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Remyelination in the medulla oblongata of adult mouse brain during experimental autoimmune encephalomyelitis

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ABSTRACT

Experimental autoimmune encephalomyelitis (EAE) is primarily used as an animal model of autoimmune demyelinating disease, multiple sclerosis. In this study, we found the proliferative rate of oligodendrocyte progenitor cells (OPCs) in the medulla elevated twofold above control levels during EAE and new generation of mature oligodendrocytes was increased as well. Although astrocytes showed hypertrophic reactive phenotype, a new generation of them was rare. Astrocyte- and tanycyte-like neural stem cells (NSCs), multipotent NSCs, did not augment their low proliferative rate. Thus, the present study demonstrates that resident OPCs derived from NSCs contribute to remyelination in the medulla oblongata in EAE mice.

1. Introduction

Multiple sclerosis (MS) is a multifactorial disease with a strong neurodegenerative atrophy or a highly prevalent demyelinating disorder in the brain and spinal cord (Martin et al., 1992; Compston and Coles, 2008). Pathological features of MS include inflammation-induced initial damage to neuroglial elements, destruction of the myelin sheath or oligodendrocyte cell body, axonal loss or damage, and astrocytic gliosis (De Stefano et al., 2001; Peterson and Fujinami, 2007). More than 80% of human patients with clinically definite MS have shown to involve the atrophy of the spinal cord and impairment of motor function (Lycklama et al., 2003). In contrast, MS is shown to be a heterogeneous disease by findings of magnetic resonance imaging (MRI). For example, lesions in the medulla oblongata are not unusual in MS patients, since it has been reported that medullary atrophy was present in 50% of cases with a clinically definite diagnosis by MRI (Brainin et al., 1987; Nakashima et al., 1999). A subpopulation of MS patients reveals clinical features of demyelinating acute vestibular syndrome due to the lesion of the medulla oblongata (Pula et al., 2013). Demyelinating lesions in the brain stem are situated more in the periaqueductal and dorsal regions in contiguity with the ventricular cerebrospinal fluid spaces (Brainin et al., 1987; Nakashima et al., 1999; Qiu et al., 2010). Case studies report that patients with progressive MS die of sudden respiratory failure due to active inflammation in the medulla oblongata (Bramow et al., 2008; Hengstman and Kusters, 2011). MRI further shows that there is a close correlation between the volume of the medulla oblongata and spinal cord damage or disability in MS (Liptak et al., 2008). Thus, recent findings have indicated that MS is a disease of the whole brain, rather than just the white matter (Reich, 2017).

EAE is the most common animal model of human demyelinating disease MS (Bynoe et al., 2007). Although there are many reports that show neuronal and glial damages in EAE animal model (Bynoe et al., 2007; Constantinescu et al., 2011), only a scattering of studies are conducted in the brain stem, especially the medulla oblongata. MRI enhanced with ultrasmall superparamagnetic iron oxide particles shows that the lesion of the inferior olives in the medulla oblongata is observed primarily in the acute phase of EAE rats, whereas the lesion of

Abbreviations: AP, area postrema; CC, central canal; APC, adenomatous polyposis coli; Cox2, cyclooxygenase-2; BrdU, bromodeoxyurigine; EAE, experimental autoimmune encephalomyelitis; EdU, 5-ethynyl-2′-deoxyuridine; FITC, fluorescein isothiocyanate isomer-I; GFAP, glial fibrillar acidic protein; GFP, green fluorescent protein; 12N, hypoglossal nucleus; Iba1, ionized calcium binding adapter molecule 1; icv, intracerebroventricular; MdV, ventral part of medullary reticular nucleus; MRI, magnetic resonance imaging; MS, multiple sclerosis; NGS, normal goat serum; NSCs, neural stem cells; MOG, myelin oligodendrocyte glycoprotein; NF-κB, nuclear factor-κB; Olig2, oligodendrocyte transcription factor 2; OPCs, oligodendrocyte precursor cells; OVLT, organum vasculosum of the lamina terminalis; PMn, paramedian reticular nucleus; PBS, phosphate-buffered saline; PBST, PBS containing 0.3% Triton X-100; pSTAT3, phosphorylated STAT3; Sol, the nucleus of the solitary tract; STAT3, signal transducer and activator of transcription factor 3; SVZ, subventricular zone; SOX2, sex determining region Y-box 2

