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Potential roles of cytokines and chemokines in human intervertebral disc degeneration: interleukin-1 is a master regulator of catabolic processes

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SUMMARY

Objective: These studies investigated cytokine and chemokine receptor profiles in nucleus pulposus (NP) cells, and the effects of receptor stimulation on mRNA levels of extracellular matrix (ECM) components, degrading enzymes and cytokine and chemokine expression.
Method: Immunohistochemistry (IHC) was performed to localise expression of CD4, CCR1, CXCR1 and CXCR2 in human NP tissue samples. Effects of cytokine and chemokine stimulation was performed to investigate effects related to ECM remodelling and modulation of cytokine and chemokine mRNA expression.
Results: IHC identified CD4, CCR1, CXCR1 and CXCR2 expression by NP cells. Differential expression profiles were observed for CD4 and CXCR2 in tissue samples from degenerate and infiltrated IVDs. *In vitro* stimulations of primary human NP cultures with IL-16, CCL2, CCL3, CCL7 or CXCL8 did not identify any modulatory effects on parameters associated with ECM remodelling or expression of other cytokines and chemokines. Conversely, IL-1 was seen to modulate ECM remodelling and expression of all other cytokines and chemokines investigated.
Conclusion: This study demonstrates for the first time that NP cells express a number of cytokine and chemokine receptors and thus could respond in an autocrine or paracrine manner to cytokines and chemokines produced by NP cells, particularly during tissue degeneration. However, this study failed to demonstrate regulatory effects on ECM genes and degradative enzymes or other cytokines and chemokines for any target investigated, with the exception of IL-1. This suggests that IL-1 is a master regulator within the IVD and may exert regulatory potential over a plethora of other cytokines and chemokines.
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List of Abbreviations: IVD, intervertebral disc; ECM, extracellular matrix; MMP, matrix metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motif; IL-, interleukin-; TNF-, tumour necrosis factor; NP, nucleus pulposus; CCL, C–C chemokine ligand; CXCL, C-X-C chemokine ligand; CD, cluster of differentiation; CCR, C–C chemokine receptor; CXCR, C-X-C chemokine receptor; PM, post-mortem; AF, annulus fibrosus; CEP, cartilaginous end plate; IHC, immunohistochemistry; TBS, Tris-buffered saline; Ig, immunoglobulin; DMEM, Dulbecco's modified eagle medium; RNA, ribonucleic acid; cDNA, complementary deoxyribonucleic acid; PCR, polymerase chain reaction; GAPDH, glyceraldehyde 3-phospate dehydrogenase; 18S, 18S ribosomal RNA; IFN, interferon; CSF, colony stimulating factor; SEM, standard error of the mean; VEGF, vascular endothelial growth factor; NF, nuclear factor; PDAR, pre-designed assay reagent.

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Introduction

In the mature intervertebral disc (IVD), tissue homeostasis is maintained by an isolated native cell population[1–3]. A continuous process of tissue remodelling occurs whereby collagen and proteoglycan synthesis is balanced by enzymatic extracellular matrix (ECM) degradation[4,5]. The native IVD cell population produces ECM degrading enzymes that facilitate degradation, and two families have been identified; the matrix metalloproteinases (MMPs)[6–8] and the a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs)[9–11].

IVD cells mediate ECM remodelling as an adaptive response to changes in the tissue microenvironment. Biochemical factors such as oxygen and the substrates and products of anaerobic metabolism[12–15], and mechanical stimuli[16–18] are seen to modulate the process, to maintain the functional integrity of tissue structures.

IVD degeneration is characterised by dysregulated ECM remodelling. Rates of ECM degradation are increased whilst ECM synthesis is decreased[4,5], resulting in the dehydration and functional failure observed in degenerate IVDs[19,20]. A number of pro-inflammatory cytokines have been implicated in the regulation of ECM remodelling. Interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α), both of which are increased in IVD degeneration[21–25] induce ECM degrading enzymes and decrease synthesis of ECM components *in vitro*[25–27].

Articular chondrocytes exhibit biochemical and morphological similarity to nucleus pulposus (NP) cells, indeed NP cells are often referred to as 'chondrocyte like'. Equilibrium between anabolic and catabolic activity in articular chondrocytes can be pushed towards catabolism by IL-1 and TNF-α stimulation[28–30], in a similar way to NP cells. Further, it has been observed that certain chemotactic cytokines: 'chemokines', also promote catabolism in articular chondrocytes. Particularly, CC and CXC chemokines are reported to simulate the release of MMP-3 and N-acetyl-β-D-glucosaminidase from articular chondrocytes[31–33].

