Remyelination Therapy for Multiple Sclerosis

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Abstract Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system characterized by infiltration of immune cells and progressive damage to myelin and axons. All therapeutics used to treat MS have been developed to target an overactive immune response, with aims to reduce disease activity. Chronic demyelinated axons are further prone to irreversible damage and death, and it is imperative that new therapies address this critical issue. Remyelination, the generation of new myelin in the adult nervous system, is an endogenous repair mechanism that restores function of denuded axons and delays their deterioration. Although remyelination can be extensive in some patients, the majority of cases limit repair only to the acute phase of disease. A significant current drive in new MS therapeutics is to identify targets that can promote remyelination by boosting endogenous oligodendrocyte precursor cells to form new myelin. Also, a number of inhibitory pathways have been identified in chronic MS lesions that prevent oligodendrocyte precursor cells from being properly recruited to demyelinated lesions or interfere with their differentiation to myelin-forming oligodendrocytes. In this review, we introduce the phenomenon of remyelination from the view of experimental models and studies in MS patients, describe a potential role in remyelination for currently available MS medications, and discuss many avenues that are being actively studied to promote remyelination. The next frontier in MS therapeutics will supplement immu-

nomodulation with agents that directly foster myelin repair, with aims to delay disease progression and recover lost neurological functions.

Keywords Oligodendrocytes · myelin · demyelination · remyelination · multiple sclerosis

Introduction

Multiple sclerosis (MS) is a chronic demyelinating disorder of the central nervous system (CNS) and a leading cause of disability in young adults. Relapsing-remitting multiple sclerosis (RRMS), characterized in the brain and spinal cord by robust inflammatory infiltration, myelin loss, and axonal damage, represents roughly 80 % of all MS cases. Development of therapeutics to treat MS have largely been aimed at reducing the aberrant immune reaction via modulation of immune cell subsets, reducing leukocyte trafficking across the blood-brain-barrier, or sequestering cells in peripheral secondary lymphoid organs. Despite tremendous advances in immunotherapy, including the first Food and Drug Administration-approved oral medication for MS, the vast majority of patients eventually develop secondary progressive MS, characterized by reduced inflammatory relapses, despite continued, irreversible neurological decline. It is believed that chronic demyelination of CNS axons leads to further axonal injury and neuronal death, and has been postulated to underlie this progression [1]. Indeed, experimental models of toxic myelin damage or myelin-interfering gene mutations result in subsequent axonal injury [2, 3]. An emerging focus in MS treatment is the development of strategies to restore lost myelin. This remarkable phenomenon, known as remyelination, is the next step in the future of MS therapeutics. In addition to the prospect of remyelination protecting denuded axons from degeneration, it is

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possible that remyelination could also restore some degree of lost neurological deficits.

In this review, we will introduce the concept of remyelination from the basis of experimental animal models, as well as human studies. We will describe evidence, albeit controversial, that currently available MS therapies may promote remyelination by mechanisms apart from their original purpose. We will explore the multitude of avenues in which remyelination can be promoted, via both intrinsic and extrinsic mechanisms. We conclude with prospects of repair in the future of MS care.

Remyelination

Viewed as its most fundamental property, the presence of concentric layers of compact myelin surrounding axons drastically increases the speed with which information can be transmitted throughout the nervous system. Segments of adjacent myelin internodes cluster sodium channels into exposed nodes of Ranvier, where saltatory conduction speeds signal transduction in an energy efficient manner. When myelin becomes stripped from axons, a process called demyelination, action potential velocity can slow by >30 times its normal, myelinated speed [4]. Demyelination causes diffuse distribution of channels, presumably as an attempt to maintain signal firing capabilities, albeit less efficiently [5].

Remyelination is the generation of new myelin sheaths around denuded axons in the adult CNS [6]. An immediate consequence of remyelination is proper redistribution of ion channels at the nodes of Ranvier and restoration of saltatory conduction [7], partially resolving an increased energy demand that is observable by reduced axonal mitochondrial content [8], and resulting in the recovery of functional deficits caused by experimental demyelination [9]. More importantly, evidence suggests that demyelinated axons are better protected from subsequent injury when they become remyelinated, perhaps by restoring proper growth factor signaling between the oligodendrocyte and the axon [10–12]. However, a recent report suggests otherwise [13]. Further evidence that the symbiotic relationship between the axon and oligodendrocyte is active and is not simply insulating, is that axons become extensively damaged when oligodendrocyte cell bodies are targeted for ablation, even in the absence of any observable demyelination [14].

