Review

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Mechanisms of remyelination: recent insight from experimental models

Abstract: Oligodendrocytes and myelin play essential roles in the vertebrate central nervous system. Demyelination disrupts saltatory nerve conduction, leading to axonal degeneration and neurological disabilities. Remyelination is a regenerative process that replaces lost myelin. However, remyelination is disrupted in demyelinating diseases such as multiple sclerosis, at least partially, due to the failure of oligodendrocyte precursor cells to differentiate into myelinating oligodendrocytes. Understanding the molecular and cellular mechanisms that impact the differentiation of oligodendrocytes and myelination may help in the development of novel therapeutic strategies for demyelinating diseases. In this review, we focus on the molecular mechanisms controlling the differentiation of oligodendrocytes during remyelination, and we discuss the function of astrocytes and microglia in animal models of demyelinating diseases.

Keywords: cuprizone; demyelination; lysolecithin; oligodendrocyte; remyelination.

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Introduction

The myelin sheath wraps axons and is a key determinant of efficient axonal function and health (1). Demyelination, the loss of myelin from intact axons, is a pathological process in the central nervous system (CNS) (2). Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS that disrupts saltatory nerve conduction, leading to axonal degeneration and neurological dysfunction (3, 4). Remyelination is a regenerative process in which new myelin sheaths are formed on demyelinated axons. However, this regenerative process is limited in MS, owing in part to the failure of adult oligodendrocyte precursor cells (OPCs) to differentiate into myelinating oligodendrocytes (5, 6). For this reason, enhancing remyelination is an attractive therapeutic strategy for preventing axonal loss in MS (7, 8). Recently, a number of signaling pathways and intracellular factors have been demonstrated to play important roles in modulating oligodendrocyte development. Here, we review the current state of knowledge on the molecular and cellular mechanisms of oligodendrocyte differentiation and remyelination, and we discuss the function of other glial cell types in remyelination.

Pathology of multiple sclerosis

MS is a chronic demyelinating disease in which autoimmune-mediated damage to myelin occurs episodically (2). The pathological hallmark of MS is the presence of focal areas (plaques) of demyelination in the CNS, with surrounding inflammation and neurodegeneration (9). Although the cause of MS is unclear, the pathogenesis of the disease appears to involve a combination of genetic susceptibility and non-genetic triggers, such as a virus, metabolic imbalance, or environmental factor (10). Most patients initially present with relapsing-remitting disease, which is marked by flare-ups of symptoms followed by periods of remission when symptoms improve or disappear. However, most patients advance to a secondary progressive disease course, during which symptoms worsen over time. Approximately 10% of patients with MS gradually worsen from the onset of the disease, without distinct periods of remission or relapse. This form of the disease is known as primary progressive MS (9, 10).

It has been reported that the MS lesion is histopathologically heterogeneous. A recent analysis of a large number of active lesions revealed four distinct patterns of immunopathology (patterns I–IV) (11, 12). Patterns I and
II show close similarities to T-cell and antibody-mediated autoimmune demyelination. Patterns III and IV are suggestive of a primary oligodendrogial dystrophy, instead of an autoimmune pathology (11, 12).

Spontaneous remyelination is known to occur after demyelination, but the efficiency of this process is limited (13, 14). Incomplete remyelination tends to occur within a transitional zone at the edge of a plaque, between normal-appearing white matter and a demyelinated center. The process of remyelination may serve to protect the axon and restore conduction velocity (15, 16). The challenges of MS research are therefore to understand why remyelination fails and develop strategies to restore myelin (17). Whatever the order of pathological events, it is clear that enhancing remyelination is a potential therapeutic strategy for alleviating disease and disability in MS (18–20).

In vivo models of demyelination and remyelination

Animal models are a helpful tool for revealing the mechanisms underlying demyelination and remyelination. These models have been developed to provide insight into a variety of aspects of human demyelinating disease. There are several established experimental approaches to induce demyelination in the rodent CNS (17).

