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Embryonic Intermediate Filaments, Nestin and Vimentin, Expression in the Spinal Cords of Rats with Experimental Autoimmune Encephalomyelitis

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Abstract

Intermediate filaments, including nestin and vimentin, are found in specific cell types in central nervous system (CNS) tissues, particularly immature glial cells and multipotent progenitor cells. In the present study, the expression patterns of nestin and vimentin in the spinal cords of rats with experimental autoimmune encephalomyelitis (EAE) and the response of cells containing filaments against acute autoimmune injury were examined by immunohistochemistry.

Nestin immunostaining was only weakly detected in vascular endothelial cells but not in any cell types in the spinal cord in normal and adjuvant-immunized rats. At the peak stage of EAE, nestin-immunoreativity was recognized in some astrocytes in the gray matter and white matter. Vimentin was immunopositive in some astrocytes and macrophages in EAE lesions, while vimentin was normally detected in ependymal cells of central canals in the rat spinal cords.

We postulate that normal animals may contain multipotent progenitor cells in the spinal cord parenchyma as well as in the subpial lesion and ependyma. Multipotent progenitor cells may activate to transform into necessary cells, including neurons, astrocytes or oligodendrocytes, depending on CNS needs. Appropriate control of progenitor cells in the injured CNS is an alternative choice for CNS remodeling.

Key words: autoimmune encephalomyelitis, nestin, vimentin, astrocyte

Introduction

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS) that is used to study human demyelinating diseases such as multiple sclerosis [10]. The clinical course of EAE is characterized by weight loss, ascending progressive paralysis, and finally, spontaneous recovery. These steps are matched by an 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 [11, 12]. Brain cells, including astrocytes, react to the inflammatory cells infiltrating in the CNS and encase the damaged lesions [8]. During this process, these cells may transform from the resting stage to the activation stage. In a few cases, it has been postulated that precursor cells may activate and transform into neuronal or glial cells around the injured lesion.

Intermediate filaments are composed of different filament proteins depending on cell type, developmental stage, and in some cases on activation stage. Three intermediate filaments, nestin, vimentin, and glial fibrillary acidic protein (GFAP), are found in specific cell types in the CNS, particularly astrocytes. Nestin and vimentin are the main intermediate filaments in immature astroglial cells, whereas maturing astrocytes contain vimentin and GFAP [6]. Replacement of nestin by vimentin and GFAP occurs during the maturation or differentiation of multipotent neural precursor into astrocytes or neurons, particularly during embryonic development [13].

Of the three intermediate filaments, expression of vimentin and GFAP is well documented in reactive astrocytes in CNS tissues, including in experimental brain injury [2], autoimmune encephalomyelitis [4], and neurodegenerative diseases such as amyotrophic lateral sclerosis [14, 15]. It has been suggested that nestin-expressing cells reflect a sustaining active stage of embryonic precursor cells, and are involved in repairing damaged CNS tissues. This phenomenon is one of the major features of EAE tissues, and is characterized by cellular infiltration of inflammatory cells, encasement of inflammatory lesions, and finally, reactive astrogliosis [8]. During recovery from EAE inflammation, it is likely that both nestin and vimentin are dynamically changed.

The aim of this study is to examine the expression of

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nestin, vimentin and GFAP in EAE lesions in the spinal cords of Lewis rats to evaluate the activation capacity of brain cells during autoimmune inflammation.

Materials and Methods

Animals

Lewis rats of both sexes (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 with a slight modification of a previously described method [12]. Briefly, each rat was injected subcutaneously and bilaterally in the hind footpads with an emulsion containing equal parts of guinea pig myelin basic protein in phosphate buffer (1 mg/ml) and complete Freund's adjuvant (CFA; *Mycobacterium tuberculosis* H37Ra, 5 mg/ml) (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, floppy tail; grade 2, mild paraparesis; grade 3, severe paraparesis; grade 4, tetraparesis or moribund condition)[12].

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 = 5) in each group 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 of paraffin-embedded spinal cords (5 ㎛) were deparaffinized and treated with 0.3% H_2O_2 in methyl alcohol for 20 minutes to block endogenous peroxidase. The sections were exposed to normal goat serum, and then incubated in optimally diluted primary antisera [mouse anti-nestin (Clone Rat 401, Chemicon International, Temecula, CA), mouse anti-vimentin (clone V9, Lab Vision corporation, Fremont, CA), and rabbit anti-GFAP (Dako, Copenhagen, Denmark)] for 1 h at room temperature. To distinguish macrophages in the CNS, mouse monoclonal anti-rat macrophage (ED1; Serotec, London, U.K.) [5] was applied to adjacent sections. The peroxidase was developed with diaminobenzidine-H_2O_2 solution (0.001% 3,3′-diaminobenzidine [Sigma] and 0.01% H_2O_2 in 0.05 M Tris-buffered saline (TBS, pH 7.4). The sections were counterstained with hematoxylin before mounting.