Remyelinated axons appear to regain proper function, although there are observable differences in myelin architecture. Most obvious is the thickness of remyelinated sheaths and length of remyelinated internodal segments. Myelin sheath thickness normally increases with axonal diameter. However, remyelinated fibers tend to have invariantly thinner sheaths around the axons of all caliber [15]. Remyelinated internodes also tend to be shorter than developmentally myelinated nodes [16]. Indeed, the ratio between the inner axonal diameter and

the outer myelin diameter (g-ratio) has become a gold standard for assessment of demyelination and remyelination in experimental models.

Remyelination is a true regenerative process. Local mature oligodendrocytes are postmitotic, and although some are spared from initial demyelination, it appears they do not contribute to myelin repair [17]. Instead, it is generally believed that remyelination is mediated through oligodendrocyte precursor cells (OPCs) in the adult CNS, identified by the expression of neuron-glial antigen 2 (NG2) and platelet-derived growth factor receptor α [18, 19]. OPCs are widespread throughout the normal CNS and form myelinating oligodendrocytes well into adulthood [20].

Evidence of Remyelination through Experimental Models

Most of what is known in regard to the choreography of remyelination has been determined by the use of several different animal models. Immune-driven demyelinating models have become standard for pre-clinical screening of candidate MS therapies. In the hallmark MS model, experimental autoimmune encephalomyelitis (EAE), animals are immunized against fragments of myelin peptides, such as myelin oligodendrocyte glycoprotein. The adaptive immune system produces myelin-reactive T cells that invade the CNS and attack myelin and axons, resulting in a characteristic ascending paralysis. Although it is possible to study remyelination in these models, it becomes challenging to interpret whether a therapy directly promotes OPCs to enhance repair or if endogenous remyelination is a consequence of treating an aberrant immune reaction. Furthermore, a positive role for the immune system in driving repair cannot be discarded, given that remyelination is impaired in recombination activating gene deficient animals that are void of functioning lymphocytes [21]. Given this ambiguity, a number of primary demyelinating models were developed to study repair mechanisms independent of an ongoing adaptive immune response. A common approach is the injection of demyelinating agents, such as the detergent lysolecithin into the spinal cord white matter, or the chelator ethidium bromide into the caudal cerebellar peduncles [22, 23]. Ultrastructural analysis reveals splitting of intraperiod lines of compact myelin within minutes, despite apparent axonal normalcy [23]. Although there are similarities between these injection paradigms, ethidium bromide lesions are often slower to remyelinate than lysolecithin injuries and are toxic to not only oligodendrocytes, but also to astrocytes [24]. Also, of note is a larger proportion of remyelination induced by Schwann cells after ethidium bromide injection [25].

An alternative, but popular method of producing CNS demyelination is through the use of the copper chelator cuprizone, which is fed to animals in the diet [26, 27]. Demyelination persists through cuprizone feeding, and remyelination

commences immediately after switching to a normal diet [27, 28]. Detection of remyelination is challenging in this model due to the nature of small-caliber axons of the frequently analyzed corpus callosum that show similar g-ratios between normal myelinated and remyelinated axons. Also, the rate of remyelination is very robust, within a week of cuprizone withdrawal, so that it is difficult to document increased remyelination that may be afforded by therapeutics.

A criticism of these described models questions the specificity in the agent used to directly impair myelin without disrupting other cell physiology. Emerging models of demyelination are taking advantage of conditional cell ablation technology to specifically target oligodendrocytes. In these models, axonal pathology follows secondary to primary oligodendrocyte death [2, 14]. Interestingly, one of these studies reports axonal damage and loss of 26 % of oligodendrocyte cell bodies, despite no widespread demyelination, further aiding support to the suggestion that oligodendrocytes confer more properties to underlying axons than just insulation [14]. Although these models will be useful in dissecting the delicate interactions between CNS cells during demyelination and perhaps remyelination, a criticism is that the extent of damage is more widespread compared to the typical focal lesion nature of MS, and as a result the behavioral phenotype of these models can be quite severe.

