Promoting Remyelination and Preventing Demyelination
New Research Goals in Finding a Therapy for Multiple Sclerosis

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Recenit Multiple Sclerosis (MS) research has made it apparent that demyelination has further consequences than its primary effects of inflammation and impaired conduction. It is now well understood that demyelination leads to significant progressive axonal and neuronal degeneration. This finding explains why patients receiving immunosuppressive therapies still show disease progression – they have unremitting demyelination because sufficient remyelination does not occur, leaving axons exposed and constantly susceptible to damage (1). Consequently, there has been a push towards researching neuroprotective and myelin repair strategies as new therapy requirements for MS patients (2). This review will focus on recent advances in promoting remyelination and preventing demyelination, with specific attention directed towards methods that utilize endogenous oligodendrocyte progenitor cells (OPCs) rather than the transplantation of exogenous ones.

Introduction

Demyelination is the destruction of myelin protein, which forms a sheath around neuronal axons. In the central nervous system (CNS) demyelination is caused by the direct attack of oligodendrocytes, which make and maintain the myelin sheath. Remyelination on the other hand, is the process in which myelin sheaths are restored to demyelinated axons. Although remyelination produces thinner and shorter myelin sheaths, functional deficits are mostly restored. This process is the normal body response to demyelination but is impaired in patients with multiple sclerosis. MS patients are therefore left with axons that are demyelinated and vulnerable to damage, resulting in neurological deficits. While the reason for remyelination failure in MS patients is still ambiguous, progress has been made in learning how remyelination occurs (1).

Remyelination involves the re-recruitment of mature oligodendrocytes, which are derived from adult central nervous system (CNS) stem cells called OPCs located in the subventricular zone (SVZ). OPCs are induced to proliferate and migrate by growth factors that are up-regulated during remyelination (3). It is thought that remyelination may be limited in MS patients because of compromised OPC differentiation and maturation processes or decreased OPC recruitment (2). This knowledge has led to much investigation focusing not only on how to prevent demyelination, but also on the proliferation, differentiation, and recruitment of OPCs to promote remyelination. There is an abundance of research that has shown the effectiveness of various compounds on these processes in animal models. The goal is to eventually find a method of promoting remyelination or inhibiting demyelination that can be used to treat MS patients in order to retain axonal function and hinder the progression of the disease.

Common Animal Models for Demyelination Disorders

To fully understand the relevance of the studies demonstrated in this review, it is important to understand the animal models for demyelination that are often used. It has been difficult to identify a model that replicates the characteristics of MS because every demyelinating model that has been found has intact remyelination processes. While the goal of MS research for remyelination techniques is to discover an intervention that will reactivate the dormant process, these models can only demonstrate at best, acceleration of the already ongoing active process or in some cases an abnormal increase in myelin or oligodendrocytes (2,4).

Some examples of models that are frequently used are the cuprizone-induced demyelination model, the experimental autoimmune encephalomyelitis (EAE) model, and the lysolecithin (LPC)-induced focal demyelination model. In the cuprizone-induced demyelination model dietary cuprizone is fed to the animal, which results in the demyelination of specific CNS tracts in a dose-dependent manner (1). In the EAE model, which is a mouse model for MS, the disease is elicited by introducing MS antigens and their adjuvants (5). In the lysolecithin model, lysolecithin is injected into the lumbar spinal cord producing a focal region of primary demyelination (6). Unfortunately, all three of these models show extensive, if not complete remyelination. While this must be taken into consideration when analyzing these studies, it should also be noted that even in MS, a disease characterized by failed or inadequate remyelination, there is evidence that in some patients complete remyelination occurs in a significant proportion of lesions (1).

How Can Remyelination be Enhanced?

