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Proinflammatory Cytokine Expression Profile in Degenerated and Herniated Human Intervertebral Disc Tissues

Mohammed F. Shamji, MD, PhD[1], **Lori A. Setton, PhD**[2], **Wingrove Jarvis, MBBS, MD, FRCS**[3], **Stephen So, BS**[2], **Jun Chen, PhD**[2], **Liufang Jing, MS**[2], **Robert Bullock, BS**[2], **Robert E. Isaacs, MD**[3], **Christopher Brown, MD**[3], and **William J. Richardson, MD**[3]

[1] Duke University, Durham, North Carolina and The Ottawa Hospital, Ottawa, Ontario, Canada;

[2] Duke University, Durham, North Carolina;

[3] Duke University Medical Center, Durham, North Carolina

Abstract

Objective—Prior reports document macrophage and lymphocyte infiltration with proinflammatory cytokine expression in pathologic intervertebral disc (IVD) tissues. Nevertheless, the role of the Th17 lymphocyte lineage in mediating disc disease remains uninvestigated. We undertook this study to evaluate the immunophenotype of pathologic IVD specimens, including interleukin-17 (IL-17) expression, from surgically obtained IVD tissue and from nondegenerated autopsy control tissue.

Methods—Surgical IVD tissues were procured from patients with degenerative disc disease (n = 25) or herniated IVDs (n = 12); nondegenerated autopsy control tissue was also obtained (n = 8) from the anulus fibrosus and nucleus pulposus regions. Immunohistochemistry was performed for cell surface antigens (CD68 for macrophages, CD4 for lymphocytes) and various cytokines, with differences in cellularity and target immunoreactivity scores analyzed between surgical tissue groups and between autopsy control tissue regions.

Results—Immunoreactivity for IL-4, IL-6, IL-12, and interferon-γ (IFNγ) was modest in surgical IVD tissue, although expression was higher in herniated IVD samples and virtually nonexistent in control samples. The Th17 lymphocyte product IL-17 was present in >70% of surgical tissue fields, and among control samples was detected rarely in anulus fibrosus regions and modestly in nucleus pulposus regions. Macrophages were prevalent in surgical tissues, particularly herniated IVD samples, and lymphocytes were expectedly scarce. Control tissue revealed lesser infiltration by macrophages and a near absence of lymphocytes.

Conclusion—Greater IFNγ positivity, macrophage presence, and cellularity in herniated IVDs suggests a pattern of Th1 lymphocyte activation in this pathology. Remarkable pathologic IVD tissue expression of IL-17 is a novel finding that contrasts markedly with low levels of IL-17 in autopsy control tissue. These findings suggest involvement of Th17 lymphocytes in the pathomechanism of disc degeneration.

Address correspondence and reprint requests to Mohammed F. Shamji, MD, PhD, The Ottawa Hospital, Division of Neurosurgery, 1053 Carling Avenue, Ottawa, Ontario K1Y 4E9, Canada. shamji@aya.yale.edu.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Shamji had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acute low back pain is among the most common reasons for which patients seek medical care, with estimates of annual economic impact as high as $200 billion in the US (1). Degeneration of the intervertebral disc (IVD) is a common cause of such pain and disability, characterized by anatomic, morphologic, and biochemical changes including altered expression of both matrix metalloproteinases and proinflammatory cytokines (2). Elevated levels of molecular mediators of inflammation have been described in pathologic disc tissue, increasing with grade of degeneration (3,4). Such findings have been observed for both interleukin-1 (IL-1) and tumor necrosis factor α (TNFα), both of which have established roles in regulating nitric oxide (NO) and prostaglandin production, metalloproteinase expression, and apoptosis, all changes that may contribute to progressive pathology of the IVD.

The inflammatory and immune activation profile exhibited by a herniated disc may be distinct from that observed in IVD degeneration and may differ further among different extents of herniation from protrusion through sequestration. Histologic evaluation of herniated disc tissue has revealed prominent infiltration of inflammatory cells, most markedly macrophages (5–10). Surgical specimens of herniated disc tissue also exhibit a greater presence of TNFα, IL-1β, and IL-6 compared with autopsy control tissue specimens (3,4), and explant cultures demonstrate a heightened release of IL-6, IL-8, prostaglandin E_2 (PGE_2), and NO from herniated disc tissue compared with control tissue (11). Recent studies have also revealed greater IL-12 and interferon-γ (IFNγ) expression in herniated disc fragments compared with bulging discs that remain contained within the disc space by an intact anulus fibrosus (12). Most of these cytokines are macrophage products, which is consistent with the greater presence of macrophages documented for herniated disc tissues (9,10). These cytokines can promote lymphocyte activation and differentiation while also recruiting additional macrophages and activating phagocytosis and secretion of proteolytic enzymes. Activated Th1 lymphocytes produce IFNγ that assists in further macrophage recruitment and activation, and the mechanism eliciting the heightened IFNγ expression in herniated disc fragments may represent a specific immune response against herniated nucleus pulposus tissue.

