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A potential role of Schwann cells in spinal nerve roots in autoimmune central nervous system diseases

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Abstract：The expression of nestin and vimentin in the spinal nerve roots of rats with experimental autoimmune encephalomyelitis (EAE) was studied to ascertain whether Schwann cells in the peripheral nerves respond to acute central nervous system autoimmune injury. Immunohistochemistry demonstrated that nestin was constitutively expressed in the dorsal roots of spinal nerves in control rats; its expression was enhanced in the spinal nerve roots of rats with EAE. Vimentin expression was weak in control rat spinal nerve roots, and it was increased in the dorsal roots of rats with EAE. It is postulated that normal animals have multipotent progenitor cells that constitutively express nestin and vimentin in the spinal nerve roots. In response to an injury of the central nervous system, these multipotent Schwann cells are activated in the spinal nerve roots through the expression of the intermediate filament proteins vimentin and nestin.

Key words : autoimmune encephalomyelitis, Schwann cells, nestin, vimentin

Introduction

The expression of intermediate filaments has been regarded as a marker of multipotent progenitor cells that can give rise to a variety of cells, including glial cells and neurons [14]. Three intermediate filaments, such as nestin, vimentin and glial fibrillary acidic protein (GFAP), are present in specific cell types in the CNS, particularly in reactive astrocytes [1, 2]. Nestin and vimentin are the main intermediate filaments in immature glial cells, whereas maturing astrocytes contain vimentin and GFAP [5]. The replacement of nestin by vimentin and GFAP occurs during the maturation or differentiation of multipotent neural precursors into astrocytes or neurons, particularly during embryonic development [14].

In our previous study, we found that astrocytes and ependymal cells constitutively express nestin and vimentin in normal spinal cords and that the number of nestin- and vimentin-positive cells increases in response to central nervous system (CNS) injury caused by experimental autoimmune encephalomyelitis (EAE) [13].

EAE is an autoimmune disease of the CNS in rats that is used to model human demyelinating diseases such as multiple sclerosis [10]. The histopathological lesions in rat EAE are characterized by cellular infiltration of the subarachnoid space, perivascular cuffing in the spinal cord parenchyma, and focal demyelination in the root entry zone during the acute stage [9, 12]. During the recovery stage of EAE, inflammatory cells are eliminated through apoptosis [6, 7, 11], and the demyelinated lesions are remyelinated by adjacent myelin-forming cells of the CNS (oligodendrocytes) or peripheral myelin-forming cells (Schwann cells) from spinal nerves [9]. We asked whether Schwann cells in the spinal nerve roots, which are derived from the neuroectoderm, are affected by spinal cord inflammation, such as the focal demyelination that occurs in EAE [12].

In this study, we re-examined the expression of nestin and vimentin in the spinal nerve roots of Lewis rats with EAE in order to evaluate the capacity of Schwann cells to become activated during autoimmune inflammation of the CNS.

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Materials and Methods

Animals

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

EAE induction

EAE was induced in Lewis rats using a slight modification of a previously described method [6, 12]. Briefly, each rat was injected subcutaneously and bilaterally in the hind footpads with an emulsion containing equal parts guinea pig myelin basic protein in phosphate buffer (1 mg/m*l*) and complete Freund's adjuvant (CFA; *Mycobacterium tuberculosis* H37Ra, 5 mg/m*l*) (Difco, Detroit, MI). Control animals received 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 [G.1], floppy tail; grade 2 [G.2], mild paraparesis; grade 3 [G.3], severe paraparesis; grade 4 [G.4], tetraparesis or moribund condition) [6, 12]. All experiments were carried out in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*.

Tissue sampling

Tissue samples were taken on days 14 and 21 post-immunization (PI), during the peak and recovery stages of EAE, respectively. Rats in each experimental group (n = 5) were sacrificed under ether anesthesia, and the spinal cords were removed. Portions of each spinal cord were processed for paraffin embedding after fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS) at pH 7.4.