Studies using these *in vivo* models of demyelination and remyelination, as well as *in vitro* culture systems, have revealed a wealth of knowledge regarding the many sequential and necessary steps involved for an OPC to remyelinate a denuded axon. First, OPCs must proliferate and migrate to the site of demyelination, driven by chemotrophic factors expressed by either activated microglia or astrocytes within the lesions [29, 30]. Thereafter, OPCs differentiate into myelinating oligodendrocytes, driven by transcription factors, such as Nkx2.2 and Olig2 that are conserved from developmental myelination [31]. Mature oligodendrocytes extend processes toward axons, make contact, enwrap axons with concentric layers of myelin membrane, and finally compact these layers into functional myelin [32].

Rodent models of demyelination convey a notion that remyelination is a robust, efficient, and rapid reparative mechanism after CNS insult. It should be noted, however, that there are intrinsic determinants which slow remyelination, apparently with the largest contributor being age. Although it has been shown that older animals can remyelinate as effectively as younger animals, this process is considerably slowed [33]. It is suggested that OPC recruitment, and subsequent differentiation, could be a cause of this decay in efficiency [34]. Experimental studies conclude that OPCs migrate to demyelinated lesions from a limited range [35, 36]. Repeat bouts of demyelination could conceivably exhaust local pools of myelin-forming OPCs, which appears not to be the case in younger animals [37]. Indeed, a clinical study from

progressive patients reported that newly remyelinated tissue is more prone to demyelination than normal-appearing white matter [38].

Recent thought-provoking evidence suggests that the underlying mechanism of decline of remyelination with aging is a reduced response of innate monocytoid immune cells (macrophages and microglia), which play a key role in remyelination [39–41]. Age-related deficiency in the monocytoid cell response could impede repair by reducing clearance of myelin debris, which is inhibitory for remyelination [41, 42]. Alternatively, monocytoid cells in the aged animal show reduced growth factors that are necessary for OPC recruitment and differentiation, such as platelet-derived growth factor and insulin-like growth factor-1, respectively [43]. Whether this explanation holds true for humans remains to be determined, although correlative evidence suggests greater remyelination efficiency at lesion edges with increased staining of macrophages/microglia [44]. Regardless, it emphasizes that the process of endogenous myelin repair, perhaps through stimulation of monocytoid cells, could be a therapeutic strategy for patients across a wide range of ages. After all, MS is currently a disease that people endure across many decades.

Endogenous Remyelination in MS Patients

Evidence of remyelination was an early pathological finding in MS histological studies. Early studies report the presence of uniformly thin, short internodes at the edges of chronic lesions, indicative of remyelination [45]. These internodes were different from the more variably thinner nodes seen in active MS plaques, which were believed to be undergoing demyelination [46]. Oligodendrocytes were identified as a presumably proliferating population of cells within demyelinating lesions, and increased numbers of cells were found at the lesion edge where remyelination was occurring [47]. The presence of both demyelination and remyelination has been observed at the edges of chronic plaques, as thinner myelin sheaths on axons are observed in the presence of myelin-phagocytizing macrophages, emphasizing the link between debris clearance and repair [12, 48, 49].

An ongoing puzzle regarding remyelination in MS is the variability with which it presents. For instance, extensive remyelination has been reported in ~20 % of cases in a post-mortem histopathological study, whereas the majority of cases showed little remyelination that typically occurred only at lesion borders [50]. Interestingly, extensive remyelination was not limited to RRMS, but also in certain patients with more progressive disease [50]. A more recent study has reported that primary progressive MS patients tend to have greater remyelination capacity than those with secondary progressive disease [38]. Intensive sampling of tissue from 2 patients with >20 years disease activity revealed that 95 % of all lesions showed at least partial remyelination, furthermore supporting that some patients

tend to extensively remyelinate despite chronic disease [44]. It is also important to note that even in reports of extensive remyelination, there is a large degree of variability in the extent of remyelination of individual plaques reported within the same patients [44, 49].