Experimental autoimmune encephalomyelitis (EAE) is a model of CNS demyelination in which paralysis is caused by an immune response to CNS antigens. Myelin oligodendrocyte glycoprotein, proteolipid protein or myelin basic protein is used as an emulsion to induce an autoimmune reaction (21). EAE has been induced in a variety of mammalian species, including mouse, rat, rabbit, pig, goat and sheep (21). It is an excellent model of inflammation and post-vaccination encephalitis in the brain, and has many characteristics of MS (18). Immunization leads to activation and expansion of peripheral antigen-specific T-cells. These cells enter the CNS, encounter the specific myelin antigen and subsequently induce disease (21, 22). Myelin protein-specific CD4+ T-cells are generally considered necessary for EAE induction, and recently, Th17 cells have also been considered important mediators of pathology (23). EAE is the most frequently used model to study autoimmune encephalitis in the CNS; however, the assessment of remyelination is problematic, since both demyelination and remyelination can proceed simultaneously (17).

Epidemiological data provide some support for a role of viruses in MS risk (24). To understand the contribution of viruses to human MS, some investigators have used models of virus-induced demyelination. Among these, Thieler’s murine encephalomyelitis virus-induced demyelination has emerged as a good model because, similarly to MS, it is based on a combined viral-immune pathogenesis (25). The most susceptible mouse strains were found to be SJL/J, DBA/1, DBA/2, PL/J and NZW (25). There are two major advantages to using the Thieler’s murine encephalomyelitis virus-induced demyelination model: (i) the disease is chronic-progressive in susceptible mice; and (ii) pathological abnormalities are limited to the CNS (26, 27). This demyelination model is also useful for studying the mechanisms of viral infection of neuronal and glial cells.

Various toxins have also been used to model MS. The most widely used toxins for inducing demyelination are bis-cyclohexanone-oxaldihydrazone (cuprizone) and lysolecithin (lysolecithin). Cuprizone ingestion in young adult mice induces a highly reproducible demyelination of distinct brain regions, especially the corpus callosum, which contains the most frequently investigated white matter tracts (28). The administration of cuprizone causes the death of oligodendrocytes, leading to subsequent demyelination (29). After 4–6 weeks of cuprizone treatment, the corpus callosum is almost completely demyelinated (30, 31). The molecular mechanisms underlying cuprizone-induced oligodendrocyte cell death are not fully understood. Cuprizone is a copper chelator that inhibits copper-dependent mitochondrial enzymes, including cytochrome oxidase and monoamine oxidase. Thus, cuprizone-induced copper deficiency in oligodendrocytes may cause mitochondrial dysfunction and a disturbance of energy metabolism, leading to demyelination (30). Cuprizone-induced demyelination is associated with a microglial and astrocytic response, similar to MS. However, the cuprizone model differs from MS and EAE in that the blood-brain barrier remains intact (29, 30). Removal of cuprizone from the diet allows significant remyelination. This model is therefore useful for studying glial reactions and interactions during remyelination in the absence of peripheral immune cells (30, 31). However, this model is not useful for studying spinal cord demyelination or remyelination because demyelination occurs only in the brain.

Lysolecithin is a demyelinating chemical that is administrated through a stereotactic injection into white matter tracts in the CNS, and is used to model focal demyelination (31, 32). The dorsal and ventrolateral funiculi of the spinal cord at the thoracolumbar level are the most common injection sites, although the corpus callosum is sometimes used as well (32). Lysolecithin injections into the spinal cord have been described in several mammalian
species, including mouse, rat, rabbit and cat (21). Demyelination occurs immediately after lysolecithin injection, with significant demyelination lasting about 7–10 days. In the acute phase, the lesion site is often infiltrated with T-cells, B-cells and macrophages. The infiltrating T-cells have been proposed to have a beneficial role in CNS repair (33). In addition, a considerable number of macrophages are observed at the lesion site (34). Substantial remyelination is evident 21 days after lesion formation (35). This model is advantageous because demyelination is rapid and remyelination has a protracted course (32).

