Strategies for myelin regeneration: lessons learned from development

Abhay Bhatt, Lir-Wan Fan, Yi Pang

Department of Pediatrics, Division of Newborn Medicine, University of Mississippi Medical Center, Jackson, MS, USA

Abstract

Myelin regeneration is indispensably important for patients suffering from several central nervous system (CNS) disorders such as multiple sclerosis (MS) and spinal cord injury (SCI), because it is not only essential for restoring neurophysiology, but also protects denuded axons for secondary degeneration. Understanding the cellular and molecular mechanisms underlying remyelination is critical for the development of remyelination-specific therapeutic approaches. As remyelination shares certain common mechanisms with developmental myelination, knowledge from study of developmental myelination contributes greatly to emerging myelin regeneration therapies, best evidenced as the recently developed human anti-Nogo receptor interacting protein-1 (LINGO-1) monoclonal antibodies to treat MS patients in clinical trials.

Key Words: oligodendrocyte; myelination; microglia; multiple sclerosis; white matter damage; spinal cord injury

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Introduction: myelin integrity and the health of axons

Myelin is a product of nervous system evolution. In some invertebrates such as cephalopods (squid, octopus), an increase in axonal diameter called "axonal gigantism" serves as a means to speed up action potentials (Hartline and Colman, 2007). The obvious disadvantage of this strategy, however, is that it takes up space. In vertebrates with a complex central nervous system (CNS), all but small axons are covered with myelin sheath, which enables action potentials to be propagated by "saltatory" conduction at up to 100 m sec⁻¹ along axons. Recently, the biological significance of myelin has broadened beyond its traditional role in axonal conductivity, to include its supportive role for axonal survival and functional integrity. This renewed view not only deepens our understanding of the biological function of myelin, but, it also challenges and expands our perspective on how myelination disorders should be treated.

Myelin supports axons in a plethora of ways, some of which are yet to be discovered. First and foremost, myelin sheath provides a physical barrier separating axons from their extracellular environment; thus, it protects axons from harmful molecules which accumulate in the extracellular milieu during pathological conditions. Hence, maintaining myelin sheath integrity is a prerequisite for the health of axons. Secondly, there is convincing evidence suggesting that OLs/myelin provide trophic support for axons. For instance, although 2', 3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase) deficient mice exhibit normal myelin sheath assembly, their CNS axons degenerate early in development (Lappe-Siefke et al., 2003). A similar finding was also observed in myelin proteolipid protein-1 (PLP-1) deficient mice; although, their axonal degeneration was not detected until 3 months of age (Griffiths et al., 1998). These findings suggest that CNPase and PLP1-mediated neuroprotection requires more than the physical integrity of myelin; rather, it appears that OLs provide trophic support for axons. Direct evidence to support this notion comes from a study demonstrating that targeted ablation of OLs in the mouse brain results in acute axonal injury, without widespread myelin degradation (Oluich et al., 2012). At present, the exact mechanisms by which OLs protect axons remain elusive. Nave has proposed that OLs may provide energy support for axons via gap junctions (Nave K., 2010). Alternatively, secreted trophic factors from OLs may support axon survival, as suggested by in vitro studies showing that OLs can produce classical neurotrophic factors (such as brain-derived neurotrophic factor, glial derived neurotrophic factor, neurotrophin 3), as well as insulin-like growth factor 1. Nevertheless, the concept that OLs/myelin are crucial for axonal health is established by substantial experimental evidence. Hence, myelin regeneration is important for not only restoring electrophysiological functions, but also protecting denuded axons from secondary degeneration, which is currently considered to be a principal neuropathology underlying most clinical symptoms in demyelination disorders.
Dysmyelination in the developing and adult CNS: some mechanisms are shared