The immune privilege of the nucleus pulposus occurs by both vascular isolation and a biochemical phenotype with the Fas ligand causing infiltrating T lymphocyte apoptosis. Support for this theory is found in regional lymph node accumulation of lymphocytes after exposure to autologous nucleus pulposus (13), lymphocyte accumulation in the IVD after anulus fibrosus injury (6), and immunoglobulin and complement membrane attack complex deposition in human herniated disc tissue (14–16). Recent studies have revealed the importance of Th17 lymphocytes in a range of inflammatory pathologies and autoimmune diseases, including rheumatoid arthritis, Crohn's disease, and multiple sclerosis (17,18). IL-17 is a proinflammatory cytokine produced primarily by Th17 lymphocytes activated in response to IL-23 production. IL-17 can promote inflammation by different signaling cascades, some modulated by other lymphocyte products such as IFNγ (19), and thereby promote the synthesis of other cytokines, proteases, NO, and PGE_2. Given the demonstrated presence of infiltrating and activated lymphocytes in herniated disc fragments and the potential importance of immune system activation by herniated disc tissues, it is of interest to determine a potential role of Th17 lymphocytes in mediating this process.

The objective of this study was to evaluate differences in the immunophenotype of the IVD fragments obtained from patients undergoing surgery for treatment of degenerative disc disease and lumbar disc herniation. The presence of Th17 lymphocyte markers in IVD pathology has not yet been investigated, and a primary goal of this study was to use immunohistochemistry to evaluate the molecular markers of different inflammatory and immune cells alongside expression of the lymphocyte product IL-17 in this surgically obtained tissue. Macrophage and lymphocyte markers as well as other mediators of inflammation known to be of relevance to IVD disease, including IL-6, IFNγ, IL-12, IL-10, and IL-4, were also evaluated to more

comprehensively characterize the inflammation pattern. All markers were evaluated in degenerated and herniated disc fragments as well as in cadaveric nucleus pulposus and anulus fibrosus tissues with little to no evidence of degeneration.

Results of this study reveal a high expression level of IL-17 in surgical tissues obtained from both degenerated and herniated IVDs, but not from autopsy tissue, suggesting a role of Th17-mediated inflammatory processes in IVD pathology. Additional results reveal the heightened presence of immune-activating IFNγ in herniated disc fragments, with concomitant greater macrophage infiltration and IL-6 expression as compared with degenerated tissues. These findings illustrate a pattern of immune activation by herniated disc tissue involving macrophage infiltration and activation, and further suggest that a novel lineage of Th17 lymphocytes may also be involved in both forms of IVD pathology.

MATERIALS AND METHODS

Participants and specimens

Human lumbar IVD tissues (surgical waste with no patient identifiers) were obtained at the time of surgery from patients undergoing surgery by 3 separate spine surgeons (REI, CB, WJR). Tissues were divided into pathology groupings corresponding to degenerative disc disease or herniated IVD and were excluded if their donors were age <18 years or had presented with a history of trauma, neoplastic disease, or previous lumbar surgery. Samples in the degenerative disc disease group contained tissues from 9 patients (mean ± SD age 60 ± 7 years; n = 25 samples), while those in the herniated IVD group contained tissues from 10 patients (mean ± SD age 32 ± 12 years; n = 12 samples). It was not possible to determine the region of tissue origin (nucleus pulposus, anulus fibrosus) for the surgical IVD samples based on morphologic appearance alone. Sex, body mass index (BMI), nonsteroidal antiinflammatory drug (NSAID) use, and previous epidural steroid injections in patients undergoing surgeries were recorded for subjects in these groups. The pattern of the patient's pain was delineated as "back only," "leg only," or "both back and leg."

Autopsy control tissue was obtained through the Surgical Training and Research Laboratory maintained by the Department of Surgery within 1–3 days of receipt of the cadaver at Duke University Medical Center. Intact IVDs were procured from 2 lumbar levels (L2–L3 and L3–L4) of 4 cadavers (mean ± age 46 ± 16 years) and separated into distinct nucleus pulposus and anulus fibrosus tissue regions. All samples were considered to be nondegenerated by examination of gross pathology (morphologic grade of 0–1 on a 0–3 grading scale).

Immunohistochemical staining

All tissue specimens were flash-frozen in liquid nitrogen following retrieval from the operating room or cadaver dissection and were subsequently cryosectioned into 8-μm sections. Tissue sections were fixed in 4% paraformaldehyde at room temperature for 10 minutes, followed by blocking with 3.75% bovine serum albumin (Gibco) and 5% goat serum (Zymed) to minimize non-specific antibody binding. All sections were incubated with one each of the following primary antibodies diluted 1:50 in blocking buffer: anti-human CD4 (sc-70660), anti-human CD68 (sc-70761), anti-human IL-4 (sc-80093), anti-human IL-6 (sc-80841), anti-human IL-12 (sc-74147), rabbit anti-human polyclonal IL-17 (sc-7927), rabbit anti-human poly-clonal IFNγ (sc-8308). All antibodies were purchased from Santa Cruz Biotechnology, and, unless otherwise indicated, all primary antibodies were mouse monoclonal antibodies. Surgical tissue sections, but not cadaveric tissues, were also incubated with anti-human IL-10 antibodies (sc-8438). Samples were washed with phosphate buffered saline and incubated for 30 minutes with Alexa Fluor 488–conjugated secondary antibodies (Molecular Probes) diluted 1:200 in blocking buffer. Tissue sections were counterstained with 0.5 mg/ml propidium iodide (Sigma-

Aldrich) at room temperature to stain the cell nuclei and were then mounted with GVA mounting medium (Zymed). Samples were imaged using an LSM 510 confocal laser scanning microscope (Zeiss) with 10× and 20× (water immersion, numerical aperture 1.2) objective lenses.

