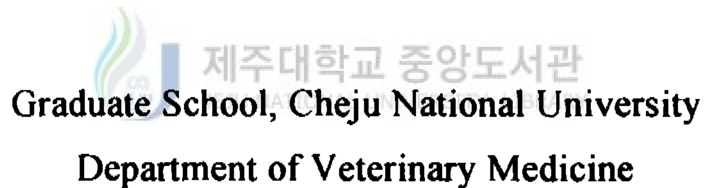
A Thesis

For The Degree of Master of Veterinary Science

Increased Expression of p53 and Bax in the Spinal Cords of Rats with Autoimmune Encephalomyelitis



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Increased Expression of p53 and Bax in the Spinal Cords of Rats with Autoimmune Encephalomyelitis

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(Supervised by Professor Hee-Seok Kim)

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Veterinary Science.

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Abstract

Increased Expression of p53 and Bax in the Spinal Cords of Rats with Autoimmune Encephalomyelitis

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To elucidate the possible role of Bcl-2, anti-apoptotic molecule, and p53 and Bax, apoptotic molecules, in the process of apoptosis in experimental autoimmune encephalomyelitis (EAE), we examined the distribution of apoptotic cells in EAE lesions by terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick end-labelling (TUNEL) and compared the expression of Bcl-2, p53 and Bax in the spinal cords of rats with EAE.

TUNEL-positive cells were usually found in the parenchyma of spinal cords of rats with EAE, and rarely found in the perivascular lesions where

most of macrophages infiltrated.

Western blot analysis showed that both p53 and Bax significantly increased in the spinal cords of EAE rats at the peak stage ($p<0.01$), and thereafter declined, but Bcl-2 did not. Immunohistochemical study showed that inflammatory cells (notably T cells) in the parenchyma express p53 and Bax, while some perivascular macrophages and brain cells, including neurons and glia, were devoid of the above molecules, and express Bcl-2.

These findings suggest that both p53 and Bax may play an important role in the elimination of T cells in the parenchyma of EAE. Furthermore, the survival of host cells, including neurons and astrocytes, and most of the inflammatory cells (mainly ED1 (+) macrophages) in perivascular lesions, may be associated with the activation of Bcl-2 in these cells.

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Key words: apoptosis, p53, Bax, Bcl-2, autoimmune encephalomyelitis.

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1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS) that is used for studying human demyelinating diseases, such as multiple sclerosis (Bonetti *et al.*, 1997; Raine, 1994). The clinical course of EAE is characterized by weight loss, ascending progressive paralysis, and finally, spontaneous recovery. These steps are matched by the inflammatory response in the CNS, which is characterized by the infiltration of T cells and macrophages, and the activation of microglia and astrocytes at the peak stage (Shin *et al.*, 1995; Moon *et al.*, 1999) Thereafter, animals with EAE were recovered the paralysis. During this process, there is a consensus that Fas-related apoptosis play an important role in the elimination of T cells (white *et al.*, 1998; Sun *et al.*, 1998)

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Programmed cell death, or apoptosis, is a fundamental biochemical process that plays an essential role in normal development and tissue homeostasis (Cohen *et al.*, 1992). Apoptosis is also utilized by the host to defend against invading cells in EAE (Schmied *et al.*, 1993; Bauer *et al.*, 1998; Kohji *et al.*, 1998).

The nuclear phosphoprotein p53 has been characterized as a growth suppressor which is expressed in normal cells (Selter and Montenarh, 1994; Mercer *et al*, 1992) and thus the activation of p53 is commonly associated with apoptosis (Eizenberg *et al.*, 1995). In case of severe DNA damage, p53

causes apoptosis, in part by promoting Bax up-regulation (Miyashita et al., 1994; Enokido *et al.*, 1996; Messemer and Brune, 1997)

Bcl-2 is an anti-apoptotic molecule that is normally expressed in neurons and cancer cells and counteract with p53 and Bax in the process of apoptosis (Reed *et al.*, 1998; Waggoner *et al.*, 1998). Bcl-2 has been identified in the inflammatory cells in multiple sclerosis lesions (Zettl *et al.*, 1998) and its animal model EAE (White *et al.*, 1998; Bonetti *et al.*, 1997), thus Bcl-2 expressing cells in the CNS continuously damage host cells via secretion of toxic molecules. Even in the possible involvement of apoptotic and anti-apoptotic molecules in the process of EAE, neurons, astrocytes and some inflammatory cells survived even though apoptotic molecules are intensely expressed in EAE lesions.

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In this study, we studied the distribution pattern of apoptosis in EAE lesions, and examined the changes of apoptosis associated molecules including, p53 and Bax, and anti-apoptotic molecule, Bcl-2, in EAE lesions. We found that both p53 and Bcl-2 is associated with the process of apoptosis of inflammatory cells and the survival of host cells in EAE lesions, respectively.