One of the key approaches currently being tested in animal models is
endogenous repair of myelin sheaths. This approach uses methods to promote the repair of myelin by already present precursor cell populations in the adult CNS, rather than by transplantation of exogenous cells (1). There are a wide variety of endogenous repair methods whose roles in remyelination are currently being investigated. These mechanisms generally fall into three categories: those that directly promote OPC differentiation; those that are found to inhibit remyelination and can be targeted to enhance remyelination; and those that are normally necessary for remyelination but could be deficient in patients with demyelinating disorders.

Directly promoting OPC differentiation

Mechanisms that directly promote OPC differentiation are currently by far the most researched methods of remyelination. This approach is attractive because if OPC differentiation mechanisms can be understood, then the hope is that they can be manipulated into therapies to promote remyelination. For example, chemokines and their receptors are being investigated for their role in MS. Of particular interest is the finding that the chemokine CXCL12 is a molecule known to mediate the migration, proliferation, and differentiation of neuronal precursor cells within the developing CNS (7). In a recent study by Patel et al. the expression of CXCL12 and its receptor CXCR4 were assessed within the demyelinating and remyelinating corpus callosum of a murine cuprizone-induced model. Two pieces of evidence were found supporting the hypothesis that CXCR4 activation is important in remyelination. It was found that antagonizing CXCR4 prevents remyelination within the corpus callosum after cuprizone exposure ended, and that in vivo CXCR4 RNA silencing inhibits remyelination after demyelination. Patel et al. concluded from their findings that up-regulation of the chemokine CXCL12 is fundamental for the differentiation of CXCR4-expressing OPCs into mature oligodendrocytes within the demyelinated model, and that if these are blocked, there is remyelination failure. This study suggests that CXCL12 and CXCR4 could be potential targets to enhance remyelination in MS patients (7).

Another receptor that when activated was found to promote oligodendrocyte formation and maturation is the thyroid hormone beta receptor. It has been known that thyroid hormones participate in oligodendrogenesis and myelination during mammalian development (8). In 2004, Fernandez et al. showed that if thyroid hormone is administered during the acute phase of MS, there is increased expression of platelet-derived growth factor alpha receptor, which restores normal levels of myelin basic protein mRNA and protein, and allows early remyelination. They also found that thyroid hormone exerts a neuroprotective effect with respect to axonal pathology (8). Unfortunately, the therapeutic potential of thyroid hormone has been challenged due to concerns of cardiac toxicity (9). However, Potter et al. found that these concerns are actually only mediated by the alpha receptor, and therefore a beta selective thyroid hormone receptor ligand could be utilized. Potter et al. has shown that GC-1, a beta selective ligand, can be used to induce differentiation of OPCs with the same success of a ligand for the alpha and beta receptor isoforms (such as the one used by Fernandez et al.). It was confirmed that the thyroid hormone receptor was up-regulated with oligodendrocyte differentiation. These findings suggest that selective control of thyroid hormones and their receptors could potentially prove to be a successful strategy in promoting remyelination in MS patients, while still avoiding the concerns accompanying non-selective TH stimulation (9).

Other compounds that are not endogenous to the model, but that can be used to enhance the function of the already present OPCs have also been found. Bordet et al. has used the fact that several growth factors have been shown to affect OPCs survival and proliferation, to find a drug that could replicate the actions of these growth factors. The lab had previously identified a class of cholesterol-oxime compounds that demonstrated neuroprotective properties. Of these compounds, olesoxime, which has been previously shown to accelerate axon regeneration and remyelination in the peripheral nervous system, was tested for its role in OPC differentiation. In the cuprizone-induced demyelinated model, olesoxime was shown to increase the number of myelinated axons in the corpus callosum, increase myelin sheath thickness, increase the number of mature oligodendrocytes, and improve the clinical course demonstrated by Rotarod scores. In lysolecithin-induced demyelination models, olesoxime had the same positive effects, and was also shown to reduce the lesion load after demyelination. It was concluded that olesoxime dose-dependently accelerates OPC differentiation by promoting their maturation in vitro. This compound also shows some promise as a therapeutic drug for MS patients because it is orally bioavailable, crosses the blood-brain barrier easily, and has already been shown to be safe for humans. However, positive evidence of olesoxime’s efficacy in animal models is needed before it can be further developed as a treatment for MS (2).

