-inflammatory action [145]. In a rabbit model of IVD herniation, the presence of TNF-α, IL-1β and MCP-1 (which has been demonstrated to be a potent macrophage chemoattractant [146]) was also analysed: TNF-α and IL-1β were detected after day 1 (via immunohistochemistry) followed by MCP-1 3 days post-injury. Infiltrating cells, mainly macrophages, were also observed after day 3 [147]. Interestingly, in a lumbar porcine model of annular incision, a significant increase in IL-8 accompanied by a decrease in IL-1 was observed in IVDs subjected to discectomy at 12 weeks post-injury, while no difference was observed in disc morphology, proteoglycan content, or in levels of IL-6 and TNF-α expression between untreated and injured IVDs. Whereas both IL-1 and IL-8 have pro-inflammatory properties, the authors propose that such discectomy procedure may be capable of initiating a repair response in the IVD, given that expression of IL-8 (an anabolic agent) is increased when the catabolic IL-1 decreases [148].

The TLR4-ligand LPS triggered inflammation when injected in a rat caudal IVD [149]. LPS injection in a rat IVD led to an increase in the levels of IL-1β, TNF-α, HMGB1 (high-mobility group box 1) and MIF (macrophage migration inhibitory factor), which correlated with morphological changes in tissue organization, namely interruption of NP/AF border, contraction of NP shape and decrease of IVD height [149]. Recently, different components of the tissue renin-angiotensin system (tRAS) (angiotensin converting enzyme, Ang II, Ang II receptor type 1, Ang II receptor type 2 and cathepsin D), that contribute to inflammation in many organs, have been found for the first time in the normal rat IVD, at both mRNA and protein levels [150]. However, the association between tRAS and IVD degeneration and its relationship to IVD inflammation has yet to be elucidated.

Most of these *in vivo* studies mainly identify and quantify inflammatory mediators in IVD, but fail to produce a mechanistic explanation of their role in either IVD degeneration or regeneration. One of the reasons for this failure may be the difficulty of unravelling the complex inflammatory mechanisms in injury models of IVD pathology. In this context, animal models of spontaneous IVD degeneration such as the sand rat [151] and both younger chondrodystrophic (with cervical or thoracolumbar IVD disease) and older non-chondrodystrophic (caudal cervical or lumbosacral IVD disease) dogs [152,153] could bring new insights to the clinic. However, as far as we are aware, the inflammatory response has not been addressed in these models. Importantly, differences between species could also bring some intricacy to this issue. For instance, notochordal cells (NC) seem to disappear in the adult human IVD, while in many other species they are retained throughout adulthood [154]. As more models become available, it is central to translate information between species and to interpret the models appropriately to understand in greater depth the process of inflammation.

4. Strategies to target and modulate inflammation towards intervertebral disc regeneration

Promoting IVD regeneration relies on restoring naive IVD properties by: (i) recovering IVD biomechanics, (ii) re-establishing cell biological activity, including production of healthy ECM, and (iii) reducing IVD-associated pain. Biological approaches focusing on IVD regeneration or IVD-associated pain relief begun in the early 1990s, and have since increased in number and diversity as reviewed elsewhere [4].

A well-balanced approach supporting tissue regeneration and control of inflammatory response could be successful in reducing IVD-associated pain. Although the molecular mechanisms behind IVD pathology and inflammatory response remain to be elucidated in detail, some inflammatory-related molecules are key targets of novel therapies in DDD [155,156]. In this section, an overview of the strategies targeting inflammatory mediators towards IVD regeneration will be given.

4.1. Injection of molecules

The more direct approaches to control inflammation in IVD are to inject regulating agents close to the IVD. Example agents are TNF-α blockers, such as infliximab, adalimumab, etanercept [136,157–160], or IL-1 inhibitors, such as IL-1 receptor antagonist (IL-1Ra) [161]. Other TNF-α blockers include a monoclonal antibody tested in herniated IVD patients, who showed less leg and back pain after antibody administration [162], and a p38-TNF-α inhibitor, which was tested in the spine to address neuroinflammation but not specifically for IVD [163]. Other strategies involve injecting corticosteroids into the IVD [164], or the anti-cholesterolemic drug simvastatin [165], which appeared to retard IVD degeneration in animal models.

The therapeutic potential of IL-1Ra for sustained attenuation of IL-1β has also been explored using poly(lactic-*co*-glycolic acid) (PLGA) microspheres as a delivery system [166]. IL-1Ra-PLGA microspheres inhibited NO production in NP cell cultures and partially restored the levels of iNOS, ADAMTS-4, MMP-13, IL-1β, IL-6 and TLR-4, which were increased in the presence in IL-1β [166].

Another candidate to control inflammation in IVDs is COX-2, which regulates PGE_2 synthesis in inflammatory conditions. Epidural injection of COX-2 inhibitors was shown to reduce pain in a rat model of IVD herniation [167]. Another approach uses platelet-rich plasma (PRP) as a therapy for degenerated IVD [168]—PRP was able to rescue chondrocyte degeneration induced by IL-1β and TNF-α [169].

Other approaches to reduce IVD-associated pain have been suggested. Resveratrol, a naturally occurring polyphenol present in red wine, was able to reduce IL-6, IL-8, MMP1, MMP3 and MMP13 expression when injected into the IVD [170]. Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid), an anthraquinone molecule derived from the rhizome of *Rheum palmatum* that exhibits anti-inflammatory activity and is used in the treatment of osteoarthritis and pain relief, was hypothesized to be a therapeutic agent for IVD through the regulation of IL-1 activity [171]. Fullerol, a derivative from fullerene and known anti-oxidant, retards cellular apoptosis and suppresses dorsal root ganglion (DRG) and neuron TNF-α-induced inflammatory responses, which when incorporated into nanoparticles is relevant for LBP treatment [172].

A very recent and promising approach to target inflammation in IVD is the inhibitor of IκB kinase-β (IKKβ), involved in NF-κB activation. The intradiscal injection of IKKβ reduced the levels of TNF-α, IL-1β and IL-6 of an injured IVD while suppressing high levels of neuropeptides within DRG neurons [173].

Despite good results in other therapeutic areas, injected molecules might be inefficient in DDD due to the short half-life of proteins in solution and the limited effect of a single protein in a complex process such as IVD degeneration [174]. Also, the risk of inducing IVD degeneration by puncturing of the IVD should be considered [175], although more recent studies describe alternative routes for molecule delivery [176]. Furthermore, given the predominantly avascular nature of IVD, systemic delivery of soluble molecules is unlikely to be effective in this situation. This view is supported by the report that the concentration of antibiotics in IVDs was undetectable in the NP of patients with IVD infection under systemic administration of antibiotics [177]. In addition, it has been shown that the rate of diffusion of antibiotics into the IVD is reduced by endplate calcification, increase in IVD size and solute molecular weight [178,179]. Moreover, although some nutrients' diffusion to the NP may occur via the endplates, the short half-life of pharmaceutical drugs or proteins can result in limited therapeutic doses that reach the NP [166].

4.2. Gene therapy

Gene therapy promises more prolonged effects in DDD, by introducing the possibility of locally modulating the expression of a specific gene and the consequent production of its protein [174]. As early as 1997, a study suggested genetic modifications as a therapy for DDD [180]. At that point, a retrovirus vector was proposed to transduce bovine chondrocytic endplate cells with IL-1Ra [180]. Cell transfection resulted in IL-1Ra production in 48 h, and injection of transfected cells in degenerated NP explants considerably reduced expression of several enzymes (such as MMP3) for two weeks after injection. This strategy aims at decreasing IL-1 mediated matrix degradation and stopping DDD progression [181]. *In vivo*, TGF-β1 transfection of rabbit IVD cells also enhanced proteoglycan synthesis for six weeks [182]. In agreement with this result, cells from human degenerated IVD transfected with TGF-β1 increased both proteoglycan and collagen production [183,184].

Gene therapy in a clinical setting may be limited by the safety of the gene transfer vector. Aspects such as exposure to high doses, long-term use, misplaced injections and the possibility of oncogenesis are key concerns when treating a chronic disease like DDD [185]. Progress in the development of more reliable viral vector constructs and in a better control of transgene expression would improve the safety of these therapies. Also, elucidation of molecular mechanisms behind the degenerative process and characterization of cell populations in IVD, as well as their role in ECM production, could bring new advancements to this field [174].

4.3. Cell-based therapies

Several cell-based therapies to stimulate IVD regeneration have been proposed in recent years: haematopoietic stem cells (HSC) [186], fetal spine cells [187], immortalized NP-cell lines [188], autologous IVD chondrocytes [189], embryonic stem cells (SC) [190], induced pluripotent SC [191], olfactory SC [192] and MSCs (derived either from bone marrow [193] or from umbilical cord blood [194]) have all been suggested to have potential for IVD repair/regeneration. NP progenitor cells were isolated from the NP (with approximately 1% frequency) and differentiated into chondrogenic and neurogenic lineages, suggesting potential for IVD regeneration [195]. Besides IVD regeneration, progenitor cells might play a protective role in regulating inflammation in IVD: rabbit NC were shown to reduce the levels of pro-inflammatory cytokines, IL-6 and IL-8, as well as iNOS, in *in vitro* co-cultures of AF cells with macrophages [196].

MSCs are one of the most attractive candidate cell types for IVD regeneration, partly because they could be autologous transplants. In a canine model, MSCs were able to increase collagen type II expression while decreasing cell apoptosis in IVD [197]. In rabbits, MSCs were able to remain in the IVD up to 24 weeks [198]. However, the number of transplanted MSCs is crucial; in the canine model 10^6 MSCs per IVD was ideal, since 10^5 MSCs resulted in decreased cell viability while 10^7 MSCs induced cell apoptosis [197]. Besides MSC multi-differentiation capacity, an associated immuno-modulatory effect has been suggested [199]. MSC role in inflammation is based on their active role as cytokine-release factories that interact directly with injured cells [200]. In this novel scenario, MSCs were shown to secrete IL-1Ra in a mouse model of lung injury [201] or produce a potent anti-inflammatory protein (TNF-α stimulated gene/protein 6, TSG-6) in a mouse infarct model [202]. Interestingly, TSG-6 was also identified as a key player in a rat model of corneal injury after MSC systemic administration [203]. After implantation of MSCs into beagle nucleotomized IVDs, the expression of Fas ligand (FasL) (a protein found in other immune privileged sites) was restored. It was suggested that MSCs either differentiated into cells expressing FasL, or stimulated the few remaining NP cells to produce this molecule—thus contributing to the recovery of immune privilege in degenerated IVDs [204]. Although the beneficial effects of these cells have been demonstrated in several models, the mechanisms behind MSC-based therapies are not clear.

