PERSPECTIVE

Myelin morphology and axon pathology in demyelination during experimental autoimmune encephalomyelitis

In the central nervous system (CNS), oligodendrocytes are responsible for myelination by wrapping around the axon and maintaining saltatory conduction. Damage to oligodendrocytes and the myelin sheath around nerves is termed demyelination.

Multiple sclerosis (MS) is an inflammatory demyelinating disease in the CNS characterized by immune-mediated disease, with autoimmune responses against myelin antigens and inflammation contributing to the pathogenesis of demyelination in the CNS (Compston and Coles, 2008). Although various genetic and/or non-genetic triggers such as viral infections, metabolism, or environmental factors have been associated with the pathogenesis of MS, the major cause of the disease remains unknown (Frohman et al., 2006; Hauser and Oksenberg, 2006; Compston and Coles, 2008; Siffrin et al., 2010). To date, it is widely accepted that immune cells attack myelinated axons in the CNS, followed by demyelination and axonal degeneration (Dutta and Trapp, 2007; Aktas et al., 2010; Herz et al., 2010). For instance, activated autoreactive T cells and myelin-specific T cells can facilitate the recruitment of macrophages by producing various cytokines and chemokines. Infiltrating inflammatory cells are activated within the CNS and interact with other immune cells and neuronal cells, resulting in oligodendroglial cell death-mediated demyelination, glial cell activation and axonal degeneration. Therefore, it has been suggested that demyelination and oligodendroglial cell death in MS is passively induced by infiltrating immune cells.

To further understand the pathology of demyelination and axonal degeneration in MS, neurological and histological investigations in different animal models that mimic some aspects of MS are needed. Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model for studying different aspects of human MS (Mix et al., 2010; Croxfor et al., 2011). The current concept of pathogenesis and progression of EAE has been based on immunological processes due to infiltrating immune cells in the CNS. In addition, the cuprizone-induced mouse model has been used to induce de- and remyelination. This model is widely accepted in the field; however, it does not involve breakdown of the blood-brain barrier or infiltration of peripheral immune cells into the CNS.

In our laboratory, we have focused on myelin morphology and axonal degeneration in two different models, the myelin oligodendrocyte glycoprotein (MOG)-induced EAE (MOG-EAE) model and the cuprizone-induced demyelinating model. Surprisingly, myelin morphology during demyelination is not fully understood. Therefore, we first examined myelin morphology in EAE mice. Transmission electron microscopy (TEM) has been traditionally used to observe myelin morphology; however, it has been suggested that destruction of myelin structure owing to sample preparation occurs often (Osawa et al., 2002). Thus, it is difficult to judge whether the loose myelin appearance is a cause of demyelination. For this reason, the morphological changes of myelin during the process of demyelination have not been well characterized. We therefore performed scanning electron microscopy (SEM), combined with the osmium-maceration method, for the detection of ultrastructural abnormalities in myelin and axons at early stages of demyelination in experimental animal models (Nomura et al., 2013). Although this technique strategically degrades protein components, membrane structures such as myelin are clearly visualized. In addition, this method allows compact myelin to remain intact during the demyelination process. Our SEM technique enables morphological changes during demyelination to be observed more accurately. In fact, this technique demonstrated a variety of abnormal myelin structures with compact lamination of myelin surrounding axons, even in EAE mice (Nomura et al., 2013).

Proof of principle of demyelination in EAE has emerged from our studies, indicating that morphological features of EAE-induced demyelination are complex (Bando et al., 2015). In the cuprizone model, histopathological changes are simple with traditional demyelination, namely loss of myelin from the nerve sheaths. In contrast, histopathological changes in MOG-EAE are complicated and different from cuprizone-induced demyelination. Surprisingly, myelin detachment and excess myelin formation, but not loose myelin, are typical myelin abnormalities at inflammatory sites in MOG-EAE mice (Bando et al., 2015). These results were also observed at non-inflammatory sites in regions of normal-appearingwhite matter (NAWA). Our results also suggest that myelin detachment from axons may be the initial step of demyelination. In addition to this morphological change, formation of excess myelin foldings, including double myelin, multiple layered and obstructive myelin, are observed in chronic EAE. These observations indicate that excess myelin formation is induced by oligodendrocyte dysfunction/dysregulation in the EAE spinal cord. Involvement of gray matter and axonal damage in NAWA has recently been reported in MS patients, indicating that axonal and neuronal damage occurs in NAWA (Bjartmar et al., 2001). Non-characteristic morphological changes to myelin, which we reported, can partly explain the pathogenesis in the NAWA. These pathological abnormalities in NAWA may contribute to clinical disability in MS patients. Therefore, it is important to understand the pathological contribution of NAWA in MS.