There are a plethora of other endogenous molecules, as well as exogenous ones that have been shown to aid in oligodendrocyte differentiation. For example, Sox17, a transcription factor known to be prominently expressed at OPC cycle exit and at the onset of differentiation, was investigated in demyelinating models. Strong evidence was shown supporting the fact that Sox17 is involved in promoting OPC differentiation that leads to OPC maturation and potentially remyelination. Up-regulation of Sox17 could again have therapeutic implications for MS (10). A final example of a molecule that has been recently found to have an effect on OPC differentiation is minocycline. In the past, minocycline has been shown to decrease the severity and progression of EAE in mice. It is now being demonstrated that minocycline also promotes remyelination via immature oligodendrocyte differentiation in various animal models by weakening microglial reactivity. Again, with further research, therapeutic treatments are a hopeful possibility (11).

Targeting natural inhibitors of remyelination

Although far less common, mechanisms have been found in models that naturally inhibit remyelination, which could potentially be targeted to...
enhance remyelination. Hyaluronan is a newly discovered example of such a molecule. Hyaluronan has been found to accumulate in demyelinated lesions in MS patients and in rodent models, and has been shown to prevent remyelination by inhibiting OPC maturation (12). Further studies have shown that murine OPCs make hyaluronan themselves. However, they also express several hyaluronidases, which are enzymes that degrade hyaluronan during growth-factor-induced oligodendrogenesis in vitro. Hyaluronidase expression fluctuates as OPCs differentiate and mature into myelinating oligodendrocytes in normal animals. Based on these findings, it was hypothesized that specific temporal patterns of hyaluronidase expression and hyaluronan turnover may be key regulators in the generation, differentiation, and maturation of oligodendrocytes. To test this hypothesis, the effects of viral mediated over-expression of one specific hyaluronidase, PH20 was analyzed. PH20 increases proliferation of OPCs but decreases differentiation into mature oligodendrocytes. It was also shown that breakdown of hyaluronan by PH20 causes inhibition of remyelination in lysolecithin-induced demyelinated mice models. Therefore, it is possible that PH20 breaks down hyaluronan, releasing products that inhibit oligodendrocyte maturation. Since PH20 expression is seen in MS lesions, it is possible that it may represent a new therapeutic target for patients (13). The assumption is that this would ensure that hyaluronan would not be destroyed and would not release its harmful products, thereby promoting remyelination. Before this is seen as a therapeutic possibility, further research is likely necessary to ensure that there are no other forms of hyaluronidase naturally present in the brain that could destroy the hyaluronans near the lesions. Otherwise this could cause the same detrimental effects of PH20. Targeting a specific hyaluronidase such as PH20 might render ineffective if there are other hyaluronidases that perform the same negative function.