In fact, myelin related neurological disorders occur in both children and adults. White matter injury (WMI) is the most common type of brain injury in premature infants, which is characterized by hypomyelination and/or delayed myelination, presumably attributed to selective injury to oligodendrocyte progenitor cells (OPCs). Other less common myelin related disorders in pediatrics include congenital myelin diseases resulting from myelin gene mutations. In adults, demyelination is a hallmark neuropathology in a number of neurological disorders, most prominently multiple sclerosis (MS), spinal cord injury (SCI), and white matter stroke. Although the etiology, pathology, and disease mechanisms vary vastly among these demyelinating disorders, spontaneous remyelination (as seen in MS or SCI) is a common finding at the early phase of disease development; however, remyelination eventually fails with disease progression. Given that OLs/myelin play critical roles in axonal health, there is a cause-effect relationship between demyelination and axonopathy. Therefore, therapies aimed at boosting myelin regeneration are of clinical significance.

In recent years, studies on post-mortem human tissue have provided invaluable information regarding the causes of myelination failure. Accumulating data suggest that there might be certain common mechanisms involved in developmental and adult myelin disorders. For example, OL differentiation block appears to be such a shared mechanism. Traditionally, it is believed that myelination deficit in WMI is caused by an insufficient number of mature OLs as a result of OPC injury. This is supported by substantial number of animal studies, yet there is limited clinical evidence. Billiards et al. (2008) recently demonstrated that in post-mortem WM lesion, OPCs are not reduced in number, but are rather stalled at immature stages. It remains to be determined whether this occurs in a particular subset of patients (e.g. mild, diffuse white matter lesion), or is a common phenomenon of WMI. As for MS, although OPC recruitment deficiency appears to be the primary cause for poor remyelination in some patients, this is not always the case. For example, in some lesions the number of OPCs is sufficient, but they fail to differentiate. This appears to be a major hurdle for remyelination in some patients (Kuhlmann et al., 2008). Interestingly, this phenomenon has also been observed in SCI. It has been shown that OPCs in the spinal cord parenchyma readily proliferate after injury; however, these OPCs fail to differentiate into mature OLs (Kotter et al., 2011). Together, these evidence points to the importance of dysregulation in OL differentiation across all major myelin disorders. Hence, elucidating the underlying mechanisms of OL differentiation failure holds a great therapeutic potential.

Perhaps, two of the best studied molecules/pathways that have been identified as crucial players in regulating developmental myelination, but subsequently found to be limiting factors for remyelination, are the Notch signaling pathway and the leucine-rich repeat and Ig-containing Nogo receptor interacting protein-1 (LINGO-1). Notch is a family of transmembrane receptors regulating the development of various cell types including OL lineage. Upon binding to its ligands (Jagged or Delta), it activates intracellular signaling cascades, leading to the release of intracellular domain of Notch from the membrane. Intracellular domain of Notch then translocates into the nucleus, where it regulates downstream genes by activating transcription factor CBF1/Su(H)/LAG1 (CSL). Both in vivo and in vitro studies have demonstrated that Notch signaling is a powerful inhibitor for OL differentiation (Piaton et al., 2010). Since notch ligands are expressed at high levels before, but are progressively reduced after, the peak of myelination, Notch signaling plays an important role in regulating the timing of myelination. However, in MS lesions, Notch ligands are re-expressed at high levels which inhibit remyelination (Juryńczyk et al., 2010). Interestingly, LINGO-1, which is an emerging molecular target for myelin regeneration therapy, was discovered in an effort to identify novel CNS-specific leucine-rich repeat (LRR) proteins. It had been known for some time that several LRR proteins (such as Nogo-66 receptor and oligodendrocyte-myelin glycoprotein) play roles in regulating axon guidance in both development and/or regeneration, and it was lately discovered that the transmembrane protein LINGO-1 belongs to this class of molecules. During early development, LINGO-1 is expressed by both neurons and OLs. Neuronal LINGO-1 inhibits axonal growth via activating ras homolog gene family member A (RhoA), while oligodendroglial LINGO-1 strongly inhibits OL differentiation and myelination, via yet to be defined mechanisms. In both immune and non-immune experimental demyelination models, LINGO-1 loss-of-function promotes in vivo remyelination through induction of OPC differentiation and neuroprotection (Mi et al., 2013). Human monoclonal anti-LINGO-1 antibody has been successfully developed, and is currently under phase-II clinical trial to treat MS. Together, this is truly an excellent example of how knowledge from developmental studies leads to novel discovery in myelin regeneration therapies.

