

Role of interleukin-17 in chondrocytes of herniated intervertebral lumbar discs

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Abstract. Lumbar disc herniation (LDH) is a common cause of lumbosacral radiculopathy. An autoimmune response to a herniated nucleus pulposus (NP) has been suggested to play an important role in the initiation of radiculopathy. Interleukin-17 (IL-17) is a cytokine associated with inflammation and autoimmunity. The presence of IL-17 has been studied in patients with LDH; however, extensive investigation into the expression of IL-17 in different disc pathologies of LDH has not yet been conducted. The aim of the present study was to investigate the role of neovascularization and hypertrophic chondrocytes in herniated intervertebral lumbar discs. Fifty-two intervertebral lumbar disc specimens were extracted from 46 patients with LDH and were subsequently classified as either contained or non-contained disc herniation (CDH and NCDH, respectively). The specimens were stained with hematoxylin and eosin or toluidine blue, or were immunostained with polyclonal antibodies to IL-17 using the streptavidin-peroxidase method. The neovascular tissue and staining results were graded to establish the histological differences between the two herniation types. The intervertebral discs (IVDs) obtained from patients with NCDH showed significantly more neovascularization and granulation tissue than the discs obtained from patients with CDH ($P<0.05$). Furthermore, hypertrophic chondrocytes were more abundant in the NCDH specimens than in the CDH specimens ($P<0.05$). Similarly, the number of IL-17-immunoreactive cells was significantly higher in the

NCDH specimens than that in the CDH specimens ($P<0.01$). In conclusion, local inflammation and autoreactive immune activation may play an important role in the pathogenesis of LDH. These results also suggest a role of chondrocytes in the repair of herniated IVDs.

Introduction

Lumbosacral radiculopathy is a relatively common clinical condition that occurs as a result of mechanical deformation and biochemical irritation caused by intervertebral disc (IVD) herniation. Although mechanical nerve root compression may contribute to the symptoms of radiculopathy, it is currently believed that an inflammatory or immune component is the key mediator of radicular pain (1,2). The nucleus pulposus (NP) tissue may be autoantigenic; this theory is supported by the accumulation of lymphocytes in regional lymph nodes following exposure to autologous NP (3) and injury to the annulus fibrosus (4). A number of animal studies applied healthy autologous NP to spinal nerve roots, as a model of non-compressive disc herniation, which induced an inflammatory and immune response resulting in neuronogial apoptosis, a decrease in nerve conduction velocity, the onset of gait abnormality, mechanical allodynia and thermal hyperalgesia (5-12). Inflammatory cells (predominantly macrophages) in herniated disc tissue that have been harvested during surgery synthesize proinflammatory mediators and cytokines, such as phospholipase A2 (13,14), leukotrienes (14), immunoglobulins (14), fibroblast growth factor (15-17), matrix metalloproteinases (18-21), nitric oxide (18-20), interleukin (IL)-1 (14,22-24), IL-4 (25), IL-6 (14,19,22,23,26,27), IL-8 (27), IL-10 (26), IL-17 (12), IL-20 (28), IL-23 (12), prostaglandin E2 (18,19,22,27,29), tumor necrosis factor- α (14,22-24,30-32), interferon γ (25), granulocyte-macrophage colony stimulating factor (22,26), nerve growth factor (21), substance P (21,33), cyclooxygenase-2 (31,32,34,35), monocyte chemoattractant protein-1 (36) and vascular endothelial growth factor (37).

Numerous studies have focused on a new subset of T cells, known as T-helper type 17 (Th17) cells, which are associated with autoimmune diseases. IL-17 is a cytokine associated with inflammation, autoimmunity (38), inflammatory bowel disease (39), psoriasis (40), rheumatoid arthritis (41) and the defense against certain bacterial and viral infections (42). IL-17A and IL-17B, along with the IL-17 receptor and IL-17

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receptor-like protein, have been found to localize in chondrocytes in the fracture callus and in the epiphyseal growth plate during an endochondral differentiation program (43).