Grading of immunostained sections

Semiquantitative grading of immunostained sections was performed in consensus by 2 graders (of MFS, SS, RB) who evaluated 9 evenly spaced separate 10× magnification fields for each tissue sample and antibody target. This strategy provided the most complete and comprehensive evaluation of the surgical tissue sample. Scores were given for degree of cellularity and cytokine immunoreactivity, as follows: for cellularity, 0 = 0–9 cells per field, 1 = 10–49 cells per field, and 2 = 50+ cells per field; for immunoreactivity, 0 = no positive cells and 1 = at least 1 positively labeled cell.

Statistical analysis

Differences in descriptors for surgical specimens between study groups (degenerative disc disease, herniated IVD) were evaluated using Student's *t*-tests for continuous data (BMI) and chi-square tests for categorical variables (sex, pain pattern, NSAID use, and history of epidural steroid administration). Differences between study groups in tissue cellularity score were also tested using a chi-square test with repeated measures on the measurement field (9 per tissue section and antibody target). Differences between study groups in immunoreactivity score were tested by one-way repeated-measures analysis of variance (ANOVA) on the measurement field. Regional intervertebral disc differences between the nucleus pulposus and the anulus fibrosus from cadaveric specimens were tested for both cellularity score (by chi-square test) and immunoreactivity score (by one-way ANOVA). *P* values less than 0.05 were considered significant.

RESULTS

Categorization of surgical specimens

Surgical IVD tissues from 19 patients (37 samples) were procured for this study and categorized as representing degenerative disc disease or herniated IVD (Table 1), with no differences in BMI or sex distribution between pathology groups. Patients with degenerative disc disease more frequently had a prominent back component to their pain, with none reporting isolated leg pain; in contrast, all patients undergoing surgery for herniated IVD experienced leg symptoms, with or without low back pain ($P < 0.01$ by chi-square test). The use of NSAIDs was similar between study groups, although more patients with herniated IVDs had received epidural steroid injections ($P < 0.01$ by chi-square test).

Tissue cellularity and cellular phenotypes

Higher cellularity scores were observed with greater frequency in herniated IVD tissues than in degenerative disc disease tissues ($P < 0.001$ by chi-square test) (Table 2). A total of 61% of examined fields from herniated IVD tissue samples had cellularity scores of 1, compared with 49% for degenerative disc disease tissue samples. Conversely, a cellularity score of 0 was observed in 23% of all measurement fields in the degenerative disc disease group, whereas such low cellularity was observed in only 9% of measurement fields in the herniated IVD group. Autopsy control tissue demonstrated regional variation in cellularity, with the anulus fibrosus samples having predominant cellularity scores of 1 (64% of examined fields), as compared with nucleus pulposus samples, which had predominant cellularity scores of 0 in a majority of fields (78% of examined fields) ($P < 0.001$ by chi-square test) (Table 2).

Remarkable CD68 immunopositivity was observed in the herniated IVD group compared with the degenerative disc disease group (mean ± SEM 93 ± 6% of fields positive versus 73 ± 6% of fields positive; $P = 0.04$ by ANOVA) (Figure 1B). These high levels contrasted with more modest infiltration of macrophages in autopsy control anulus fibrosus and nucleus pulposus tissues (mean ± SEM 7 ± 5% of fields positive versus 24 ± 9% of fields positive, respectively; $P = 0.13$ by ANOVA) (Figure 1A). No difference was observed between the degenerative disc disease and herniated IVD groups in CD4 lymphocyte surface antigen positivity, with <20% of all measurement fields staining positively for CD4. Conversely, lymphocyte positivity was virtually absent in both nucleus pulposus and anulus fibrosus regions of autopsy control tissue.

Cytokine positivity

Significantly greater expression of IL-4, IL-6, IL-12, and IFNγ was observed in herniated disc fragments than in degenerative disc tissue ($P < 0.05$ by ANOVA) (Figures 2 and 3). The fraction of positively labeled fields was low for these cytokines (average of <40% of examined fields). Similarly, IL-10 expression was low in both surgical tissue groups, with no difference between pathologies (data not shown). These results for surgical tissues stand in contrast to the very low levels of expression of IL-4, IL-6, and IL-12 in autopsy control tissues from both the nucleus pulposus and anulus fibrosus regions, with immunopositivity in <5% of all examined fields (Figures 2 and 3). The Th1 cytokine IFNγ was also similarly expressed in nucleus pulposus tissue and anulus fibrosus tissue (mean ± SEM 14 ± 9% and 1.5 ± 1.8%, respectively; $P = 0.15$ by ANOVA), although values for immunopositivity in the nondegenerated nucleus pulposus tissue overlapped with levels in the degenerated surgical tissues.

