Th17 Cell Frequency and IL-17 Concentration Correlate With Pre- and Postoperative Pain Sensation in Patients With Intervertebral Disk Degeneration

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Abstract

Numerous studies have revealed the presence of T helper 17 (Th17) cells in pathologic intervertebral disk (IVD) tissues and the contribution of Th17-associated cytokines to the development of this disease. However, the pre- and postoperative changes in the proportion of Th17 cells and the concentration of IL-17 in the peripheral blood of patients with IVD degeneration are not clear. The levels of Th17 frequency and the interleukin-17 (IL-17) concentration in peripheral blood from patients and volunteers were examined by flow cytometry and by enzyme-linked immunosorbent assay (ELISA), respectively. The clinical results were evaluated using the visual analogue scale (VAS). These results were subjected to a correlation analysis. Compared with the normal controls, the proportion of Th17 cells and the concentration of IL-17 were significantly increased preoperatively in patients with IVD degeneration. Postoperatively, the levels of Th17 cells and the expression of IL-17 were dramatically decreased. The correlation analysis of the VAS pain scores, Th17 cell frequency, and IL-17 concentration, including the pre- and postoperative levels and the changes induced by the surgery, revealed a positive correlation. The authors’ results explain the contribution of Th17 cells and IL-17 to the pain sensation experienced by patients with IVD degeneration. These 2 factors may be good indicators for the evaluation of the surgical outcome of patients with lumbar disk herniation.

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Degeneration of the human intervertebral disk (IVD) is a widespread and debilitating disorder characterized by pronounced anatomic and biological changes in a process that is strongly associated with aging. Low back pain in humans, which is an increasingly common and costly health problem, is mainly caused by IVD degeneration. Bobechko and Hirsh demonstrated that the nucleus pulposus, which is the largest avascular tissue in vivo, is recognized as a foreign antigen, and an autoimmune response producing inflammation and sustained lumbocaudal pain is induced once the immune system is prominently exposed to this antigen. Furthermore, Macnab reported that the numbness experienced by patients with low back pain is caused only by compression of the disks and that the main factor leading to pain is inflammation. Subsequently, various proinflammatory and inflammatory cytokines, such as nitric oxide (NOx), prostaglandin E2 (PGE2), interleukin-6 (IL-6), interleukin-8 (IL-8), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) have been found within and around herniated disks.

T helper 17 (Th17) cells are a new T-cell lineage characterized by the production of IL-17 and other abundant proinflammatory cytokines, including TNF-α, IL-22, and IL-26. Gabr demonstrated that IL-17 is expressed in degenerated IVD tissues and showed that the production of NOx, PEG2, and IL-6 is up-regulated by the addition of Th17-associated cytokines to the culture medium of nucleus pulposus cells. Furthermore, recent work by the authors’ group revealed that the levels of Th17 lymphocytes and IL-17 are increased in degenerated IVD tissues. The correlation analysis of the visual analogue scale (VAS) pain scores, PGE2, Th17 frequency, and levels of IL-17 suggested that the increase in the number of Th17 cells and the overexpression of IL-17 contribute to the low back pain experienced by patients with lumbar disk herniation. The authors have also provided a potential explanation for the involvement of the CCL20-CCR6 system in the migration of Th17 cells to degenerated IVD tissues and explained the contribution of Th17-associated cytokines to the development of degenerated disks.

However, all of the results discussed above were obtained from samples collected preoperatively. The changes in the Th17 cell frequency, the expression of IL-17, and the positive correlation of these factors with the VAS scores postoperatively have not been reported. In the current study, the authors aimed to discover the variation in the Th17 cell frequency, the expression of IL-17, and the VAS scores and the interrelationship between the pre- and postoperative variables in patients with protruded and herniated IVD.