Previous studies have examined the association between IL-17 and disc diseases, particularly lumbar disc herniation (LDH). According to the findings of those studies, IL-17 participates in the local inflammatory response in an autologous NP model of radiculopathy (44). The presence of IL-17 has been examined in herniated disc tissues of patients with LDH, demonstrating the involvement of Th17 lymphocytes in disc degeneration (45,46). IL-17 is currently being used as a marker of inflammation and immune activation. There is, however, limited research on the expression of IL-17 in different disc pathologies in LDH. The present study investigated the differences in IL-17 expression among patients with different types of LDH. Specimens were collected from patients who had undergone surgery and were investigated through pathological and immunological observations.

Materials and methods

Patients. IVD specimens from 46 patients who had received conservative therapy for ≥ 3 months between March and October 2009 were obtained through primary lumbar discectomy for radiculalgia. The subjects comprised 24 men and 22 women aged 18-76 years (mean age, 45.2 ± 13.1 years) (Table I). Patients showed either no improvement in radiculopathy or initial improvement during conservative therapy, which was, however, followed by relapse. Patients were assessed according to symptoms, physical examination (sensation, muscle strength and straight leg raising test) and image data. Certain patients also underwent the femoral nerve stretch test if it was considered that the L3/4 herniated lumbar disc could affect the femoral nerve. The study protocol was approved by the Ethics Committee of Tianjin Hospital (Tianjin, China). Herniation was present at L3/4 in 3 discs, L4/5 in 32 discs and L5/S1 in 17 discs. The presence or absence of perforation of the posterior longitudinal ligament could not be conclusively confirmed by magnetic resonance imaging. The type of herniation was, therefore, confirmed during the surgery, based on whether or not the annulus fibrosus was broken. The patients were divided into two groups: The contained disc herniation (CDH) and the non-contained disc herniation (NCDH) groups. Specimens collected during surgery were examined by immunopathology.

Preparation of pathological specimens. IVDs were harvested during discectomy and specimens underwent light microscopic examination. The specimens for light microscopy were fixed in 10% formalin and embedded in paraffin. Paraffin sections (5- μ m-thick) were cut and stained with hematoxylin and eosin and toluidine blue. Surgical specimens were qualitatively graded according to whether or not inflammatory changes existed in the herniation (47) (Fig. 1) and hypertrophic chondrocytes were present in the specimens (Fig. 2), as shown by the amount of toluidine blue staining.

Immunohistochemistry of IL-17. Immunohistochemistry of IL-17 was performed according to the streptavidin-peroxidase (SP) method, using an SP-9001 Rabbit SP kit (Tianjin Haoyang Biological Manufacture Co., Ltd., Tianjin, China).

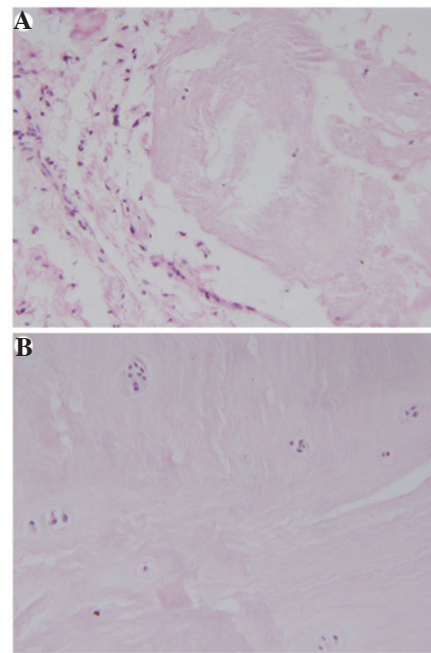


Figure 1. Classification of surgical specimens based on pathological findings. (A) Grade 1, inflammatory cell infiltration and neovascularization in the herniated tissue (H&E staining; original magnification, x200). (B) Grade 2, no inflammatory cell infiltration or neovascularization in the herniated tissue (H&E staining; original magnification, x200). H&E, hematoxylin and eosin.