Is remyelination a recapitulation of myelination in development?

The so called recapitulation hypothesis, first proposed by Franklin (Franklin, 1999), believes that remyelination is a recapitulation of myelination in development. Myelination and remyelination share a common objective, that is, to invest axons with myelin sheath, which is not simply a physical barrier, but rather a means to increase axonal conduction velocity. As a consequence of evolutionary economy, myelination and remyelination would be expected to share the same mechanisms. In fact, both developmental myelination and remyelination involve the same OL developmental milestones, i.e., OPC proliferation, migration (or recruitment), differentiation, and myelination. Despite these similarities, there are also several significant differences noted between normal myelinated and remyelinated axons. For example, in developmental myelination, there is a strict relationship be-
tween the thickness of myelin sheath and axonal diameters (for those large than 10 um), known as g ratio. In remyelinated axons, myelin sheath is found to be often thinner than their developmental counterparts. Most importantly, the cytoarchitecture of nodal domains in remyelinated axons appears to be different also from that of healthy myelinated axons. The true significance of these differences in relation to myelination mechanisms remains an open question. In brief, the general consensus is that remyelination needs to re-run the program of developmental myelination, albeit facing a formidable task to overcome a challenging environment with many inhibitory cues.

Some remyelination limiting factors play important roles in developmental myelination

During the past two decades, great progress has been made in our understanding of the cellular and molecular mechanisms underlying myelination in development. In brief, myelination in development consists of two inter-related programs: the development of OL lineage, and the formation of myelin sheath. The development of OLs is a tightly regulated process encompassing a series of cellular events, including OL fate commitment, OPC proliferation, migration, and differentiation. These developmental milestones are highly orchestrated between intrinsic (i.e., transcription factors, TF) and extrinsic (e.g., growth factors, cytokines, axonal surface molecules, etc.) factors. TFs involved in OL development are well studied using genetic rodent models. It is known that except for Olig1 (Paes de Farisa et al., 2014), all known TFs, including Olig2, the homeodomain TF Nkx2.2, Sox10, Tcf4, myelin gene regulatory factor (MRF), Zinc finger protein 191 (Zfp191), and transcription factor Ying Yang (YY1) appear to be critical in maintaining sufficient OL population (Emery, 2010). Moreover, several of these factors specifically regulate OPC differentiation. For instance, the number of OPCs appears normal in MRF mutant mice, but they fail to differentiate into mature OLs. The significance of most other TFs in myelin disorders, however, remains largely elusive.

Many growth factors/cytokines and hormones (especially sex steroids and thyroid hormones) are known to modulate OL differentiation and/or myelination. Since they have been extensively reviewed elsewhere, all of them will not be discussed here. Perhaps the most important extrinsic factors regulating OL differentiation and myelination in development are adhesion molecules, which are mostly expressed on the axonal surface and are inhibitory in nature. For example, laminins, semaphorins 3A, contactin, axonal cell adhesion molecule L1, the polysialylated neuronal cell adhesion molecule (PSA-NCAM), LINGO-1 and the Notch-1 receptor ligand Jagged1, are inhibitory cues for myelination. As discussed above, these inhibitory cues play important roles in the timing of myelination during development, so that their expression levels are progressively down-regulated once active myelination is initiated, and remain at low levels in the adult brain. The discovery that some of these molecules are re-expressed in demyelination lesions may provide novel therapeutic targets. In addition to Notch and LINGO-1, emerging evidence suggests that other factors, such as semaphorins and laminins, are also up-regulated in MS plaques (Williams et al., 2007).