### Materials and Methods

#### Patients and Controls

A total of 80 patients with lumbar disk degeneration (median age, 45 years; range, 29 to 66 years) and 40 healthy adults (median age, 27 years; range, 19 to 40 years) from the Qi Lu Hospital of Shandong Province in China were enrolled in this study. Patients were divided into 2 groups based on the radiology results and the clinical findings during surgery: the protrusion group (intact annulus fibrosus; n=40) and the extrusion group (ruptured annulus fibrosus; n=40). Some of the control volunteers experienced strained lumbar muscles during the course of the study, but the radiology results determined that these patients did not exhibit disk degeneration.

Early on the morning preoperatively, 4 mL of fasting blood was drawn from patients using a heparin sodium anticoagulant tube. As a control, 4 mL of fasting blood was drawn from the healthy volunteers. All of the patients had had microendoscopic discectomy surgery for just 1 segment of the lumbar vertebra.

The patient selection criteria were the following: (1) having typical sciatica; (2) positive for Lasegue’s sign; (3) reduction in the height of the degenerated disk as revealed by spinal radiography, reduced signal of the involved nucleus pulposus as determined by T2-weighted magnetic resonance imaging, and no signs of calcification on the computed tomography scan; and (4) no tuberculosis, rheumatoid arthritis, or a recent history of glucocorticoid use. The determination of the disk degeneration grade was made according to the Pfirrmann grading system based on the magnetic resonance imaging signal intensity, disk structure, distinction between nucleus and annulus, and disk height, as shown in the Table. The experimental protocol was approved by the Shandong University Human Subjects Institutional Review Board, and an informed consent form was signed by each patient prior to the use of his or her blood samples.

#### Pain Assessment

The visual analogue scale (VAS) was used to evaluate the level of low back pain experienced by patients diagnosed with degenerated IVD, and this scale was applied 1 day preoperatively and again 2 weeks postoperatively. The discomfort experienced by the control subjects during the study was also assessed using this scale (Table). In every case, the level responsible for the symptomatology of the spinal cord was determined through clinical symptomatology, physical examination, and magnetic resonance imaging of the lumbar spine.

#### Flow Cytometry

Heparinized peripheral whole blood (400 µL) and an equal volume of Roswell Park Memorial Institute 1640 medium were incubated for 5 hours at 37°C and 5% CO₂ in the presence of 25 ng/mL phorbol myristate acetate (PMA) (LK-CS0001; LiankeBio, Hangzhou, China), 1 µg/mL ionomycin (LK-CS0002; LiankeBio), and 1.7 µg/mL monensin (LK-CS0004; LiankeBio, Hangzhou, China). As a control, 4 mL of fasting blood was drawn from the healthy volunteers. All of the patients had had microendoscopic discectomy surgery for just 1 segment of the lumbar vertebra.

More details on the experimental protocol, methods, and analysis can be found in the Table.
Enzyme-linked Immunosorbent Assay (ELISA)

The serum concentrations of IL-17 in patients with IVD disease were determined using the Human IL-17 Quantikine ELISA kit (eBioscience, BMS2017) according to the manufacturer’s protocol. All of the procedures were conducted at room temperature, and the mean absorbance of the standards and samples was determined from duplicates. The color reaction was assayed at 450 nm using a Varioskan flash Enzyme-linked Immunosorbent Assay (ELISA) kit (Thermo Fisher Scientific, Waltham, Massachusetts).

Statistical Analysis

Data are expressed as the means±standard deviations (SDs). The statistical evaluation of the flow cytometry and ELISA data was performed by one-way analysis of variance with a post hoc analysis. A bivariate correlation was used to determine the relationships between the VAS scores, the frequency of Th17 cells, and the levels of IL-17. All tests were performed using SPSS version 16.0 software (SPSS, Inc, Chicago, Illinois). Differences with a $P$ value less than .05 were considered statistically significant.