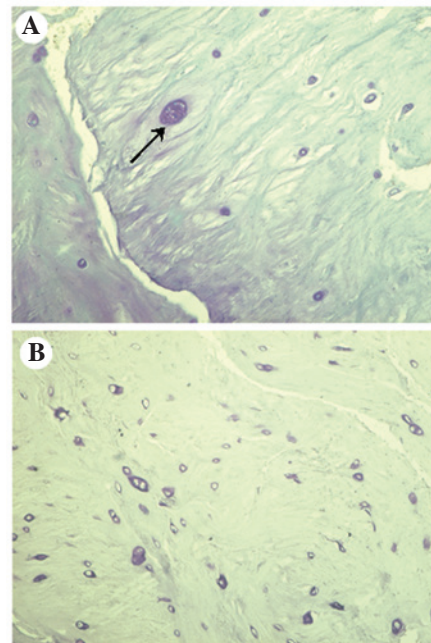


Figure 2. Classification of surgical specimens based on toluidine blue staining. (A) Grade 1, high toluidine blue metachromasia, clustering of chondrocytes and hypertrophic chondrocytes (highlighted by arrow) in herniated tissue (toluidine blue staining; original magnification, x200). (B) Grade 2, low toluidine blue metachromasia and absence of hypertrophic chondrocytes in herniated tissue (toluidine blue staining; original magnification, x200).

The sections were baked at 60°C for 1 h, deparaffinized, rehydrated, immersed in 3% H₂O₂ in phosphate-buffered saline (PBS) for 10 min, heated in a sodium citrate bath in a microwave oven, washed in PBS and blocked in 10% sheep

Table I. Clinical characteristics of the 46 patients with lumbar disc herniation.

Case no.	Gender	Age, years	Level (side)	Duration of sciatica, months	SLR test, degrees	Sensory disturbance of lower leg	Muscle weakness of lower leg
1	F	22	L5/S1 (R)	6	40	+	+
2	M	23	L4/5 (L)	48	50	-	-
3	M	28	L4/5 (R)	24	40	+	-
			L5/S1 (R)				
4	F	55	L4/5 (L)	2	45	+	+
5	F	53	L4/5 (L)	1	30	+	+
6	M	53	L4/5 (R)	2	70	+	-
			L5/S1 (R)				
7	M	46	L5/S1 (R)	0.2	50	+	-
8	M	44	L4/5 (R)	2	50	-	+
9	F	21	L5/S1 (L)	4	30	+	-
10	F	46	L5/S1 (L)	5	40	-	-
11	F	46	L4/5 (R)	5	30	+	+
12	F	41	L5/S1 (L)	3	50	+	+
13	F	36	L4/5 (R)	1	40	+	+
			L5/S1 (R)				
14	M	52	L4/5 (R)	1.2	50	+	+
15	M	18	L4/5 (R)	1	10	+	+
16	M	42	L5/S1 (R)	10	50	+	
17	M	56	L4/5 (L)	1	45	+	+
18	M	56	L4/5 (L)	2	70	+	+
19	F	44	L4/5 (L)	1	30	+	+
			L5/S1 (R)				
20	F	52	L3/4 (R)	0.3	FNST ⁺	+	+
21	F	47	L5/S1 (R)	4	50	+	+
22	M	20	L4/5 (R)	6	20	+	-
23	M	39	L4/5 (L)	30	30	+	-
24	M	54	L4/5 (R)	0.3	40	-	-
25	M	34	L4/5 (R)	4	20	+	+
26	M	54	L4/5 (R)	2	30	+	+
27	F	44	L4/5 (L)	2	30	+	+
28	M	42	L3/4 (R)	60	FNST ⁺	+	+
29	M	58	L4/5 (R)	2	40	+	+
30	M	47	L3/4 (L)	48	FNST ⁻	+	+
31	M	52	L4/5 (L)	3	40	+	+
			L5/S1 (R)				
32	F	40	L5/S1 (R)	0.2	50	+	+
33	F	39	L4/5 (L)	12	30	+	+
			L5/S1 (R)				
34	F	56	L4/5 (L)	3	40	+	+
35	M	76	L4/5 (R)	6	30	+	+
36	F	67	L4/5 (R)	2	70	+	+
37	F	68	L4/5 (R)	8	20	+	+
38	F	63	L4/5 (L)	2	0	+	+
39	F	40	L4/5 (L)	36	30	+	+
40	M	48	L4/5 (L)	36	70	+	-
41	M	21	L5/S1 (L)	9	20	+	-
42	F	43	L4/5 (L)	0.5	80	+	-
43	M	52	L4/5 (L)	0.7	30	+	+
44	F	52	L5/S1 (R)	0.3	30	+	+
45	F	39	L4/5 (L)	12	30	+	+
46	M	52	L5/S1 (L)	0.5	30	+	+

F, female; M, male; R, right side; L, left side; FNST, femoral nerve stretch test; SLR, straight leg raise.