2. Materials and Methods

2.1. Animals

Lewis rats of both sexes (7-12 weeks old) were obtained from the Korea Research Institute of Bioscience and Biotechnology, KIST (Taejeon, Korea) and bred in our animal facility.

2.2. EAE induction

EAE was induced in Lewis rats with a slight modification of a previously described method (Shin *et al.*, 1995). Briefly, each rat was subcutaneously injected in the hind foot pads bilaterally with an emulsion containing an equal part of fresh rat spinal cord homogenates in phosphate buffer (mg/ml) and complete Freund's adjuvant (CFA; Mycobacterium tuberculosis H37Ra, 5 mg/ml; Difco). Control animals received either CFA only. Immunized rats were observed daily for clinical signs of EAE. Clinically, EAE was separated into five stages: grade 0, no signs; grade 1, floppy tail; grade 2, mild paraparesis; grade 3, severe paraparesis; grade 4, tetraparesis or moribund condition (Ohmori *et al.*, 1992; Shin *et al.*, 1995).

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2.3. Antisera

The following antisera were used in this study: mouse anti-p53 (Santa

Cruz, CA), mouse anti-Bax (Santa Cruz), rabbit anti-Bcl-2 (Santa Cruz), mouse anti-ED1 (Serotec, London, UK) for labeling macrophages, and rabbit anti-glial fibrillary acidic protein (GFAP) (Dakopatte, Copenhagen, Denmark) for staining astrocytes.

2.4. Tissue sampling

Tissue samples were taken on days 14 and 21 post-immunization (PI), during the peak and recovery stages of EAE, respectively. Experimental rats (n=3) in each group were sacrificed under ether anesthesia, and the spinal cords were removed and frozen in a deep freezer (-70 ℃) for protein analysis. Pieces of the spinal cords were processed for paraffin embedding after fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4.

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2.5. Western blot analysis

Frozen spinal cords were thawed at room temperature, minced, lysed in a buffer of 40 mM Tris-HCl, pH 7.4, 120 mM NaCl, 0.1% Nonidet P-40 (polyoxyethylene [9] p-t-octyl phenol) containing the protease inhibitors, leupeptin (0.5 μg/ml), PMSF (1 mM), and Aprotinin (5 μg/ml), and homogenized with a Tissue-Tearor (Biospec, USA). The homogenate was sonicated three times (5 sec at 4 ℃) and centrifuged at 14,000×g for 20 min. The supernatant was diluted with electrophoretic sample buffer to obtain a protein concentration of 3 μg/μl, and heated at 100 ℃ for 5 min. Samples

were electrophoresed under denaturing conditions in sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) using a discontinuous procedure (Laemmli, 1970). Stacking gels were 4.5% polyacrylamide and separating gels were 15% polyacrylamide. Paired mini-gels (Mini-protein II cell, Bio-Rad Laboratories, U.S.A.) were loaded with 30 μg protein per well. The protein concentration was estimated using Bradford's method (1970). After electrophoresis, mini-gels were equilibrated in a transfer buffer (100 mM Tris base, 192 mM glycine and 20% v/v methanol at pH 7.3). The proteins were then electrotransferred in the transfer buffer to a PROTRAN® nitrocellulose transfer membrane (Schleicher and Schuell, Keene N. H., USA) overnight at 4 °C and 30 Volts. The transferred proteins were visualized by staining the membrane for 10 min with Brilliant Blue R-250 (Sigma, St. Louis, USA). The nitrocellulose membrane was blocked with 5% (w/v) nonfat dry milk and 0.1% Tween 20 in Tris-buffered saline (TBS) at room temperature for 2 hrs, and later washed three times in 0.1% Tween 20/TBS (T-TBS). The p53, Bax, and Bcl-2 binding was detected by incubating the membrane in a moist chamber overnight at 4 °C, with the primary antibody (mouse anti-p53, mouse anti-Bax and rabbit anti-Bcl-2 (Santa Cruz Biotechnology) diluted with TBS-T, 1:1000). After three washed with T-TBS, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG secondary antibody (Vector Laboratories, Burlingame, CA), and diluted in T-TBS 1:2000 for 1 hr at room temperature. The membrane was then washed

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three times with T-TBS and rinsed once with TBS. Visualization was achieved using Amersham ECL reagents (Arlington heights, IL). Quantification was performed by a densitometer (M GS-700 imaging Densitometer, Bio-Rad, U.K.).

2.6. Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL)

DNA fragmentation was detected by *in situ* nick end-labeling, as described in the manufacturer's instructions (Intergen, Purchase, N.Y.).

In brief, the paraffin sections were deparaffinized, rehydrated, and washed in PBS. The sections were treated with 0.02% proteinase K (Sigma, St. Louis, M.O.) for 15 min at room temperature, and blocked with 3% hydrogen peroxide in PBS for 10 min and washed with PBS. Tissue were incubated with equilibration buffer for 5 min and reacted with TdT enzyme for 60 min at 37 °C, and then reaction was stopped in stop buffer for 15 min. Finally, sections were reacted with peroxidase-labeled anti-digoxigenine anti-body for 60 min. Positive cells were visualized using a diaminobenzidine substrate kit and counter-stained with hematoxylin.