The location of remyelinating lesions tends to also play considerable importance, as subcortical lesions tend to remyelinate better than periventricular lesions [49, 50]. Lesions of the cerebellum tend to show persistent demyelination with little remyelination [49]. There are also notable differences in remyelination in white *versus* gray matter. An assessment of remyelination in both white and gray matter lesions from the same patients revealed a greater extent of repair in the gray matter [51]. The explanations for anatomical differences in remyelination are presently speculative, but they are an active area of exploration.

Despite the exceptions with extensive remyelination, it appears that remyelination, in general, is more prominent in the early stages of disease. A direct comparison of acute and chronic lesions revealed evidence of remyelination in 80.7 % of early lesions, with only 60 % in chronic lesions [49]. In early lesions, OPCs are found in greater density immediately outside of demyelinated plaques than in normal-appearing white matter; significantly fewer OPCs are seen in chronic lesions [52]. The latter smaller pool of chronic stage OPCs lack markers of cell proliferation and appear not to mature to myelinating oligodendrocytes, particularly in the center of the lesions [53]. However, another report found that not all oligodendrocyte lineage cells in chronic lesions remain stalled as OPCs. “Premyelinating” oligodendrocytes, defined as being positive for the marker proteolipid protein 1 and extending processes, have also been reported in chronic MS lesions [54]. These processes extend toward axons and even spiral around them, although they do not enwrap them with compact layers of myelin [54]. It is now appreciated that reduced numbers of lesion-associated OPCs, whether through reduced migration, decreased proliferation, or inhibited differentiation to myelinating oligodendrocytes, culminates in remyelination failure in chronic MS. The role of other cell types contributing to this deficiency, particularly the aforementioned slowing of the monocytoïd cell response [41], is awaited with anticipation.

In summary, remyelination is undisputed to occur in MS. Some patients remyelinate better than others, but the majority of patients would require some therapeutic intervention. Understanding why some patients resist remyelination failure could prove to be useful for the identification of new drug targets.

Evidence of Remyelination with Current MS Therapeutics

A number of disease-modifying therapies in MS have been developed, mainly to reduce ongoing inflammation. It is

debated whether some of these medications may also act on the CNS to directly promote myelin repair. Here we describe 2 of these cases in detail, and we also discuss what is known in regard to the emerging medications that are currently being tested in clinical trials.

Glatiramer Acetate

Glatiramer acetate (GA) is a mixture of 40–100 residue polymers (randomly assigned with *L*-alanine, *L*-glutamate, *L*-lysine, and *L*-tyrosine) that was approved for treatment of MS in the late 1990s and is currently used as first-line therapy [55]. GA is known to exert immunomodulatory properties through shifting CD4⁺ T cells into an anti-inflammatory, T_H2 phenotype, increasing the numbers of regulatory T (Treg) cells [56–59], and polarizing monocytoïd cells into an M2 subtype with an anti-inflammatory and potentially reparative phenotype [58]. Apart from an immunoregulatory role, increased numbers of OPCs and remyelination after GA treatment have been observed in both EAE and lysolecithin demyelination [60–62]. In tissue culture, secretory products from GA-polarized CD4⁺ T cells increase the number of OPCs from earlier precursors [61, 63]. The mechanism of GA conferring remyelination properties appears to result from increased growth factor expression, either from T_H2 or M2 polarized cells or as a bystander effect on resident CNS cells [58, 61, 63, 64]. Although the exact mechanism is unclear, it is also possible that antibodies raised against GA, commonly observed in GA-treated MS patients, interact with oligodendrocytes to promote repair [65, 66].