Expression of IL-17 was notably different, being high in cells of both herniated IVD and degenerative disc disease tissues, with a nonsignificant trend toward greater immunoreactivity in herniated IVD specimens (mean ± SEM 91 ± 7% versus 74 ± 6%; $P > 0.05$ by ANOVA) (Figure 3). This was markedly higher than that in nondegenerated cadaveric tissues, despite regional differences in IL-17 immunopositivity (mean ± SEM 52 ± 13% in the nucleus pulposus region versus 6 ± 4% in the anulus fibrosus region; $P < 0.01$ by ANOVA). Figure 4 shows representative samples used for immunohistochemical evaluation of IVD specimens.

DISCUSSION

This study investigated the involvement of inflammatory mediators through the study of lumbar degenerative, herniated, and cadaveric nondegenerated IVD tissues. Prior studies have contributed to understanding of inflammatory processes in pathologic IVD, demonstrating elevated production of prostaglandins, leukotrienes, and thromboxane in herniated disc tissues (11,20–22). Numerous studies also have revealed either higher expression or simply the presence of the proinflammatory cytokines TNFα, IL-1α, and/or IL-1β, IFNγ, and IL-6 in protruded, extruded, or sequestered disc tissues (3,4,12,20,21,23–25).

Our findings of increased levels of IFNγ and IL-6 are consistent with prior work and provide new quantitative evidence of higher expression in herniated IVD tissues than in degenerated disc tissues, with both pathologic tissues exhibiting expression patterns generally higher than in autopsy control anulus fibrosus or nucleus pulposus specimens. While the presence of IL-4 and IL-12 had been documented previously in herniated disc fragments (12), the present study extends that to demonstrate greater expression in herniated IVDs, with magnitudes of expression that are again much greater than levels noted in nondegenerated lumbar IVD tissues. This grouping of inflammatory mediators includes macrophage products that promote lymphocyte activation and differentiation as well as lymphocyte products that further recruit and activate macrophages toward phagocytosis and proteolytic enzyme secretion. Taken together, the findings of greater IL-4, IL-6, IL-12, and IFNγ expression in herniated disc fragments suggests that immune lymphocyte activation of the Th1 lineage is an important event

mediating disc herniation–associated pathology. This may take place subsequent to exposure of nucleus pulposus material to the systemic circulation.

This work helps clarify involvement of the alternative Th17 lymphocyte pathway and the IL-17 cytokine in IVD pathology. Nondegenerated anulus fibrosus tissues exhibited virtually no expression of IL-17, while the nondegenerated nucleus pulposus tissue regions showed immunoreactivity for IL-17 in >50% of examined fields. Surprisingly, pathologic IVD tissues exhibited even greater IL-17 expression (in >70% of fields). While it was not possible to distinguish between anulus fibrosus and nucleus pulposus tissue sources in the surgical IVD samples, the substantial presence of IL-17 in surgical but not autopsy control specimens suggests that autoreactive Th17 lymphocytes may be involved in IVD pathology, perhaps in synergy with IFNγ (19). While the biologic effects of IL-17 on IVD cells remain unclear, studies of other cell types suggest that IL-17 promotes NO synthase and cyclooxygenase 2 up-regulation, NO and PGE_2 production, and IL-6 release (19,26). Further work must investigate the presence and activity of IL-17 receptors in IVD cells that may implicate IL-17 involvement in differentially regulating the 2 different IVD pathologies studied here. The modest expression of IL-17 in nonpathologic autopsy control nucleus pulposus tissue may suggest subclinical degenerative changes in healthy adults as the nucleus pulposus tissue loses immune privilege.

While IL-6 production occurs in various cell types, work by Takada and coworkers (27) showed that macrophages are the major cell in disc herniation–associated pathology. IL-6 helps mediate the acute-phase response to injury, promoting monocyte differentiation into macrophages and activating maturation of both B- and T-lineage lymphocytes. Further, in the context of symptomatic radiculopathy, IL-6 can induce PGE_2-mediated allodynia in experimental rat models of radiculopathy (28), with delayed onset of such sensitivity in IL-6–knockout mice (29). Another macrophage product is IL-12, a heterodimeric cytokine that stimulates T lymphocyte differentiation along the Th1 lineage, activates differentiated Th1 lymphocytes toward IFNγ production, and enhances cytotoxic activity of natural killer cells. Noncontained herniated disc tissue expresses 3-fold more IL-12 than contained herniated disc tissue (12), reflecting a more prominent role of macrophages in resorption of the heterotopic herniated disc tissue. In the current study, findings of greater IL-6 and IL-12 expression in herniated IVD tissues were consistent with the substantial CD68-positive macrophage population in herniated IVD specimens, as compared with the degenerated and autopsy control tissue sources. The findings that CD4-positive lymphocyte populations were limited and equivalent in presence among the degenerative disc disease and herniated IVD groups are consistent with work by Habtemariam and coworkers (30) revealing that in the chronic phase of disc herniation, beyond 21 days, lymphocytes were scarce and present in fewer than one-fourth of samples.