In vitro studies showed that MSCs repress IgG production of peripheral blood lymphocytes co-cultured with IVD fragments from the same donors [205]. MSC influence in IVD inflammatory response has not been fully dissected until now due to its multifactorial complexity and time dependence [205]. Human MSCs were able to downregulate gene expression of pro-inflammatory cytokines (IL-3, IL-6, IL-11, IL-15, TNF-α) and MMPs when in co-culture with rat NP cells [198].

In humans, two clinical trials took advantage of autologous MSCs, albeit with controversial results. MSCs were either directly injected in NP [206] or implanted in the IVD after seeding in collagen sponges [207]. In the first case, when MSCs were directly injected in patients diagnosed with DDD, but with preserved external AF and persistent LBP, the lumbar pain was strongly reduced after three months. However, no improvement on IVD height was detected by imaging [206]. Injection of MSCs in degenerated IVDs seems to promote an analgesic effect, due to trophic effects, which can occur quicker than detecting possible regenerative effects [206]. Given this, the authors suggest that the MSCs exhibited immuno-modulatory properties. The second case reports the implantation of MSCs after seeding in collagen sponges [207]. Two years post-surgery, the published results reported relief or disappearance of LBP and improvement of the vacuum phenomenon (gas in the intervertebral space, associated with intervertebral regressive degeneration). However, besides the small number of patients used (two), this study also lacks experimental details such as the controls used and effective number of cells transplanted [207]. More recent studies propose IVD injection of umbilical cord-derived MSCs as a promising therapy to overcome chronic discogenic LBP [208]. In this study, pain and lumbar function were recovered after cell transplantation and preserved over a 2-year follow-up period; however, only two patients were studied. Another recent study injected bone marrow concentrate cells into 26 patients [209]. In the 1-year follow-up study, the majority of the patients showed improvement of pain score and reduced impairment, with only some of them presenting IVD rehydration. The authors emphasize the use of critical unmanipulated cell doses. Usually, MSC-based therapies involve cell expansion *in vitro* to obtain sufficient cell numbers, but this *in vitro* manipulation risks modifying their receptor expression and can introduce contaminants.

One interesting feature of MSCs is their capacity to migrate into injured tissues and participate in the regenerative process, interacting with the surrounding environment through secretion of numerous molecules such as growth factors, cytokines and chemokines [210]. Nevertheless, contrary to leukocyte migration and haematopoietic SC homing, the mechanisms that regulate MSC migration to injured sites

are not well characterized [211]. *In vitro*, in a pro-inflammatory environment stimulated by TNF-α, MSCs migrate towards SDF-1, RANTES and MDC gradients, amongst others [212]. Furthermore, MMPs and their inhibitors have also been shown to enhance MSC migratory capacity [213]. In a recent study, MSCs were recruited *in vitro* by conditioned medium from IVDs cultured under degenerative-simulated conditions [214]. CCL5/RANTES has been identified as a key chemoattractant released by degenerative IVD in organ culture [215]. Moreover, CXCL12/SDF-1 delivery in IVD organ cultures promotes MSC recruitment towards NP, especially if MSCs were harvested from young donors [216]. This does not exclude the possibility that other cytokines involved in IVD degeneration pathogenesis, namely TNF-α and IL-1β, might play a role in regulation of MSC recruitment to the IVD [181]. A hypothetical migration route of high-proliferative cells lateral to the epiphyseal plate and the outer border region of the IVD was recently described [217]—if this route is confirmed, new strategies envisaging IVD regeneration may be attempted.

5. Future perspectives

Recent findings from *in vitro* studies, animal models and clinical trials have started to unveil the role of inflammation in IVD degeneration. However, no evidence for a beneficial role of inflammation in maintaining homeostasis has been presented, owing to the difficulty in studying IVD tissue homeostasis. In other tissues, such as bone [218,219] or cardiovascular tissue [220], the control of inflammation has already proven to be critical in shifting the degeneration/regeneration balance towards regeneration. In particular, our group has focused on modulating inflammation in bone [5,221–224]. Hence, we believe that novel therapies for DDD should aim at restoring the homeostatic inflammatory conditions in the disc, rather than totally inhibiting inflammation, thus enabling endogenous repair mechanisms to operate.

Our group has recently shown that incorporating fibrinogen, a well-known inflammatory protein, into a biomaterial leads to increased bone formation [5]. *In vitro* studies have shown that fibrinogen-modified biomaterial stimulates NK cell-mediated MSC recruitment without affecting the MSC differentiation marker alkaline phosphatase [222]. Moreover, a broad analysis of macrophage-secreted factors showed that fibrinogen modified macrophage response, leading to a downregulation of the expression of inflammatory cytokines and a stimulation in the production of growth factor [224]. Factors such as MIP-1δ, platelet-derived growth factor-BB, bone morphogenetic protein (BMP)-5 and BMP-7 were significantly promoted by fibrinogen [224], which may impact tissue regeneration.

Recent advances in development biology also highlight the crucial role of immune cells. An efficient nuclear reprogramming to obtain induced pluripotent stem (iPS) cells was shown to require activation of an innate response [225] and was achieved via activation of TLR3 in the work of Yamanaka and colleagues [226]. The importance of this finding in physiological situations remains unclear, but it is becoming increasingly evident that activation of the immune response, particularly the innate response, may contribute to regulation of stem cell behaviour [225,227]. Moreover, it has been known for a long time that the post-inflammatory wound repair process recapitulates basic phenomena that occur during embryogenesis [228]. Therefore, future studies should focus on trying to understand what happens early in development, to discover more cues on how to modulate inflammation in a disease situation.

Although diverse studies have presented data on inflammatory key players in IVD, the inherent variability and contradictions arising from the different *in vitro* culture conditions and animal models used in these studies may be hampering translation of the research to a clinical setting. The standardization of methods, the correlation of results with different IVD clinical problems, the use of alternative *ex vivo* models (based on organotypic cultures or bioreactors) and the use of more physiologically accurate *in vivo* models of IVD degeneration could bring further advances to the IVD research field.

For IVD regeneration therapies to succeed, it will be important to address IVD degeneration together with inflammation. Until now, most studies have focused on only one of these two aspects. An integrated strategy, which addresses both the synergistic interplay that exists between the multiple factors associated with IVD degeneration and balances the inflammatory response, could be a step closer to the success of IVD regenerative strategies and bring relief for those suffering from LBP.

References

1. Andersson GB. 1999 Epidemiological features of chronic low-back pain. *Lancet* **354**, 581–585. (doi:10.1016/S0140-6736(99)01312-4)
2. Schwarzer AC, Aprill CN, Derby R, Fortin J, Kine G, Bogduk N. 1995 The prevalence and clinical features of internal disc disruption in patients with chronic low back pain. *Spine* (*Phila Pa 1976*) **20**, 1878–1883. (doi:10.1097/00007632-199509000-00007)
3. Koes BW, Van Tulder MW, Peul WC. 2007 Diagnosis and treatment of sciatica. *Br. Med. J.* **334**, 1313–1317. (doi:10.1136/bmj.39223.428495.BE)
4. Hughes SP, Freemont AJ, Hukins DW, Mcgregor AH, Roberts S. 2012 The pathogenesis of degeneration of the intervertebral disc and emerging therapies in the management of back pain. *J. Bone Joint Surg. Br.* **94**, 1298–1304. (doi:10.1302/0301-620X.94B10.28986)
5. Santos SG *et al*. 2013 Adsorbed fibrinogen leads to improved bone regeneration and correlates with differences in the systemic immune response. *Acta Biomater.* **9**, 7209–7217. (doi:10.1016/j.actbio.2013.04.008)
6. Sun Z, Zhang M, Zhao XH, Zhao ZH, GAO Y, Samartziz D, Wang HQ, Luo ZJ. 2013 Immune cascades in human intervertebral disc: the pros and cons. *Int.J. Clin. Exp. Pathol.* **6**, 1009–1014.
7. Medzhitov R. 2008 Origin and physiological roles of inflammation. *Nature* **454**, 428–435. (doi:10.1038/nature07201)
8. Urban JP, Smith S, Fairbank JC. 2004 Nutrition of the intervertebral disc. *Spine* (*Phila Pa 1976*) **29**, 2700–2709. (doi:10.1097/01.brs.0000146499.97948.52)
9. Moore RJ. 2006 The vertebral endplate: disc degeneration, disc regeneration. *Eur. Spine J.* **15**(Suppl. 3), S333–S337. (doi:10.1007/s00586-006-0170-4)
10. Benneker LM, Heini PF, Alini M, Anderson SE, Ito K. 2005 2004 Young Investigator Award winner: vertebral endplate marrow contact channel

occlusions and intervertebral disc degeneration. *Spine* (*Phila Pa 1976*) **30**, 167–173. (doi:10.1097/01.brs.0000150833.93248.09)