Immunohistochemistry

Sections (thickness, 5 μm) of paraffin-embedded spinal cords with spinal nerve roots were deparaffinized and treated with 0.3% H_2O_2 in methyl alcohol for 20 min to block endogenous peroxidase. The sections were exposed to normal goat serum, and then incubated for 1 h at room temperature with one of the following optimally diluted primary antisera: mouse anti-nestin (clone Rat 401; Chemicon International, Temecula, CA, U.S.A.), mouse anti-vimentin (clone V9; Lab Vision Corp., Fremont, CA, U.S.A.), and rabbit anti-GFAP (Dako, Copenhagen, Denmark). To distinguish macrophages in the CNS, mouse monoclonal anti-rat activated macrophage (ED1; Serotec, London, U.K.) [4] was applied to adjacent sections. After three washes, the sections were incubated with the appropriate biotinylated second antibody, followed by formation of the avidin-biotin peroxidase complexes using the Elite kit (Vector, Burlingame, CA). Peroxidase was developed with a diaminobenzidine substrate kit (Vector). Before mounting, the sections were counterstained with hematoxylin.

Results

The clinical course and histopathological findings of EAE were precisely described in our previous report [13]. In this report, we focused on the expression of

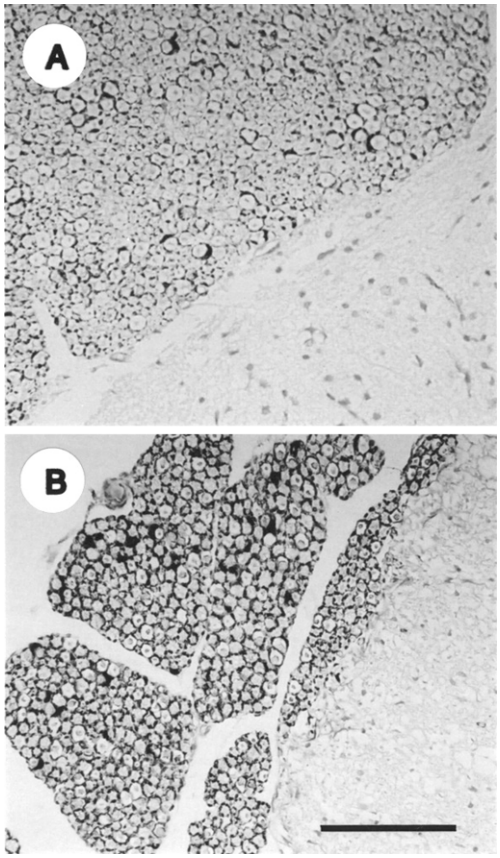

Fig. 1. Immunostaining for nestin in the dorsal roots of spinal nerves of CFA-immunized control rats (A) and rats with EAE (day 14 P.I.) (B). Nestin immunostaining was found in the Schwann cells in control dorsal roots (A) and was enhanced in the Schwann cells of rats with EAE (B). Counterstained with hematoxylin. Scale bar = 100 μm.

