INFLAMMATION IN DISC HERNIATION MAY BE A RESPONSE TO NUCLEUS PULPOSUS CELL CYTOKINE PRODUCTION AND NOT PRIMARY INFILTRATION OF INFLAMMATORY CELLS

Introduction
It has been proposed that nucleus pulposus (NP) by itself or in combination with compression causes nerve root inflammation. Nucleus pulposus has experimentally been shown to induce increased vascular permeability, myelin changes, attraction of leukocytes and intravascular coagulation. It has also been found that TNF-α is produced by nucleus pulposus cells and may play an important role in the pathophysiological mechanisms of sciatica. The aim of this study was to clarify the inflammatory properties of NP in attracting inflammatory cells and in production of inflammatory mediators such as cytokines in normal and herniated nucleus pulposus.

Materials and methods
Series 1: In an autologous experimental model in rats disc herniation was created by disc puncture at L4-L5 level in 5 rats, causing the nucleus pulposus to leak out to the epidural space. Five rats served as control animals and surgery was performed in the same way except for the disc puncture. The rats were anaesthetized after 7 days and the exposed nerve root was harvested. Immunohistochemical staining with antibodies against ED 2 (marker for macrophages), HIF 48 (marker for granulocytes), OX 19 (marker for T-lymphocytes) and OX 39 (marker for activated macrophocytes) was performed.

Series 2: Nucleus pulposus was harvested from pig lumbar discs and the cells were separated and cultured. After 3-4 weeks the cells were fixed and the presence of TNF-α, IL-1β, IL-6, IFN-γ were determined by immunohistochemistry.

Series 3: Intervertebral disc material was obtained from 7 patients (age: 28-75 years) without any history of sciatic pain. One disc was obtained from each patient. Herniated disc material was obtained from 9 patients with sciatica undergoing discectomy. The presence of TNF-α, IL-1β, IL-6 and IFN-γ was determined by immunohistochemistry of all the specimens.

The study was approved by the Human Research Ethics Committee and the Animal Care Committee of the University of Gothenburg, Sweden.

Result
Series 1: Macrophages were present in the nerve root in 4/5 of the nucleus pulposus exposed rats and in 4/5 in the control animals. Granulocytes were present in the nerve root or outside the nerve root in 3/5 and 2/5 of the NP and control animals respectively. T-lymphocytes and activated lymphocytes were present in 1/5 and 0/5 of the NP animals and neither of these two antibodies were found in the 5 control animals.

Series 2: TNF-α, IL-1β, IL-6 and IFN-γ were present in more than 95 % of nucleus pulposus cells in culture cell.

Series 3: In the normal intervertebral discs, positive staining for TNF-α was present in the cells in 6/7 individuals. IL-6 and IFN-γ staining was present in disc cells from all the patients. IL-1β was found in or around NP cells in 4/7 patients.

In the herniated disc material IL-6 and IFN-γ was found in all nine patients, TNF-α was found in cells in 7/9 patients and IL-1β was present in cells or in the surrounding of NP cells in 8/9 patients.

Discussion
This study showed that the number of inflammatory cells in the nerve root, DRG and surrounding tissue in a rat model after NP exposure was not markedly different from control animals. In both the NP and control animals macrophages were the most common inflammatory cells. Macrophages were present in 80 % of the animals, while granulocytes and lymphocytes were sparsely found in both groups. The data also showed that nucleus pulposus cells from pigs in cell culture, cells in normal human intervertebral discs and herniated disc material produce cytokines without previous stimulation. TNF-α, IL-6 and IFN-γ was clearly present in nucleus pulposus cells in culture and in normal intervertebral discs. IL-1β was present in a lower number of cells in the human intervertebral discs and also more diffuse in the cultured NP cells than the other cytokines. In the herniated disc material no obvious differences between the four investigated cytokines in distribution or percentage of cells that stained positive were found.

These findings may suggest that the inflammatory reaction seen in and around nerve roots both in patients and experimental models of disc herniation may be related to chemically induced reactions rather than an activation of the immune system. This is in agreement with previous studies where the number of macrophages in patients with disc herniation is only slightly increased and other inflammatory cells are rare. In an experimental pig model the treatment with doxycyclin (which blocks TNF-α, IL-1β and IFN-γ) has been shown to prevent the effects of nucleus pulposus on nerve conduction velocity. These results suggest that the findings of cytokine producing cells in nucleus pulposus may be relevant in the pathophysiological mechanisms of sciatica. However, other substances may also contribute to the induced nerve fiber injury and vascular changes either as primary initiators or in response to one or several cytokines produced by NP cells.

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References

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