11. Adams MA, Roughley PJ. 2006 What is intervertebral disc degeneration, and what causes it? *Spine* (*Phila Pa 1976*) **31**, 2151–2161. (doi:10.1097/01.brs.0000231761.73859.2c)
12. Battie MC, Videman T, Kaprio J, Gibbons LE, Gill K, Manninen H, Saarela J, Peltonen L. 2009 The Twin Spine Study: contributions to a changing view of disc degeneration. *Spine J.* **9**, 47–59. (doi:10.1016/j.spinee.2008.11.011)
13. Cook CE, Taylor J, Wright A, Milosavljevic S, Goode A, Whitford M. 2013 Risk factors for first time incidence sciatica: a systematic review. *Physiother. Res. Int.* **19**, 65–78. (doi:10.1002/pri.1572)
14. Cheung KM, Karppinen J, Chan D, Ho DWH, Song Y-Q, Sham P, Cheah KSE, Leong JCY, Luk KDK. 2009 Prevalence and pattern of lumbar magnetic resonance imaging changes in a population study of one thousand forty-three individuals. *Spine* (*Phila Pa 1976*) **34**, 934–940. (doi:10.1097/BRS.0b013e3181a01b3f)
15. Hristova GI, Jarzem P, Ouellet JA, Roughley PJ, Epure LM, Antoniou J, Mwale F. 2011 Calcification in human intervertebral disc degeneration and scoliosis. *J. Orthop. Res.* **29**, 1888–1895. (doi:10.1002/jor.21456)
16. Desanto J, Ross JS. 2011 Spine infection/inflammation. *Radiol. Clin. N. Am.* **49**, 105–127. (doi:10.1016/j.rcl.2010.07.018)
17. Burke JG, Watson RW, Mccormack D, Dowling FE, Walsh MG, Fitzpatrick JM. 2002 Intervertebral discs which cause low back pain secrete high levels of proinflammatory mediators. *J. Bone Joint Surg. Br.* **84**, 196–201. (doi:10.1302/0301-620X.84B2.12511)
18. Lee RS, Kayser MV, Ali SY. 2006 Calcium phosphate microcrystal deposition in the human intervertebral disc. *J. Anat.* **208**, 13–19. (doi:10.1111/j.1469-7580.2006.00504.x)
19. Gruber HE, Norton HJ, Sun YB, Hanley EN. 2007 Crystal deposits in the human intervertebral disc: implications. *Spine J.* **7**, 444–450. (doi:10.1016/j.spinee.2006.08.015)
20. Feinberg J, Boachie-Adjei O, Bullough PG, Boskey AL. 1990 The distribution of calcific deposits in intervertebral discs of the lumbosacral spine. *Clin. Orthop. Relat. Res.* **254**, 303–310.
21. Kalya S, Rosenthal AK. 2005 Extracellular matrix changes regulate calcium crystal formation in articular cartilage. *Curr. Opin. Rheumatol.* **17**, 325–329. (doi:10.1097/01.bor.0000160783.14798.10)
22. Masuda I, Ishikawa K, Usuku G. 1991 A histologic and immunohistochemical study of calcium pyrophosphate dihydrate crystal deposition disease. *Clin. Orthop. Relat. Res.* **263**, 272–287.
23. Stutz A, Golenbock DT, Latz E. 2009 Inflammasomes: too big to miss. *J. Clin. Investig.* **119**, 3502–3511. (doi:10.1172/JCI40599)
24. Weiler C, Nerlich AG, Bachmeier BE, Boos N. 2005 Expression and distribution of tumor necrosis factor alpha in human lumbar intervertebral discs: a study in surgical specimen and autopsy controls. *Spine* (*Phila Pa 1976*) **30**, 44–53; discussion 54. (doi:10.1097/01.brs.0000174529.07959.c0)
25. Noble PW. 2002 Hyaluronan and its catabolic products in tissue injury and repair. *Matrix Biol.* **21**, 25–29. (doi:10.1016/S0945-053X(01)00184-6)
26. Vaday GG, Lider O. 2000 Extracellular matrix moieties, cytokines, and enzymes: dynamic effects on immune cell behavior and inflammation. *J. Leukoc. Biol.* **67**, 149–159.
27. Eyre DR, Muir H. 1977 Quantitative analysis of types I and II collagens in human intervertebral discs at various ages. *Biochim. Biophys. Acta* **492**, 29–42. (doi:10.1016/0005-2795(77)90211-2)
28. Eyre DR, Dickson IR, Van Ness K. 1988 Collagen cross-linking in human bone and articular cartilage. Age-related changes in the content of mature hydroxypyridinium residues. *Biochem. J.* **252**, 495–500.
29. Sivan SS, Hayes AJ, Wachtel E, Caterson B, Merkher Y, Maroudas A, Brown S, Roberts S. 2014 Biochemical composition and turnover of the extracellular matrix of the normal and degenerate intervertebral disc. *Eur. Spine J.* **23**(Suppl. 3), S344–S353. (doi:10.1007/s00586-013-2767-8)
30. Eyre DR, Muir H. 1976 Types I and II collagens in intervertebral disc. Interchanging radial distributions in annulus fibrosus. *Biochem. J.* **157**, 267–270.
31. Brinckmann J, Notbohm H, Müller PK. 2005 *Collagen: primer in structure, processing and assembly*, vol. 247, p. 252. Berlin, Germany: Springer.
32. Ippolito E, Ponseti IV. 1981 Juvenile kyphosis: histological and histochemical studies. *J. Bone Joint Surg. Am.* **63**, 175–182.
33. Gruber HE, Ingram JA, Hanley Jr EN. 2007 Morphologic complexity of the pericellular matrix in the annulus of the human intervertebral disc. *Biotech. Histochem.* **82**, 217–225. (doi:10.1080/10520290701713999)
34. Roberts S, Menage J, Duance V, Wotton SF. 1991 Type III collagen in the intervertebral disc. *Histochem. J.* **23**, 503–508. (doi:10.1007/BF01041176)
35. Culav EM, Clark CH, Merrilees MJ. 1999 Connective tissues: matrix composition and its relevance to physical therapy. *Phys. Ther.* **79**, 308–319.
36. Clouet J *et al*. 2009 Identification of phenotypic discriminating markers for intervertebral disc cells and articular chondrocytes. *Rheumatology* **48**, 1447–1450. (doi:10.1093/rheumatology/kep262)
37. Tow BP, Hsu WK, Wang JC. 2007 Disc regeneration: a glimpse of the future. *Clin. Neurosurg.* **54**, 122–128.
38. Mio F *et al*. 2007 A functional polymorphism in COL11A1, which encodes the alpha 1 chain of type XI collagen, is associated with susceptibility to lumbar disc herniation. *Am. J. Hum. Genet.* **81**, 1271–1277. (doi:10.1086/522377)
39. Roberts S, Ayad S, Menage PJ. 1991 Immunolocalisation of type VI collagen in the intervertebral disc. *Ann. Rheum. Dis.* **50**, 787–791. (doi:10.1136/ard.50.11.787)
40. Melrose J, Smith SM, Appleyard RC, Little CB. 2008 Aggrecan, versican and type VI collagen are components of annular translamellar crossbridges in the intervertebral disc. *Eur. Spine J.* **17**, 314–324. (doi:10.1007/s00586-007-0538-0)
41. Eyre DR, Wu JJ, Fernandes RJ, Pietka TA, Weis MA. 2002 Recent developments in cartilage research: matrix biology of the collagen II/IX/XI heterofibril network. *Biochem. Soc. Trans.* **30**, 893–899. (doi:10.1042/BST0300893)
42. Brinckmann J *et al*. 2005 Interleukin 4 and prolonged hypoxia induce a higher gene expression of lysyl hydroxylase 2 and an altered cross-link pattern: important pathogenetic steps in early and late stage of systemic scleroderma? *Matrix Biol.* **24**, 459–468. (doi:10.1016/j.matbio.2005.07.002)
43. Eyre DR, Matsui Y, Wu JJ. 2002 Collagen polymorphisms of the intervertebral disc. *Biochem. Soc. Trans.* **30**, 844–848. (doi:10.1042/BST0300844)
44. Onnerfjord P, Khabut A, Reinholt FP, Svensson O, Heinegard D. 2012 Quantitative proteomic analysis of eight cartilaginous tissues reveals characteristic differences as well as similarities between subgroups. *J. Biol. Chem.* **287**, 18 913–18 924. (doi:10.1074/jbc.M111.298968)
45. Johnson WE, Caterson B, Eisenstein SM, Roberts S. 2005 Human intervertebral disc aggrecan inhibits endothelial cell adhesion and cell migration *in vitro*. *Spine* (*Phila Pa 1976*) **30**, 1139–1147. (doi:10.1097/01.brs.0000162624.95262.73)
46. Gotz W, Barnert S, Bertagnoli R, Miosge N, Kresse H, Herken R. 1997 Immunohistochemical localization of the small proteoglycans decorin and biglycan in human intervertebral discs. *Cell Tissue Res.* **289**, 185–190. (doi:10.1007/s004410050864)
47. Hildebrand A, Romaris M, Rasmussen LM, Heinegård D, Twardzik DR, Border WA, Ruoslahti E. 1994 Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. *Biochem. J.* **302**, 527–534.
48. Reed CC, Iozzo RV. 2002 The role of decorin in collagen fibrillogenesis and skin homeostasis. *Glycoconj. J.* **19**, 249–255. (doi:10.1023/A:1025383913444)
49. Gruber HE, Ingram JA, Hoelscher GL, Zinchenko N, Hanley Jr EN, Sun Y. 2009 Asporin, a susceptibility gene in osteoarthritis, is expressed at higher levels in the more degenerate human intervertebral disc. *Arthritis Res. Ther.* **11**, R47. (doi:10.1186/ar2660)
50. Kizawa H *et al*. 2005 An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. *Nat. Genet.* **37**, 138–144. (doi:10.1038/ng1496)
51. Chakravarti S. 2002 Functions of lumican and fibromodulin: lessons from knockout mice. *Glycoconj. J.* **19**, 287–293. (doi:10.1023/A:1025348417078)
52. Bengtsson E, Morgelin M, Sasaki T, Timpl R, Heinegard D, Aspberg A. 2002 The leucine-rich repeat protein PRELP binds perlecan and collagens and may function as a basement membrane anchor. *J. Biol. Chem.* **277**, 15 061–15 068. (doi:10.1074/jbc.M108285200)