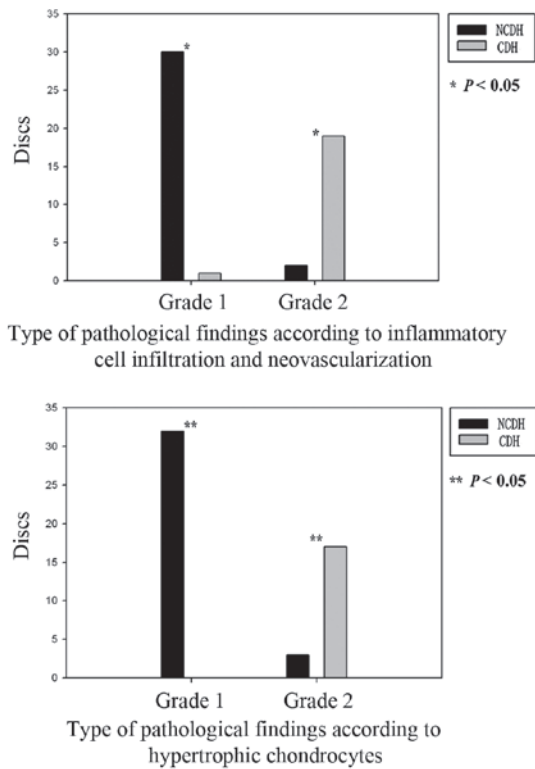


Figure 3. Comparison of surgical and pathological findings. CDH, contained disc herniation; NCDH, non-contained disc herniation.

serum for 15 min at room temperature. The sections were then incubated with primary rabbit polyclonal anti-human IL-17 antibodies (cat. no. sc-7927; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), and diluted 1:50 in a blocking solution at 4°C in a humidified chamber overnight. Incubation with biotinylated secondary antibody (Tianjin Hao Yang Biological Manufacture Co. Ltd., Tianjin, China) was performed for 15 min at room temperature, and an avidin-biotin alkaline phosphatase complex reaction was performed for 15 min, also at room temperature. Color was developed with diaminobenzidine, and the sections were counterstained with hematoxylin for 1 min. Tris-buffered saline was used as a negative control instead of the primary antibodies, and the conditions were selected so that there was no signal in the negative control samples. Five slices of IVDs were used to determine the number of IL-17-immunoreactive cells per 0.015625 mm² of histological section for each evaluation. The evaluation was blinded with respect to the grouping of the specimens.

Statistical analysis. The statistical significance of the differences in the neovascularization, granulation tissue and hypertrophic chondrocytes between the two groups was assessed using the χ^2 test. The number of IL-17-immunoreactive cells in the herniated IVDs was compared by an independent-samples t-test. $P < 0.05$ indicated a statistically significant difference.

Results

Baseline characteristics. In Table I the clinical conditions of the patients have been introduced. Six patients suffered

