Spontaneous Regression Mechanisms of Lumbar Disc Herniation

Role of apoptosis and macrophages during disc tissue resorption

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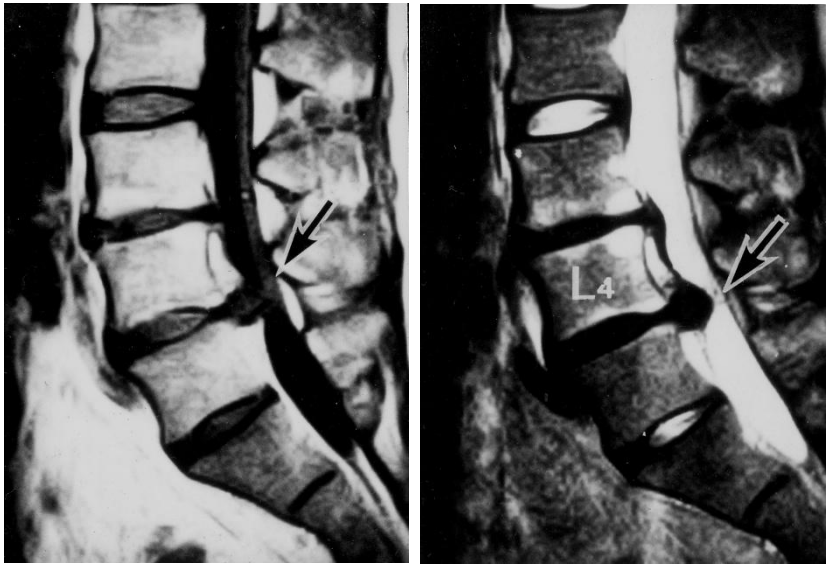
Introduction

- Spontaneous regression of lumbar disc herniation has already been demonstrated by diagnostic imaging with tools such as MRI.

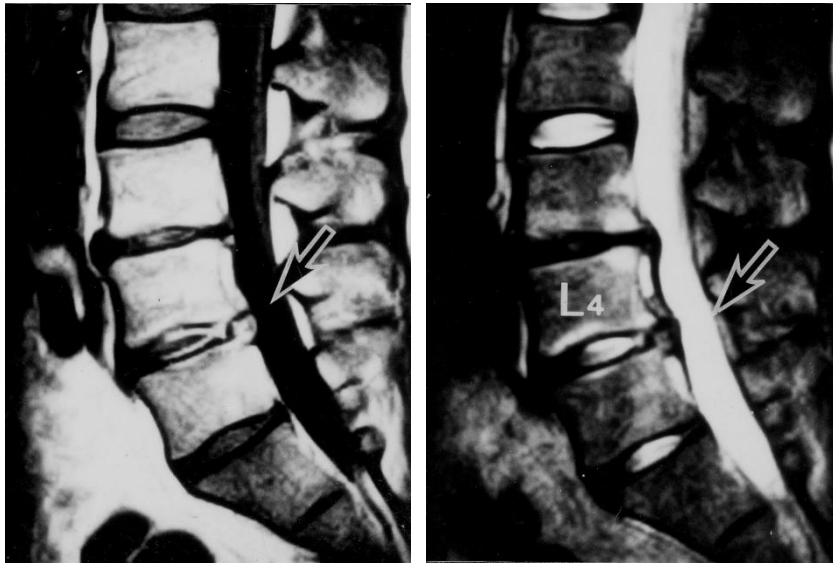
at first examination



T1-W after Gd-DTPA injection

T2-W

after 3months



T1-W after Gd-DTPA injection

T2-W

- The inflammatory response around herniated tissue in the epidural space is believed to play a major role in the spontaneous regression of herniated lumbar disc.

Following recent advances in diagnostic imaging techniques such as magnetic resonance imaging (MRI), reports have appeared on the spontaneous regression of lumbar disc herniation. These studies have highlighted the role played by the inflammatory response around the hernia in spontaneous regression. However, much remains unclear about the mechanism responsible for spontaneous regression of lumbar disc herniation. Particularly little is known about the function of macrophages, which appear to play a role in the breakdown and phagocytosis of the extracellular matrix in herniated tissue, and the fate of chondrocytes. In the present study, we examined surgical lumbar disc herniation specimens by light and electron microscopy, and investigated the localization and function of macrophages using immuno-histochemical techniques, and the presence of apoptotic cells in herniated tissue using terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL).