2.7. Immunohistochemistry

Five-micron thick sections of paraffin-embedded spinal cords were deparaffinized in xylene and rehydrated by decreasing ethanol concentrations

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(100%, 95%, 80%, 70%, and 50%), ending in deionized water. Endogenous peroxidase activity was blocked by a 20 min incubation in 3% hydrogen peroxide in methyl alcohol, followed by incubation of the sections in 10mM citrate buffer (pH 6.0) for 20 min. Citrate buffer was preheated in a microwave oven until boiling. The sections were washed with distilled water and phosphate-buffered saline (PBS), and incubated with non-immune goat serum to block non-specific binding of the secondary antibody. The sections were incubated in optimally diluted primary antisera, including mouse anti-p53 (1:200), mouse anti-Bax (1:200 dilution, Santa Cruz Biotechnology) and rabbit anti-Bcl-2 (1:200) for at least 1 hr at room temperature. To determine the cell type, either rabbit anti-GFAP for astrocytes or ED1 for macrophages were applied to adjacent sections. After three washes in PBS, the sections were incubated with biotinylated anti-rabbit or anti-mouse antibody, and then with the avidin-biotin complex reagent (Vector, Burlingame, CA) and diaminobenzidine as chromogens (Sigma Co., USA). All incubations were carried out at room temperature. Slides counter-stained with hematoxylin were dehydrated and mounted in balsam (Sigma Co., USA).

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2.8 Quantitative Analysis

The immunoreactivity of p53 and Bcl-2 in the spinal cords of rats was performed as described previously (Krajewska *et al.*, 1997; Moon *et al.*, 1999)

Immunoreactivity of positive cells was expressed as negative (0), weak (1+), moderate (2+), strong (3+), very intense (4+) in the spinal cords of each group (n=3). Also, distribution of immunoreactive expression in the positive cell was expressed as nucleus (N), cytoplasm (C), both nucleus and cytoplasm (NC).

2.9. Statistical analysis

Western blot was further analyzed among groups (n=3) using a Student-Newman-Keuls Multiple Comparison's test. Differences with a *P*-value < 0.05 were considered as significant.

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3. Results

3.1. Clinical observation of EAE

The clinical course of EAE is shown in Fig. 1. EAE rats immunized with spinal cord homogenates developed floppy tail (G1), and showed severe paresis (G3) on days 14 PI (Fig. 1). All rats were recovered subsequently. Histological examination was showed that a large number of inflammatory cells infiltrated in the perivascular lesions and parenchyma of the rat spinal

cords with EAE at the peak stage (Fig. 2B). In the normal and CFA-immunized control spinal cords, no infiltrating cells were detected in the spinal cord parenchyma (Fig. 2A)

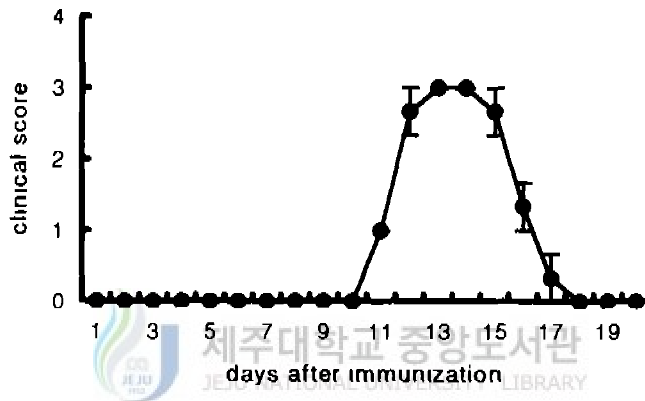

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Fig. 1. Clinical course of EAE in Lewis rats. Injecting 200mg of homogenized rat spinal cord on day 0 induced EAE. The symbol (•) indicates the mean clinical score for a group (n=3) on the day indicated.