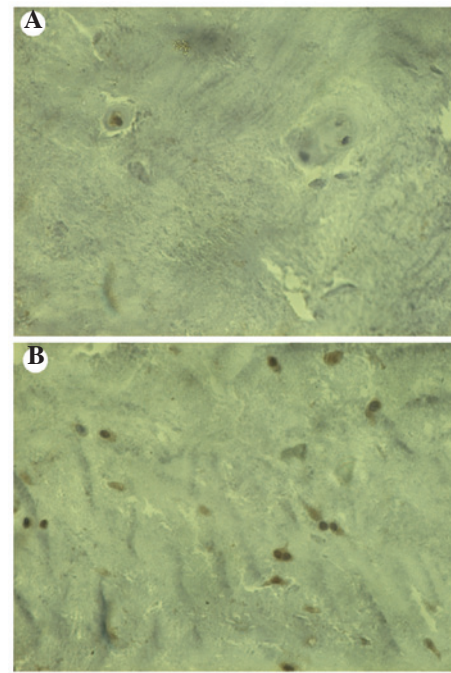


Figure 4. Herniated disc tissue analyzed by immunohistochemistry for IL-17. (A and B) IL-17-immunoreactive cells in (A) contained disc herniation and (B) non-contained disc herniation tissues (original magnification, x400). IL-17, interleukin 17.

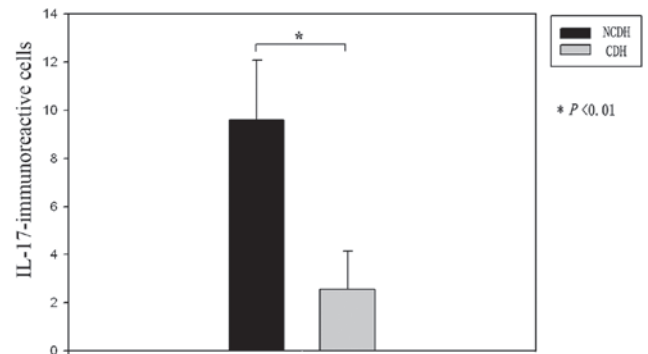


Figure 5. IL-17-immunoreactive cells. The number of IL-17-immunoreactive cells in tissues of the NCDH group was higher than that in tissues of the CDH group. CDH, contained disc herniation; NCDH, non-contained disc herniation.

from two segments of lumbar disc herniation so the age and male/female ratio could be compared in all patients.

Differences in the pathological results from the specimens collected from patients with LDH. Thirty-one discs were classified as NCDH and 21 as CDH, based on the position of the IVD that was observed during the surgery (Table II). Neovascularization and granulation tissues were observed in 30 out of 31 (97%) herniated tissues in the NCDH group. Hypertrophic chondrocytes were detected in the IVD specimens from the entire NCDH group (Fig. 3).

Pattern of distribution of IL-17-immunoreactive cells in the IVDs. IL-17 immunoreactivity was observed in the IVD specimens from both the NCDH and CDH groups. The

Table II. Summary of surgical and pathological findings in the 46 patients with lumbar disc herniation.

Case no.	Level (side)	Type of hernia	Blood vessels around hernia	Hypertrophic chondrocytes
1	L5/S1 (R)	NC	1	I
2	L4/5 (L)	C	2	II
3	L4/5 (R)	C	2	I
	L5/S1 (R)	C	2	II
4	L4/5 (L)	C	2	II
5	L4/5 (L)	NC	1	I
6	L4/5 (R)	C	2	II
	L5/S1 (R)	C	2	II
7	L5/S1 (R)	NC	1	II
8	L4/5 (R)	C	1	II
9	L5/S1 (L)	C	2	II
10	L5/S1 (L)	C	2	II
11	L4/5 (R)	NC	1	I
12	L5/S1 (L)	NC	1	I
13	L4/5 (R)	C	2	I
	L5/S1 (R)	C	2	I
14	L4/5 (R)	NC	1	I
15	L4/5 (R)	C	2	II
16	L5/S1 (R)	C	2	II
17	L4/5 (L)	C	2	II
18	L4/5 (L)	NC	1	II
19	L4/5 (L)	C	2	II
	L5/S1 (R)	NC	2	I
20	L3/4 (R)	NC	1	I
21	L5/S1 (R)	NC	1	I
22	L4/5 (R)	NC	1	I
23	L4/5 (L)	NC	2	II
24	L4/5 (R)	NC	1	I
25	L4/5 (R)	NC	1	I
26	L4/5 (R)	NC	1	I
27	L4/5 (L)	C	2	II
28	L3/4 (R)	NC	1	I
29	L4/5 (R)	NC	1	I
30	L3/4 (L)	NC	1	I
31	L4/5(L)	C	2	II
	L5/S1 (R)	C	2	II
32	L5/S1 (R)	NC	1	I
33	L4/5(L)	NC	1	I
	L5/S1 (L)		1	I
34	L4/5 (L)	NC	1	I
35	L4/5 (R)	NC	1	I
36	L4/5 (R)	NC	1	I
37	L4/5 (R)	C	2	II
38	L4/5 (L)	NC	1	I
39	L4/5 (L)	NC	1	I
40	L4/5 (L)	C	2	II
41	L5/S1 (L)	NC	1	I
42	L4/5 (L)	NC	1	I
43	L4/5 (L)	NC	1	I
44	L5/S1 (R)	NC	1	I
45	L4/5 (L)	NC	1	I
46	L5/S1 (L)	NC	1	I