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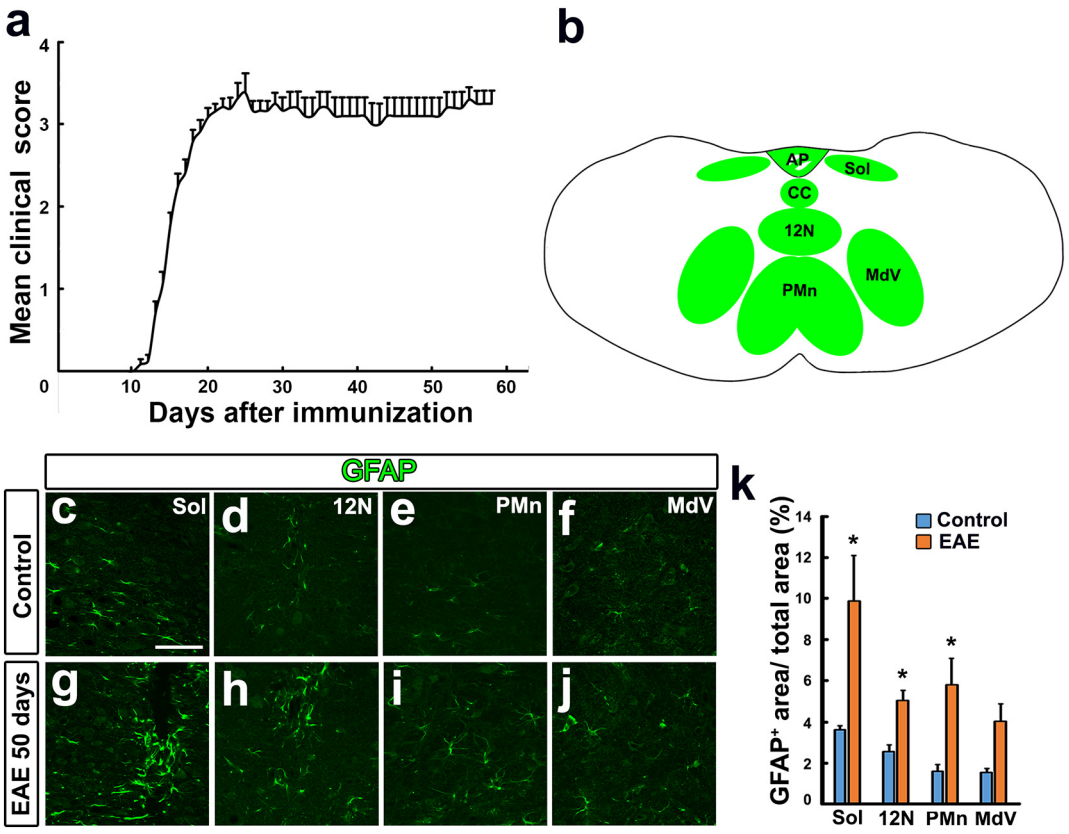

Fig. 1. EAE mice displayed a moderate clinical course that began day 12 after induction (a). Schematic representation of a sagittal brain section illustrating brain regions studied: AP, CC, Sol, 12N, MdV, and PMn (b). The immunohistochemistry of GFAP showed reactive astrocytes in EAE mice, but such astrocytes were not observed in the control (c–j). The quantitative analysis revealed that GFAP$^+$ area was significantly increased medullary regions examined (k). Scale bars = 50 (a) and 10 (bottom in a') μm. Data were expressed as the mean (± SE) of 4 animals. *$p < 0.05$ between control and EAE by unpaired Student *t*-test.