53. Haglund L, Ouellet J, Roughley P. 2009 Variation in chondroadherin abundance and fragmentation in the human scoliotic disc. *Spine* (*Phila Pa 1976*) **34**, 1513–1518. (doi:10.1097/BRS.0b013e3181a8d001)
54. Akhatib B, Onnerfjord P, Gawri R, Ouellet J, Jarzem P, Heinegard D, Mort J, Roughley P, Haglund L. 2013 Chondroadherin fragmentation mediated by the protease HTRA1 distinguishes human intervertebral disc degeneration from normal aging. *J. Biol. Chem.* **288**, 19 280–19 287. (doi:10.1074/jbc.M112.443010)
55. Melrose J, Smith S, Ghosh P, Whitelock J. 2003 Perlecan, the multidomain heparan sulfate proteoglycan of basement membranes, is also a prominent component of the cartilaginous primordia in the developing human fetal spine. *J. Histochem. Cytochem.* **51**, 1331–1341. (doi:10.1177/002215540305101010)
56. Oegema Jr TR, Johnson SL, Aguiar DJ, Ogilvie JW. 2000 Fibronectin and its fragments increase with degeneration in the human intervertebral disc. *Spine* **25**, 2742–2747. (doi:10.1097/00007632-200011010-00005)
57. Kadler KE, Hill A, Canty-Laird EG. 2008 Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. *Curr. Opin. Cell Biol.* **20**, 495–501. (doi:10.1016/j.ceb.2008.06.008)
58. Leiss M, Beckmann K, Giros A, Costell M, Fassler R. 2008 The role of integrin binding sites in fibronectin matrix assembly *in vivo*. *Curr. Opin. Cell Biol.* **20**, 502–507. (doi:10.1016/j.ceb.2008.06.001)
59. Yu J, Fairbank JC, Roberts S, Urban JP. 2005 The elastic fiber network of the anulus fibrosus of the normal and scoliotic human intervertebral disc. *Spine* (*Phila Pa 1976*) **30**, 1815–1820. (doi:10.1097/01.brs.0000173899.97415.5b)
60. Yu J, Winlove PC, Roberts S, Urban JP. 2002 Elastic fibre organization in the intervertebral discs of the bovine tail. *J. Anat.* **201**, 465–475. (doi:10.1046/j.1469-7580.2002.00111.x)
61. Johnson EF, Chetty K, Moore IM, Stewart A, Jones W. 1982 The distribution and arrangement of elastic fibres in the intervertebral disc of the adult human. *J. Anat.* **135**, 301–309.
62. Ishii Y, Thomas AO, Guo XE, Hung CT, Chen FH. 2006 Localization and distribution of cartilage oligomeric matrix protein in the rat intervertebral disc. *Spine* (*Phila Pa 1976*) **31**, 1539–1546. (doi:10.1097/01.brs.0000221994.61882.4a)
63. Rutges J, Creemers LB, Dhert W, Milz S, Sakai D, Mochida J, Alini M, Grad S. 2010 Variations in gene and protein expression in human nucleus pulposus in comparison with annulus fibrosus and cartilage cells: potential associations with aging and degeneration. *Osteoarthritis Cartilage* **18**, 416–423. (doi:10.1016/j.joca.2009.09.009)
64. Gruber HE, Bornstein P, Sage EH, Ingram JA, Zinchenko N, Norton HJ, Hanley EN. 2008 Disruption of the thrombospondin-2 gene alters the lamellar morphology but does not permit vascularization of the adult mouse lumbar disc. *Arthritis Res. Ther.* **10**, R96. (doi:10.1186/ar2483)
65. Dicesare PE, Morgelin M, Mann K, Paulsson M. 1994 Cartilage oligomeric matrix protein and thrombospondin 1. Purification from articular cartilage, electron microscopic structure, and chondrocyte binding. *Eur. J. Biochem.* **223**, 927–937. (doi:10.1111/j.1432-1033.1994.tb19070.x)
66. Le Maitre CL, Freemont AJ, Hoyland JA. 2004 Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J. Pathol.* **204**, 47–54. (doi:10.1002/path.1608)
67. Vo NV, Hartman RA, Yurube T, Jacobs LJ, Sowa GA, Kang JD. 2013 Expression and regulation of metalloproteinases and their inhibitors in intervertebral disc aging and degeneration. *Spine J.* **13**, 331–341. (doi:10.1016/j.spinee.2012.02.027)
68. Weiler C, Nerlich AG, Zipperer J, Bachmeier BE, Boos N. 2002 2002 SSE Award Competition in Basic Science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption. *Eur. Spine J.* **11**, 308–320. (doi:10.1007/s00586-002-0472-0)
69. Pockert AJ, Richardson SM, Le Maitre CL, Lyon M, Deakin JA, Buttle DJ, Freemont AJ, Hoyland JA. 2009 Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration. *Arthritis Rheum.* **60**, 482–491. (doi:10.1002/art.24291)
70. Nagase H. 2012 The ADAMTS family of metalloproteinases. In *Extracellular matrix: pathobiology and signalling* (ed. N Karamanos). Berlin, Germany: De Gruyter.
71. Richardson SM, Doyle P, Minogue BM, Gnanalingham K, Hoyland JA. 2009 Increased expression of matrix metalloproteinase-10, nerve growth factor and substance P in the painful degenerate intervertebral disc. *Arthritis Res. Ther.* **11**, R126. (doi:10.1186/ar2793)
72. Le Maitre CL, Freemont AJ, Hoyland JA. 2006 Human disc degeneration is associated with increased MMP 7 expression. *Biotech. Histochem.* **81**, 125–131. (doi:10.1080/10520290601005298)
73. Gruber HE, Ingram JA, Hanley Jr EN. 2005 Immunolocalization of MMP-19 in the human intervertebral disc: implications for disc aging and degeneration. *Biotech. Histochem.* **80**, 157–162. (doi:10.1080/10520290500387607)
74. Feng H, Danfelter M, Stromqvist B, Heinegard D. 2006 Extracellular matrix in disc degeneration. *J. Bone Joint Surg. Am.* **88**(Suppl. 2), 25–29. (doi:10.2106/JBJS.E.01341)
75. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM. 2000 Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. *Spine* (*Phila Pa 1976*) **25**, 3005–3013. (doi:10.1097/00007632-200012010-00007)
76. Roughley PJ. 2004 Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. *Spine* (*Phila Pa 1976*) **29**, 2691–2699. (doi:10.1097/01.brs.0000146101.53784.b1)
77. Marom B, Rahat MA, Lahat N, Weiss-Cerem L, Kinarty A, Bitterman H. 2007 Native and fragmented fibronectin oppositely modulate monocyte secretion of MMP-9. *J. Leukoc. Biol.* **81**, 1466–1476. (doi:10.1189/jlb.0506328)
78. Sztrolovics R, White RJ, Poole AR, Mort JS, Roughley PJ. 1999 Resistance of small leucine-rich repeat proteoglycans to proteolytic degradation during interleukin-1-stimulated cartilage catabolism. *Biochem. J.* **339**, 571–577. (doi:10.1042/0264-6021:3390571)
79. Arroyo AG, Iruela-Arispe ML. 2010 Extracellular matrix, inflammation, and the angiogenic response. *Cardiovasc. Res.* **86**, 226–235. (doi:10.1093/cvr/cvq049)
80. Virri J, Sikk S, Gronblad M, Tolonen J, Seitsalo S, Kankare J, Karaharju EO. 1994 Concomitant immunocytochemical study of macrophage cells and blood vessels in disc herniation tissue. *Eur. Spine J.* **3**, 336–341. (doi:10.1007/BF02200147)
81. Jiang D, Liang J, Noble PW. 2007 Hyaluronan in tissue injury and repair. *Annu. Rev. Cell Dev. Biol.* **23**, 435–461. (doi:10.1146/annurev.cellbio.23.090506.123337)
82. Morwood SR, Nicholson LB. 2006 Modulation of the immune response by extracellular matrix proteins. *Arch. Immunol. Ther. Exp.* **54**, 367–374. (doi:10.1007/s00005-006-0043-x)
83. Abdollahi-Roodsaz S, Joosten LA, Roelofs MF, Radstake TRDJ, Matera G, Popa C, van der Meer JWM, Netea MG, van den Berg WB. 2007 Inhibition of toll-like receptor 4 breaks the inflammatory loop in autoimmune destructive arthritis. *Arthritis Rheum.* **56**, 2957–2967. (doi:10.1002/art.22848)
84. Quero L *et al*. 2013 Hyaluronic acid fragments enhance the inflammatory and catabolic response in human intervertebral disc cells through modulation of toll-like receptor 2 signaling pathways. *Arthritis Res. Ther.* **15**, R94. (doi:10.1186/ar4274)
85. Sorokin L. 2010 The impact of the extracellular matrix on inflammation. *Nat. Rev. Immunol.* **10**, 712–723. (doi:10.1038/nri2852)
86. Jones P, Gardner L, Menage J, Williams GT, Roberts S. 2008 Intervertebral disc cells as competent phagocytes *in vitro*: implications for cell death in disc degeneration. *Arthritis Res. Ther.* **10**, R86. (doi:10.1186/ar2466)
87. Nerlich AG, Weiler C, Zipperer J, Narozny M, Boos N. 2002 Immunolocalization of phagocytic cells in normal and degenerated intervertebral discs. *Spine* **27**, 2484–2490. (doi:10.1097/00007632-200211150-00012)
88. Phillips KL, Chiverton N, Michael AL, Cole AA, Breakwell LM, Haddock G, Bunning RAD, Cross AK, Le Maitre CL. 2013 The cytokine and chemokine expression profile of nucleus pulposus cells: implications for degeneration and regeneration of the intervertebral disc. *Arthritis Res. Ther.* **15**, R213. (doi:10.1186/ar4408)
89. Gabr MA *et al*. 2011 Interleukin-17 synergizes with IFNgamma or TNFalpha to promote inflammatory mediator release and intercellular adhesion

molecule-1 (ICAM-1) expression in human intervertebral disc cells. *J. Orthop. Res.* **29**, 1–7. (doi:10.1002/jor.21206)