These established experimental approaches can induce demyelination in the rodent CNS. However, a single model is unlikely to accurately mimic all pathological and clinical features of human MS, because MS is a complex disease with an unclear etiology. Indeed, as described above, the pathological lesions in MS show distinct features. Some appear to be due to autoimmune tissue destruction, while others are suggestive of a primary oligodendrocyte dystrophy, reminiscent of virus or toxin-induced demyelination rather than autoimmunity (12).

Toxin-induced demyelination models appear to be suitable for studying remyelination because disease kinetics are predictable and the lesion site is known (30). In the following sections, we discuss recent advances in our knowledge of the mechanisms of remyelination obtained mainly from studies on models of toxin-induced demyelination.

Functional roles of astrocytes and microglia in remyelination

Although the early phase of MS is characterized by remyelination and recovery of function, remyelination eventually becomes limited for unclear reasons (5, 13, 14). It has been estimated that around 70% of MS lesions that remain demyelinated contain plentiful OPCs (9, 36). This suggests that insufficient OPC differentiation at least partially contributes to poor remyelination in MS. A detailed understanding of the molecular mechanisms of remyelination and oligodendrocyte differentiation is therefore necessary for the development of new treatment strategies.

Remyelination can be classified into four distinct stages: (i) OPC proliferation; (ii) migration of OPCs towards the demyelinated axons; (iii) OPC differentiation; and (iv) interaction of premature oligodendrocytes with the denuded axon (37). These stages are regulated by various extracellular factors and intracellular signals. Glial cells and many extracellular signals play crucial roles in these various stages. Demyelination is accompanied by the appearance of astrocytes and microglia. Astrocytes and microglia are considered to play beneficial as well as detrimental roles in MS (38–40) (Figure 1). Astrogliosis is related to the glial scar, which is a physical barrier around demyelinated lesions in chronic stages of MS. The glial scar prevents both OPCs and axons from entering demyelinated plaques, thereby inhibiting remyelination (39). This inhibition is mediated by astrocyte-derived chondroitin sulfate proteoglycans (41). Astrocytes also release ephrins, which bind to receptors on regenerating axons, inducing collapse of growth cones (42). In addition, astrocytes express tumor necrosis factor-α (TNF-α) a cytokine implicated in the pathogenesis of MS (38). The expression of TNF-α is positively correlated with demyelinating activity and oligodendrocyte pathology in MS (43). It has been suggested that a number of neurotrophic factors produced by astrocytes, including insulin-like growth factor-1 (IGF-1), fibroblast growth factor 2 (FGF2), brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF), support the differentiation of OPCs (39, 44), as will be described in more detail later in this review.

A number of studies suggest detrimental as well as beneficial roles of macrophages/microglia in demyelinating diseases (32, 45). Recent studies have demonstrated that under specific conditions, macrophages/microglia develop into different phenotypes, termed M1 and M2 (40). The M1 phenotype releases destructive pro-inflammatory mediators and causes damage to healthy tissues. In contrast, the M2 phenotype possesses protective properties and promotes tissue remodeling and repair (46, 47). Whether microglia polarize into the M1 or M2 phenotype may depend on mediators in the microenvironment and the extent of injury (32). The harmful properties include the production of cytokines, such as TNF-α, as well as glutamate and reactive oxygen species (40, 48, 49). The efficient removal of degenerated myelin by microglia, in contrast, is necessary for remyelination.

Microglial phagocytosis requires recognition of targets through phagocytic receptors. Among the many phagocytic receptors involved in MS, triggering receptor expressed on myeloid cells-2 (TREM-2) is the best studied (50, 51). TREM-2 is a member of the TREM family of innate immune receptors. It has been reported that TREM-2 facilitates repair within the murine CNS by clearing cellular debris during demyelination (51, 52). Olah et al. performed genome-wide gene expression analysis of microglia from the corpus callosum during demyelination and remyelination in the mouse cuprizone model (53). In that study, they reported that microglia displayed a phenotype associated with the phagocytosis of myelin debris...
and apoptotic cells as well as with the recruitment of OPCs to the lesion site through the expression of cytokines and chemokines. Skripuletz et al. reported that astrocyte loss caused impaired recruitment of microglia and clearance of damaged myelin (54). They further showed that recruitment of activated microglia was mediated through C-X-C motif chemokine 10 up-regulation in astrocytes, suggesting that the crosstalk between astrocytes and microglia in the demyelinating lesion was essential for remyelination.