Fingolimod

Fingolimod is the first Food and Drug Administration-approved oral medication for the treatment of RRMS. It is a sphingosine-1-phosphate (S1P) receptor modulator, acting as an antagonist for endogenous S1P and causes receptor downregulation. There are 5 S1P receptors, and fingolimod binds with various efficacies to S1P₁₋₃ and S1P₅ in most body organs and cell types. Important for MS and other autoimmune diseases, fingolimod blocks S1P-mediated T cell egress from peripheral lymph nodes, reducing blood lymphocyte availability of autoreactive T cells to further target the CNS [67]. Because S1P receptors are found on a variety of cell types, and fingolimod has been shown to readily cross the blood-brain-barrier, considerable research both *in vitro* and *in vivo* has assessed the outcome of this medication directly on cells in the CNS. In isolation, fingolimod protects OPC survival, but inhibits their differentiation, the latter of which can be counteracted with growth factors [68, 69]. With longer treatment in culture, initial process retraction mediated through S1P₅ is overcome with process extension mediated through S1P₁ [70, 71]. The most compelling *in vitro* evidence for a beneficial effect on remyelination is observed in

coculture systems. Fingolimod had no effect on oligodendrocyte lineage cells in basal conditions, however, after lyssolecithin induced demyelination, fingolimod enhanced remyelination in both cell aggregate and organotypic slice culture models [72, 73]. Whether this effect was mediated directly through OPCs/oligodendrocytes, or whether a more important role exists for modulating astrocytic or microglial cells remains to be determined [72–74]. Interestingly, although fingolimod appears to protect myelinated fibers from demyelination, particularly as an early treatment strategy, direct evidence for remyelination has yet to be observed *in vivo*, including in the cuprizone and lyssolecithin demyelination models [74–76]. More studies are needed to assess the role of fingolimod, if any, in myelin repair.

Emerging MS Therapeutics

Available data on the mechanisms of MS therapeutics that are currently in clinical trial stages reveal effects on the CNS that are protective, whereas they are not necessarily linked to directly promoting repair. One example is orally administered dimethyl fumarate (BG-12), which reduced disease burden with an impressive safety profile in phase III clinical trials [77, 78]. Although BG-12 appears to confer cytoprotective effects by protecting against oxidative stress [79], a direct effect of remyelination in the cuprizone model was not observed [80]. The immunomodulator laquinimod, which also reduced disease activity at the phase III clinical trial stage [81], showed protective CNS responses in the cuprizone model, possibly through downregulation of an astrocytic proinflammatory response [82]. Scarce data is available regarding the repair capacity of agents at various stages of development. A detailed analysis of these agents assessing remyelination potential is strongly needed.

As methods improve to assess remyelination in humans, testing direct repair effects of candidate therapies will not have to rely solely on animal models of disease. For instance, magnetized transfer imaging is emerging as a tool that correlates to remyelination in animal models and post-mortem tissue; this method points to a potential remyelination capacity with the second-line therapy known as natalizumab [83].

Targets for Remyelination Therapy

Many targets involved in either boosting the endogenous oligodendrocyte response or overcoming inhibitors of cell differentiation have been identified as targets for putative remyelination therapies (Fig. 1). These strategies can largely be divided between cell intrinsic and extrinsic mechanisms, and here we provide examples of each.