90. Huang KY, Lin RM, Chen WY, Lee CL, Yan JJ, Chang MS. 2008 IL-20 may contribute to the pathogenesis of human intervertebral disc herniation. *Spine* (*Phila Pa 1976*) **33**, 2034–2040. (doi:10.1097/BRS.0b013e31817eb872)
91. Smith LJ, Chiaro JA, Nerurkar NL, Cortes DH, Horava SD, Hebela NM, Mauck RL, Dodge GR, Elliott DM. 2011 Nucleus pulposus cells synthesize a functional extracellular matrix and respond to inflammatory cytokine challenge following long-term agarose culture. *Eur. Cell Mater.* **22**, 291–301.
92. Hoyland JA, Le Maitre C, Freemont AJ. 2008 Investigation of the role of IL-1 and TNF in matrix degradation in the intervertebral disc. *Rheumatology* (*Oxf.*) **47**, 809–814. (doi:10.1093/rheumatology/ken056)
93. Seguin CA, Pilliar RM, Roughley PJ, Kandel RA. 2005 Tumor necrosis factor-alpha modulates matrix production and catabolism in nucleus pulposus tissue. *Spine* (*Phila Pa 1976*) **30**, 1940–1948. (doi:10.1097/01.brs.0000176188.40263.f9)
94. Seguin CA, Pilliar RM, Madri JA, Kandel RA. 2008 TNF-alpha induces MMP2 gelatinase activity and MT1-MMP expression in an *in vitro* model of nucleus pulposus tissue degeneration. *Spine* (*Phila Pa 1976*) **33**, 356–365. (doi:10.1097/BRS.0b013e3181642a5e)
95. 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)
96. Walter BA, Korecki CL, Purmessur D, Roughley PJ, Michalek AJ, Iatridis JC. 2011 Complex loading affects intervertebral disc mechanics and biology. *Osteoarthritis Cartilage* **19**, 1011–1018. (doi:10.1016/j.joca.2011.04.005)
97. Freemont AJ, Watkins A, Le Maitre C, Baird P, Jeziorska M, Knight MTN, Ross ERS, O'Brien JPJ, Hoyland A. 2002 Nerve growth factor expression and innervation of the painful intervertebral disc. *J. Pathol.* **197**, 286–292. (doi:10.1002/path.1108)
98. Melrose J, Roberts S, Smith S, Menage J, Ghosh P. 2002 Increased nerve and blood vessel ingrowth associated with proteoglycan depletion in an ovine anular lesion model of experimental disc degeneration. *Spine* (*Phila Pa 1976*) **27**, 1278–1285. (doi:10.1097/00007632-200206150-00007)
99. Kawakami M, Weinstein JN, Chatani K, Spratt KF, Meller ST, Gebhart GF. 1994 Experimental lumbar radiculopathy. Behavioral and histologic changes in a model of radicular pain after spinal nerve root irritation with chromic gut ligatures in the rat. *Spine (Phila Pa 1976)* **19**, 1795–1802. (doi:10.1097/00007632-199408150-00002)
100. Olmarker K, Blomquist J, Stromberg J, Nannmark U, Thomsen P, Rydevik B. 1995 Inflammatogenic properties of nucleus pulposus. *Spine (Phila Pa 1976)* **20**, 665–669. (doi:10.1097/00007632-199503150-00006)
101. Lotz JC, Ulrich JA. 2006 Innervation, inflammation, and hypermobility may characterize pathologic disc degeneration: review of animal model data. *J. Bone Joint Surg. Am.* **88**(Suppl. 2), 76–82. (doi:10.2106/JBJS.E.01448)
102. Kobayashi S *et al*. 2009 Ultrastructural analysis on lumbar disc herniation using surgical specimens: role of neovascularization and macrophages in hernias. *Spine* (*Phila Pa 1976*) **34**, 655–662. (doi:10.1097/BRS.0b013e31819c9d5b)
103. Ahn SH, Ahn MW, Byun WM. 2000 Effect of the transligamentous extension of lumbar disc herniations on their regression and the clinical outcome of sciatica. *Spine* (*Phila Pa 1976*) **25**, 475–480. (doi:10.1097/00007632-200002150-00014)
104. Ikeda T, Nakamura T, Kikuchi T, Umeda S, Senda H, Takagi K. 1996 Pathomechanism of spontaneous regression of the herniated lumbar disc: histologic and immunohistochemical study. *J. Spinal Disord.* **9**, 136–140. (doi:10.1097/00002517-199604000-00009)
105. Gezici AR, Ergun R. 2009 Spontaneous regression of a huge subligamentous extruded disc herniation: short report of an illustrative case. *Acta Neurochir.* (*Wien*) **151**, 1299–1300. (doi:10.1007/s00701-009-0370-x)
106. Takada T, Nishida K, Maeno K, Kakutani K, Yurube T, Doita M, Kurosaka M. 2012 Intervertebral disc and macrophage interaction induces mechanical hyperalgesia and cytokine production in a herniated disc model in rats. *Arthritis Rheum.* **64**, 2601–2610. (doi:10.1002/art.34456)
107. Matsui Y, Maeda M, Nakagami W, Iwata H. 1998 The involvement of matrix metalloproteinases and inflammation in lumbar disc herniation. *Spine* (*Phila Pa 1976*) **23**, 863–868; discussion 868–869. (doi:10.1097/00007632-199804150-00005)
108. Murai K, Sakai D, Nakamura Y, Nakai T, Igarashi T, Seo N, Murakami T, Kobayashi E, Mochida J. 2010 Primary immune system responders to nucleus pulposus cells: evidence for immune response in disc herniation. *Eur. Cell Mater.* **19**, 13–21.
109. Wang H *et al*. 2013 Inflammatory cytokines induce NOTCH signaling in nucleus pulposus cells: implications in intervertebral disc degeneration. *J. Biol. Chem.* **288**, 16 761–16 774. (doi:10.1074/jbc.M112.446633)
110. Kepler CK, Markova DZ, Dibra F, Yadla S, Vaccaro AR, Risbud MV, Albert TJ, Anderson DG. 2013 Expression and relationship of proinflammatory chemokine RANTES/CCL5 and cytokine IL-1beta in painful human intervertebral discs. *Spine* (*Phila Pa 1976*) **38**, 873–880. (doi:10.1097/BRS.0b013e318285ae08)
111. Le Maitre C, Hoyland J, Freemont A. 2007 Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1beta and TNFalpha expression profile. *Arthritis Res. Ther.* **9**, R77. (doi:10.1186/ar2275)
112. Le Maitre CL, Freemont AJ, Hoyland JA. 2005 The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. *Arthritis Res. Ther.* **7**, R732–R745. (doi:10.1186/ar1732)
113. Abe Y, Akeda K, An HS, Aoki Y, Pichika R, Muehleman C, Kimura T, Masuda K. 2007 Proinflammatory cytokines stimulate the expression of nerve growth factor by human intervertebral disc cells. *Spine* (*Phila Pa 1976*) **32**, 635–642. (doi:10.1097/01.brs.0000257556.90850.53)
114. Miyahara K, Ishida T, Hukuda S, Horiike K, Okamoto M, Tojo H. 1996 Human group II phospholipase A2 in normal and diseased intervertebral discs. *Biochim. Biophys. Acta* **1316**, 183–190. (doi:10.1016/0925-4439(96)00023-3)
115. Wang J *et al*. 2013 Tumor necrosis factor alpha- and interleukin-1beta-dependent induction of CCL3 expression by nucleus pulposus cells promotes macrophage migration through CCR1. *Arthritis Rheum.* **65**, 832–842. (doi:10.1002/art.37819)
116. Kokubo Y *et al*. 2008 Herniated and spondylotic intervertebral discs of the human cervical spine: histological and immunohistological findings in 500 en bloc surgical samples. Laboratory investigation. *J. Neurosurg. Spine* **9**, 285–295. (doi:10.3171/SPI/2008/9/9/285)
117. Takahashi H, Suguro T, Okazima Y, Motegi M, Okada Y, Kakiuchi T. 1996 Inflammatory cytokines in the herniated disc of the lumbar spine. *Spine* (*Phila Pa 1976*) **21**, 218–224. (doi:10.1097/00007632-199601150-00011)
118. Kang JD, Georgescu HI, Mcintyre-Larkin L, Stefanovic-Racic M, Donaldson III WF, Evans CH. 1996 Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. *Spine* (*Phila Pa 1976*) **21**, 271–277. (doi:10.1097/00007632-199602010-00003)
119. Ahn SH, Cho YW, Ahn MW, Jang SH, Sohn YK, Kim HS. 2002 mRNA expression of cytokines and chemokines in herniated lumbar intervertebral discs. *Spine* (*Phila Pa 1976*) **27**, 911–917. (doi:10.1097/00007632-200205010-00005)
120. Henriksson H, Thornemo M, Karlsson C, Hägg O, Junevik K, Lindahl A, Brisby H. 2009 Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region: a study in four species. *Spine* **34**, 2278–2287. (doi:10.1097/BRS.0b013e3181a95ad2)
121. Miyamoto H, Saura R, Harada T, Doita M, Mizuno K. 2000 The role of cyclooxygenase-2 and inflammatory cytokines in pain induction of herniated lumbar intervertebral disc. *Kobe J. Med. Sci.* **46**, 13–28.
122. Shamji MF *et al*. 2010 Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. *Arthritis Rheum.* **62**, 1974–1982. (doi:10.1002/art.27444)
123. Zhang W, Nie L, Wang Y, Wang X, Zhao H, Dongol S, Maharjan S, Cheng L. 2013 CCL20 secretion from

the nucleus pulposus improves the recruitment of CCR6-expressing Th17 cells to degenerated IVD tissues. *PLoS ONE* **8**, e66286. (doi:10.1371/journal.pone.0066286)