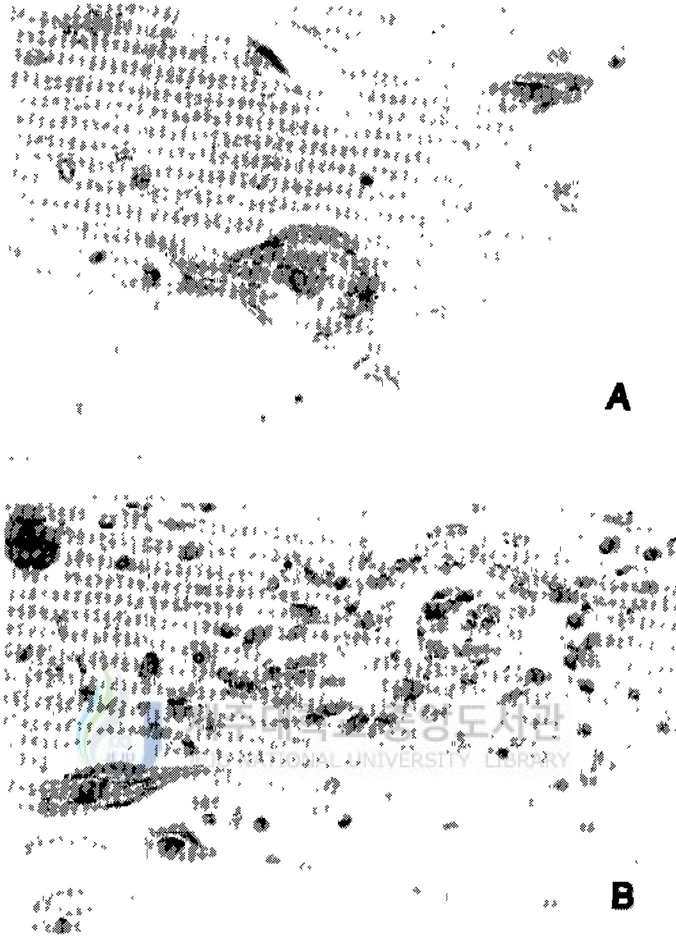

Fig 2 Histological findings in the spinal cord of rats. There were no histological changes in the normal rat spinal cord (A), while the Grade 3 EAE rat spinal cord (B) was showed intercellular edema, perivascular cuffing, and inflammatory cells (H-E stain, ×132)

3.2. Detection of apoptosis

During the peak stage of EAE, apoptotic cells were scattered throughout

the spinal cord parenchyma, but were rarely found in perivascular lesions (Fig 3A). The TUNEL reaction was barely seen in neurons and glial cells, suggesting that host cells escape death in autoimmune CNS inflammation (Table 1).

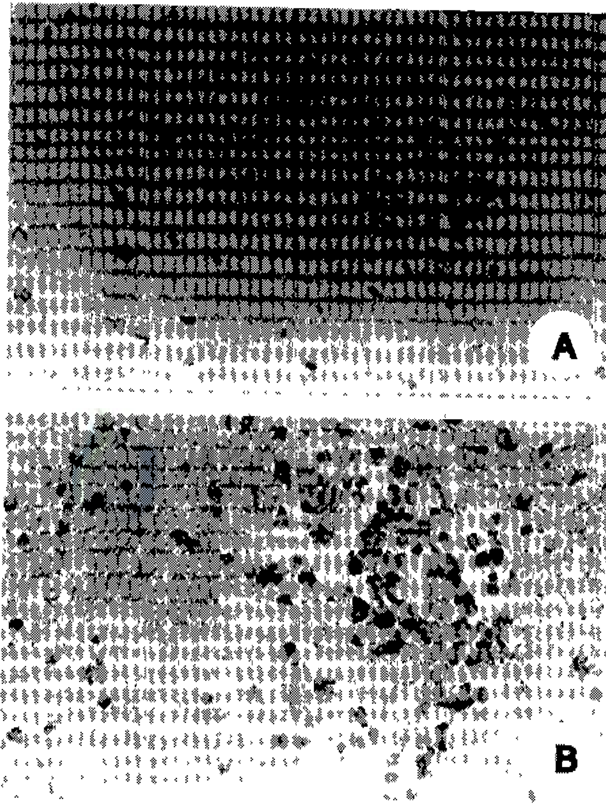

Fig 3. Localization of immunoreactivity to TUNEL (A) and to ED1 (B) in the spinal cords of rats with EAE. TUNEL-positive cells were scattered throughout the spinal cord parenchyma, but were rarely found in perivascular lesion (A) ED1-positive cells were mainly found in perivascular lesion (B), and were relatively distant from TUNEL-positive cells

A and B. counterstained with hematoxylin, Magnification, ×132

Table 1. Immunohistochemical localization of TUNEL and ED1-positive inflammatory cells in the spinal cords of rats with EAE.

	Location	Normal[a]	CFA[a]	EAE[a] (D14PI)	Recovery[a] (D21PI)
TUNEL+ cells[b]	Perivascular	–	–	+	+
	Parenchyma	–	–	+++	++
ED1+ cells[b]	Perivascular	–	–	+++	+
	Parenchyma	–	–	+	+

[a] Three animals were examined in each group by 3 blind observers.

[b] Normal and EAE spinal cord sections were analyzed using an apoptosis detection kit and immunohistochemistry using antibodies to detect specific markers. Stained sections were scored on the number of cells per field that were positive. The number of positive cells was defined in the average of 5 randomly chosen 100× fields: -, no positive cells; +, <10 cells per field; ++, <30 cells; +++, ≥30 cells.