Results

Clinical and histological observation of EAE

Rats immunized with myelin basic protein and CFA developed floppy tail (G1) on days 9 to 11 PI, and showed hindlimb paralysis (G3) on days 12 to 15 PI. All rats subsequently recovered.

Histological examination revealed few if any inflammatory cells in the spinal cords of rats immunized with CFA. At the peak stage of EAE (day 14 PI), a large number of inflammatory cells infiltrated the perivascular lesions and parenchyma of spinal cords in rats with EAE. Thereafter, inflammatory cells declined in number at the recovery stage (day 21 PI). These findings are largely consistent with our previous reports [1, 8].

Enhanced expression of nestin in EAE lesions.

In normal rat spinal cords, nestin immunostaining was only visible in some vascular endothelial cells, while neurons, astrocytes and ependymal cells were negative for nestin in this staining protocol (Fig. 1, A). These findings were similar to those in CFA-immunized control rats. At the peak stage of EAE (day 14 PI), a striking change occurred: a population of astrocytes in the gray matter and in the white matter expressed nestin(Fig. 1, B). These cells were typically negative for nestin in normal rats. At this time, a group of radial glial cells in the subpial region showed intense nestin immunostaining. At the recovery stage of EAE (day 21 PI), the nestin immunostaining pattern was similar to the pattern at the peak stage of EAE, with fewer nestin-positive astrocytes. These findings suggest that the spinal cord constitutively expresses a multipotent cell type that is nestin-positive.

Vimentin expression in macrophages and CNS cells in EAE lesions.

In normal rat spinal cord, vimentin was expressed in some astrocytes and ependymal cells (Fig. 2, A). With the infiltration of inflammatory cells in the spinal cord (EAE, grade 3, day 14 PI), vimentin immunoreactivity (Fig. 2, B) was found in round cells surrounding blood vessels, which were positive for ED1 (Fig. 2, C). Other cell types, including ependymal cells and astrocytes, showed intense immunoreactivity at this stage of EAE. Increased vimentin immunoreactivity was detected in astrocytes, ependymal cells, and vessels at the recovery stage of EAE.

Discussion

In the present study, expression of nestin and vimentin was evaluated in the spinal cords of normal, CFA-immunized control, and EAE-affected rats. Astrocytes in the gray matter that are usually negative for both nestin and vimentin in normal adult rats were found to express nestin and/or vimentin in rats with EAE. This finding is in part

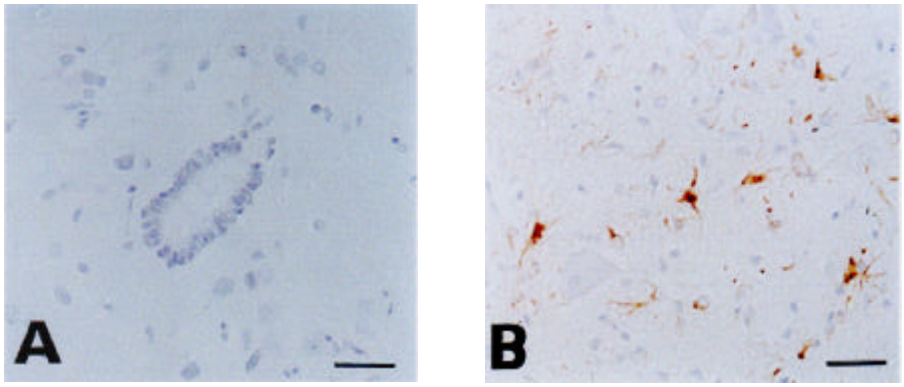

Fig. 1. Immunohistochemical staining of nestin in the spinal cords of normal rats (A) and rats with EAE (B). In normal rat spinal cord (A), some vascular endothelial cells, but not astrocytes and ependymal cells, were immunostained with nestin. In EAE lesions, nestin was detected in many process-bearing cells (probably astrocytes) in the gray matter and in the white matter. A and B: A representative section from three different animals in each group. Counterstaining with hematoxylin. Scale bar = 30 (m. B (EAE, G.3) was obtained at day 14 post-immunization.

