The development of myelin repair agents for treatment of multiple sclerosis
Progress and challenges

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Keywords: myelin, multiple sclerosis, remyelination, oligodendrocyte, neurodegeneration

Abbreviations: MS, multiple sclerosis; CNS, central nervous system; BBB, blood brain barrier; IFN, interferon beta; OL, oligodendrocyte; OPC, oligodendrocyte precursor cell; SVZ, subventricular zone; RXR, retinoid X receptor; EAE, experimental autoimmune encephalomyelitis; LINGO-1, leucine rich repeating and Ig containing NOGO receptor interacting protein-1; LPC, lyssolecithin; ARR, annualized relapse rate; EDSS, expanded disability status scale

Multiple sclerosis (MS) is an inflammatory demyelinating disorder which affects the central nervous system. Multiple sclerosis treatment has traditionally focused on preventing inflammatory damage to the myelin sheath. Indeed, all currently available disease modifying agents are immunomodulators. However, the limitations of this approach are becoming increasingly clear, leading to the exploration of other potential therapeutic strategies. In particular, targeting the endogenous remyelination system to promote replacement of the lost myelin sheath has shown much promise. As our understanding of remyelination biology advances, the realization of a remyelinating therapeutic comes closer to fruition. In our review, we aim to summarize the limitations of the current immune focused treatment strategy and discuss the potential of remyelination as a new treatment method. Finally, we aim to highlight the challenges in the identification and development of such therapeutics.

Introductory Comments

Multiple sclerosis is an autoimmune disease distinguished by the primary pathological hallmarks of central nervous system (CNS) demyelinated lesions associated with inflammatory infiltrates. The prevalence of MS varies greatly worldwide but is estimated to affect between 2 and 150 per 100,000 people, depending on the country or specific population, making it one of the most common disabling disorders. The white matter lesions associated with MS are classically described as resulting from myelin-reactive lymphocytes crossing the blood brain barrier (BBB) and entering the brain and spinal cord. Once in the CNS these cells drive an immune response which damages oligodendrocytes, the cells responsible for the production of myelin, and strips axons of the myelin sheath.

Demyelination results in interrupted axonal signal conduction as well as secondary axonal degradation which manifests as a combination of any or all of the following symptoms: blurred vision, muscle stiffness, muscle weakness, tremor, fatigue, vertigo and cognition impairment. Based on the pattern of symptom appearance, MS can be classed as one of either relapsing remitting MS or progressive MS. The most common form of MS, relapsing remitting, is characterized by acute inflammatory attacks associated with bouts of disability, separated by periods of remission where no symptoms are apparent. However, this often develops into secondary progressive MS which is characterized by worsening disability accompanied by a shortened or absent remission phase. Primary progressive MS, in which there is progressive clinical deterioration from the onset of the disease, can also occur but is less common. To date, MS therapeutics have only been successful in reducing the relapse rate in relapsing remitting forms of MS. No disease modifying agents which effectively halt the progressive forms of MS have been identified. In the following sections we aim to summarize the current immunomodulating therapies available, the search for new remyelinating MS therapeutics and some of the potential challenges in identifying and assessing such therapeutics.

Current Therapies

The current therapeutic strategy for MS is aimed at preventing inflammatory damage to the CNS through the use of immunomodulating drugs. Interferon β (IFNβ), glatiramer acetate, natalizumab and Fingolimod are the leading therapies currently used in the management of MS symptoms. Fingolimod, the most recently approved of these, is a sphingosine analog which interacts with sphingosine 1-phosphate receptors on lymphocytes to reduce autoreactive cell infiltration into the CNS. IFNβ has been shown to have inhibitory effects on the proliferation of leukocytes, antigen presentation and T-cell migration across the blood-brain barrier, but it is only partially effective at delaying disease progression. Glatiramer acetate is a synthetic polymer originally designed to be...
It has been hypothesized that impaired OPC differentiation, rather than migration or proliferation is the primary underlying cause of the reduced remyelination competence seen in MS. 

Pathological evidence from human post mortem tissue appears to support this hypothesis. It has been shown that MS lesions are populated with OPCs, but that these cells lose the ability to differentiate into mature oligodendrocytes. Unsurprising then are findings which show that the white matter lesion microenvironment contains factors which inhibit OPC differentiation.

Based on this evidence, research has been focused on ways to overcome OPC differentiation failure and spark endogenous remyelination.