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In serial sections, ED1 immunoreactivity was localized in the perivascular inflammatory cells during the peak stage of EAE (Fig. 3B), while vessels and neurons were negative. ED1-positive cells in the perivascular EAE lesions were mainly macrophages.

3.3. Western blot analysis of p53, Bax, Bcl-2 in EAE

Little of p53 immunoreactivity was identified in normal and adjuvant sensitized spinal cords (Fig. 4A). p53 immunoreactivity was significantly increased (about 8 fold) in the rat spinal cords with EAE at the peak stage (day 14 PI, G3) (O.D. value; 51.16±12.31, $p<0.01$), as compared with adjuvant sensitized control (O.D. value; 6.08±2.32), and declined thereafter (day 21 PI, R0) (Fig. 4A). Bax immunoreactivity was significantly increased (about 5 fold) in the rat spinal cords with EAE at the peak stage (day 14 PI, G3) (O.D. value; 33.87.08±8.65, $p<0.01$), as compared with adjuvant sensitized control (O.D. value; 6.39±1.39), and declined thereafter (day 21 PI, R0) (Fig. 4B). Contrary to p53 and Bax, Bcl-2 was slightly expressed in the normal and adjuvant sensitized spinal cords of rats, and was not significantly changed in EAE tissues (Fig. 4C).

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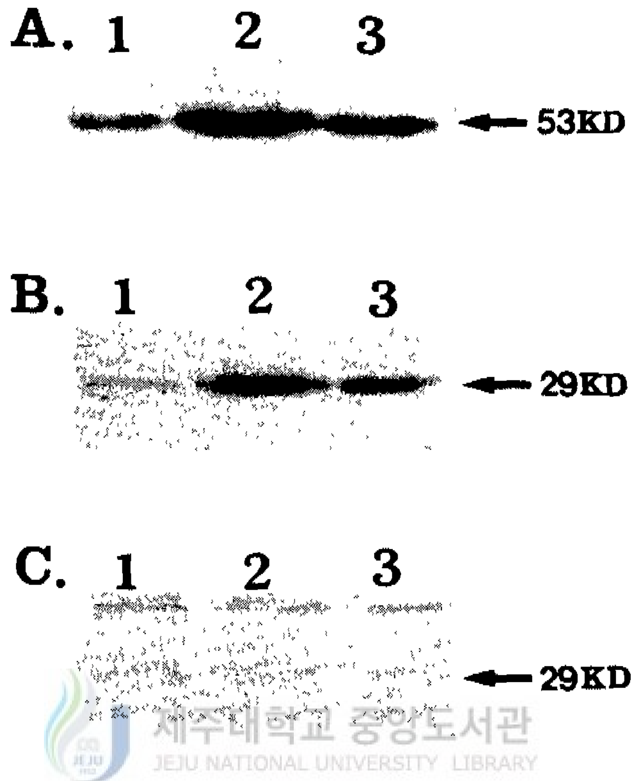

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Fig. 4. Western blot analysis of p53 (A), Bax (B), and Bcl-2 (C) in the spinal cords of rats with adjuvant-immunized control and EAE. Minimal amounts of p53 and Bax were identified in the adjuvant-immunized spinal cords (A, B, lane 1). In the spinal cords with EAE, both p53 and Bax were increased in the spinal cords of rats with grade 3 paralysis (G3) (A, B, lane 2), but its expression decreased during the EAE recovery stage (R0) (A, B, lane 3). Contrary to the apoptotic p53 and Bax, Bcl-2 was weakly expressed in the adjuvant-immunized spinal cords of rats (C, lane 1), and was not significantly changed in the spinal cords with grade 3 paralysis (G3) (C, lane 2) and recovery stage (R0) (C, lane 3) in EAE.

3.4. Immunohistochemical detection of p53, Bax and bcl-2 in EAE

p53, a pro-apoptotic protein, was weakly expressed on some host cells (especially, neurons) in the normal spinal cords (Fig. 5A, B). It was present mainly in the cytoplasm, as reported in a previous in vitro paper (Eizenberg *et al.*, 1996) (Fig. 5, A). In EAE lesions, p53 was seen in some inflammatory cells in the perivascular cuffing, as well as in the parenchyma (probably T cells), while its expression increased in neurons and glial cells in EAE lesions (Fig. 5). In adjacent section immunostained with ED1, some p53 (+) cells in the perivascular and parenchyma EAE lesions were ED1+ macrophages. Their expression appeared mainly in the nuclei, suggesting that these cells were activated (Fig. 5, C) (Table 2). In frozen section immunostained with R73 (monoclonal anti-rat T cell receptor alpha and beta), small, p53-positive cells in the parenchyma were identical to T cells (data not shown).

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Bax also was seen diffusely in some neurons and neuroglial cells in the normal rat spinal cords (Fig. 6, A, B). It was expressed on the inflammatory cells in the perivascular and parenchyma EAE lesion (Fig. 6, C) (Table 2). In an adjacent section stained with TUNEL method, a few p53 (+) and Bax (+) cells in parenchyma EAE lesions matched TUNEL (+) cells (data not shown). The intensity of Bax immunoreactivity always weaker than that of p53, and thus was not included in the immunohistochemical evaluation.