Activated Th1 lymphocytes can produce IFNγ, which was initially described for its role in specific antiviral and antitumor immune responses but is now recognized for the ability to recruit macrophages to a site of inflammation by promoting adhesion and migration, and further activating lysosome activity. Several investigators (12) have demonstrated heightened protein expression of IFNγ in uncontained or symptomatic herniated disc tissue, concluding that this represents activation of an immune response against a previously immune-privileged material. The finding of greater IFNγ immunoreactivity among herniated disc specimens in this study confirms those results, identifying a potent mediator that could explain the greater macrophage infiltration in such tissue samples.

While these data are encouraging in identifying potential inflammatory and immune cytokines involved in IVD pathology, the cellular sources of the investigated cytokines have not been rigorously characterized, nor has their role in mediating primary degenerative processes or a secondary postinflammatory pain phenotype. Animal models of disc degeneration with incorporation of genetic modifications in select cytokines could provide further understanding

about the mechanisms regulating those molecular mediators required for histologic degeneration and subsequent manifestations of pain.

In summary, the findings of this study support the hypothesis that immune system activation and Th1- and Th17-related cytokines are coincident with pathology in the herniated and degenerated IVD. Both types of IVD pathology demonstrated substantial evidence of macrophage infiltration as compared with nondegenerated autopsy control tissues, with even greater macrophage presence documented in the herniated IVD tissue specimens. This pattern of cell expression was consistent with the greater presence of the macrophage products, IL-6 and IL-12, in these tissues. These findings, together with those of elevated expression of the CD4 lymphocyte marker and various lymphocyte products in these tissues, provide further support for the hypothesis that nucleus pulposus tissue contact with the systemic circulation in disc herniation leads to lymphocyte activation with secretion of IFNγ and consequent heightened macrophage recruitment. These new findings of substantial expression of IL-17 in pathologic disc tissues may implicate the Th17 lineage of lymphocytes in the pathomechanism of disc degeneration. Tissue-level consequences of this recruitment could include desirable nucleus pulposus tissue resorption and undesirable inflammatory peripheral neuropathy manifesting as sciatica. The present results identify novel pathways against which local therapy can be targeted to treat both degenerative disc disease and disc herniation radiculopathy.

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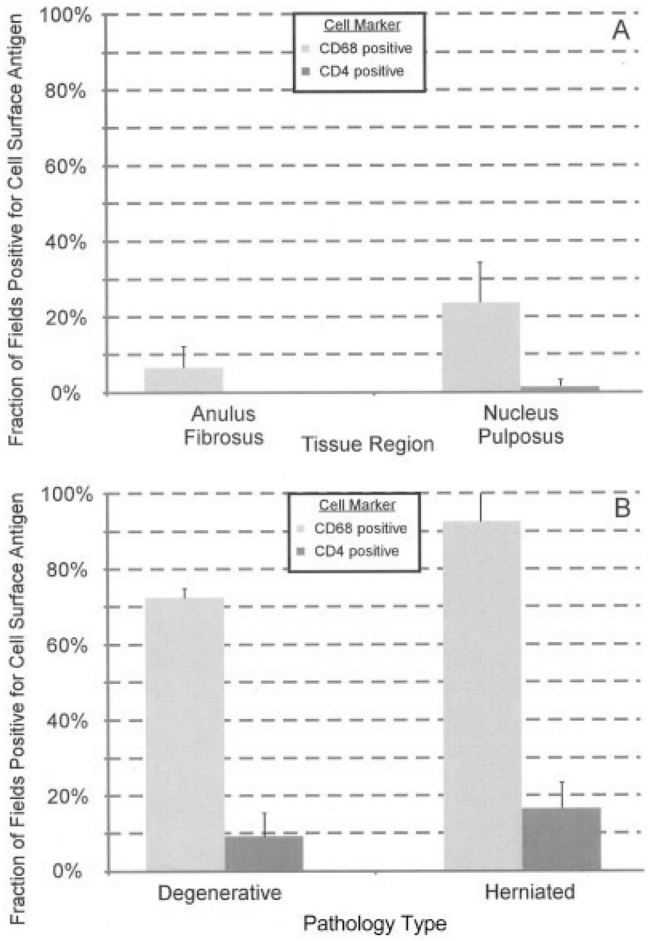

Figure 1.

Expression of cell surface antigens for macrophages (CD68) and lymphocytes (CD4) in nondegenerated human anulus fibrosus and nucleus pulposus autopsy control tissues (n = 8 each) **(A)** and in surgically obtained specimens from degenerated (n = 25) and herniated (n = 12) intervertebral disc (IVD) tissues **(B)**. A similar presence of macrophages was observed in nondegenerated nucleus pulposus and anulus fibrosus tissues ($P = 0.13$ by repeated-measures analysis of variance [ANOVA]). Neither type of tissue demonstrated significant lymphocyte infiltration. A significantly greater presence of macrophages was observed in the herniated IVD tissues than in the degenerated IVD tissues ($P = 0.04$ by repeated-measures ANOVA). While the herniated IVD group showed a trend toward more CD4 expression, both groups had few positive fields and no difference was observed between the pathoanatomies. Values are the mean and SEM from 9 fields evaluated for each sample.