Patients and Methods

Thirty-nine patients were studied who showed appreciable enhancement within the herniated tissue and surrounding area on preoperative gadolinium- enhanced MRI.

Male : Female = 21 :18

Age : 18 to 76 years old
(average 38.0 years old)

- L3/4 disc : 1 disc
- L4/5 disc : 20 discs
- L5/S1 disc : 18 discs

Microdiscectomy

Intraoperative assessment of the hernia based on the classification of McCulloch et al. showed

- subligamentous extrusion (SE) in 23 patients
- transligamentous extrusion(T) in 8 patients
- sequestration(S) in 8 patients

↓

The specimens collected during surgery were assigned for examination by either light or electron microscopy.

Examination-1 :

* Histological study :H-E stain
•Immunohistocheical study: ABC method (Vectastain)

Antisera

* Anti-human macrophage CD68 (Dako Co., code no. M0814)
* Anti-interleukin 1 (IL-1β) (CalbiochemCo.; code no. 76900105)
* Anti-inducible nitric synthase (i-NOS) (Affnity Bioreagents Inc., code no. RB130300)
* Anti-human matrix metalloprothease-3(MMP-3) (Lot No. ISO 6, Fuji Chemical Industries, LTD.)

Examination-2

* Electron Microscopical Study

Pre-fxation: 2.5%-glutaraldehyde
Post-fxation : Osmic acid
Infiltration : QY-1
Embedding : Epoxy resin

Examination-3

* TUNEL Method

[Apop tag kit (Oncor)]

1. Deproteinization : Proteinase K (20μg/ml)
2. Removal of endogeneous peroxidase: 2%H_2O_2
3. Application of equilibration buffer
4. Application of working strength TdTenzyme
5. Application of anti-digoxigenin-peroxidase
6. Color development : 0.05% DAB

Results

* Macrophage Infiltration
[Anti-human macrophage CD68 (+)]