In recent years, a number of neurotrophins, cytokines and other growth factors derived from astrocytes and microglia have been suggested to support the migration, proliferation and differentiation of OPCs (55, 56). It is well known that astrocytes and microglia produce IGF-1 and FGF2. IGF-1 is a growth factor that has been studied most extensively in the CNS, being produced by astrocytes and microglia to promote remyelination (57). In addition, interleukin-1β-deficient mice lacking IGF-1 production by
microglia/macrophages and astrocytes show profoundly delayed OPC differentiation into mature oligodendrocytes, resulting in the failure of proper remyelination (58). These data support a potential protective and regenerative role of IGF-1. In comparison, the function of FGF2 in myelination is controversial. Although an initial report indicated that FGF2 arrests OPC differentiation in vivo (59), subsequent studies demonstrated that FGF2 was required for proper myelination (60, 61). It has been reported that FGF2 is higher in MS patients with both relapsing-remitting and secondary progressive disease types (62). Although FGF2 signaling could contribute to remyelination in MS, further investigation on the action of FGF2 is necessary. Platelet-derived growth factor (PDGF) is well studied in oligodendrocytes because its receptor, PDGFRα, is a phenotypic marker for OPCs (44, 63). PDGF expression in astrocytes driven by the Gfap promoter results in enhanced proliferation of OPCs following focal demyelinating lesions induced by lysolecithin or cuprizone (64, 65). Furthermore, it has been reported that a single intracerebral microinjection of PDGF accelerates the rate of remyelination in a toxin-induced demyelination model (66). CNTF can promote oligodendrocyte survival and maturation by activating astrocytes (67, 68). In a recent study from our lab, the role of CNTF derived from microglia in remyelination in the cuprizone model was investigated by using minocycline, which inhibits microglial activation. Microglial CNTF displayed a protective role in the remyelination phase (31). Other studies have also demonstrated the production of other neurotrophic factors, including BDNF and neurotrophin-3, by astrocytes and microglia (32, 44). After spinal cord injury, transplanted grafts of fibroblasts expressing neurotrophin-3 and BDNF increase proliferation of oligodendrocytes and promote myelination of the regenerating axon (69).

**Signaling mechanisms in OPC differentiation**

Recently, a series of signaling pathways, including Wnt, Notch, bone morphogenetic protein (BMP) and Shh, have been shown to play important roles in regulating oligodendrocyte development and myelination (70) (Figure 1). The Wnt signaling pathway is indispensable in development, metabolism and maintenance of stem cells (71, 72). The Wnt/β-catenin/Tcf pathway has been considered to play a negative role in oligodendrocyte maturation and remyelination in a toxin-demyelination mouse model (73). Transcription factor 4 transduces Wnt/β-catenin signals to activate downstream target genes (71). Wnt3a and its downstream effector, β-catenin, are expressed in chronic active MS plaques (74). Interestingly, contrasting observations, showing a positive effect of Wnt signaling in myelination/remyelination, have also been reported (75, 76). It appears that the canonical Wnt signaling pathway plays a more complex role in regulating myelination than previously thought. Additional research is required to clarify the role of Wnt signaling in MS (77).

Notch signaling has been shown to regulate oligodendrocyte development. OPCs express Notch1, and neurons express its ligand, Jagged1 (78). Binding of Jagged1 to Notch1 induces the expression of hairy and enhancer of split 5 (Hes5), which blocks the maturation of OPCs (79). Furthermore, deletion of Notch1 enhances oligodendrocyte differentiation and remyelination after lysolecithin-induced injury in mice (80, 81). These results suggest that Notch1 signaling plays an inhibitory role in oligodendrocyte maturation. However, divergent effects of Notch signaling have also been reported (82). The glycosyl phosphatidylinositol-anchored neural cell adhesion molecule F3/contactin, which is specifically expressed on axons, activates a Notch signaling pathway that promotes oligodendrocyte maturation and myelination (82). Further studies are required to elucidate the functions of Notch signaling in OPC development.