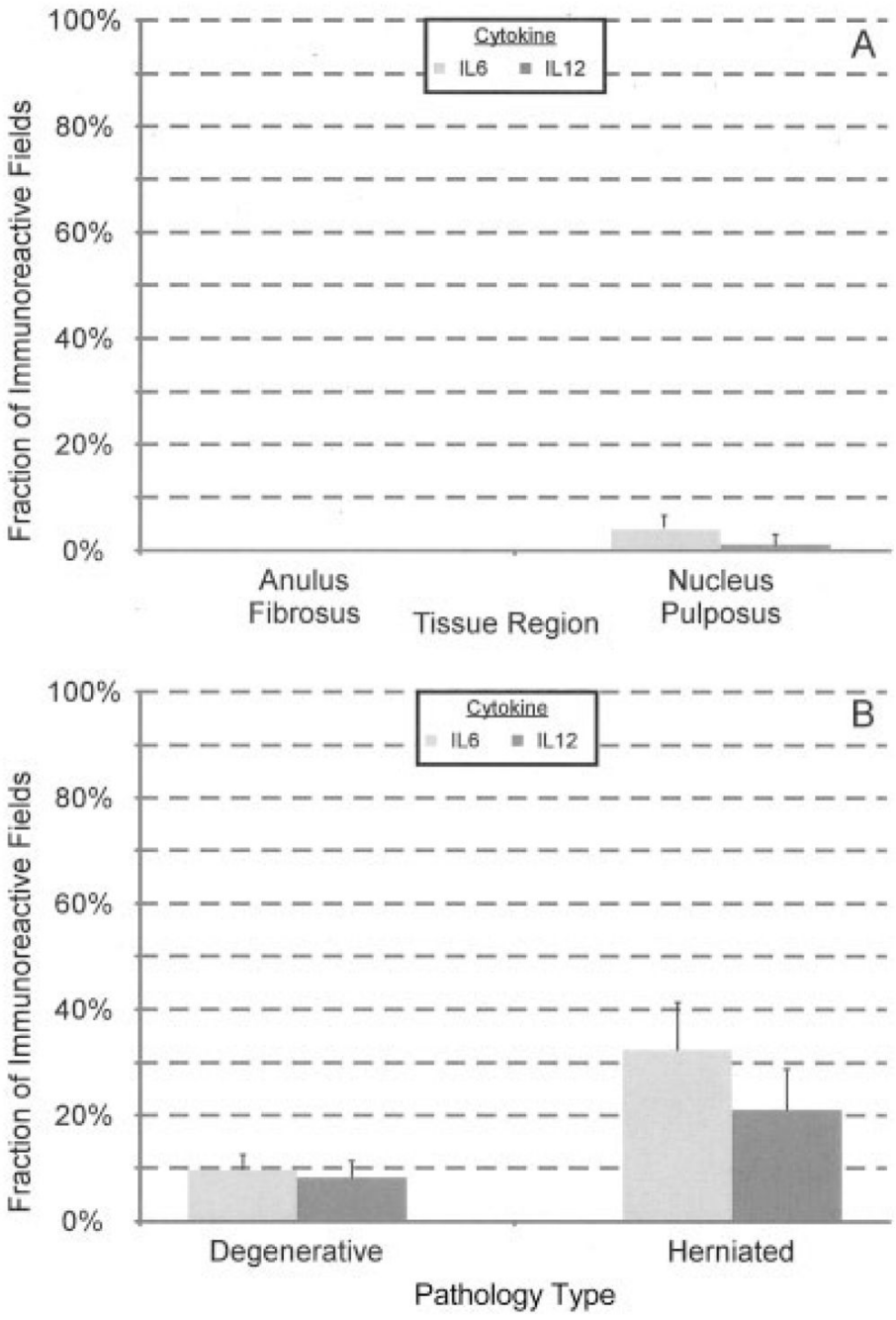

Figure 2.

Expression of macrophage products interleukin-6 (IL-6) and IL-12 in nondegenerated human anulus fibrosus and nucleus pulposus autopsy control tissues (n = 8 each) **(A)** and in surgically obtained specimens from degenerated (n = 25) and herniated (n = 12) intervertebral disc (IVD) tissues **(B)**. Neither anulus fibrosus nor nucleus pulposus tissues demonstrated significant expression of either cytokine, nor were there any differences observed between the two disc regions (*P* not significant by repeated-measures analysis of variance [ANOVA]). Significantly higher expression of IL-6 and IL-12 was observed in the herniated IVD tissues than in the degenerated IVD tissues ($P < 0.05$ by repeated-measures ANOVA). Values are the mean and SEM from 9 fields evaluated for each sample.