Encouragingly, multiple influential pathways or signals have now been discovered which regulate OPC differentiation. Wnt, Notch and retinoid X receptor (RXR) signaling have all been shown to be regulators of OPC differentiation and to play an important role in myelogenesis and/or remyelination. These pathways are particularly interesting as they have already been extensively studied in other settings, and there are pharmacological tools available to manipulate these pathways. Promisingly, some of these pharmacological tools have already been used to demonstrate proof of principle that these pathways are valid therapeutic targets for demyelinating disorders. Activation of RXR with the agonist 9-cis-erotic acid has been shown to enhance remyelination in vivo and in vitro, inhibition of WNT signaling through GSK-3β antagonism stimulate OPC differentiation and remyelination in vitro, and Notch inhibition has been shown to alleviate experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Given that these pathways are also areas of interest for anti-cancer therapeutics, there may be a concerted effort to quickly develop and test drugs which target these pathways in the context of MS.

Perhaps the most important of these recently identified OPC differentiator regulators is leucine rich repeating and Ig domain containing Nogo receptor interacting protein-1 (LINGO-1). LINGO-1 was first identified as a CNS specific transmembrane protein which interacts with the Nogo-66 receptor complex and acts as a strong inhibitor of axonal sprouting. More recently, LINGO-1 has been shown to negatively affect myelination and to inhibit OPC differentiation. LINGO antagonism has been shown to enhance remyelination in EAE as well as ameliorating the paralytic symptoms seen in this model. Importantly LINGO-1 antagonism has also been shown to promote CNS remyelination in two toxin induced models of demyelination, and it appears that this is achieved through direct regulation of OPC differentiation.

This encouraging data has led to a LINGO-1 antagonist (BBIB033) entering clinical trials for MS. The outcome of these trials will be a watershed moment for remyelinating therapeutics as it will offer proof of concept that an agent which promotes remyelination is beneficial in MS. Should LINGO-1 antagonism be shown to have marked benefit clinically, it will become the prototypical “remyelinating agent.” Other potential therapies are thus likely to follow the same path of pre-clinical development, and to be evaluated on the same criteria that LINGO-1 antagonism has been shown to meet. A LINGO-1 antagonist was...
brought to the clinic on the back of robust effects in animal models, its clinical utility is often limited. In this regard, it is completely unknown whether these models can act as reliable predictors of clinical efficacy.

**Challenges of Identification and Development of Remyelinating Therapeutics**

Preclinical testing of MS therapeutics has historically relied on models which aim to simulate the immunological component of MS. However, models of demyelination which are independent of inflammation have increased in popularity as the search for remyelinating therapeutics intensifies. None of these models are without weaknesses and one should be aware of the limitations of these models for assessing remyelinating therapeutics.

The classic model for identifying and assessing MS therapeutics has been EAE. Initially described in the 1930s in primates, EAE has been studied extensively, but only some versions reproduce significant demyelination.50 EAE was later adapted for rodents to facilitate MS research. Classically EAE is described as CD4+ Th1 cell response against myelin, which is typically induced by injecting one of the myelin components, MBP, MOG or PLP, with an adjuvant to induce an auto-aggressive response against myelin. Less frequently EAE is induced by adoptive transfer of CD4+CD8- myelin reactive cells to a naive animal. Typically EAE manifests as a distinct hindlimb paralysis accompanied by white matter lesions with inflammatory cell infiltrates. This model is extremely flexible and by varying the myelin component or animal strain, relapsing remitting, chronic progressive or acute forms of EAE can be induced.51 Studies using this model have shown that by dampening the immune response or modulating the infiltration of white cells into the CNS, the severity of EAE can be reduced.52 Indeed, fingolimod, glatiramer acetate, IFN-β and natalizumab have all been shown to have efficacy in some model of EAE.44,45 For years, EAE has proven to be the basis for the greater understanding of the immunological component of MS as well as becoming the standard test for identifying immunomodulating MS therapies.

The question remains, however, as to whether EAE holds much value in investigating remyelination therapeutics. One of the EAE model’s greatest strengths lies in its flexibility and reproducibility across multiple species. The variances in species and auto-antigens used to study EAE result in differences in the pathology of EAE, meaning that one must carefully choose the correct model to avoid misleading or uninformative results. For example, it is known that MBP induced EAE in Lewis rats has a large inflammatory component but CNS demyelination is absent or highly restricted,53 making this model unsuitable for investigating remyelinating therapeutics. It is now known that all forms of rodent EAE result in inflammatory infiltrates into the CNS but only some versions reproduce significant demyelination.54 In particular, remyelination in MOG induced EAE in C57B6 mice is frequently studied, but the suitability of this model for assessing a purely remyelinating therapeutic still remains questionable. The paralytic symptoms seen in this form of EAE are likely due to a mixture of edema, loss of axonal integrity and demyelination rather than resulting exclusively from persistent demyelination.55,56 Careful interpretation of results is therefore required as it is not sufficient to rely on the degree of functional recovery of symptoms as indicative of remyelination. Extra diligence is needed to ensure that a therapy which ameliorates EAE is truly enhancing remyelination and not just simply acting to influence the onset of EAE. This becomes even more important when it is taken into consideration that drug interventions are often given before EAE symptoms develop.57,58 Even extensive historical analysis may not be enough to conclusively confirm a remyelinating effect as it can be difficult to assess the extent of demyelination and remyelination independent of the impact of axonal loss and inflammatory infiltrates. Perhaps most worrying however, is the notoriety that EAE is gaining as a poor predictor of clinical efficacy.59 Many interventions have been shown to ameliorate symptoms in this model, but few have been successful clinically, raising concerns over this model’s suitability for therapeutic assessment.