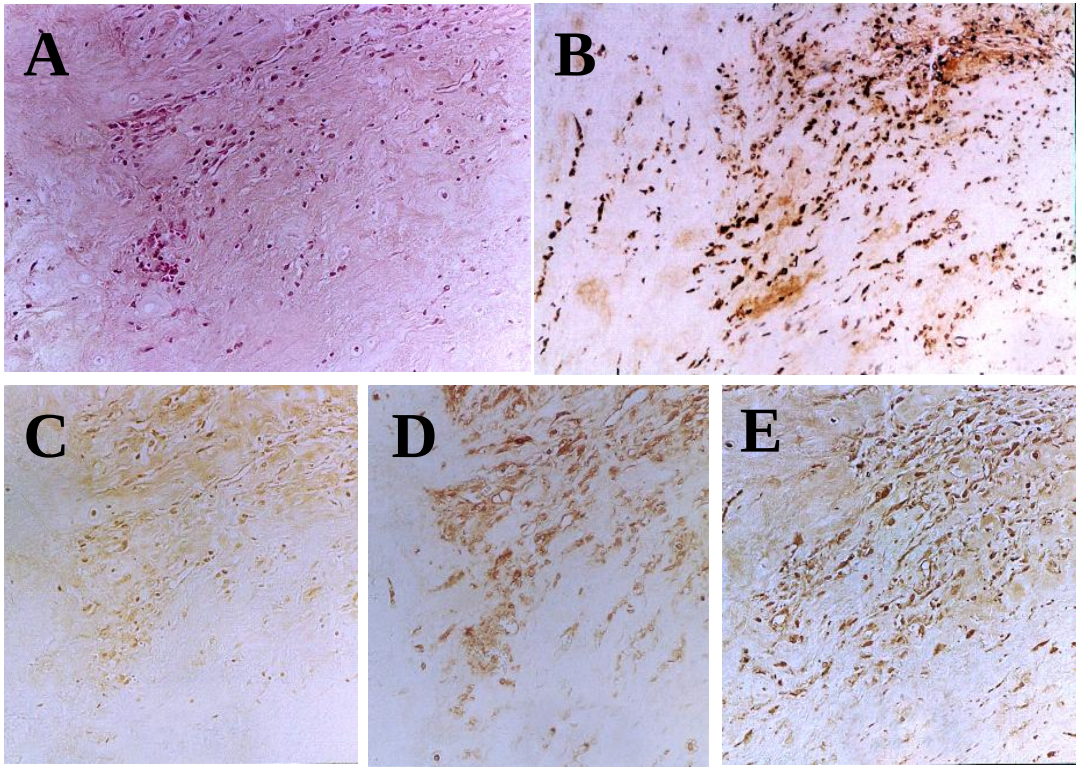


Fig.1. Histological and immunohistochemical sections of SE hernia specimen. (A) HE, (B) CD68, (C) IL-1β (D) i-NOS, (E) MMP-3.

• Comparison of the H&E-stained sections with those immunostained using CD68 showed vascularization and macrophage infiltration of the posterior longitudinal ligament in all of the patients with SE hernias (Fig.1A,B). However, vascularization and macrophage infiltration of the hernia tissue were evident in only 5 of the 23 patients (21.7%) from this group. Examination of serial sections confirmed that macrophages were positive for IL-1β (Fig1C), i-NOS (Fig1D), and MMP-3 (Fig1E). Then, whereas IL-1β and MMP-3 staining was confined within the disc cells, the staining extended to the extracellular matrix surrounding macrophages.

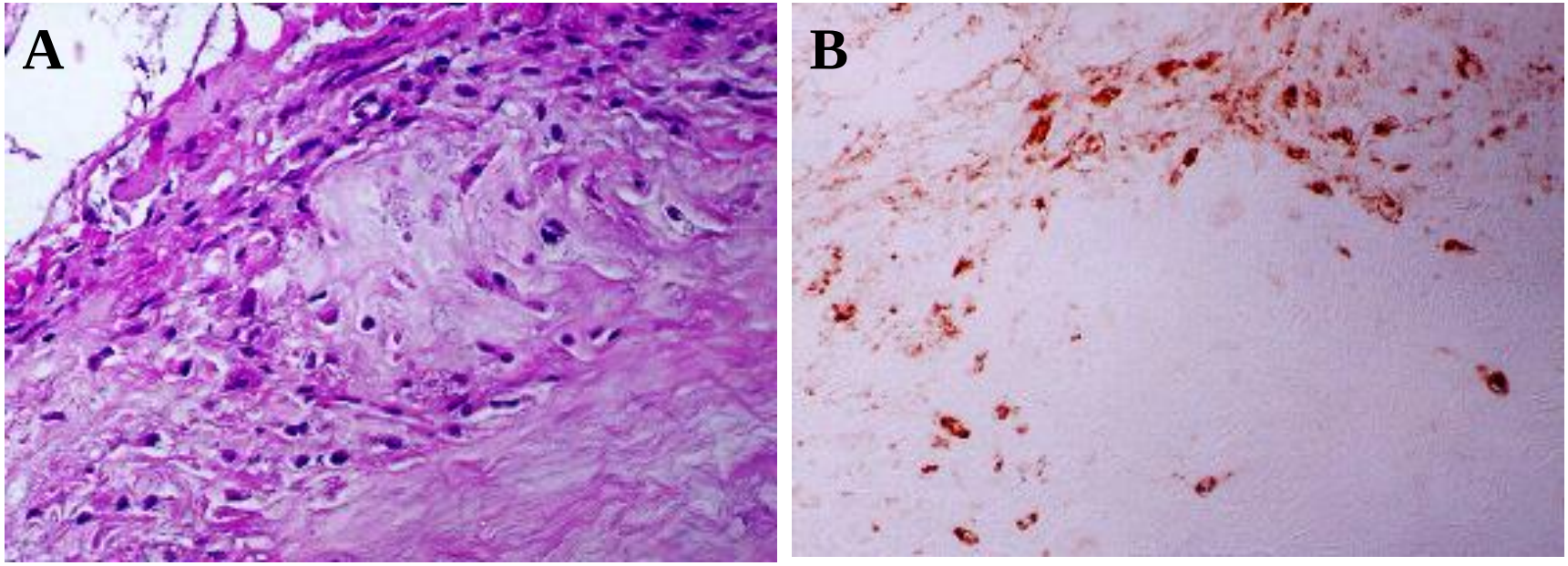


Fig.2. Histological and immunohistochemical sections of T hernia specimen. (A) HE, (B) CD68