**RESULTS**

**Changes in the Th17 Levels**

Representative flow cytometry data are shown in Figure 1. The lymphocyte (Figure 1A) and CD3+ T cell (Figure 1B) subsets were gated. The proportion of Th17 cells (CD3-IL-17+) was determined from the CD3- cell subset (Figures 1C-G). The statistical analysis of the flow cytometry data obtained pre- and postoperatively for the protrusion and the extrusion groups is shown in Figures 1H and 1I, respectively. Compared with the healthy controls (1.002±0.337%), the percentage of peripheral Th17 cells was significantly increased in the 2 patient groups (2.231±0.380% and 4.490±0.671%, respectively; $P<0.001$). Compared with the preoperative results, the frequency of circulating Th17 cells in the 2 groups postoperatively was significantly decreased (1.113±0.194% vs 2.23±0.380% and 1.836±0.145% vs 4.490±0.671%, respectively; $P<0.001$). These results indicate that the frequency of Th17 cells in the peripheral blood of patients with degenerated IVD was significantly increased preoperatively and that this frequency was significantly decreased postoperatively.

**Changes in IL-17 Serum Levels**

The IL-17 expression followed a pattern similar to that observed in the analysis of the Th17 cell frequency. The statistical analysis of the IL-17 ELISA data obtained for the protrusion and the extrusion groups is shown in Figures 2A and 2B, respectively. Compared with the level of IL-17 in the healthy controls (1.317±0.731 pg/mL), the levels of IL-17 were significantly higher in the 2 groups of patients with degenerated IVD preoperatively (5.994±1.587 and 13.444±1.655 pg/mL, respectively; $P<0.001$). The level of IL-17 in the 2 groups of patients with degenerated IVD were significantly decreased postoperatively (2.574±0.469 and 5.058±1.93 pg/mL, respectively; $P<0.001$). These results indicate that the levels of IL-17 in the peripheral blood of patients diagnosed with degenerated IVD were significantly increased preoperatively and that the IL-17 levels significantly decreased postoperatively, similarly to the changes observed in the frequency of Th17 cells.

**Changes in the VAS Scores**

The statistical analysis of the VAS data obtained for the protrusion and the

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**Table 1: Summary of Characteristics in the Study Population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Protrusion Group</th>
<th>Extrusion Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Mean±SD age, y</td>
<td>39.63±6.91</td>
<td>50.58±8.09</td>
<td>26.80±4.19</td>
</tr>
<tr>
<td>Age range, y</td>
<td>29-55</td>
<td>35-66</td>
<td>19-40</td>
</tr>
<tr>
<td>Male/female, No.</td>
<td>20/20</td>
<td>22/18</td>
<td>20/20</td>
</tr>
<tr>
<td>Average degeneration grade</td>
<td>III</td>
<td>IV</td>
<td>II</td>
</tr>
<tr>
<td>Mean±SD VAS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>6.44±1.25</td>
<td>7.85±0.92</td>
<td>0.57±0.53</td>
</tr>
<tr>
<td>Postoperative</td>
<td>2.17±0.86</td>
<td>2.73±1.15</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviation: VAS, visual analogue score.*
extrusion groups is shown in Figures 3A and 3B, respectively. Compared with the healthy controls (0.570±0.525), the pain intensity experienced by the 2 patient groups preoperatively, as represented by the VAS scores, was significantly increased preoperatively (6.440±1.247 and 7.850±0.925, P<.0001). The VAS scores for the protrusion and the extrusion groups significantly decreased postoperatively (2.170±0.857 and 2.730±1.151, respectively) compared with the preoperative scores. Combined with the flow cytometry and ELISA results, this outcome suggests that there may be a correlation between the amount of Th17 cells, the IL-17 concentration, and the pain intensity.

### Correlation of VAS Pain Scores With Th17 Cell Frequency and IL-17 Concentration Preoperatively

The Pearson correlation coefficients for the correlation of the VAS pain scores, the Th17 cell frequency, and the IL-17 concentration preoperatively are shown in Figure 4A. A positive correlation was found between the VAS pain scores, the Th17 cell frequency, and the IL-17 concentration: Th17 proportion vs IL-17 concentration (r=0.723, P<.01), Th17 proportion vs VAS scores (r=0.805, P<.01) and IL-17 concentration vs VAS scores (r=0.675, P<.01). These results indicate that Th17 cells may be the chief source of IL-17 in the peripheral blood of patients with IVD degeneration and that the levels of Th17 cells and the expression of IL-17 are positively related to the intensity of the pain experienced by the patients.