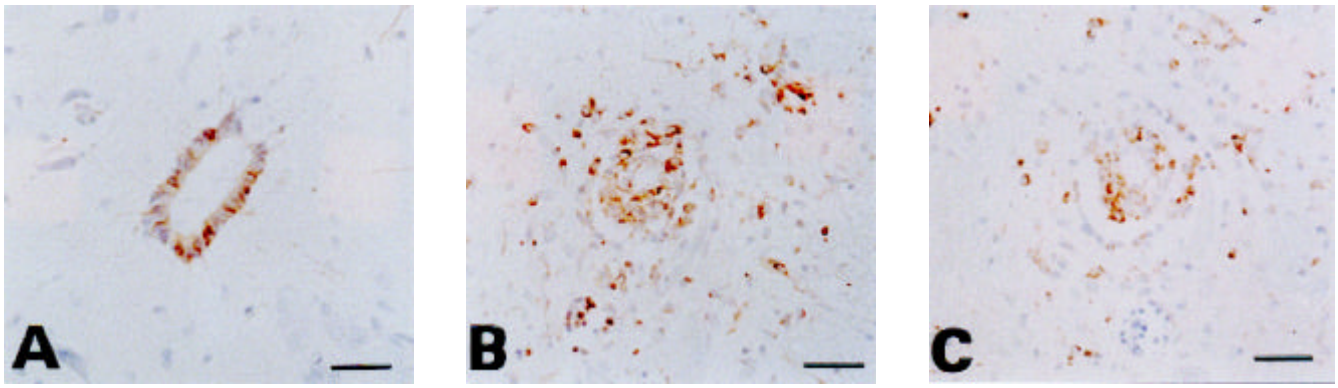

Fig. 2. Immunohistochemical staining of vimentin in the spinal cords of normal rats (A) and rats with EAE (B, C). In normal rat spinal cord (A), some ependymal cells were immunostained with vimentin. In EAE lesions, vimentin (B)was detected in many inflammatory cells in the perivascular cuffing which were positive for ED1(C). A-C: Representative sections from three different animals in each group. Counterstaining with hematoxylin. Scale bar = 30 (m. B and C (EAE, G.3) was obtained at day 14 post-immunization.

Table 1. Immunohistochemical localization of nestin and vimentin in the spinal cord of normal and EAE-affected rats

	Nestina			Vimentin		
	Normal	EAE(G.3)	EAE(R.0)	Normal	EAE(G.3)	EAE(R.0)
Neuron	−b	−	−	−	−	−
Glial cells with processes	−	++	+	+	++	+
Ependyma	−	+	+	+	+++	++
Vascular Endothelial cells	+	+	+	+	++	+
Inflammatory cells	NDc	−	−	MDc	+++	+

a Three different sections from three animals were examined in each group by two blind observers.

b The presence of immunoreactive cells in the spinal cords of each group is expressed as negative (-), under 10 cells positive (+), 10 to 30 cells/section (++), over 30 cells/section (+++).

c ND ; There were no inflammatory cells in the spinal cords of normal rats.

d Rat spinal cords were obtained at day 14 post-immunization (pi) (EAE G.3) and day 21 pi (EAE, R.0)

consistent with previous studies that have reported vimentin expression in astrocytes and macrophages in EAE lesions [4], and nestin expression in astrocytes and ependymal cells in EAE lesions [3]. The latter focused on the remyelination capacity of the CNS after an EAE attack. Consequently, we postulate that glial elements along the central canal and subpial regions are potentially multipotent in adult CNS tissues. These cell types are easily activated in response to CNS attack, such as inflammation, and readily generate new cells, which may transform into oligodendroglial, astroglial and/or neuronal cells.

In this model of EAE, the majority of nestin-positive cells were found in the gray matter and in the white matter, where neuronal loss is not evident in acute lesions. As a result, the phenotypic change from nestin-positive cells into neurons remains unclear. However, neuronal loss has been confirmed in the dorsal horn of gray matter in a mouse EAE model [9] in which the disease is more chronic. We do not exclude the possibility that chronic inflammation in rat EAE results in neuronal loss, and subsequently nestin-positive cells may replace those cells. However, it is not likely that nestin-positive cells in the spinal cord parenchyma (particularly the ventral horn) are involved in neuron turnover at the peak stage of EAE, because no neuron loss occurs in this lesion in the acute rat EAE model.

There is general agreement that nestin-positive multipotent cells may switch their phenotype to either astrocytes or oligodendrocytes, depending on stimulation factors [2,3]. In the present study, it is likely that some of the increased GFAP immunoreactivity with elongated processes originated from precursor cells that were nestin or vimentin positive.

These results suggest that normal animals may contain multipotent progenitor cells in the spinal cord parenchyma, as well as in the subpial lesion and in ependyma, which are derived from neuroectoderm at the embryonic stage. During injury, such as autoimmune inflammation, multipotent progenitor cells may activate and be ready to transform into necessary cells, including neurons, astrocytes or oligodendrocytes, depending on CNS needs. Appropriate control of progenitor cells in the injured CNS is an alternative for CNS remodeling.

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