Remyelination inhibitory factors derived from the extracellular matrix

Certain remyelination inhibitory factors are not involved in development, but are rather disease-specific. Most of these factors are extracellular matrix molecules (ECM). A major class of ECM is chondroitin sulphate proteoglycans (such as the neuron-glial antigen, NG2), which are often found at the edge of active and expanding white matter lesions in MS. Their inhibitory nature for myelination has been demonstrated in both experimental autoimmune encephalomyelitis (EAE) animal models as well as in vitro (Lau et al., 2013). Hyaluronan, a glycosaminoglycan, is yet another important ECM identified within MS lesions, which is found to inhibit OPC maturation and remyelination. Hyaluronan is also secreted by astrocytes within MS plaques (Hanafy et al., 2011). Finally, if demyelination is the principal pathology, then a large amount of myelin debris is present in the extracellular matrix, which is well known to inhibit remyelination. Since microglia/macrophages are responsible for the clearance of myelin debris, and they are known to secrete a diverse range of trophic factor/cytokines affecting OL development, they may play crucial roles in remyelination (will be discussed below).

The involvement of microglia/macrophage in developmental myelination and myelin repair

In addition to serving as professional scavengers in the CNS, microglia are increasingly recognized as key players in early neural development. Much is known about the roles of microglia in neuronal development, such as controlling neuronal numbers and synaptic pruning; however, their roles in OL development and/or myelination are not firmly established. We have shown that microglia-conditioned culture medium not only provides strong support for OPC survival, but also greatly enhances their differentiation as well as myelination in vitro (Pang et al., 2000, 2013). In vivo, depletion of microglia on postnatal day 4 (before myelination) by liposome-encapsulated clodronate significantly reduces myelination in the corpus callosum (the largest white matter track in the brain) of postnatal day 21 rat brain (unpublished observations), suggesting that microglia may play an active role in OL development and/or myelination. Microglia are invaded myeloid progenitor cells from the peripheral during early development, and they share same progenitors with macrophage. In MS, macrophages seem to play a beneficial role in remyelination, as they are often found to be abundant at the peripheral of lesion plaques with active remyelination. In addition, depletion of macrophages in the lysolecithin-induced demyelination model negatively affects remyelination (Kotter et al., 2001). The fact that microglia often exhibit an activated, amoeboïd-like morphology in the early postnatal period, suggests that their functional roles in developmental...
myelin regeneration strategies

Broadly, emerging myelin regeneration therapies fall into two strategies. The first is aimed at promoting OL differentiation by targeting intrinsic blockers (e.g., Notch and LINGO-1), or alternatively, by forcing OL to mature (e.g., using growth factors such as IGF-1, CNTF, and/or thyroid hormones, etc.). These strategies, however, are based on the assumption that there are enough OPCs in the lesion plaques. Although this seems to be true in some patients, it is likely that in other lesions the number of OPCs is not sufficient. For example, repeated relapse-remission in MS could exhaust the OPC pool. In this case, a second strategy, cell transplantation, might provide a better solution. A handful of cell types (e.g., stem cells, OPCs, olfactory ensheathing cells, Schwann cells, etc.) hold potentials for this purpose. It is worth mentioning that cell transplantation provides not only cell sources for remyelination, but also trophic supports as well as immunomodulatory effects (Ben-Hur, 2011). Numerous excellent reviews have been devoted to this topic, and thus will not be discussed further.

Finally, neuroinflammation is present not only in myelin disorders, but also in a diverse range of CNS disorders, including neurodegenerative disorders. It is increasingly recognized that the functional roles of microglia activation in neurodegenerative disorders are quite diverse, but not exclusively neurotoxic as we previously believed. Many immunosuppressive reagents, such as IFN-beta, glucocorticoids, and blocking antibodies for GM-CSF, profoundly affect microglia and macrophage function. However, therapies specifically targeting microglia/macroglia are lacking. It is tempting to speculate that once the roles of microglia in these CNS disorders are better elucidated, immunomodulatory reagents targeting microglia/macroglia may represent a novel class of drugs for myelin regenerative therapy.

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References