Subligamentous extrusion (SE) :	5 / 23 discs (21.7%)
Transligamentous extrusion (TE):	8 / 8 discs (100%)
Sequestration (S)	: 8 / 8 discs (100%)

In all of the patients with T and S hernias, macrophages were seen inside the hernia tissue, with many being present around newly formed blood vessels (Fig.2) .

* Appearance of Apoptotic Cells in Hernia Tissue

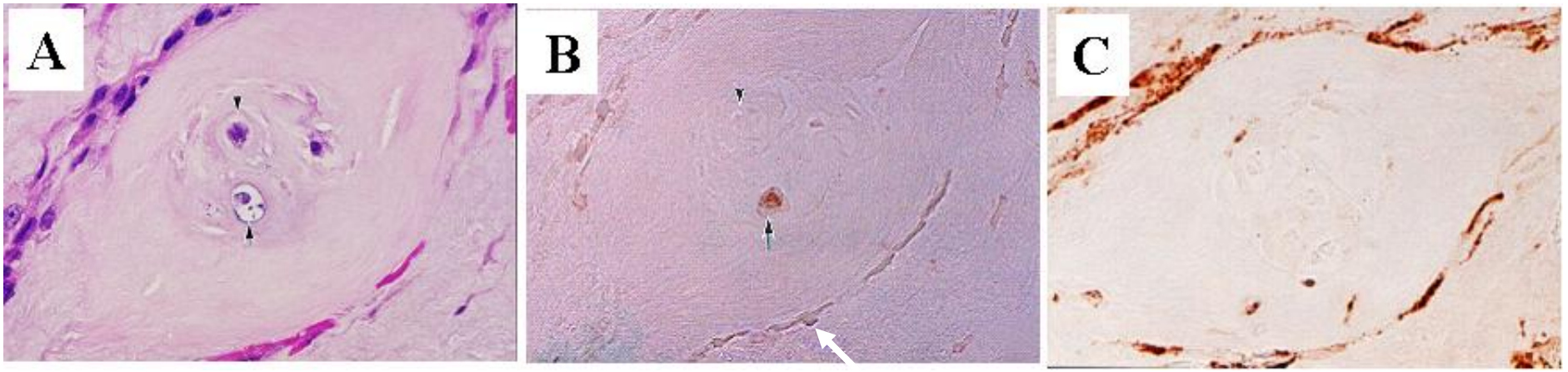
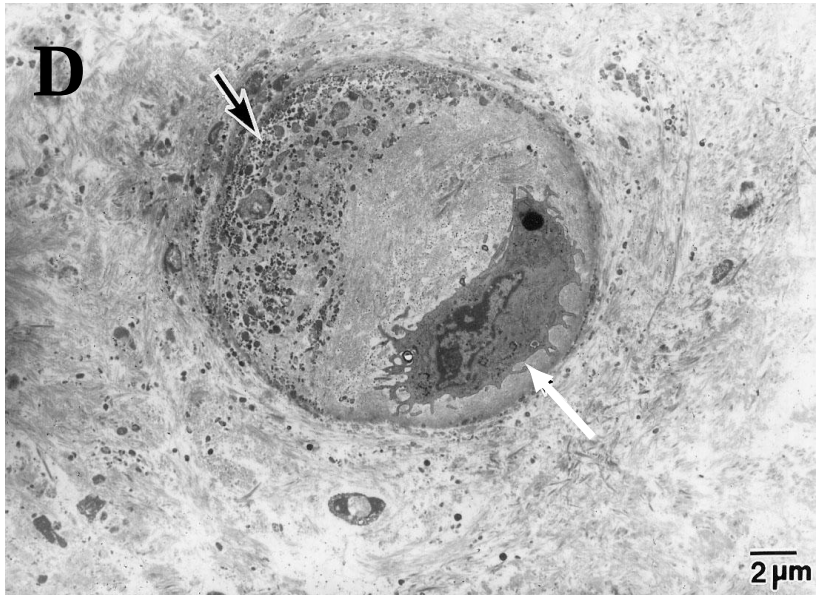