Bcl-2, which is expressed in some neurons and glial cells in the normal spinal cord, was localized diffusely in the cells of perivascular cuffing as well

as of the parenchyma, while its expression increased in neurons and some glial cells in EAE (Fig. 3, A) (Table 2).

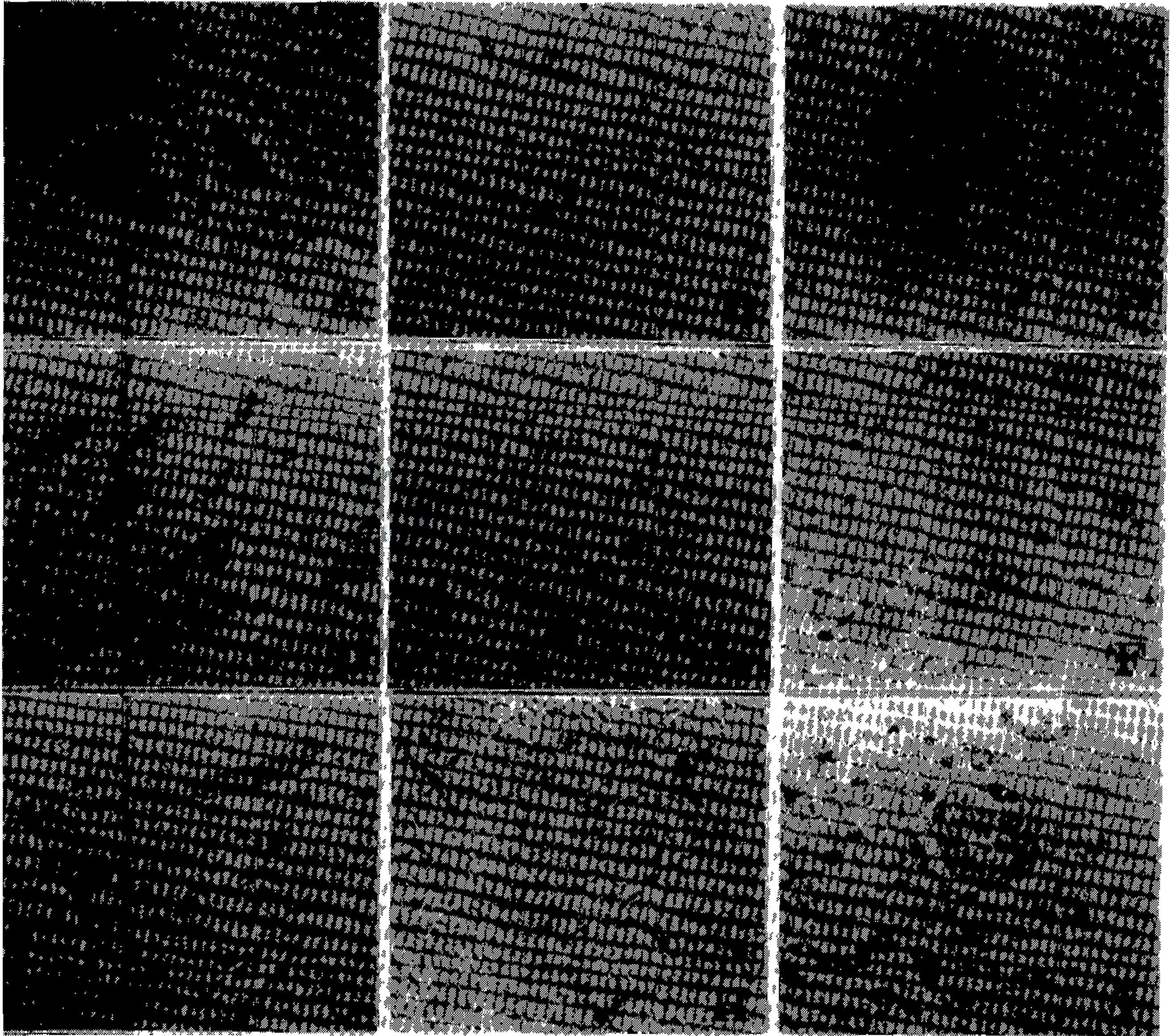

Fig. 5. Immunohistochemical detection of p53 (A-C), Bax (D-F) and Bcl-2 (G-I) in the spinal cords of rats with normal (A, D, G), adjuvant-immunized control (B, E, F), and peak stages of EAE (C, F, I). p53 (+) reaction was present mainly in the cytoplasm of neurons in the normal spinal cords of rats (A). Few p53 (+) glial cells was found in the spinal cords of rats with adjuvant-immunized control (B, arrows). p53 (+) reaction was seen on inflammatory cells in the perivascular cuffing as well as in the parenchyma, but was mainly found in the parenchyma (C, arrows), as compared with the perivascular lesions.