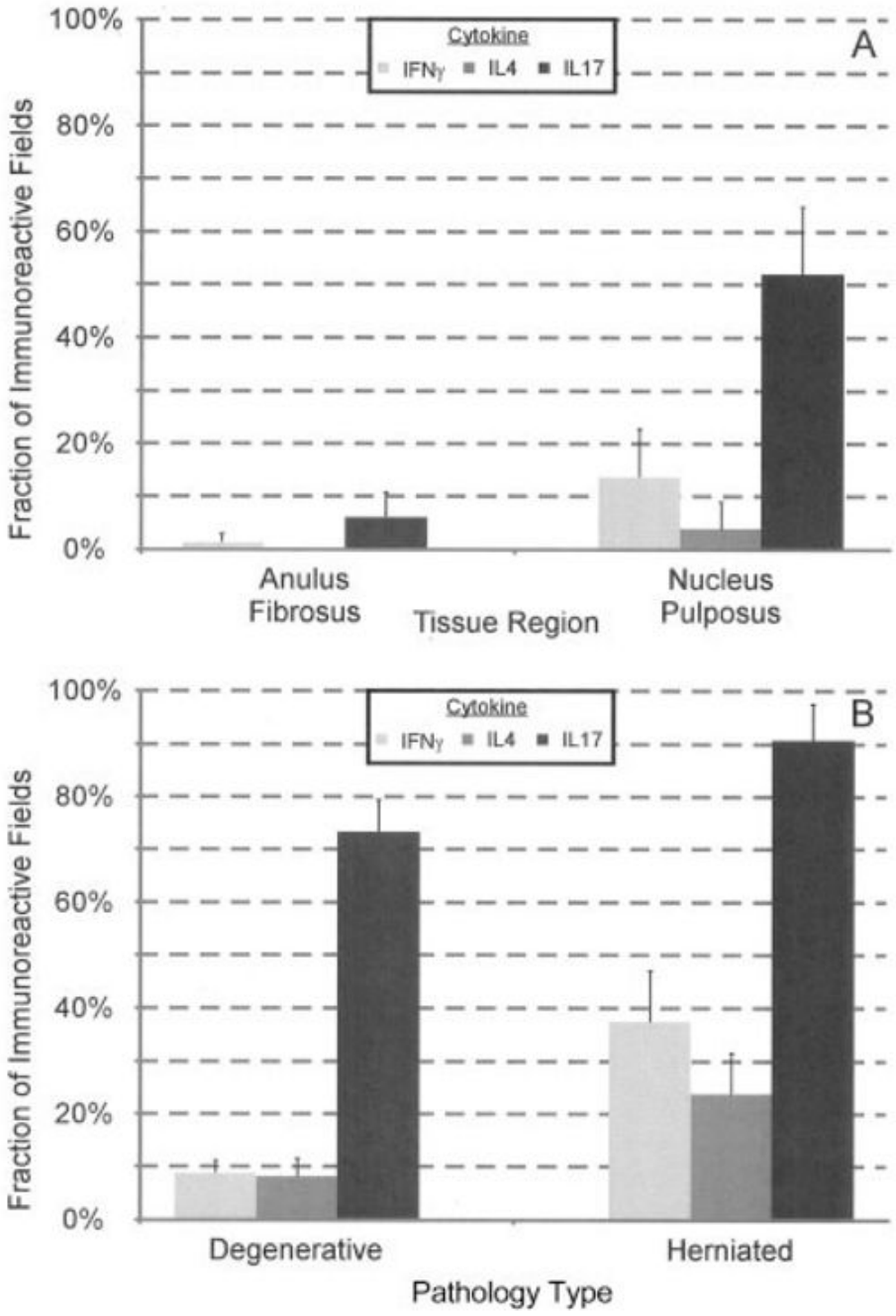

Figure 3.

Expression of lymphocyte products interferon-γ (IFNγ), interleukin-4 (IL-4), and IL-17 in nondegenerated human anulus fibrosus and nucleus pulposus autopsy control tissues (n = 8 each) **(A)** and in surgically obtained specimens from degenerated (n = 25) and herniated (n = 12) intervertebral disc (IVD) tissues **(B)**. Significantly higher expression of IL-17 was observed in nucleus pulposus tissues than in anulus fibrosus tissues ($P < 0.01$ by repeated-measures analysis of variance [ANOVA]). No difference in expression of IL-4 or IFNγ was observed between the 2 disc regions. Significantly higher expression of IFNγ and IL-4 was observed in the herniated IVD tissues than in the degenerated IVD tissues ($P < 0.05$ by repeated-measures ANOVA). While both groups had substantially more fields that were positive for IL-17, no

significant difference was observed between the two pathoanatomies. Values are the mean and SEM from 9 fields evaluated for each sample.