To determine what happens to axons following morphological changes of myelin, we further examined axonal pathology in EAE mice. We found that the abnormal morphology of myelin in the spinal cord of EAE mice triggered axonal degeneration and morphological changes of axonal organelles, including axoplasmic reticulum-like structures (ARLS) and mitochondria (Bando et al., 2015). For example, development of ARLS and the accumulation of mitochondria in axons of the spinal cord are observed in focal axonal degeneration during EAE. In addition, the number of Y-shaped mitochondria and small mitochondria increased in spinal cord axons of EAE mice, indicating mitochondrial dysfunction. With respect to mitochondrial shape and size, Drp1/Dlp1 (mitochondrial fission-related protein) and MFN1 (mitochondrial fusion-related protein) are associated with mitochondrial fission/fusion machinery in the EAE spinal cord. Furthermore, abnormalities to myelin structures observed in EAE mice are similar to those observed in human MS brains. These observations indicate the central role of mitochondria and ARLS in axonal degeneration to the pathogenesis of MS. Our findings strongly suggest that detachment of myelin from axons and excess myelin formation are essential for the initial demyelination process. It seemed to be oligodendrocyte pathy (**Figure 1**). Although dysregulation of the immune system has been the main focus of MS pathology, our findings suggest that oligodendrocytes are a considerable new target for MS therapy.

In conclusion, we have proposed a new concept of myelin

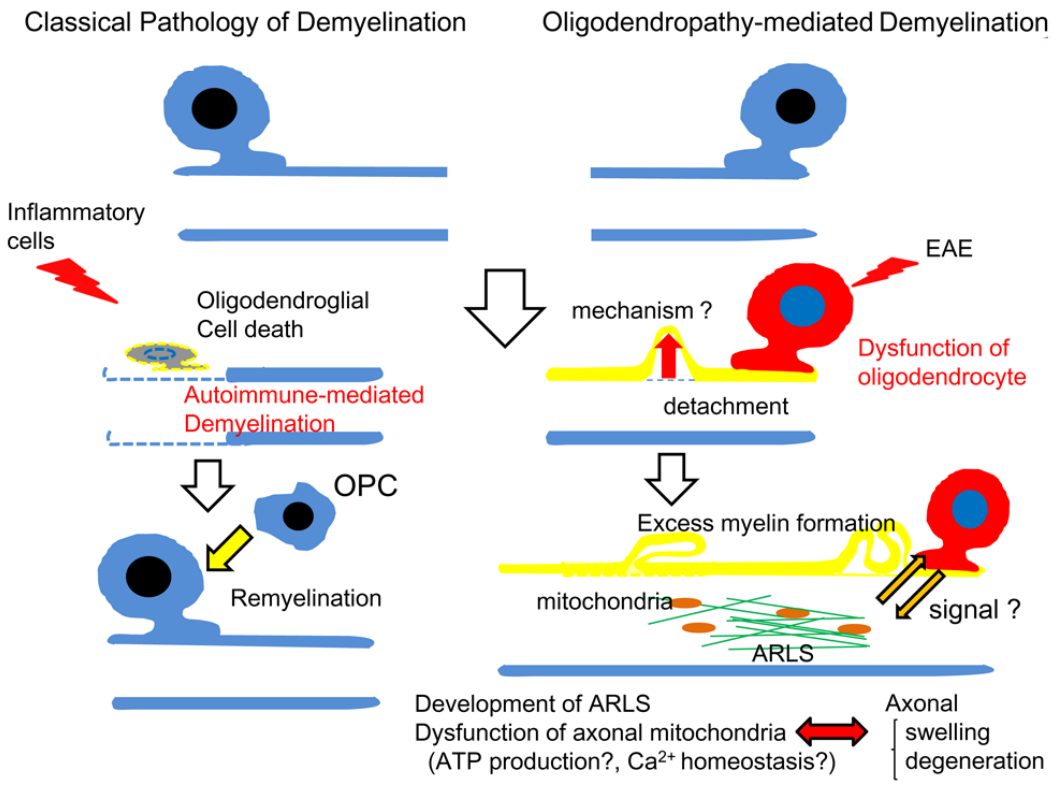

Figure 1 Schematic mechanisms of demyelination.
Left: Classical pathology of demyelination. Inflammatory cells or Inflammatory cytokines attack either myelin or oligodendrocytes which can induce autoimmue-mediated demyelination. After that, oligodendrocyte precursor cells remyelinate the axons. OPC: Oligodendrocyte precursor cells. Right: Oligodendropathy-mediated demyelination. EAE induces dysfunction of oligodendrocytes including excess myelin folding. Besides of this event, accumulation of mitochondria and development of ARLS are also induced, followed by axonal injury. ARLS: Axoplasmic reticulum-like structures; ATP: adenosine triphosphate; EAE: experimental autoimmune encephalomyelitis.

abnormalities followed by axonal degeneration in EAE mice and human MS patients. In EAE and MS, myelin abnormalities and morphological changes in axonal ARLS and mitochondria may be a critical step in axonal degeneration. Therefore, understanding oligodendroglial behavior in demyelination and remyelination may open new avenues for the treatment of MS.

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