BMP signaling negatively regulates oligodendrocyte differentiation and myelination. Exogenous administration of BMP4, a member of the BMP family, inhibits OPC differentiation via binding to its receptor (83). Intravenous infusion of BMP4 into the mouse brain during demyelination increases the proliferation of OPCs. In contrast, infusion of Noggin, an extracellular antagonist of BMP4, increases the density of mature oligodendrocytes in the region undergoing remyelination (84). After the BMP receptor is activated, the downstream R-Smad/Smad4/p300 complex targets and activates the expression of differentiation inhibitors, such as Id2 and Id4, to repress myelination (85). These results indicate an important role for BMP signaling in modulating the maturation of OPCs and remyelination.

Sonic hedgehog (Shh) is a member of the hedgehog family of signaling molecules. Its downstream effectors, glioma-associated oncogene 1 (Gli1) and Gli2, are required for OPC differentiation (86, 87). Gli1 is increased during the early inflammatory period and mediates Shh-induced neural progenitor differentiation in EAE and MS (88). Shh influences the expression of Olig2, which is required for the development of oligodendrocytes (89). Although Gli1 and Gli2 are expressed during OPC differentiation, it is unclear how these effectors regulate or interact with Olig1.
and Olig2 (70). Seifert et al. reported that Shh is expressed in macrophages, endothelium and astrocytes during remyelination in demyelinated lesions; however, the molecular mechanisms of Shh signaling in EAE remain unclear (90). In contrast, Fancy et al. were unable to detect Shh during remyelination within lesion areas in a rat model of toxin-induced demyelination, suggesting that Shh is not essential for remyelination (91). Leucine-rich repeat and immunoglobulin domain-containing 1 (LINGO-1) is a transmembrane signaling protein which has been implicated in the inhibition of axonal regeneration (92). It inhibits oligodendrocyte differentiation and myelination through the activation of RhoA (93). It has been demonstrated that LINGO-1 antagonism enhances OPC differentiation in demyelinated lesions in rat and mouse models of EAE and toxin-induced demyelination (94). This suggests that LINGO-1 antagonism may have therapeutic potential in demyelination.

Endogenous factors regulating oligodendrocyte differentiation

Several transcription factors such as Olig1 and Olig2 play important roles in the differentiation of OPCs, as described above. The intracellular localization of Olig1 changes dynamically during remyelination (95). Olig1 in OPCs translocates from the cytoplasm to the nucleus in early remyelinating lesions in EAE, as well as at the edge of MS lesions (95). Olig2 is expressed in OPCs and mature oligodendrocytes, and plays an important role in the differentiation of neural progenitor cells into oligodendrocytes (96). Islam et al. showed that proliferation and differentiation of local and/or recruited Olig2-expressing progenitors contribute to remyelination in cuprizone-induced demyelinated lesions (97). Disruption of Olig2 leads to a lack of NG2-positive OPCs, and decreases the number of oligodendrocytes in the spinal cord (98). The Sox family transcription factors Sox8 and Sox10 are known to regulate oligodendrocyte differentiation (99, 100). It has been reported that Olig1 and Sox10 can complex together to synergistically activate Mbp transcription via conserved DNA sequence motifs in the promoter region (101). A homeodomain transcription factor, NK2 homeobox 2, is expressed in OPCs in white matter regions during development, and its expression is up-regulated after demyelination (91, 102). The bHLH transcription factor achaete-scute complex homolog 1 (Ascl1)/Mash1 is also required for remyelination, and selective deletion of Ascl1/Mash1 in OPCs prevents remyelination (103). Zinc finger protein 488 is an oligodendrocyte-specific zinc finger transcription factor (104). A recent study showed that zinc finger protein 488 overexpression promotes remyelination after cuprizone-induced demyelination in mice by enhancing oligodendrocyte differentiation in the subventricular zone (105). OPC differentiation is also regulated by epigenetic mechanisms (106, 107).