### Correlation of VAS Pain Scores With Th17 Cell Frequency and IL-17 Concentration Postoperatively

The Pearson correlation coefficients for the correlation of the VAS pain scores, the Th17 cell frequency, and the IL-17 concentration postoperatively are shown in Figure 4B. A positive correlation was found between the VAS pain scores, the Th17 cell frequency, and the IL-17 concentration: Th17 proportion vs IL-17 concentration (r=0.551, P<.01), Th17 proportion vs VAS scores (r=0.536, P<.01), and IL-17 concentration vs VAS scores (r=0.507, P<.01). The results revealed that the proportion of Th17 cells, the concentration of IL-17, and the VAS pain scores all decreased postoperatively and that a positive correlation existed between these factors.

### Correlation of the Changes Observed in the VAS Pain Scores, Th17 Cell Frequency, and IL-17 Concentration Due to Surgery

The changes in the VAS pain scores, Th17 cell frequency, and IL-17 concentration were obtained by subtracting the postoperative results from the preoperative results. The Pearson correlation coefficients for the correlation of the changes in the VAS pain scores, Th17 cell frequency, and IL-17 concentration postoperatively are shown in Figure 4C. A positive cor-
relation was found between the changes in the VAS pain scores, Th17 cell frequency, and IL-17 concentration: changes in the Th17 proportion vs changes in the IL-17 concentration \( (r=0.729, P<.01) \), changes in the Th17 proportion vs changes in the VAS scores \( (r=0.732, P<.01) \), and changes in the IL-17 concentration vs changes in the VAS scores \( (r=0.536, P<.01) \). These correlation results provide insight into the roles that Th17 cells and IL-17 cytokines play in the intensity of the pain experienced by patients with degenerated IVD. The concentration of Th17 cells and the expression of IL-17 may serve as a predictive value of the pain sensation postoperatively.

**Discussion**

In the current study, the authors used flow cytometry and ELISA to demonstrate that the frequency of circulating Th17 cells and the concentration of IL-17 are significantly increased in the peripheral blood of patients with IVD degeneration preoperatively. Postoperatively, the proportion of Th17 cells and the concentration of IL-17 were dramatically decreased. To investigate whether the pain sensation is related to the proportion of Th17 cells and the IL-17 concentration, the authors analyzed the correlations between the VAS pain scores, the Th17 cell frequency, and the IL-17 concentration, including the pre- and postoperative levels and the changes induced by the surgery. The results from this study indicate that a positive correlation exists between the Th17 cell frequency, the IL-17 concentration, and the VAS pain scores in patients with IVD degeneration both pre- and postoperatively.

As is well known, Th17 cells are a new T-cell subset characterized by the production of IL-17, which is a proinflammatory cytokine capable of abundantly inducing other inflammatory cytokines, including TNF-α, IL-22, and IL-26. Since the discovery of this T-cell subset, many studies have revealed that Th17 cells play a major role in the pathogenesis of many immune-mediated diseases, including psoriasis, rheumatoid arthritis, inflammatory bowel disease, and asthma. In the current study, the authors confirmed that the number of Th17 cells and the expression of IL-17 are both markedly increased in the peripheral blood of patients with IVD degeneration and that a positive correlation exists between these factors. The authors speculated that Th17 cells are responsible for the increased IL-17 concentration and that the proliferation of these cells is positively correlated with the VAS pain scores experienced by patients with IVD degeneration.

Previous studies have proposed that disk herniation is an immune-mediated inflammatory process based on the particular anatomical structure. According to immunologists and Burnet’s clonal selection theory, the nucleus pulposus, which is the largest avascular tissue in vivo, exists in an immunoprivileged state and cannot be recognized by the immune system after it has been prominently exposed.
Instead, the nucleus pulposus elicits an immune response that stimulates autointoxication and subsequently leads to chronic inflammation and sustained lumbocrural pain. In the lesions of extruded and herniated disks, noticeable elevations were observed in the levels proinflammatory cytokines, including NOx, PGE2, IL-6, and IL-8,17 and increases were also observed in the levels of cytokines that are important for the regulation of pathologies, including IFN-γ, TNF-α, and IL-1β.18

Coincidentally, Macnab4 reported that the numbness experienced by patients with degenerated IVD is caused by mechanical compression only, whereas inflammation is the main factor leading to pain.