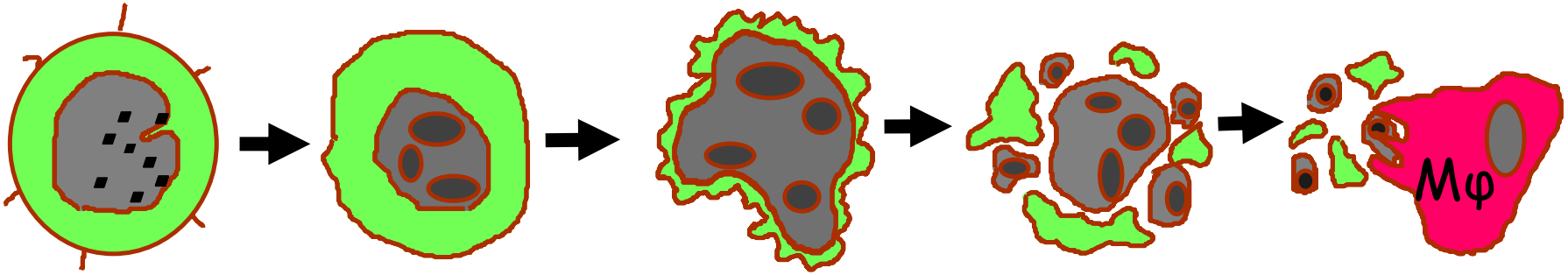
Fig.3. HE (A), TUNEL (B) and CD68 (C) sections of disc hernia specimen.

- HE and TUNEL showed apoptosis of chondrocyte-like cells in the herniated tissue (arrow). Macrophages were positive for CD68.

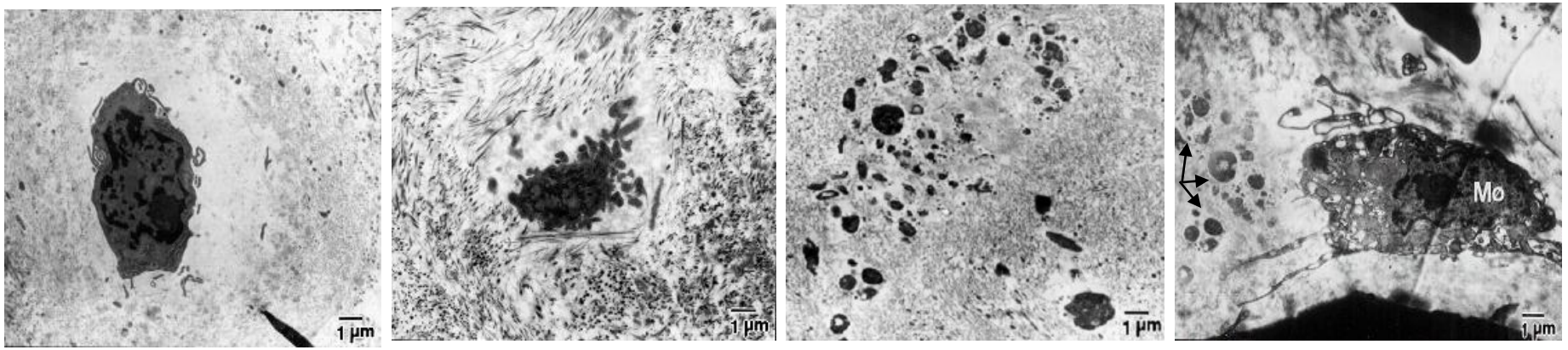


- Electron microscopy (D) showed an alive (white arrow) and apoptosis (black arrow) of chondrocyte-like cell were noted inside the lacuna.