Unlike EAE, which aims to approximate the pathophysiology of MS, toxin induced demyelination is used to study demyelination and remyelination in isolation of an inflammatory environment. Therefore, the interpretation and analysis of a remyelinating effect should be more straightforward than with EAE. Several toxins have been shown to induce CNS demyelination, including but not restricted to, ethidium bromide, lyssolecithin and cuprizone. Of these three, the two most extensively studied models, and the two models in which LINGO-1 antagonism has proven to be effective, are cuprizone diet-induced demyelination and lyssolecithin-induced focal demyelination. Lyssolecithin (LPC) is a known potent demylating agent which is long established as causing demyelinated focal lesions upon injection into the spinal cord.60 Since then LPC has been shown to cause demyelination in other central nervous system regions61 as well as exhibiting demyelinating properties when applied to the peripheral nervous system.62,63 In the context of CNS remyelination, LPC-induced spinal cord lesions still appear to be the model of choice as the unidirectional path of axons in the spinal cord makes it easy to assess myelination and axon numbers by microscopy. Upon LPC injection, measurable demyelination can be seen within hours.64 Remyelination occurs over the following weeks,65 with the rate of recovery depending on the age of the animals.66 There appears to be relatively little axonal loss in this model,67 with LPC appearing to function by acting primarily on oligodendrocytes at the concentrations typically used in these studies. Although oligodendrocytes have been shown to be particularly sensitive to LPC toxicity,68 it is uncertain why this is the case. This model provides a platform for the study of inherent remyelination ability, as well as providing a window of opportunity for assessment of treatments which accelerate the repair process.

More recently LPC has been applied to in vitro organotypic or multicellular systems,69,70 where it reproduces the cycle of demyelination and remyelination seen in vivo. In particular, organotypic-based models are becoming a popular research tool as a first pass system to identify potential remyelinating therapeutics before progressing to expensive and laborious in vivo studies. Jarjour et al.71 present a comprehensive review of these in vitro demyelination models, some of which use LPC as demyelinating toxin.
Cuprizone-induced demyelination is rapidly becoming the standard model for studying remyelination biology. A powdered cuprizone diet of 0.2% w/w over a 6–8 week period produces a reliable myelin insult in several different brain regions, notably the hippocampus,65,66 the cortex67-69 and the corpus callosum.70,71 The corpus callosum is the most studied of these regions, primarily because it is a large white matter tract which is easy to identify and to assess levels of myelin. Although first described in the 1960s,72 the exact mechanism of cuprizone demyelination is unclear. Copper toxicity,73 mitochondrial dysfunction74 and inhibition of OPC differentiation75 have all been suggested to play a role. Whatever the cause of demyelination, upon removal of cuprizone from the diet, remyelination spontaneously occurs over a period of weeks.76 Similar to LPC-induced demyelination, this model has provided a platform for basic remyelination biology as well as the assessment of interventions which may accelerate the spontaneous repair process.

Although the toxin-induced models of demyelination have offered valuable insights into the basic biology of remyelination, there are some caveats which may cast doubt over their ability to act as an indicator of clinical efficacy.

First, any positive remyelinating effects recorded are in isolation of an inflammatory environment. Inflammation, at the very least, has a role to play in the pathophysiology of MS. Any pro-myelin therapy will have to overcome an inflammatory environment to be of clinical efficacy. Before being confident of an agent's clinical relevance one has to balance the knowledge that toxin models have minimal inflammation, while the standard inflammatory model used for MS therapies is likely to be unsuitable for identifying remyelinating agents. Therefore efficacy in either one, or both, of these models, does not necessarily guarantee clinical relevance of an agent.

Second, neither toxin-induced model has a true functional readout of demyelination or remyelination. The ultimate aim of a remyelinating therapeutic is to reverse or at least halt the functional deficits seen in MS. In order to achieve this, saltatory conduction must be restored in freshly remyelinated axons, yet CNS function is rarely studied in these models. Unlike EAE there are no gross behavioral changes which can be easily assessed. There have been some subtle changes noted in the behavior of cuprizone-treated mice, including prepulse inhibition deficit102 and changes to motor coordination,103,104 but it needs to be fully elucidated if these deficits are directly and exclusively the result of demyelination.