124. Kim JH, Studer RK, Sowa GA, Vo NV, Kang JD. 2008 Activated macrophage-like THP-1 cells modulate anulus fibrosus cell production of inflammatory mediators in response to cytokines. *Spine* (*Phila Pa 1976*) **33**, 2253–2259. (doi:10.1097/BRS.0b013e318182c35f)
125. Park JY, Kuh SU, Park HS, Kim KS. 2011 Comparative expression of matrix-associated genes and inflammatory cytokines-associated genes according to disc degeneration: analysis of living human nucleus pulposus. *J. Spinal Disord. Tech.* **24**, 352–357. (doi:10.1097/BSD.0b013e3181fee4df)
126. Kepler CK, Markova DZ, Hilibrand AS, Vaccaro AR, Risbud MV, Albert TJ, Anderson DG. 2013 Substance P stimulates production of inflammatory cytokines in human disc cells. *Spine* (*Phila Pa 1976*) **38**, E1291–E1299. (doi:10.1097/BRS.0b013e3182a42bc2)
127. Burke JG, Rw GW, Conhyea D, McCormack D, Dowling FE, Walsh MG, Fitzpatrick JM. 2003 Human nucleus pulposis can respond to a pro-inflammatory stimulus. *Spine* (*Phila Pa 1976*) **28**, 2685–2693. (doi:10.1097/01.BRS.0000103341.45133.F3)
128. Kim JH, Studer RK, Vo NV, Sowa GA, Kang JD. 2009 p38 MAPK inhibition selectively mitigates inflammatory mediators and VEGF production in AF cells co-cultured with activated macrophage-like THP-1 cells. *Osteoarthritis Cartilage* **17**, 1662–1669. (doi:10.1016/j.joca.2009.06.004)
129. Sinclair SM, Shamji MF, Chen J, Jing L, Richardson WJ, Brown CR, Fitch RD, Setton LA. 2011 Attenuation of inflammatory events in human intervertebral disc cells with a tumor necrosis factor antagonist. *Spine* (*Phila Pa 1976*) **36**, 1190–1196. (doi:10.1097/BRS.0b013e3181ebdb43)
130. Studer RK, Vo N, Sowa G, Ondeck C, Kang J. 2011 Human nucleus pulposus cells react to IL-6: independent actions and amplification of response to IL-1 and TNF-alpha. *Spine* (*Phila Pa 1976*) **36**, 593–599. (doi:10.1097/BRS.0b013e3181da38d5)
131. 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-kappaB. *Am. J. Pathol.* **182**, 2310–2321. (doi:10.1016/j.ajpath.2013.02.037)
132. Yu ZG, Xu N, Wang WB, Pan SH, Li KS, Liu JK. 2009 Interleukin-1 inhibits Sox9 and collagen type II expression via nuclear factor-kappaB in the cultured human intervertebral disc cells. *Chin. Med. J.* (*Engl.*) **122**, 2483–2488.
133. Doita M, Kanatani T, Harada T, Mizuno K. 1996 Immunohistologic study of the ruptured intervertebral disc of the lumbar spine. *Spine* (*Phila Pa 1976*) **21**, 235–241. (doi:10.1097/00007632-199601150-00015)
134. Bachmeier BE, Nerlich AG, Weiler C, Paesold G, Jochum M, Boos N. 2007 Analysis of tissue distribution of TNF-α, TNF-α-receptors, and the activating TNF-α-converting enzyme suggests activation of the TNF-α system in the aging intervertebral disc. *Ann. NY Acad. Sci.* **1096**, 44–54. (doi:10.1196/annals.1397.069)
135. Risbud MV, Shapiro IM. 2014 Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat. Rev. Rheumatol.* **10**, 44–56. (doi:10.1038/nrrheum.2013.160)
136. Goupille P, Mulleman D, Paintaud G, Watier H, Valat JP. 2007 Can sciatica induced by disc herniation be treated with tumor necrosis factor alpha blockade? *Arthritis Rheum.* **56**, 3887–3895. (doi:10.1002/art.23051)
137. Purmessur D, Walter BA, Roughley PJ, Laudier DM, Hecht AC, Iatridis J. 2013 A role for TNFalpha in intervertebral disc degeneration: a non-recoverable catabolic shift. *Biochem. Biophys. Res. Commun.* **433**, 151–156. (doi:10.1016/j.bbrc.2013.02.034)
138. Le Maitre CL, Pockert A, Buttle DJ, Freemont AJ, Hoyland JA. 2007 Matrix synthesis and degradation in human intervertebral disc degeneration. *Biochem. Soc. Trans.* **35**, 652–655. (doi:10.1042/BST0350652)
139. Igarashi T, Kikuchi S, Shubayev V, Myers RR. 2000 Exogenous tumor necrosis factor-alpha mimics nucleus pulposus-induced neuropathology: molecular, histologic, and behavioral comparisons in rats. *Spine* **25**, 2975–2980. (doi:10.1097/00007632-200012010-00003)
140. Kauppila LI. 1995 Ingrowth of blood vessels in disc degeneration. Angiographic and histological studies of cadaveric spines. *J. Bone Joint Surg. Am.* **77**, 26–31.
141. Hess A *et al*. 2011 Blockade of TNF-alpha rapidly inhibits pain responses in the central nervous system. *Proc. Natl Acad. Sci. USA* **108**, 3731–3736. (doi:10.1073/pnas.1011774108)
142. Cuellar JM, Borges PM, Cuellar VG, Yoo A, Scuderi GJ, Yeomans DC. 2012 Cytokine expression in the epidural space: a model of non-compressive disc herniation-induced inflammation. *Spine* (*Phila Pa 1976*) **38**, 17–23. (doi:10.1097/BRS.0b013e3182604baa)
143. Rousseau MA, Ulrich JA, Bass EC, Rodriguez AG, Liu JJ, Lotz JC. 2007 Stab incision for inducing intervertebral disc degeneration in the rat. *Spine* (*Phila Pa 1976*) **32**, 17–24. (doi:10.1097/01.brs.0000251013.07656.45)
144. Anderson DG, Izzo MW, Hall DJ, Vaccaro AR, Hilibrand A, Arnold W, Tuan RS, Albert TJ. 2002 Comparative gene expression profiling of normal and degenerative discs: analysis of a rabbit annular laceration model. *Spine* (*Phila Pa 1976*) **27**, 1291–1296. (doi:10.1097/00007632-200206150-00009)
145. Sobajima S, Kompel JF, Kim JS, Wallach CJ, Robertson DD, Vogt MT, Kang JD, Gilbertson LG. 2005 A slowly progressive and reproducible animal model of intervertebral disc degeneration characterized by MRI, X-ray, and histology. *Spine* (*Phila Pa 1976*) **30**, 15–24. (doi:10.1097/00007632-200511150-00023)
146. Baggiolini M, Dewald B, Moser B. 1997 Human chemokines: an update. *Annu. Rev. Immunol.* **15**, 675–705. (doi:10.1146/annurev.immunol.15.1.675)
147. Yoshida M, Nakamura T, Sei A, Kikuchi T, Takagi K, Matsukawa A. 2005 Intervertebral disc cells produce tumor necrosis factor alpha, interleukin-1beta, and monocyte chemoattractant protein-1 immediately after herniation: an experimental study using a new hernia model. *Spine* (*Phila Pa 1976*) **30**, 55–61. (doi:10.1097/01.brs.0000149194.17891.bf)
148. O'Neill CW, Liu JJ, Leibenberg E, Hu SS, Deviren V, Tay BK-B, Chin CT, Lotz JC. 2004 Percutaneous plasma decompression alters cytokine expression in injured porcine intervertebral discs. *Spine J.* **4**, 88–98. (doi:10.1016/S1529-9430(03)00423-6)
149. Rajan N, Bloom O, Maidhof R, Stetson N, Sherry B, Levine M, Chahine NO. 2012 Toll-like receptor 4 (TLR4) expression and stimulation in a model of intervertebral disc inflammation and degeneration. *Spine* (*Phila Pa 1976*) **38**, 1343–1351. (doi:10.1097/BRS.0b013e31826b71f4)
150. Morimoto R, Akeda K, Iida R, Nishimura A, Tsujii M, Obata S, Kasai Y, Uchida A, Sudo A. 2012 Tissue renin-angiotensin system in the intervertebral disc. *Spine* (*Phila Pa 1976*) **38**, E129–E136. (doi:10.1097/BRS.0b013e31827b8c89)
151. Gruber HE, Hanley Jr EN. 2002 Ultrastructure of the human intervertebral disc during aging and degeneration: comparison of surgical and control specimens. *Spine* (*Phila Pa 1976*) **27**, 798–805. (doi:10.1097/00007632-200204150-00004)
152. Bach FC, Willems N, Penning LC, Ito K, Meij BP, Tryfonidou MA. 2014 Potential regenerative treatment strategies for intervertebral disc degeneration in dogs. *BMC Vet. Res.* **10**, 3. (doi:10.1186/1746-6148-10-3)
153. Bergknut N *et al*. 2013 Intervertebral disc disease in dogs—part 1: a new histological grading scheme for classification of intervertebral disc degeneration in dogs. *Vet. J.* **195**, 156–163. (doi:10.1016/j.tvjl.2012.05.027)
154. Alini M *et al*. 2008 Are animal models useful for studying human disc disorders/degeneration? *Eur. Spine J.* **17**, 2–19. (doi:10.1007/s00586-007-0414-y)
155. Roberts S, Butler RC. 2005 Inflammatory mediators as potential therapeutic targets in the spine. *Curr. Drug Targets* **4**, 257–266. (doi:10.2174/1568010053586372).
156. Wuertz K, Vo N, Kletsas D, Boos N. 2012 Inflammatory and catabolic signalling in intervertebral discs: the roles of NF-kappaB and MAP kinases. *Eur. Cell Mater.* **23**, 103–119; discussion 119–120.
157. Genevay S, Viatte S, Finckh A, Zufferey P, Balague F, Gabay C. 2010 Adalimumab in severe and acute sciatica: a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum.* **62**, 2339–2346. (doi:10.1002/art.27499)
158. Genevay S, Stingelin S, Gabay C. 2004 Efficacy of etanercept in the treatment of acute, severe sciatica:

a pilot study. *Ann. Rheum. Dis.* **63**, 1120–1123. (doi:10.1136/ard.2003.016451)