Bax (+) reaction was present mainly in the cytoplasm of neurons in the normal spinal cords of rats (D). Some Bax glial cells are found in the spinal cords of rats with adjuvant-immunized control (E, arrow). Bax was seen on inflammatory cells in the perivascular cuffing as well as in the parenchyma of EAE (F, arrows).

Bcl-2 was present mainly in the cytoplasm of neurons in the normal spinal cords of rats (G). Some Bcl-2 (+) glial cells are found in the spinal cords of rats with adjuvant-immunized control (H, arrows). Bcl-2 (+) cells were mainly found on inflammatory cells in the perivascular lesion (I), as compared with the parenchymal lesion.

A-I: counterstained with hematoxylin, Magnification: A-I: ×132. All tissues except normal were collected at days 13-14 post-immunization of adjuvant or brain tissue immunization.

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Table 2. Immunoreactivity to Bcl-2 and p53 in the spinal cords of rats with normal, adjuvant-immunized control and EAE.

Antibody	Cell type	Normal [a]	CFA [a]	EAE [a] (D12-14PI)	Recovery [a] (D21PI)
Bcl-2	ED1-positive inflammatory cells *	– [b] / – [c]	– / –	0 – 4+ / N, NC	0 – 4+ / N, NC
	ED1-negative inflammatory cells **	– / –	– / –	0 – 3+ / N, NC	0 – 3+ / N, NC
	Glial cells ***	0 – 3+ / N, C	0 – 3+ / N, C	0 – 3+ / C, NC	0– 3+ / C, NC
	Neurons	0 – 3+ / C	0 – 3+ / C	0 – 4+ / C	0 – 4+ / C
P53	ED1-positive inflammatory cells *	– / –	– / –	0 – 3+ / N, NC	0 – 3+ / N, NC
	ED1-negative inflammatory cells **	– / –	– / –	0 – 4+ / N, NC	0 – 4+ / N, NC
	Glial cells ***	0 – 2+ / N, C	0 – 3+ / N, C	0 – 3+ / C, NC	0 – 2+ / C, NC
	Neurons	0 – 2+ / C	0 – 2+ / C	0 – 2+ / C	0 – 2+ / C

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[a] Three animals were examined in each group.

[b] The intensity of immunoreactive cells is expressed as negative (0), weak (1+), moderate (2+), strong (3+), very intense (4+) of positive cells in the spinal cords of each group.

[c] Distribution of expression in the cell. N, nucleus; C, cytoplasm; NC, both nucleus and cytoplasm.

* ED1-positive inflammatory cells are macrophages/activated microglia.

** ED1-negative cells are inflammatory cells (probably T cells) except ED1-positive macrophages/activated microglia.

••• Glial cells imply all unidentified non-neuronal cells in the parenchyma, which may include GFAP (+) astrocytes, oligodendrocytes and inactivated microglia.

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4. Discussion

This study reports for the first time that some survival genes, including Bcl-2, rescue brain cells from apoptosis in the course of EAE, and some apoptotic genes may regulate cell apoptosis. In contrast to the brain cells, invading inflammatory cells underwent apoptosis through the lack of Bcl-2 and the intense increase of apoptotic molecules, including p53 and Bax.

The tumor suppressor gene p53, which is able to induce growth arrest or apoptosis in DNA-damaged cells, is one of the well-known apoptotic molecules in many cultured cells. Although all cells expressing p53 do not undergo apoptosis, a lot of apoptotic cells showed nuclear localization of p53 suggesting that this molecule may be activated. In this study, we found that some brain cells, which were not apoptosis, expressed p53 in the cytoplasm, while some inflammatory cells showed nuclear staining of p53 and were positive for TUNEL. If p53 increase in the cells *in vitro* or *in vivo*, Bax, one of the other death signals, will increased and induced apoptosis of the cell (Miyashita *et al.*, 1994; Selvakumaran *et al.*, 1994). In this study, Bax immunoreactivity was shown the similar pattern of p53.

Bcl-2 is an anti-apoptotic molecule that allows neuronal cells to survive *in vitro* (Melkova *et al.*, 1997). It is known as an anti-apoptosis factor. Consistent with previous findings, multiple sclerosis lesions have been to contain Bcl-2 expressing T lymphocytes, which may continuously damage the

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brain tissues (Zettl *et al*., 1998). Furthermore, Bcl-2 expressing oligodendroglia and astrocytes do not undergo apoptosis in EAE (Bonetti *et al*., 1997). In this study, we postulate that increased expression of Bcl-2 mediate the survival of neurons and some glial cells. It is one possible reason that brain cells, including neurons and glial cells, survive autoimmune injury in the CNS. Our finding that some inflammatory cells do not undergo apoptosis in perivascular lesions, suggests that in these cells, during the peak stage of EAE, the anti-apoptotic Bcl-2 predominate, rather than the death signals. Similar findings are consistently identified in cancer cells (Waggoner *et al*., 1998) and multiple sclerosis lesions (Bonetti and Raine, 1997). Our findings are further supported by the observation that effector cells, such as oligodendroglia expressing many death signals including Fas, do not undergo apoptosis in the murine EAE model, while homing inflammatory cells are selectively vulnerable to the death signals associated with apoptosis (Bonetti *et al*., 1997).