Histone deacetylases (HDACs) are nuclear enzymes involved in transcriptional repression. HDAC1 and HDAC2 are required for oligodendrocyte formation (107, 108). It has been reported that genetic deletion of both Hdac1 and Hdac2 in oligodendrocyte lineage cells results in the stabilization and nuclear translocation of β-catenin, which inhibits oligodendrocyte differentiation by repressing Olig2 expression (108).

MicroRNAs have recently emerged as mediators of proliferation and differentiation of neural progenitor cells (109). Dugas et al. showed that miR-219 is required for normal oligodendrocyte differentiation and myelination (110). Furthermore, some specific microRNAs, including miR-138, miR-9, miR-23 and miR-19b, were recently found to participate in the regulation of oligodendrocyte differentiation and myelin maintenance, as well as in the pathogenesis of MS (111).

Negative regulators of OPC differentiation, such as Id2, Id4, Hes1 and Hes5, have been detected in OPCs (112–114). It has been reported that Id2 and Id4 mediate the inhibitory effects of BMP4 on oligodendrocyte differentiation through interaction with Olig1 and Olig2 (113). Hes expression is usually induced by Notch signaling via the RBP-Jκ transcription complex, and is involved in the developmental regulation of various neural cell types (115). Kondo et al. reported that Hes5 mRNA decreases as OPCs proliferate, and overexpression of Hes5 inhibits oligodendrocyte differentiation (112). Hes1 functions downstream of growth factors to maintain oligodendrocyte lineage cells in the early progenitor stage, and exerts suppressive effects on the maturation of the oligodendrocyte lineage (116).

Pre-clinical approaches for enhancing remyelination

Much less is known about the factors resulting in the failure of remyelination during chronic stages of MS. However, it has been reported that chronic MS lesions contain significant numbers of OPCs (36). Thus, it appears that remyelination fails during chronic stages of MS because of the
inability of OPCs to differentiate. Accordingly, there is considerable interest in the pathways inhibiting OPC differentiation, such as Wnt, Notch and LINGO-1 (73, 79, 94). These molecules have been considered negative factors in remyelination (73, 79, 92). The Wnt inhibitor XAV939 enhances oligodendrocyte differentiation and myelination by stabilizing Axin2, an intracellular target of Wnt (20). EAE mice treated with a γ-secretase inhibitor, MW167, which prevents Notch signaling, exhibited improved remyelination and axon survival (117). Clinical trials with the LINGO-1 antagonist BIIB033 have begun to evaluate whether it can promote oligodendrocyte differentiation and CNS repair in MS patients (19). In addition, it has been recently reported that geissoschizine methyl ether in the Uncaria hook, a galenical constituent of the traditional Japanese medicine yokukansan (Yi-gan san) improves remyelination after cuprizone-induced demyelination in the medial prefrontal cortex of adult mice, although their pharmacological properties have not yet been fully investigated (118). Pharmacologically encouraging remyelination by enhancing endogenous OPC differentiation may have substantial therapeutic potential in MS (119).

Conclusions

Remyelination is a regenerative process following demyelination; however, this process is disrupted in demyelinating diseases. For this reason, enhancing remyelination is an attractive therapeutic strategy for demyelinating diseases. There are no remyelination-enhancing therapies clinically available at present; however, research in this field is progressing. As described in this review, the differentiation of OPCs is regulated by the actions of extracellular factors and intracellular signals. Stimulating the endogenous repair process by targeting these effectors is therefore an attractive therapeutic strategy for demyelinating diseases. Toxin-induced demyelination models are well established for studying remyelination in rodents; however, these models lack the autoimmune component of human MS. Furthermore, a single model is unlikely to accurately mimic all of the pathological and clinical features of human MS. Future studies aimed at enhancing remyelination should conduct integrated analysis using various animal models.

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References