Intrinsic Targets

Notch Signaling

The Notch-Jagged signaling pathway is important for the timing of myelination in the developing nervous system [84]. Prior to myelination, Notch1 receptors on OPCs interact with Jagged1 signals on axons, activating the canonical pathway that increases the downstream effector Hes5. In turn, Hes5 inhibits OPC differentiation until axon Jagged1 downregulation prompts the onset of myelination [85, 86]. It has been demonstrated that re-expression of the Notch1-Jagged1-Hes5 pathway in active MS plaques may contribute to stalled OPC differentiation [87]. Jagged1, which is expressed by human astrocytes *in vitro* via stimulation with transforming growth factor- β 1, was reported to be expressed in active demyelinating, but not remyelinated lesions [87]. There is conflicting evidence regarding a role for canonical Notch-Jagged in remyelination of the adult CNS. OPCs express Notch1 in an experimental demyelinating model, despite complete remyelination; Notch1 expression was also unlinked to slower remyelination with aging [88]. Although remyelination was reported in EAE after treatment with a Notch inhibiting γ -secretase inhibitor, markers of immune infiltration were also reduced [89], meaning a direct effect on remyelination is challenging to interpret. A further EAE study showed that while astrocytes appear to be a major contributor of Jagged1 ligand, consistent with an earlier report [87], Notch1 was surprisingly expressed exclusively by oligodendrocytes associated with remyelinated lesions, and not with lesions absent of remyelinated activity [90]. In contrast, a study reported a beneficial effect of conditionally ablating Notch1 in oligodendrocytes after lyssolecithin demyelination to the corpus callosum, despite reduced proliferation of progenitors [91]. It is now appreciated that while canonical Notch1 signaling via Jagged1 prevents OPC differentiation, noncanonical signaling mediated through Contactin interacting with Notch1 promotes differentiation [92]. Surprisingly, Contactin was reported to be expressed by demyelinated axons in MS, an opposite expectation considering the stalled differentiation of OPCs reported in chronic MS [93]. An explanation for this discrepancy was revealed when Notch1 intracellular domain, a cleaved form of Notch, was found trapped within the cytoplasm of OPCs; translocation of Notch1 intracellular domain into the nucleus is required for noncanonical promotion of differentiation [94]. It was discovered that TIP30, which inhibits the nuclear transporter Importin, was aberrantly expressed in these OPCs explaining why these cells fail to differentiate, despite the presence of Contactin [93]. Because Notch has now been associated with both promoting and inhibiting OPC differentiation via noncanonical and canonical signaling, respectively, it appears that any future for targeting Notch as a candidate remyelination therapeutic would have to counteract increased TIP30 expression [94].

Fig. 1 Intrinsic and extrinsic competitors in remyelination. Oligodendrocyte precursor cells (OPCs) need to be recruited to demyelinated lesions and further undergo maturation to myelin-forming oligodendrocytes for remyelination to occur. Attractive cues (Sema3F) compete with repulsive cues (Sema3A), chondroitin sulfate proteoglycan (CSPGs) for recruitment to lesions. Factors promoting oligodendrocyte differentiation (retinoid X receptor [RXR]- γ , noncanonical Notch1-Contactin signaling) compete with inhibitors of differentiation (TIP30, Wnt- β -catenin signaling, canonical Notch1-Jagged1 signaling, hyaluronan, CSPGs, LINGO-1) to impact OPC maturation. Boosting the endogenous OPC response or overcoming lesion-associated inhibitors serve as attractive strategies to promote repair in multiple sclerosis (MS). 9-cis retinoic acid (RA)

Wnt Signaling

Another identified negative regulator of oligodendrocyte differentiation is the canonical β -catenin-Wnt signaling pathway. In the developing spinal cord and telencephalon, Wnt signaling controls the timing of oligodendrocyte maturation by inhibiting progenitors in an immature state, without changing proliferation of precursors [95–97]. In contrast, Wnt signaling promotes neural differentiation, presumably to prevent oligodendrocyte maturation before axons are appropriate for myelination [98]. In agreement with this, SOX17, one of the identified transcription factors that regulate OPC cell cycle exit and differentiation, directly antagonizes β -catenin [99]. One identified mechanism controlling this regulation is the histone deacetylases 1/2, which compete with β -catenin for interaction with oligodendrocyte-specific transcription factor TCF4 and promote oligodendrocyte differentiation [100]. TCF4 has been reportedly expressed in lesion-specific adult white matter, as well as developmental human tissue; experimental overexpression of OPC specific β -catenin results in impaired remyelination without affecting recruitment [101]. Another known inhibition pathway of oligodendrogenesis, the bone morphogenic proteins, has been shown to act downstream of Wnt and also to cause global histone acetylation, counteracting the efforts of the histone deacetylases [102, 103]. The notion that Wnt/ β -catenin act distinctively as inhibitors of myelination has recently been challenged by a finding that inhibiting Wnt prevented expression of mature

myelin markers and myelination [104]. It is postulated that perhaps Wnt signaling first regulates the timing of oligodendrocyte maturation by inhibiting differentiation, but then switches to a driving force in the later stages of myelination [104]. Additional work is needed to explain this discrepancy.