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In conclusion, we suggest that Bcl-2 may play an essential role in the survival of host cells, as well as most of ED (+) perivascular inflammatory cells in autoimmune CNS inflammation, while the inflammatory cells in the parenchyma may undergo apoptosis through the contribution of p53 and Bax.

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초 록

자기면역성뇌척수염에서 apoptosis 유발인자 p53와 apoptosis 억제인자 Bcl-2의 발현

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면역학적으로 격리된 부위로 알려진 뇌에서 침입된 이물질이 어떤 과정을 거쳐 제거되는지를 규명하기 위하여 랫트에 자기면역성뇌척수염 (Experimental autoimmune encephalomyelitis, EAE)을 유도한 후 척수조직을 면역조직화학적으로 검사하였다.

Apoptosis 확인은 terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick end-labelling (TUNEL) 방법을 이용하여 EAE의 병변에서 apoptotic cell의 분포를 조사하였다 Apoptosis에 관여하는 인자 p53 과 Bax, 그리고 apoptosis 억제인자인 Bcl-2는 Western blot을 통해 양적 변화를 조사하였고 동시에 면역조직화학적 검사를 통해 발현 세포를 확인한 후 연속절편에서

Apoptosis 양상과 비교 검토하였다

EAE에 이환된 랫트의 척수조직에서 TUNEL양성의 apoptosis세포는 대부분 실질내에 관찰된 반면 다수의 ED1양성의 macrophage가 침윤된 혈관 주위에는 매우 드물게 확인되었다

Western blot 결과 p53과 Bax는 대조군에 비해 심한 마비기 (EAE, G3)에 발현이 유의성 있게 증가하였고 (p<0.01), 마비에서 회복되면서 이들의 발현은 감소하였다 그러나 Bcl-2는 대조군에 비해 마비기에도 유의성 있는 변화는 보이지 않았다

면역염색결과, p53과 Bax의 발현은 대부분의 실질내 염증세포 (대부분의 T-cell)에서 발현하였고, 혈관주위의 macrophage 와 brain cell (neuron, glial cell)에서는 매우 드물게 확인되었다 그러나 Bcl-2는 혈관주위의 염증세포 (주로 macrophage)와 neuron 및 glial cell 에서도 발현하였다

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이상의 결과를 종합해 볼 때, apoptosis를 유도하는 p53과 Bax는 EAE에 이환된 랫트의 척수조직에서 염증세포(특히, T-cell)의 apoptosis에 깊게 관여할 것으로 추정된다. 동시에 신경세포 및 혈관주위의 일부 염증세포들은 Bcl-2등의 apoptosis 억제인자의 활성화로 인해 죽지않고 보호될 것으로 생각된다

주요어. apoptosis, p53, Bax, Bcl-2, 자기면역성뇌척수염

감사의 글

이 결실을 얻을 수 있도록 물심양면으로 도와주시고, 걱정해주신 부모님께 감사드리며, 저에게 학문적 가르침 뿐만아니라, 성인으로서의 도리를 가르쳐 주신 신태균 교수님께 감사드립니다. 그리고, 저의 논문심사 뿐만아니라, 항상 깊은 이해와 격려로써 지도해주신 김희석 교수님과 위명복 교수님께 깊이 감사드립니다 또한, 정성으로 저의 논문을 심사해주신 일본 동경도립신경과학연구소 Yoh Matsumoto 박사님께 감사드립니다.

제가 수의학이란 학문을 접하며 지금까지 저에게 많은 가르침을 주신 박전홍 교수님, 배종희 교수님, 이경갑 교수님, 임윤규 교수님, 이영재 교수님, 이두식 교수님, 정종태 교수님, 우호춘 교수님께 감사드리고, 이 논문에 조언과 격려를 아끼지 않으신 일본 동경도립신경과학연구소 지영흔 박사님과 그 동안 저에게 과분한 격려와 성원을 주신 제주경마장 이용덕 진료과장님, 한림대학교 환경생명과학연구소 진재광 선생님, 일본 농공대학 김승준 선생님께 진심으로 감사드리며, 대학원 생활을 활기차고 열심히 할 수 있도록 도와준 용철이 형, 성선, 김정훈 조교, 김정훈 수의사와 경헌, 성진, 종훈, 창근, 태철, 실험실 후배들, 종철, 미정, 광협, 희철, 경숙, 대원, 도현, 영승, 지윤, 상우에게도 감사의 마음을 전합니다

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문 창 종

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