Chemokine expression within the IVD has been shown by a number of previous studies[34–39], and recently we reported that the native NP cell population is a source of many cytokines and chemokines within degenerate and prolapsed human IVDs[40]. Further, we demonstrated that expression of CCL2, CCL7 and CXCL8 increased concordant with histological degeneration[40] and hence, chemokine expression may be linked to disease severity in chronic low back pain (LBP) associated with degeneration and prolapse of the IVD. However, expression alone is not sufficient to implicate chemokines as biological mediators in the pathogenesis of IVD degeneration or prolapse. Their roles within the IVD have not been well investigated and it remains unclear which cells may respond to chemokine signalling and also whether chemokine signalling may elicit effects on ECM remodelling. Our previous investigation putatively identified receptor expression by NP cells (at the mRNA level) for CD4 (IL-16 signalling receptor[41–44]), CCR1, CXCR1 and CXCR2 along with co-expression of chemokine ligands, suggesting an autocrine or paracrine signalling mechanism.

This study aimed to address the hypothesis that cytokines and chemokines modulate NP cell behaviour in the human IVD. Specifically, we aimed to identify the expression of cytokine and chemokine receptors by NP cells to determine the capacity of NP cells to respond to chemokine signalling in degenerate and prolapsed human IVDs. Further, we aimed to determine the effects of stimulation of identified receptors on primary human NP cell metabolism, and to determine the inter-relationships between cytokine and chemokine expression within the IVD.

Materials & methods

Tissue samples processing and grading

Thirty six human lumbar IVD tissue samples were obtained for use in this study, either at surgery or post-mortem examination (PM) with informed consent of the patient or relatives (Table I). Local ethics approval was given for this work by Sheffield Research Ethics Committee (09/H1308/70). Eight post-mortem IVDs were recovered from two donors with no recorded history of IVD disease or LBP. Twenty eight NP samples were recovered from patients undergoing microdiscectomy for alleviation of root pain related to IVD prolapse, samples were selected to provide representation of non-degenerate, degenerate and infiltrated samples from discs received with sufficient NP tissue for study. Representative tissue samples were fixed in 10% v/v neutral buffered formalin (Leica, Milton Keynes, UK) and processed to paraffin wax on a Shandon Elliott Duplex Processor. Haematoxylin and eosin stained sections were evaluated independently by two researchers (KLEP & CLLM) to determine the extent of degenerative tissue changes. Sections were scored numerically between 0 and 12 based on the presence of cell clusters, fissures, loss of demarcation and haematoxophilia; a score of 0–3 indicates histologically non-degenerate IVDs and a grade of ≥4 indicates evidence of degeneration, as described

Table I
Tissue Donor and Sample Classification Details. S, Surgical; PM, Post-mortem. Surgically obtained prolapsed tissue samples were considered to be from 'intact' IVDs when no signs of AF or CEP rupture were evident (protrusion type of prolapse).* denotes tissue sample used for NP cell isolation. Grades are histologically determined grade of degeneration, Infiltrated means infiltrating immune cells were observed

Ref.	Source	Age (y)	Level	Intact	Cell isolation	IHC	Grade	Infiltrated
1	S	42	L4/L5	No		Y	3.50	Y
2	S	40	L5/S1	Yes	*	Y	4.50	Y
3	S	25	L4/L5	Yes		Y	5.70	N
4	S	48	L4/L5	Yes	*	Y	4.70	Y
5	S	–	–	–	*	Y	4.20	Y
6	S	62	L4/L5	Yes	*	Y	7.50	N
7	S	32	L5/S1	Yes	*	Y	2.90	N
8	S	53	L4/L5	No		Y	3.50	Y
9	S	40	L5/S1	Yes		Y	6.20	N
10	S	66	L5/S1	–		Y	4.50	Y
11	S	34	L4/L5	Yes		Y	9.20	N
12	S	29	L4/L5	Yes	*	N	7.80	Y
13	PM	45	L4/L5	Yes		Y	2.00	N
14	PM	45	L4/L5	Yes		Y	3.50	N
15	PM	45	L4/L5	Yes		Y	5.00	N
16	S	48	L4/L5	No		Y	8.40	N
17	S	26	L5/S1	No		Y	3.90	N
18	S	33	L5/S1	Yes		Y	4.50/7.40	Y
19	PM	–	L1/L2	Yes		Y	5.50	N
20	PM	–	L4/L5	Yes		Y	8.50	N
21	PM	–	L5/S1	Yes		Y	8.00	N
22	PM	–	L3/L4	Yes		Y	8.50	N
23	PM	–	L2/L3	Yes		Y	9.00	N
24	S	36	L5/S1	Yes		Y	5.90	N
25	S	–	–	–		Y	5.00	Y
26	S	44	L5/S1	Yes		Y	4.40	Y
27	S	52	L4/L5	Yes		Y	5.40	N
28	S	–	L4/L5	No		Y	4.00	Y
29	S	38	L5/S1	No		Y	4.50	Y
30	S	28	L4/L5	Yes	*	Y	7.40	N
31	S	43	L5/S1	No	*	N	5.5	N
32	S	40	L3/L4	Yes	*	N	7.4	N
33	S	45	L4/L5	No	*	N	2.6	Y
34	S	47	L2/L3	No	*	N	5.40	Y
35	S	39	L5/S1	Yes	*	N	3.9	Y
36	S	19	L5/S1	No	*	Y	5.20	N
n=					13	30		

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