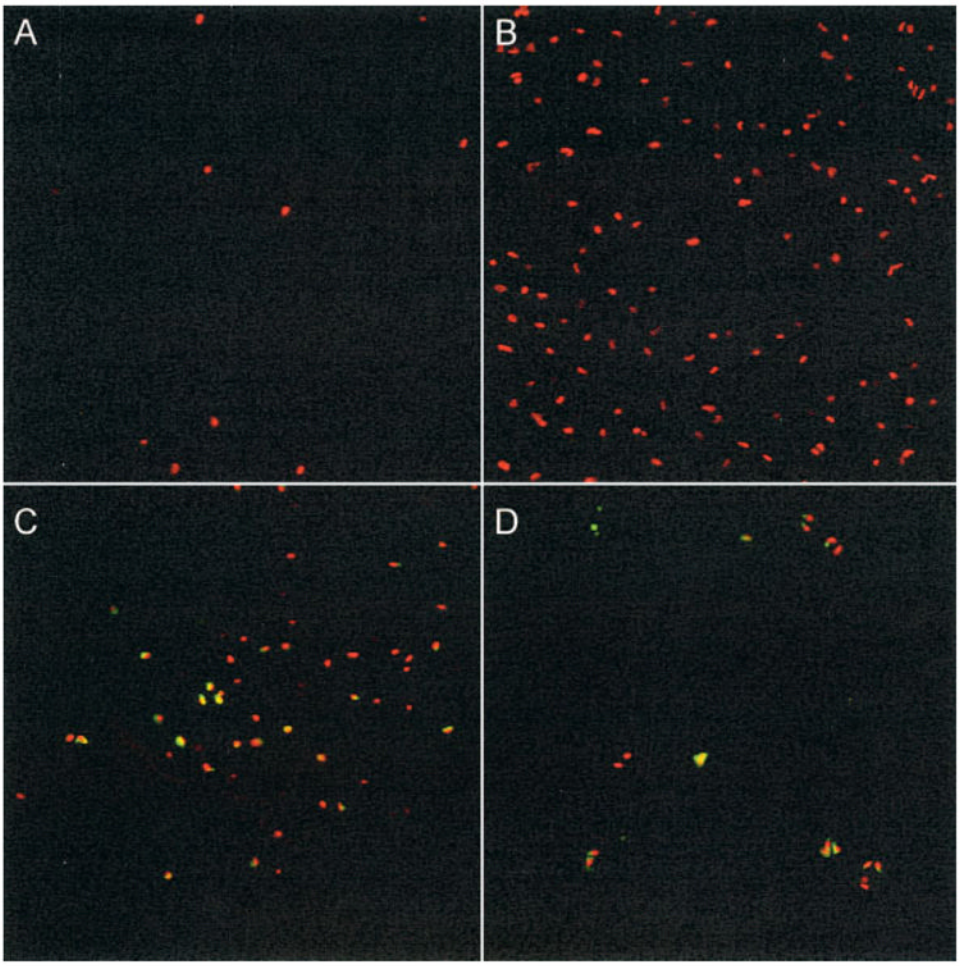

Figure 4.

Representative samples used for immunohistochemical evaluation of intervertebral disc (IVD) specimens. Scores were given for degree of cellularity and cytokine immunoreactivity, as follows: for cellularity, 0 = 0–9 cells per field, 1 = 10–49 cells per field, and 2 = 50+ cells per field; for immunoreactivity, 0 = no positive cells and 1 = at least 1 positively labeled cell. **A,** Interleukin-17 (IL-17) staining of degenerated IVD tissue with cellularity score of 0 and immunoreactivity score of 0. **B,** IL-10 staining of degenerated IVD tissue with cellularity score of 2 and immunoreactivity score of 0. **C,** CD68 staining of herniated IVD tissue with cellularity score of 1 and immunoreactivity score of 1. **D,** IL-17 staining of herniated IVD tissue with cellularity score of 1 and immunoreactivity score of 1.

Table 1

Demographic and clinical characteristics of the tissue donors*

Variable	Degenerative disc disease tissue	Herniated IVD tissue	P†	Cadaver tissue
No. of donors	9	10	–	4
No. of samples	25	12	–	8
Age, mean ± SD years	60 ± 7	32 ± 12	<0.01‡	46 ± 16
Age range, years	49–70	19–56	–	29–64
Men, %	12	33	0.18§	NA
BMI, mean ± SD kg/m^2	30 ± 6	28 ± 8	0.32‡	NA
Pain pattern, no. of samples			<0.01§	NA
Back only	15	0	–	–
Leg only	0	6	–	–
Both back and leg	10	5	–	–
NSAID use, %	64	83	0.28§	NA
Epidural steroid injection, %	48	92	<0.01§	NA

*NA = not available; BMI = body mass index; NSAID = nonsteroidal antiinflammatory drug.

†Degenerative disc disease tissue versus herniated intervertebral disc (IVD) tissue.

‡By Student's *t*-test.

§By chi-square test.

Table 2

Molecular phenotype of IVD tissue by tissue pathology or tissue region*

	Cadaver tissue			Surgically obtained tissue		
	Anulus fibrosus	**Nucleus pulposus**	***P*†**	**Degenerative disc disease**	**Herniated IVD**	***P*†**
Cellularity score‡			<0.001			<0.001
0	27	78	–	23	9	–
1	64	21	–	49	61	–
2	9	1	–	28	30	–

*Values are the percentage of measurement fields with each cellularity score for nondegenerated anulus fibrosus (n = 8) and nucleus pulposus (n = 8) autopsy control tissue samples and for surgically obtained degenerated disc disease (n = 25) and herniated intervertebral disc (IVD) (n = 12) tissue samples.

†By chi-square test.

‡0 = 0–9 cells per field; 1 = 10–49 cells per field; 2 = 50+ cells per field.