RXR Signaling

The 2 examples previously described highlight signaling pathways in which OPCs are inhibited from differentiating into myelinating oligodendrocytes. What are the pathways that promote remyelination? One recently described system highlights the retinoid X receptor (RXR) signaling pathway as a positive driving force for repair [105]. The RXRs are nuclear receptors that homodimerize or heterodimerize with other nuclear receptors, including retinoid acid receptors, vitamin D receptors, and others [106]. Microarray analysis comparing stages of demyelination and remyelination revealed that all 3 members of the RXR family had increased transcription during the remyelinating phase; 1 member, RXR- γ , was upregulated exclusively during remyelination in both an animal model and in MS tissue [105]. Activators of the RXRs, such as 9-cis-retinoic acid (RA), promote OPC maturation *in vitro*, as well as remyelination *in vivo*. A recent report highlights that chronic demyelination with cuprizone increases expression of retinoic acid synthesizing enzyme retinaldehyde dehydrogenase type 1, and subsequently RXR β exclusively in astrocytes

[107]; outcomes of remyelination were not measured in this study, thus it is unclear if astrocytic RXR β results from a consequence of demyelination or as a compensatory response to attempt repair despite continued cuprizone treatment [107]. One report suggests a role for remyelination with supplementation of vitamin D3 [108], which could be explained by dimeric interaction of vitamin D receptors and RXRs, triggering a reparative phenotype. Vitamin D has become a popular agent in MS, linked through a negative correlation with exposure and MS risk, and for its known immunomodulatory properties [109]. It would be interesting to compare activators of RXRs (such as 9-cis-retinoid acid) to activators of their binding partners, such as vitamin D, to further tease apart their relative contributions to repair.

Extrinsic Targets

Semaphorins

Class 3 semaphorins (Sema) are known to exert opposite effects on OPCs through their neuropilin receptors. Sema3F acts as a positive guidance and mitogenic factor, whereas Sema3A is a negative guidance molecule for both embryonic, as well as adult OPCs [110, 111]. Indeed, injecting Sema3A into demyelinated white matter results in impaired remyelination [112]. An apparent contradictory finding is that Sema3A, as well as Sema3F, are upregulated in MS lesions and in models of experimental demyelination [113]. What seems more important is the relative proportion of the 2 molecules, and although correlative, there is a higher proportion of Sema3F than Sema3A in remyelinating tissue [113]. Shifting the balance away from Sema3A toward Sema3F may be a promising avenue of exploration with prospects of greater OPC recruitment to lesions, as well as a capacity for repair.

LINGO-1

LINGO-1 is a nervous system-specific transmembrane protein that interacts with the Nogo-66 receptor 1 and p75 signaling molecule [114]. LINGO-1 is expressed by oligodendrocytes and exerts control over developmental myelination *in vitro* and *in vivo*, through homophilic self-interactions and a RhoA-mediated mechanism [115, 116]. LINGO-1 is also expressed on axons, inhibiting OPC differentiation and myelination, but it is regulated by the nerve growth factor [117]. Function blocking LINGO-1 antibodies have been successful in reducing axonal damage, promoting remyelination, and restoring function after EAE, as well as in lysolecithin and cuprizone demyelination models [118, 119], despite achieving brain concentrations of <0.5 % of that in the blood [120]. Anti-LINGO-1 antibodies are now being introduced into humans as the first remyelinating therapy trial. As a true pioneer in the human translation of

remyelinating therapies, anti-LINGO-1 will pave the way for more remyelination therapies to reach the clinic.

Extracellular Matrix Molecules

The extracellular matrix (ECM), consisting of dense networks of proteoglycans, large molecular weight saccharide polymers, link proteins, and tenascins, are now appreciated to have a much broader range of functioning than simply providing structure and stability to CNS tissue. Components of the ECM radically change throughout development, and particularly after injury, to impact a number of functions, including cell migration and differentiation [121]. A body of evidence now supports that ECM changes, including the proteases that shape the ECM, impact the outcome of repair after demyelination. The matrix metalloprotease-9 is required for oligodendrocyte process extension *in vitro*, and knockout mice have reduced remyelination capacity, explained in part by a reduction in clearance of the shed form of the inhibitory proteoglycan NG2 [122, 123]. A high-molecular weight form of hyaluronan is produced by astrocytes in chronic MS lesions, as well as in experimental models, inhibiting OPC maturation *in vitro* and remyelination *in vivo*, signaling via a TLR2/MyD88 mechanism [124, 125].