In the current study, the authors confirmed that the Th17 proportion and the IL-17 concentration are significantly increased in the 2 groups of patients preoperatively and that the levels were markedly decreased postoperatively; in addition, this decrease was accompanied by a decrease in the VAS scores. Combined with the previous results, the authors speculate that a feedback loop exists that plays an important role in the process of disk degeneration and lumbocrural pain sensation. Once the degenerated disks become protruded or herniated, they can secrete the chemotactic factor CCL20, which is a specific ligand for CCR6 that is specifically expressed on the surface of Th17 cells. The CCL20/CCR6 system plays an important role in the trafficking of Th17 cells to degenerated disks.10

The protruded or herniated disks then activate the body’s immune response to produce more Th17 cells and IL-17 cytokines in the peripheral blood of patients with IVD degeneration, and this effect provides a rich source of Th17 cells that can be trafficked into the local degenerated disks. After the Th17 cells are trafficked to the lesion areas via chemotaxis, the cells can secrete many inflammatory cytokines to yield a complex cytokine milieu that includes the other cytokines produced by macrophages and other inflammatory cells. This cytokine milieu promotes the inflammatory process and stimulates the production of CCL20, which results in the trafficking of more Th17 cells to the inflammatory lesion. The result of the feedback loop is the formation of the cytokine milieu, which plays an important role in lumbocrural pain. When corrective surgery is performed on patients with IVD degeneration, the initiator of the feedback loop (ie, the degenerated disks) is removed and the loop is blocked; thus, the cause of the compression and a large portion of the inflamed tissues will be removed. Furthermore, the activated immune system is blocked, which results in the marked decrease in the production of Th17 cells and IL-17 postoperatively. Ultimately, the lumbocrural pain experienced by patients is also significantly improved.

To investigate whether pain sensation is related to the production of Th17 cells and the concentration of IL-17 cytokines in the peripheral blood of patients with IVD degeneration, the authors analyzed the correlations between these 3 factors pre- and postoperatively. The results revealed a positive correlation between the VAS pain scores, the Th17 cell frequency, and the IL-17 concentration, and these positive correlations were obtained for the pre- and postoperative levels and for the changes induced by the surgery. Previous studies have reported that the TNF-α levels are correlated with the VAS scores 6 weeks and 12 months postoperatively but not preoperatively, which indicates that the level of TNF-α can determine the clinical outcome of patients with lumbar inflammation.

Figure 4: Correlations between the visual analogue scale (VAS) scores, the T helper 17 (Th17) cell frequency, and the interleukin-17 (IL-17) concentration levels pre- and postoperatively and changes intraoperatively. The positive correlations are shown in A (a: r=0.723; b: r=0.805; c: r=0.675; P<.01), B (a: r=0.551; b: r=0.536; c: r=0.507; P<.01), and C (a: r=0.729; b: r=0.732; c: r=0.536; P<.01), respectively.
The results from the current study indicate that the Th17 cell frequency and the IL-17 levels may be a better indicator for the evaluation of the surgical outcome of patients with lumbar disk herniation.

In the near future, the immune-induced inflammation may be reduced by either blocking the binding of CCR6 to Th17 cells during the initial process of IVD degeneration or reducing the CCL20 production by modifying the cytokine milieu in the degenerated disks. These treatments may prevent Th17 cell trafficking via the CCL20/CCR6 chemotactic pathway and thus have the potential to cure or prevent inflammation and significantly improve the lumbocervical pain experienced by patients with IVD degeneration. Although the mechanisms responsible for chronic lumbocervical pain are poorly understood, this study may aid the prediction of the clinical effectiveness of diskectomy surgery and the exploration of novel therapeutic approaches.

REFERENCES