159. Olmarker K, Rydevik B. 2001 Selective inhibition of tumor necrosis factor-alpha prevents nucleus pulposus-induced thrombus formation, intraneural edema, and reduction of nerve conduction velocity: possible implications for future pharmacologic treatment strategies of sciatica. *Spine* (*Phila Pa 1976*) **26**, 863–869. (doi:10.1097/00007632-200104150-00007)
160. Okoro T, Tafazal SI, Longworth S, Sell PJ. 2010 Tumor necrosis alpha-blocking agent (etanercept): a triple blind randomized controlled trial of its use in treatment of sciatica. *J. Spinal Disord. Tech.* **23**, 74–77. (doi:10.1097/BSD.0b013e31819afdc4)
161. Muller-Ladner U, Roberts CR, Franklin BN, Gay RE, Robbins PD, Evans CH, Gay S. 1997 Human IL-1Ra gene transfer into human synovial fibroblasts is chondroprotective. *J. Immunol.* **158**, 3492–3498.
162. Autio RA, Karppinen JNiinimäki J, Ojala R, Veeger N, Korhonen T, Hurri H, Tervonen O. 2006 The effect of infliximab, a monoclonal antibody against TNF-α, on disc herniation resorption: a randomized controlled study. *Spine* **31**, 2641–2645. (doi:10.1097/01.brs.0000244616.64962.9e)
163. Tobinick E. 2006 Spinal delivery of p38: TNF-alpha inhibitors. *PLoS Med.* **3**, e511. (doi:10.1371/journal.pmed.0030511)
164. Ragab AA, Woodall Jr JW, Tucci MA, Wingerter SA, Fosnaugh AW, Franklin LN, Benghuzzi HA. 2009 A preliminary report on the effects of sustained administration of corticosteroid on traumatized disc using the adult male rat model. *J. Spinal Disord. Tech.* **22**, 473–478. (doi:10.1097/BSD.0b013e31818d5e55)
165. Zhang H, Wang L, Park JB, Park P, Yang VC, Hollister SJ, La Marca F, Lin C-Y. 2009 Intradiscal injection of simvastatin retards progression of intervertebral disc degeneration induced by stab injury. *Arthritis Res. Ther.* **11**, R172. (doi:10.1186/ar2861)
166. Gorth DJ, Mauck RL, Chiaro JA, Mohanraj B, Hebela NM, Dodge GR, Elliott DM, Smith LJ. 2012 IL-1ra delivered from poly(lactic-co-glycolic acid) microspheres attenuates IL-1β-mediated degradation of nucleus pulposus *in vitro*. *Arthritis Res. Ther.* **14**, R179. (doi:10.1186/ar3932)
167. Kawakami M, Matsumoto T, Hashizume H, Kuribayashi K, Tamaki T. 2002 Epidural injection of cyclooxygenase-2 inhibitor attenuates pain-related behavior following application of nucleus pulposus to the nerve root in the rat. *J. Orthop. Res.* **20**, 376–381. (doi:10.1016/S0736-0266(01)00114-0)
168. Nagae M *et al*. 2007 Intervertebral disc regeneration using platelet-rich plasma and biodegradable gelatin hydrogel microspheres. *Tissue Eng.* **13**, 147–158. (doi:10.1089/ten.2006.0042)
169. Wu C-C, Chen W-H, Zao B, Lai P-L, Lin T-C, Lo H-Y, Shieh Y-H, Wu C-H, Deng W-P. 2011 Regenerative potentials of platelet-rich plasma enhanced by collagen in retrieving pro-inflammatory cytokine-inhibited chondrogenesis. *Biomaterials* **32**, 5847–5854. (doi:10.1016/j.biomaterials.2011.05.002)
170. Wuertz K, Quero L, Sekiguchi M, Klawitter M, Nerlich A, Konno S, Kikuchi S, Boos N. 2011 The red wine polyphenol resveratrol shows promising potential for the treatment of nucleus pulposus-mediated pain *in vitro* and *in vivo*. *Spine* **36**, 1373–1384. (doi:10.1097/BRS.0b013e318221e655)
171. Li H, Liang C, Chen Q, Yang Z. 2011 Rhein: a potential biological therapeutic drug for intervertebral disc degeneration. *Med. Hypotheses* **77**, 1105–1107. (doi:10.1016/j.mehy.2011.09.013)
172. Liu Q, Jin L, Shen FH, Balian G, Li XJ. 2013 Fullerol nanoparticles suppress inflammatory response and adipogenesis of vertebral bone marrow stromal cells—a potential novel treatment for intervertebral disc degeneration. *Spine J.* **13**, 1571–1580. (doi:10.1016/j.spinee.2013.04.004)
173. Kobori S *et al*. 2014 Inhibiting IkappaB kinase-beta downregulates inflammatory cytokines in injured discs and neuropeptides in dorsal root ganglia innervating injured discs in rats. *Spine* (*Phila Pa 1976*) **39**, 1171–1177. (doi:10.1097/BRS.0000000000000374)
174. Vadalà G, Sowa GA, Kang JD. 2007 Gene therapy for disc degeneration. *Expert Opin. Biol. Ther.* **7**, 185–196. (doi:10.1517/14712598.7.2.185)
175. Carragee EJ, Don AS, Hurwitz EL, Cuellar JM, Carrino JA, Herzog R. 2009 2009 ISSLS prize winner: does discography cause accelerated progression of degeneration changes in the lumbar disc: a ten-year matched cohort study. *Spine* (*Phila Pa 1976*) **34**, 2338–2345. (doi:10.1097/BRS.0b013e3181ab5432)
176. Vadala G, Russo F, Pattappa G, Schiuma D, Peroglio M, Benneker LM, Grad S, Alini M, Denaro V. 2013 The transpedicular approach as an alternative route for intervertebral disc regeneration. *Spine* (*Phila Pa 1976*) **38**, E319–E324. (doi:10.1097/BRS.0b013e318285bc4a)
177. Gibson MJ, Karpinski MR, Slack RC, Cowlishaw WA, Webb JK. 1987 The penetration of antibiotics into the normal intervertebral disc. *J. Bone Joint Surg. Br.* **69**, 784–786.
178. Motaghinasab S, Shirazi-Adl A, Urban JP, Parnianpour M. 2012 Computational pharmacokinetics of solute penetration into human intervertebral discs—effects of endplate permeability, solute molecular weight and disc size. *J. Biomech.* **45**, 2195–2202. (doi:10.1016/j.jbiomech.2012.06.033)
179. Ferguson SJ, Ito K, Nolte LP. 2004 Fluid flow and convective transport of solutes within the intervertebral disc. *J. Biomech.* **37**, 213–221. (doi:10.1016/S0021-9290(03)00250-1)
180. Wehling P, Schulitz KP, Robbins PD, Evans CH, Reinecke JA. 1997 Transfer of genes to chondrocytic cells of the lumbar spine. Proposal for a treatment strategy of spinal disorders by local gene therapy. *Spine* **15**, 1092–1097. (doi:10.1097/00007632-199705150-00008)
181. Le Maitre CL, Hoyland JA, Freemont AJ. 2007 Interleukin-1 receptor antagonist delivered directly and by gene therapy inhibits matrix degradation in the intact degenerate human intervertebral disc: an *in situ* zymographic and gene therapy study. *Arthritis Res. Ther.* **9**, R83. (doi:10.1186/ar2282)
182. Nishida K, Kang JD, Gilbertson LG, Moon S-H, Suh J-K, Vogt MT, Robbins PD, Evans CH. 1999 Modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an *in vivo* study of adenovirus-mediated transfer of the human transforming growth factor beta 1 encoding gene. *Spine* (*Phila Pa 1976*) **24**, 2419–2425. (doi:10.1097/00007632-199912010-00002)
183. Lee YJ, Kong MH, Song KY, Lee KH, Heo SH. 2008 The relation between Sox9, TGF-β1, and proteoglycan in human intervertebral disc cells. *J. Kor. Neurosurg. Soc.* **43**, 149–154. (doi:10.3340/jkns.2008.43.3.149)
184. Tan Y, Hu Y, Tan J. 2003 Extracellular matrix synthesis and ultrastructural changes of degenerative disc cells transfected by Ad/CMV-hTGF-beta 1. *Chin. Med. J.* (*Engl.*) **116**, 1399–1403.
185. Leckie S *et al*. 2012 Injection of AAV2-BMP2 and AAV2-TIMP1 into the nucleus pulposus slows the course of intervertebral disc degeneration in an *in vivo* rabbit model. *Spine* **12**, 7–20. (doi:10.1016/j.spinee.2011.09.011)
186. Haufe SMW, Mork AR. 2006 Intradiscal injection of hematopoietic stem cells in an attempt to rejuvenate the intervertebral discs. *Stem Cells Dev.* **15**, 136–137. (doi:10.1089/scd.2006.15.136)
187. Quintin A, Schizas C, Scaletta C, Jaccoud S, Gerber S, Osterheld MC, Juillerat L, Applegate LA, Pioletti DP. 2008 Isolation and *in vitro* chondrogenic potential of human foetal spine cells. *J. Cell Mol. Med.* **13**, 2559–2569. (doi:10.1111/j.1582-4934.2008.00630.x)
188. Iwashina T, Mochida J, Sakai D, Yamamoto Y, Miyazaki T, Ando K, Hotta T. 2006 Feasibility of using a human nucleus pulposus cell line as a cell source in cell transplantation therapy for intervertebral disc degeneration. *Spine* **31**, 1177–1186. (doi:10.1097/01.brs.0000217687.36874.c4)
189. Hohaus C, Ganey TM, Minkus Y, Meisel HJ. 2008 Cell transplantation in lumbar spine disc degeneration disease. *Eur. Spine J.* **17**(Suppl. 4), S492–S503. (doi:10.1007/s00586-008-0750-6)
190. Sheikh H, Zakharian K, De La Torre RP, Facek C, Vasquez A, Chaudhry GR, Svinarich D, Peter-Cruet MJ. 2009 *In vivo* intervertebral disc regeneration using stem cell-derived chondroprogenitors: laboratory investigation. *J. Neurosurg.* **10**, 265–272. (doi:10.3171/2008.12.SPINE0835)
191. Chen J, Lee EJ, Jing L, Christoforou N, Leong KW, Setton LA. 2013 Differentiation of mouse induced pluripotent stem cells (iPSCs) into nucleus pulposus-like cells *in vitro*. *PLoS ONE* **8**, e75548. (doi:10.1371/journal.pone.0075548)
192. Murrell W, Sanford E, Anderberg L, Cavanagh B, Mackay-Sim A. 2009 Olfactory stem cells can be induced to express chondrogenic phenotype in a rat intervertebral disc injury model. *Spine J.* **9**, 585–594. (doi:10.1016/j.spinee.2009.02.011)
193. Sakai D, Mochida J, Iwashina T, Watanabe T, Nakai T, Ando K, Hotta T. 2005 Differentiation of

mesenchymal stem cells transplanted to a rabbit degenerative disc model: potential and limitations for stem cell therapy in disc regeneration. *Spine* **30**, 2379–2387. (doi:10.1097/01.brs.0000184365.28481.e3)