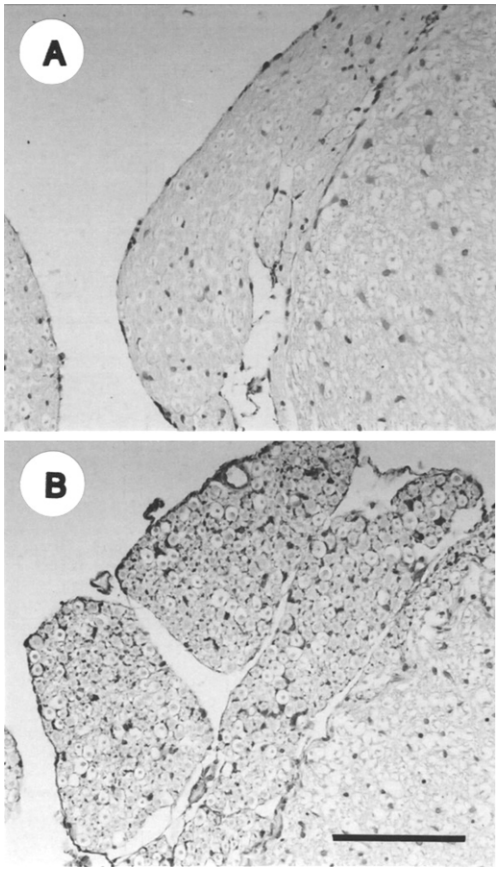

Fig. 2. Immunostaining for vimentin in the dorsal roots of spinal nerves of CFA-immunized control rats (A) and rats with EAE (day 14 P.I.) (B). Vimentin was weakly expressed in the Schwann cells of control rats (A), while its expression intensely increased in the Schwann cells of rats with EAE (B). Counterstained with hematoxylin. Scale bar = 100 μm.

Table 1. Immunohistochemical analysis of nestin and vimentin expression in Schwann cells of the spinal nerve roots in CFA-immunized control rats and EAE-affected rats

	CFA-immunized control[a]	EAE[a]	
		Peak stage (G.3)	Recovery stage (R.0)
Nestin	++[b]	+++	++
Vimentin	+	+++	++

[a]Rat spinal cords were obtained at day 14 post-immunization (P.I.) (Control and EAE [G.3]) and day 21 P.I. (EAE, R.0)
[b]Three different sections from three animals were examined in each group by two blind observers. The intensity of the immunoreactivity was categorized as weak (+), moderate (++), or intense (+++).

intermediate filament proteins in the spinal nerve roots of rats with EAE.

Schwann cells in the dorsal roots of the spinal nerves of CFA-immunized control rats were nestin-positive (Fig. 1A). Nestin immunoreactivity was increased in the Schwann cells of rats with EAE at the peak stage (G.3), suggesting that Schwann cells are activated during autoimmune CNS inflammation (Fig. 1B).

Vimentin was also constitutively expressed in Schwann cells in the dorsal roots of CFA-immunized control rats (Fig. 2A), but the immunoreactivity was weak. In rats with EAE, vimentin was intensely immunostained in the Schwann cells of the dorsal roots at the peak stage (G.3) and slightly declined at the recovery stage (Fig. 2B). The results of the immunohistochemical analysis of the distributions of nestin and vimentin are summarized in Table 1.

Discussion

Using the rat EAE model, we found a functional switch in Schwann cells in the dorsal roots of the spinal nerves in response to inflammation. In control rats, both nestin and vimentin were constitutively expressed in Schwann cells, although the nestin immunoreactivity was more intense than the vimentin immunoreactivity. In response to EAE-induced autoimmune CNS injury, the expression of both nestin and vimentin increased in the Schwann cells, suggesting that Schwann cells react to and are possibly involved in the inflammation. These findings also suggest that the activation of Schwann cells is manifested through changes in the expression levels of the intermediate filament proteins nestin and vimentin. It has been shown that in mice infected with Theiler's murine encephalomyelitis virus (WW strain), demyelination and subsequent remyelination occur in the spinal cord parenchyma, although the source of the Schwann cells was unknown [3]. The authors suggested that myelin-producing cells in the spinal cord parenchyma might arise from multipotent parenchymal cells, or might migrate from the spinal nerve roots.

In our recent study [7], we found that the transplantation of olfactory bulb tissue into hemisected spinal cords promoted functional recovery and that a large mass of Schwann cells were located in the vicinity of the transplant during the recuperative phase. This suggests that some spinal nerve root cells might proliferate in damaged spinal cords, or that Schwann

cells from the transplanted olfactory bulb filled the space. In either case, Schwann cells might contribute to the remodeling of mechanical damage to the CNS, possibly by secreting neurotrophic factors.

Collectively, these findings indicate that nestin- and vimentin-positive Schwann cells from the dorsal roots of spinal nerves contribute to the repair process after a CNS injury, such as that caused by EAE, or other neurodegenerative changes. The appropriate control of the progenitor cells in the spinal nerve roots is an alternative mechanism for CNS remodeling.

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