Recent attention has been given to the chondroitin sulfate proteoglycan (CSPG) family, which impairs OPC adhesion and morphological differentiation *in vitro* via a Rho kinase mechanism and can be overcome with enzymatic degradation of CSPG glycosaminoglycan side chains with a bacterial enzyme called chondroitinase ABC [126, 127]. Our group has reported an increase in CSPGs within lesions after lysolecithin demyelination, which are cleared during correspondent endogenous remyelination [127]. Treatment with xyloside, which disrupts CSPG synthesis, reduces CSPG load at the lesion site, increases the number of mature oligodendrocytes, and enhances the remyelination response [127]. Because it is known that CSPGs also inhibit axonal regeneration, and perturbation of CSPGs promotes axonal sprouting [128, 129], it could be argued that increased remyelination after xyloside treatment is a consequence of increased axonal targets for new sheath development and not per se true remyelination. However, at least in spinal cord injury models, the kinetics of axonal sprouting and OPC invasion are discordant, suggesting that the 2 events are independent and CSPG disruption could indeed directly promote remyelination [130].

Therapeutically targeting ECM components, particularly CSPGs, could be achieved through a variety of means. Apart from enzymatic degradation through proteases or chondroitinase ABC, interfering with enzymes involved in CSPG biosynthesis, such as chondroitin polymerizing factor or xylosyltransferase-1, have been effective in promoting axon growth in the presence of CSPGs [131, 132]. Alternatively, the receptors that bind CSPGs, including protein tyrosine

phosphatase- σ , leukocyte common antigen-related phosphatase, or Nogo-66 receptor 1 and 3, could potentially be targets for the development of antagonistic inhibitors [133–135]. It is important to note that the majority of work investigating CSPG-mediated inhibition has been focused on growth of neurites after traumatic injuries, and inhibition of OPCs by CSPGs has only recently been put in the spotlight [126, 127, 130]. Whether similar or different mechanisms mediate CSPG inhibition of neurons *versus* OPCs awaits further study.

Conclusion

The future is promising for new therapeutics in MS. New immunomodulators, including orally administered BG-12 and teriflunomide, have shown impressive effects in reducing relapse rates in patients with RRMS [136]. However, immunomodulation is not enough to drive MS treatment into the next frontier. Generation of new myelin, fostered by boosting endogenous OPC recruitment and differentiation, or by overcoming lesion-associated inhibitors of OPCs, will change therapy from purely suppressing an aberrant immune reaction to directly promoting myelin regeneration. It is most likely that the 2 streams of therapy will work in concert to synergistically increase outcome measures for MS patients. This is particularly important because remyelinated areas in MS are subject, and indeed they are more prone, than normal-appearing white matter to new inflammatory demyelination [38]. There are many avenues aimed to directly promote myelin repair, as well as the emergence of techniques available to quantitate remyelination in MS patients. We believe that the future of MS trials will evaluate current or upcoming, particularly orally available, immunomodulation strategies accompanied by agents to facilitate endogenous OPC repair of lesions. Although not described here, there is a multitude of exogenous stem cell transplantation strategies being considered to replace lost cells or aid the endogenous repair stream. Primary outcomes of reducing new lesion formation and relapse rate will be accompanied by existing lesion repair and at best will delay or prevent disease progression.

There are still many challenges ahead for remyelination therapies. With axons that are severely compromised or lost, would axon tracts conduct impulses sufficient to re-establish functions? With so many inhibitors present within lesions, would the neutralization of 1 be sufficient to overcome a hostile microenvironment? What roles do neighboring cells, including microglia and astrocytes, play in the remyelination process? Nonetheless, inherent negative cues can be overcome naturally because remyelination is a normal occurrence in the MS lesion. The remarkable nature of remyelination will only become more exciting as new therapies are brought to human trials.

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