194. Chon BH, Lee EJ, Jing L, Setton LA, Chen J. 2013 Human umbilical cord mesenchymal stromal cells exhibit immature nucleus pulposus cell phenotype in a laminin-rich pseudo-three-dimensional culture system. *Stem Cell Res. Ther.* **4**, 120. (doi:10.1186/scrt331)
195. Erwin WM, Islam D, Eftekarpour E, Inman RD, Karim MZ, Fehlings MG. 2013 Intervertebral disc-derived stem cells: implications for regenerative medicine and neural repair. *Spine* **38**, 211–216. (doi:10.1097/BRS.0b013e318266a80d)
196. Kim JH, Moon HJ, Lee JH, Kim JH, Kwon TH, Park YK. 2012 Rabbit notochordal cells modulate the expression of inflammatory mediators by human annulus fibrosus cells cocultured with activated macrophage-like THP-1 cells. *Spine* (*Phila Pa 1976*) **37**, 1856–1864. (doi:10.1097/BRS.0b013e3182579434)
197. Serigano K, Sakai D, Hiyama A, Tamura F, Tanaka M, Mochida J. 2010 Effect of cell number on mesenchymal stem cell transplantation in a canine disc degeneration model. *J. Orthop. Res.* **28**, 1267–1275. (doi:10.1002/jor.21147)
198. Miyamoto T, Muneta T, Tabuchi T, Matsumoto K, Saito H, Tsuji K, Sekiya I. 2010 Intradiscal transplantation of synovial mesenchymal stem cells prevents intervertebral disc degeneration through suppression of matrix metalloproteinase-related genes in nucleus pulposus cells in rabbits. *Arthritis Res. Ther.* **12**, R206. (doi:10.1186/ar3182)
199. Yoo K *et al*. 2009 Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. *Cell Immunol.* **259**, 150–156. (doi:10.1016/j.cellimm.2009.06.010)
200. Prockop DJ, Oh JY. 2012 Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. *Mol. Ther.* **20**, 14–20. (doi:10.1038/mt.2011.211)
201. Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG. 2007 Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc. Natl Acad. Sci. USA* **104**, 11 002–11 007. (doi:10.1073/pnas.0704421104)
202. Lee RH *et al*. 2009 Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell* **5**, 54–63. (doi:10.1016/j.stem.2009.05.003)
203. Oh JY, Roddy GW, Choi H, Lee RH, Ylostalo JH, Rosa RH, Prockop DJ. 2010 Anti-inflammatory protein TSG-6 reduces inflammatory damage to the cornea following chemical and mechanical injury. *Proc. Natl Acad. Sci. USA* **107**, 16 875–16 880. (doi:10.1073/pnas.1012451107)
204. Hiyama A, Mochida J, Iwashina T, Omi H, Watanabe T, Serigano K, Tamura F, Sakai D. 2008 Transplantation of mesenchymal stem cells in a canine disc degeneration model. *J. Orthop. Res.* **26**, 589–600. (doi:10.1002/jor.20584)
205. Bertolo A, Thiede T, Aebli N, Baur M, Ferguson S, Stoyanov J. 2011 Human mesenchymal stem cell co-culture modulates the immunological properties of human intervertebral disc tissue fragments *in vitro*. *Eur. Spine J.* **20**, 592–603. (doi:10.1007/s00586-010-1662-9)
206. Orozco L, Soler R, Morera C, Alberca M, Sanchez A, García-Sancho J. 2011 Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study. *Transplantation* **92**, 822–828. (doi:10.1097/TP.0b013e3182298a15)
207. Yoshikawa T, Ueda Y, Miyazaki K, Koizumi M, Takakura Y. 2010 Disc regeneration therapy using marrow mesenchymal cell transplantation: a report of two case studies. *Spine* **35**, E475–E480. (doi:10.1097/BRS.0b013e3181cd2cf4)
208. Pang X, Yang H, Peng B. 2014 Human umbilical cord mesenchymal stem cell transplantation for the treatment of chronic discogenic low back pain. *Pain Phys.* **17**, E525–E530.
209. Pettine KA, Murphy MB, Suzuki RK, Sand TT. 2014 Percutaneous injection of autologous bone marrow concentrate cells significantly reduces lumbar discogenic pain through 12 months. *Stem Cells* **33**, 146–156. (doi:10.1002/stem.1845)
210. Meirelles L, Fontes AM, Covas DT, Caplan AI. 2009 Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytok. Growth Factor Rev.* **20**, 419–427. (doi:10.1016/j.cytogfr.2009.10.002)
211. Karp JM, Leng Teo GS. 2009 Mesenchymal stem cell homing: the devil is in the details. *Cell Stem Cell* **4**, 206–216. (doi:10.1016/j.stem.2009.02.001)
212. Ponte A, Marais E, Gallay N, Langonne A, Delorme B, Herault E, Charboard P, Domenech J. 2009 The *in vitro* migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. *Stem Cells* **25**, 1737–1745. (doi:10.1634/stemcells.2007-0054)
213. Tondreau T, Meuleman N, Stamatopoulos B, De Bruyn C, Delforge A, Dejeneffe M, Martiat P, Bron D, Lagneaux L. 2009 *In vitro* study of matrix metalloproteinase/tissue inhibitor of metalloproteinase production by mesenchymal stromal cells in response to inflammatory cytokines: the role of their migration in injured tissues. *Cytotherapy* **11**, 559–569. (doi:10.1080/14653240903051541)
214. Illien-Jünger SPG, Peroglio M, Benneker LM, Stoddart MJ, Sakai D, Mochida J, Grad S, Alini M. 2012 Homing of mesenchymal stem cells in induced degenerative intervertebral discs in a whole organ culture system. *Spine* **37**, 1865–1873. (doi:10.1097/BRS.0b013e3182544a8a)
215. Pattappa G, Peroglio M, Sakai D, Mochida J, Benneker LM, 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; discussion 136.
216. Pereira CL *et al*. 2014 The effect of hyaluronan-based delivery of stromal cell-derived factor-1 on the recruitment of MSCs in degenerating intervertebral discs. *Biomaterials* **35**, 8144–8153. (doi:10.1016/j.biomaterials.2014.06.017)
217. Henriksson H, Svala E, Skioldebrand E, Lindahl AMD, Brisby H. 2012 Support of concept that migrating progenitor cells from stem cell niches contribute to normal regeneration of the adult mammal intervertebral disc. *Spine* **37**, 722–732. (doi:10.1097/BRS.0b013e318231c2f7)
218. Claes L, Recknagel S, Ignatius A. 2012 Fracture healing under healthy and inflammatory conditions. *Nat. Rev. Rheumatol.* **8**, 133–143. (doi:10.1038/nrrheum.2012.1)
219. Mountziaris PM, Spicer PP, Kasper FK, Mikos AG. 2011 Harnessing and modulating inflammation in strategies for bone regeneration. *Tissue Eng. B* **17**, 393–402. (doi:10.1089/ten.teb.2011.0182)
220. Boccafoschi F, Mosca C, Cannas M. 2012 Cardiovascular biomaterials: when the inflammatory response helps to efficiently restore tissue functionality? *J. Tissue Eng. Regen. Med.* **8**, 253–267. (doi:10.1002/term.1526)
221. Torres AL, Santos SG, Oliveira MI, Barbosa MA. 2013 Fibrinogen promotes resorption of chitosan by human osteoclasts. *Acta Biomater.* **9**, 6553–6562. (doi:10.1016/j.actbio.2013.01.015)
222. Almeida CR, Vasconcelos DP, Goncalves RM, Barbosa MA. 2012 Enhanced mesenchymal stromal cell recruitment via natural killer cells by incorporation of inflammatory signals in biomaterials. *J. R. Soc. Interface* **9**, 261–271. (doi:10.1098/rsif.2011.0357)
223. Oliveira MI, Santos SG, Oliveira MJ, Torres AL, Barbosa MA. 2012 Chitosan drives anti-inflammatory macrophage polarisation and pro-inflammatory dendritic cell stimulation. *Eur. Cell Mater.* **24**, 136–152; discussion 152–153.
224. Maciel J, Oliveira MI, Colton E, McNally AK, Oliveira C, Anderson JM, Barbosa MA. 2014 Adsorbed fibrinogen enhances production of bone- and angiogenic-related factors by monocytes/macrophages. *Tissue Eng. A* **20**, 250–263. (doi:10.1089/ten.tea.2012.0439)
225. Lee J, Sayed N, Hunter A, Au KF,, Wong WH, Mocarski ES, Pera RR, Yakubov E, Cooke JP. 2012 Activation of innate immunity is required for efficient nuclear reprogramming. *Cell* **151**, 547–558. (doi:10.1016/j.cell.2012.09.034)
226. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda M, Yamanaka S. 2007 Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872. (doi:10.1016/j.cell.2007.11.019)
227. O'Neill LAJ. 2012 Transflammation: when innate immunity meets induced pluripotency. *Cell* **151**, 471–473. (doi:10.1016/j.cell.2012.10.006)
228. Raghow R. 1994 The role of extracellular matrix in postinflammatory wound healing and fibrosis. *FASEB J.* **8**, 823–831.

Glossary

ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
AF	Annulus fibrosus
BMP	Bone morphogenic protein
C/EBPβ	CCAAT/enhancer-binding protein beta
CCL	Chemokine ligand
CEP	Cartilaginous endplates
COX	Cyclooxygenase
CPPD	Calcium pyrophosphate dihydrate
CXCL9	Monokine induced by gamma interferon
DDD	Degenerative disc disease
DRG	Dorsal root ganglion
ECM	Extracellular matrix
FasL	Fas ligand
FGF	Fibroblast growth factor
fHA	Hyaluronic acid fragments
GAG	Glycosaminoglycan
GAP	Growth-associated protein
GDF-1	Growth and differentiation factor-1
GM-CSF	Granulocyte macrophage colony-stimulating factor
HA	Hyaluronic acid
HMGB1	High-mobility group protein box 1
HSC	Haematopoietic stem cells
ICAM-1	Intracellular adhesion molecule-1
ICE	IL-1β-converting enzyme
IFN-γ	Interferon-γ
IKKβ	3-Phosphoinositide-dependent protein kinase-1-mediated IκB kinase β
IL	Interleukin
IL-1Ra	Interleukin-1 receptor antagonist
iNOS	Inducible nitric oxide synthase
iPS	Induced pluripotent stem cells
IVD	Intervertebral disc
LBP	Low back pain
LFA	Lymphocyte function-associated antigen
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinases
MCP	Monocyte chemoattractant protein
MDC	Macrophage-derived chemokine
MIF	Macrophage migration inhibitory factor
MIP	Macrophage inflammatory protein
MMP	Metalloproteinase
mRNA	Messenger ribonucleic acid
MSC	Mesenchymal stromal cells
NALP3	NACHT, LRR and PYD domains-containing protein 3
NC	Notochordal cells
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor
NLRP3	NOD-like receptor family, pyrin domain containing 3
NO	Nitric oxide
NP	Nucleus pulposus
PGE_2	Prostaglandin E2
PGF2α	Prostaglandin F2 alpha
PLA2	Calcium-dependent phospholipase A2
PLGA	Poly(lactic-co-glycolic acid)
PRP	Platelet-rich plasma
RANTES	Regulated on activation, normal T-cell expressed and secreted
Rhein	4,5-Dihydroxyanthraquinone-2-carboxylic acid
SC	Stem cells
Sox-9	Transcription factor Sox-9
TGF	Transforming growth factor
TGS-6	TNF-α stimulated gene/protein 6
TIMP	Tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor alpha
TrkA	High affinity NGF receptor
tRAS	Tissue renin-angiotensin system