R, right side; L, left side; NC, non-contained disc herniation; C, contained disc herniation.

number of IL-17-immunoreactive cells in the IVD specimens from patients in the NCDH group was significantly greater than that in the IVD specimens from patients in the CDH group (Figs. 4 and 5).

Discussion

LDH is the most common cause of sciatica, a condition that severely limits the ability to perform daily activities. Although there is a high incidence of LDH in society, controversy still surrounds the pathogenesis and treatment of the disease. In 1962, Naylor proposed that chronic lower back pain could be due to an autoimmune mechanism (48). In the last few decades, numerous studies have proposed a possible immunological mechanism for nerve injury in LDH. By acting as a biochemical or immunological irritant, herniated disc material could contribute to a patient's clinical signs and symptoms (13-15).

Previous studies have provided evidence for the neovascularization and infiltration of macrophages and other inflammatory cells into herniated disc tissues (12,49). In the current study, IVDs from patients with NCDH had significantly more neovascularization and granulation tissue than patients with CDH. This is consistent with results from previous studies (37,47), which showed that annulus fibrosus disruption could induce changes in the microenvironment surrounding herniated disc tissues. The present study evaluated the differences in neovascularization between the CDH and NCDH groups; however, no post-treatment follow-up evaluations were performed. A recent study has shown that severe histodegeneration in LDH is associated with enhanced neovascularization and a potentially spontaneous regression of the herniated tissue (50). The process of angiogenesis in the degenerated IVD affects the pre- and postoperative quality of life of the patients and may also serve as a predictive factor for postoperative results (51).

Through histological observations of intact rabbit IVDs, Kim *et al* (52) confirmed that chondrocytes in the NP originate from the cartilage endplate. Following the identification of chondrocytes in IVDs, an increasing number of studies have focused on the effects of chondrocyte apoptosis on physiological changes in IVDs (52,53). The frequency of chondrocyte apoptosis in the sequestered NP (SNP) and in the remaining NP (RNP) was the same (53). The pathways involved in chondrocyte apoptosis in the SNP and the RNP differed among individuals and included intrinsic and/or extrinsic pathways (53). In the current study, hypertrophic chondrocytes were more prevalent in specimens from the NCDH group than in specimens from the CDH group.

Immunohistochemical analysis showed that IL-17 was expressed in human herniated IVD tissues. IL-17 is a cytokine associated with inflammation and autoimmunity. The number of IL-17-immunoreactive cells found in the specimens from patients with NCDH was significantly higher than that found in the specimens from patients with CDH.

The present study had a few limitations. Each group contained only a small number of cases, including patients of different ages; however, the intensity of pain or IL-17 expression in each patient was not compared. The results of this study suggest that the expression of IL-17 in herniated disc tissue may

be a cause of lower back pain in LDH; however, further studies should be conducted in order to investigate the pathophysiological mechanisms of herniated disc tissue-induced pain.

In conclusion, the present results demonstrate that neovascularization, granulation tissue and hypertrophic chondrocytes are found in herniated disc tissues. IL-17 is expressed in human IVDs. Following its expression and secretion in inflamed human herniated disc tissues, IL-17 acts in an autoimmune manner to regulate inflammation and angiogenesis during the healing process. The present study demonstrates that IL-17 contributes to the pathogenesis of human IVD herniation by promoting autoimmune inflammation, chemotaxis and angiogenesis.

Acknowledgements

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