the cerebellum and spinal cord is observed during the chronic phase (Chin et al., 2009). In rodents, a significant number of immune cells is observed in the circumventricular organs, such as the area postrema (AP), subfornical organ, organum vasculosum of the lamina terminalis, and the median eminence in EAE-induced animals (Schulz and Engelhardt, 2005). The CVOs are the specialized brain regions that lack the general blood-brain barrier and have a high vascular permeability (Miyata and Morita, 2011; Miyata, 2015, 2017). Although inflammation and subsequent damages are considered to occur in the medulla oblongata of EAE animals, the study about remyelination in this brain region is lacking.

The discovery of neural stem cells (NSCs) in the adult rodent (Reynolds and Weiss, 1992) and human CNS (Kirschenbaum et al., 1994) sheds light on new perspectives for self-repair of brain damage. NSCs are located at different sites in the adult brain: the subventricular zone (SVZ), subgranular zone, and central canal (CC) of the spinal cord (Weiss et al., 1996; Doetsch et al., 1999). Multiple lines of evidence have demonstrated under pathological conditions that NSCs and oligodendrocyte progenitor cells (OPCs) contribute to myelin repair (Calza et al., 1998; Franklin, 2002; Michailidou et al., 2015). For instances, neural progenitor cells derived from NSCs in the SVZ usually migrate along the rostral migratory stream to the olfactory bulb in rodents or to the striatum in humans, while their OPCs mostly populate at the adjacent corpus callosum (Seri et al., 2006). Both NSCs and OPCs contribute to the replacement of oligodendrocytes in EAE mice (Picard-Riera et al., 2002; Menn et al., 2006) and MS patients (Nait-Oumesmar et al., 1999, 2007). It is shown in cuprizone-treated mice that remyelination in the corpus callosum surrounding the SVZ results from NSC-derived OPCs rather than resident OPCs (Xing et al., 2014; Akkermann et al., 2016). In EAE animals, however, proliferation and differentiation of NSCs in the SVZ have a minor contribution in remyelination, as compared to the main effectors, resident OPCs (Grade et al., 2013). Another source of NSCs is shown to be present within the ependymal layer of the CC in the spinal cord, but they are mostly quiescent or low proliferative activity during normal conditions (Horner et al., 2000; Covacu and Brundin, 2016). NSCs in the spinal cord are progressively activated in response to damages of the spinal cord, but the contribution of NSCs to supply astrocytes is far greater than to do oligodendrocytes in EAE (Brundin et al., 2003; Zawadzka et al., 2010) and injured mice (Barnabé-Heider et al., 2010).

Previous our study has demonstrated in normal condition that NSCs in the AP and CC of the medulla oblongata are able to supply many oligodendrocytes and sparse numbers of neurons and astrocytes into adjacent medullary regions, such as the nucleus of the solitary tract (Sol) and hypoglossal nucleus (12N) (Furube et al., 2015; Miyata, 2015). In this study, we aimed to elucidate whether NSCs and OPCs in the medulla oblongata contribute to repair processes of oligodendrocytes during demyelinating pathological condition by using EAE mouse. We examined the proliferation of NSCs and OPCs and used a genetic fate mapping with *Nestin-CreERT2/CAG-CAT$^{loxP/loxP}$-EGFP* transgenic mice in the AP and CC, and their adjacent medullary regions; the Sol, 12N, paramedian reticular nucleus (PMn), and ventral part of the medullary reticular nucleus (MdV). We show (1) that astrocytic gliosis occurs in the medulla oblongata, (2) that proliferation of OPCs was significantly increased at early phase of EAE, but it was returned to control levels at late phase of EAE, (3) that proliferation of NSCs was not changed at both early and late phases of EAE, and (4) that differentiation to mature oligodendrocytes is promoted by EAE. Thus, the present study demonstrates that resident OPCs derived from NSCs are activated to contribute to remyelination in the medulla oblongata in