INTRODUCTION
With recent advances in imaging techniques, spontaneous regression of herniated intervertebral discs has been increasingly reported. Histologic investigations revealed the presence of inflammatory granulation in the surgically resected herniated intervertebral discs, suggesting that the inflammatory response of the host is involved in the process of herniated disc resorption. However, it remains controversial as to whether the inflammation is induced from direct chemical irritation of the nucleus pulposus material, or secondary to an autoimmune response to the nucleus pulposus. Immune response is initiated by processing and presentation of antigens by dendritic cells (DC). Thus, analysis of the DC system is fundamental to address the immune reaction. In this study, we immunohistochemically analyzed the surgical specimens of the ruptured intervertebral disc using a series of monoclonal antibodies (mAbs) for lymphocyte, monocyte, macrophage and DC markers.

MATERIALS AND METHODS
Patients and Specimens. The herniated portion of the disc was collected from 21 patients who underwent surgery for ruptured lumbar disc herniation. The patients were 11 women and 10 men with an average age of 48.3 years (range, 20-74). Disc specimens obtained from a patient with pyogenic spondylitis were also used for reference. Specimens were sliced into small pieces, immediately frozen in liquid nitrogen.

Antibodies. MAbs used were: L26 (anti-CD20, B lymphocyte), UCHL-1 (anti-CD45RO, T lymphocyte), TD4D5 (anti-CD4, T lymphocyte), TD3A2 (anti-CD8, T lymphocyte and lymphoid DC3), Kp-1 (anti-CD68, monocyte, macrophage and myeloid DC3), 2LPM19c (anti-CD11b, monocyte), KB90 (anti-CD11c, mature macrophage), WM54 (CD33, mature monocyte), NA1/34 (anti-CD1a, CD34+ cell-derived DC1), B-B20 (anti-CD40, macrophage and DC3), BB1 (anti-CD80, mature DC5), and BU63 (anti-CD86, mature DC5).

Immunohistochemistry. Thin cryostat sections were made and fixed in cold acetone, then blocked for endogenous peroxidase activity by 3% hydrogen.

Evaluation. The extent of cell infiltration was divided into none, marginal, and extensive infiltration. The reactivity to mAbs was scored on the basis of positive cell number: (-) absent, (+/-) few, (+) common, and (++) abundant.

RESULTS
Of the specimens from 21 patients with disc herniation, marginal cell infiltration was found in those from 4 patients and extensive cell infiltration from 7 patients. Also, extensive cell infiltration was found in the specimens from a patient with pyogenic spondylitis. Thus, a set of consecutive sections from these 12 patients were subjected to immunostaining. The inflammatory infiltrates in the 11 herniated discs exhibited a characteristic immunophenotype (Table 1). None of the inflammatory infiltrates contained lymphocyte (CD20, CD45RO, CD4, CD8), mature monocyte (CD33), CD34+ cell-derived DC (CD1a), or mature DC expressing co-stimulatory molecules, CD80 and CD86, whereas all the infiltrates abundantly contained CD68-positive cells. The infiltration of CD11c and CD40-positive cells varied between cases from absent to abundant. The disc sections of a pyrogenic spondylitis showed cellular infiltrates expressing various immunophenotypes except CD1a, CD80 and CD86.

DISCUSSION
Infiltration of DC in the lesion is thought to reflect the interaction between the host immune system and the antigen as observed in autoimmune diseases, tumors, and transplantation. Immunohistochemical analysis of the ruptured intervertebral discs exhibited inflammatory infiltrates in 11 out of 21 cases, which contained cells with no expression of the immunophenotypic markers of mature DC including CD1a and co-stimulatory molecules. Abundant infiltration of CD68 positive cells that lacked CD33 but various extent of CD11c and CD40 likely represents a range of differentiation from monocytes to macrophages and immature myeloid DC. These findings are consistent with the immunophenotype of chemically-induced inflammation rather than that of antigen-specific immune response.

REFERENCES