Morphological Changes of Apoptotic Cells



Induction of apoptosis | *Chromatin conduction* | *Formation of apoptotic body* | *Fragmentation of nucleus* | *Phagocytosis*



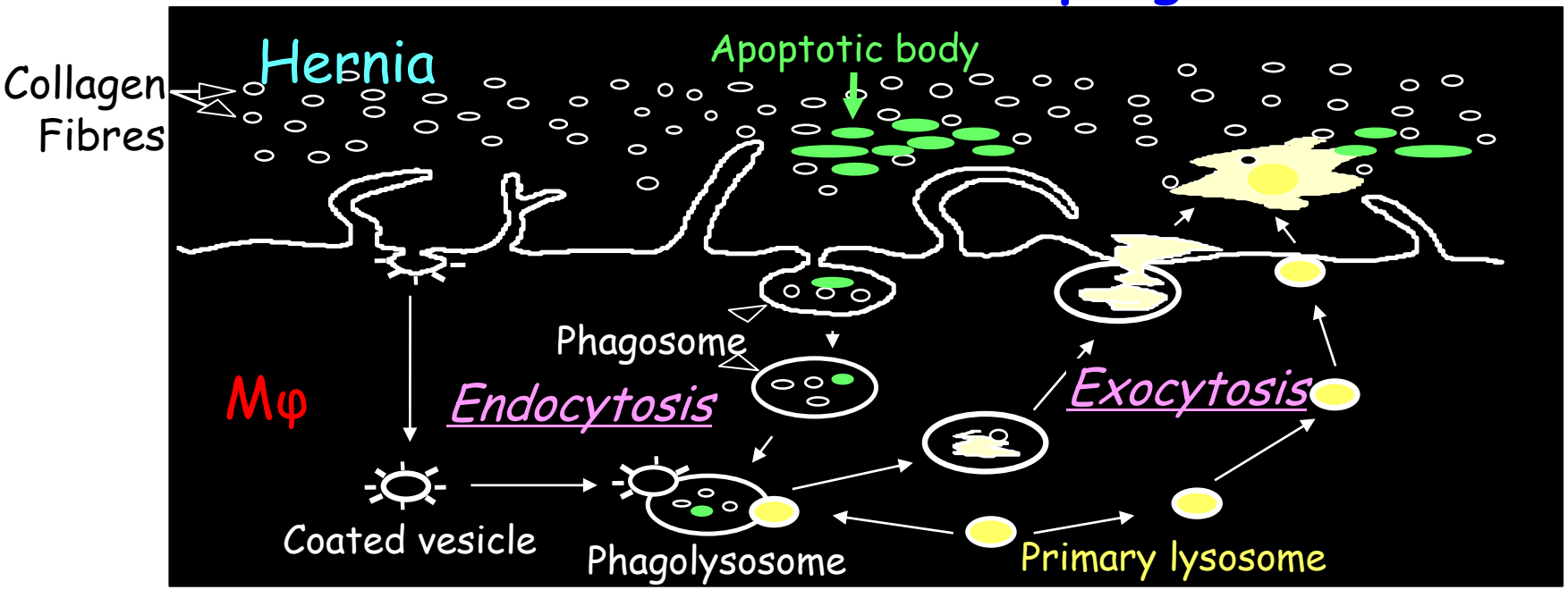
Apoptotic Changes of Disc Cells in the Hernia

- In the apoptotic cells, the cell membrane was curved, the nucleus was pyknotic, and the fragmented cells were being phagocytozed by macrophages.