Typically the success of a remyelinating therapeutic in vivo is defined by assessment of myelin in post-mortem tissue through the use of electron microscopy, classic histological stains such as luxol fast blue or immunofluorescent staining against myelin components. While these tools can demonstrate that myelin sheath has been restored, there is an underlying assumption that the ensheathed axons are once again functional. In truth, these histological assessments reveal little of the integrity or functionality of the ensheathed axons.65

Restoration of CNS conduction velocities, combined with histological confirmation of myelin, would constitute compelling evidence of the benefit of a remyelinating agent. Demonstrable functional deficit rescue by a therapy may also increase the probability of therapy efficacy translating from animal model to clinical trial. Return of normal signal transmission indicates that the myelin sheath has not only been restored but also that the underlying axons have been returned to functional norms and that remyelination has indeed acted as a neuroprotectant. Interestingly, it has already been shown that axon conduction is impaired in cuprizone demyelination,105 reaffirming its suitability as demyelination model. However, conduction does not return to normal levels even after complete remyelination106,107 as measured by the typical histological markers thus, further investigation is required. This finding also suggest that restoration of the myelin sheath in the cuprizone model occurs subsequent to significant axonal damage and remyelination alone is not sufficient to reverse functional deficits. In contrast, LPC demyelination has been shown to cause conduction block, which is restores to normal upon remyelination.108 It should be noted that LINGO-1 antagonism has been shown to have functional benefits as measured by conduction velocity,109 bolstering the hopes that it will display clinical efficacy, although this property appears to have been largely undervalued in comparison to data gathered from histological analysis.

The models currently available are imperfect. EAE models approximate the immunological components of MS but they may not be appropriate for assessing remyelinating therapeutics. On the surface, targeted testing of remyelinating therapeutics in toxin induced demyelination appears to be more rational strategy, but it is underemphasised if these models can act as good predictors of clinical efficacy. However, the value of these models in providing insight into the biology of myelination processes, and identifying promising therapeutic leads should not be invalidated because of this uncertainty.

BIIB035 is the first remyelinating therapeutic to enter clinical trials, largely based on its successes in the aforementioned preclinical models. In the coming years it is hoped that several other drugs will join BIIB035 at this stage of development. The judgment of efficacy of a remyelinating therapeutic may prove to be less straightforward than that of an immunomodulator as MS symptoms can result from a mixture of axonal loss, demyelination and inflammation. Post mortem tissue is not readily available for study, biopsies are difficult to obtain and it is unknown how or if remyelination will correlate with MS symptoms. Imaging techniques which visualize the extent of remyelination could offer the solution to this problem. Progress is being made in this area,115,116 but we are still some time away from reliably monitoring remyelination clinically.

How then, can the impact of a remyelinating therapeutic be measured if we cannot easily quantify remyelination directly and are uncertain to what degree efficacy will correlate with symptoms? One possible solution is to assess new remyelinating agents in combination with existing immunomodulatory treatments. The current disease modifying agents are known to reduce the frequency of relapses, as measured by the annualised relapse rate (ARR). As relapses are linked to an immune response, a remyelination therapeutic, with a mechanism independent of immunomodulation, is unlikely to affect the ARR score. A second scoring
system, the expanded disability status scale (EDSS), is used to quantify disability in MS. Increasing EDSS scores, indicative of worsening disability, are typically seen over the course of MS, particularly in the progressive phases. It is known that the current worsening disability, are typically seen over the course of MS, because disability largely results from the loss of chronically demyelinated axons, rather than primary inflammation. A remyelinating therapeutic, which theoretically repairs the damage caused by inflammation and prevents secondary axonal loss from this damage, should therefore reduce the EDSS score. Whether a remyelinating therapeutic alone would be powerful enough to overcome the inflammatory damage in MS and reduce the EDSS score is unknown. This raises the possibility of a two pronged treatment strategy: combining a remyelinating agent with an immunomodulator. This would at least reduce the confounding factor of inflammation, while allowing for assessment of any synergistic effects. The already established EDSS scoring system could, perhaps, act as a proxy to measure the impact of the remyelinating agent. As functional improvements are not seen with an immunomodulator alone, any halt or reversal in neurological deterioration as measured by the EDSS score would most likely be attributable to the remyelinating agent.

In conclusion, there is large scale on-going research activity focusing on the development of remyelinating therapeutics. There are now several established models which are useful for studying remyelination biology which have helped identify multiple drug targets, but, at this stage, it is simply unknown if efficacy in these established models will translate into tangible clinical benefits. It is only now, with the first targeted remyelinating therapeutic entering clinical trials that we will begin to learn what refine- ments of these models are needed to improve successful crossover from pre-clinical to clinical stage. Moreover, it needs to be taken into account that the previously used methodology for assessing immunomodulating MS therapeutics may not be applicable for the determination efficacy in the context of remyelinating agents.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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