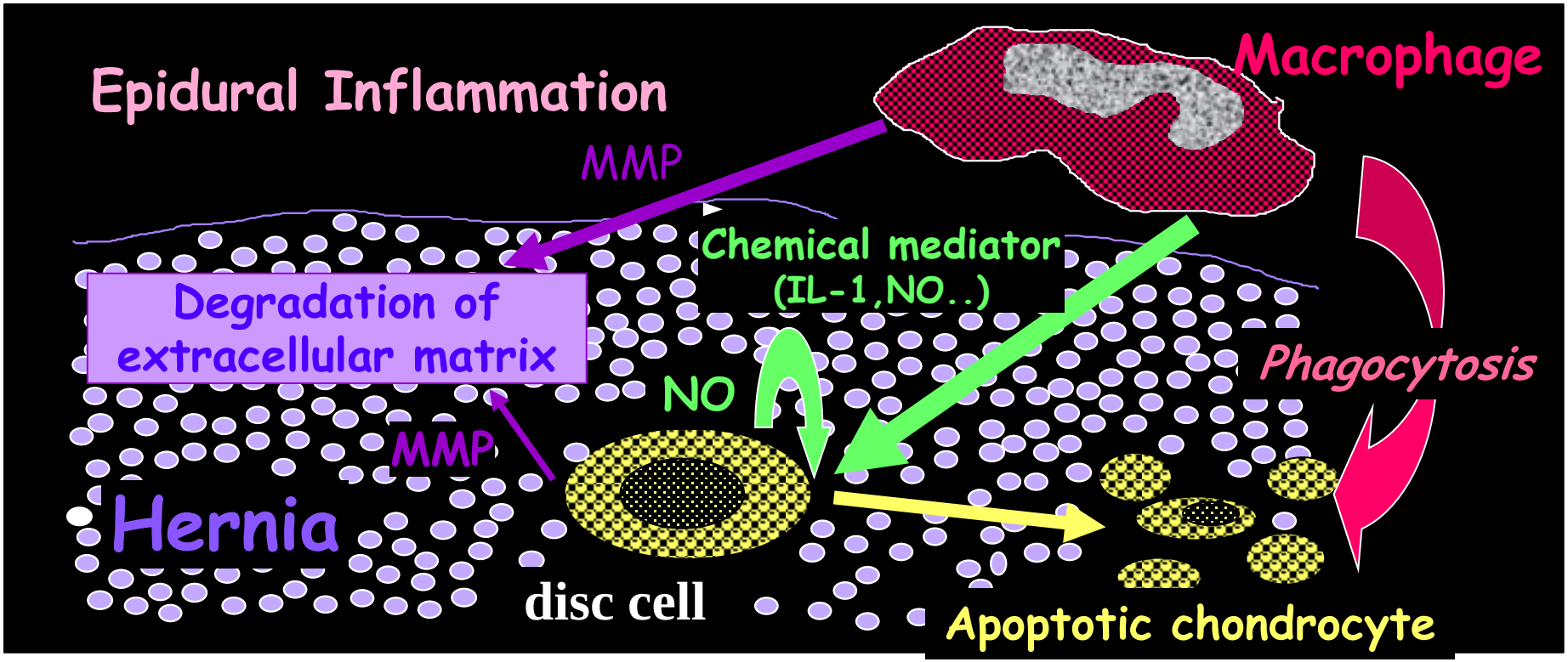
Discussion

The inflammatory response that occurs around hernia tissue in the epidural space is believed to play a major role in the spontaneous regression of lumbar disc hernias. Although many studies have investigated the mechanism of disc degeneration by assessing the chemical mediators produced by disc cells within the disc, few have focused on the chemical mediators produced by macrophages at sites of inflammation around the hernia.

Function of Macrophage



• Electron microscopy showed an abundance of macrophages around the capillaries inside the hernia tissue, and degradation and phagocytosis of collagen fibers was noted inside the hernia.



The present study showed the presence of numerous macrophages within the hernia tissue, along with newly formed blood vessels. IL-1β, i-NOS, and MMP-3 were co-expressed by these macrophages. This suggests that inflammatory cytokines, such as IL-1, and nitric oxide produced by macrophages are involved in the onset of inflammation around the hernia, and that stimulation by these chemical mediators causes the production and release in macrophages of proteases, such as MMP, which break down and digest the extracellular matrix of cartilage.

Conclusions

- The macrophages that infiltrate herniated tissue play an important role in the breakdown and removal of extracellular matrix, and in clearing away apoptotic chondrocytes, and in the process, control the immune response by producing various chemical mediators.

- In addition, the large quantity of nitric oxide(NO) released by macrophages is also thought to contribute to the apoptosis of chondrocytes.