Deficiency of molecules normally necessary for remyelination

In regards to promoting remyelination there has also been some focus on identifying components of the cell that are necessary for normal remyelination, which could potentially be deficient in patients with demyelinating disorders. The role of iron has been the subject of much research in recent years (14,15,16). It has been concluded by some that iron is essential to myelin production by showing that reduced iron in the diet is associated with hypomyelination (14). In other experiments, it was shown that iron levels might affect oligodendrocyte development at early developmental stages, and that myelin composition is altered by limited iron. These changes in myelin that were induced in mice by iron deficiency, could be reversed by a single injection of apotransferrin (16). Schulz et al. went on to show that astrocytes are the source of iron for oligodendrocytes, and that efflux of iron from these cells is necessary for remyelination in a demyelinated model. A major iron transporter in astrocytes, ferroportin (Fpn), was analyzed to determine whether or not astrocytes deliver iron to oligodendrocytes during situations where high amounts of iron are needed, such as during remyelination. Astrocyte-specific Fpn knockout mice were created and were induced with localized demyelination using intraspinal LPC. Fpn knockout mice showed significantly reduced remyelination as compared to the wildtype control mice, suggesting that astrocytes do indeed provide iron to oligodendrocytes during remyelination (15). The clinical implications of this research must be further analyzed as it is still inconclusive whether or not iron deficiency is a problem in MS patients. Even if it is found that many patients are iron deficient, it is unlikely that they are completely void of iron transport from astrocytes to oligodendrocytes since the function of iron is widespread and essential for normal brain function. Therefore research must be directed towards determining if there is a dose-dependent relationship between iron efflux to oligodendrocytes, and remyelination. Even with the vast research pre-
sented on possible mechanisms of promoting remyelination, we do not conclusively understand how remyelination works in the human body. As a result, it is impossible to explain why remyelination fails in patients with MS. However, with the continuation of research similar to that presented above, and with the improvement of animal models of demyelination, a more comprehensive and conclusive explanation is in sight. Given the plethora of research in this specific area of Multiple Sclerosis, it is now agreed upon that promoting remyelination should be a requirement of future MS therapies.

The Opposite Approach: Can Demyelination be Prevented?

While remyelinating axons is an important research endeavor to develop ways to treat MS patients and slow disease progression, methods of preventing the disease entirely are also highly desirable research goals. In the past there has been a lot of emphasis on suppressing the immune system in MS patients in order to prevent oligodendrocytes from being attacked. These efforts are unlikely to prove effective as they do not target specific components of the MS immune response, but rather cause widespread immunosuppression (17). In addition to researching therapeutic strategies to target these specific MS immune response pathways there has been a push towards discovering other means of inhibiting demyelination.

Intrathecal methotrexate (ITMTX) is a drug that has proven to be somewhat effective on progressive MS patients due to its anti-inflammatory properties (18). It has been hypothesized however, that the benefits of ITMTX could go beyond these properties because patients who do not respond to other anti-inflammatory drugs, do benefit from receiving ITMTX. In order to test this hypothesis, ITMTX was introduced in a non-inflammatory, cuprizone-induced demyelinated model. It was found that ITMTX inhibits demyelination and astrogliosis in the corpus callosum of these animals (19). While these findings by no means demonstrate that MS can be avoided or cured, they do demonstrate that demyelination could potentially be inhibited by non-immunosuppressive means, thereby avoiding systemic immunosuppressive side effects and potentially providing a more pathology specific response (17).

Similarly, it has also been shown that different levels of expression of endogenous molecules and their receptors can inhibit the process of demyelination without the use of immunosuppressant drugs. For example, galanin, a neuropeptide with multiple regulatory roles in the nervous system was investigated for its myelin protective role in cuprizone-induced demyelinated models. Overexpression of galanin in galanin transgenic (Gal-Tg) mice significantly inhibited demyelination compared to that in wildtype mice. In addition, it was found that expression levels of Galanin Receptor 1 (GalR1) in Gal-Tg mice were highly activated after demyelination, and GalR2 was up-regulated later in the re-myelination period. These data suggest that there is potential pharmacological significance for molecules that can activate galanin receptors in MS patients (20). A different lab also researched this phenomenon based on the fact that galanin expression is specifically up-regulated in microglia in MS lesions. Using the EAE model, it was found that over-expression of galanin in transgenic mice eliminated the disease entirely, and that loss-of-function mutations in galanin or its receptor increased the progression and severity of the disease (21). These experiments show that what was found in cuprizone-induced demyelinated models also holds true when antigens against myelin cause onset of the disease.

Finally, it is important to consider the research that is being done to improve therapies that target the immune system of MS patients. Efforts are being made to engineer therapies that are more specific to the pathology of the disease, rather than those that attack the entire system (17). Ideally, this will create therapies that are not only more effective, but that will also have fewer side effects.

In rats induced with EAE, it was demonstrated that introduction of autantibodies could improve the clinical effects of the disease. In the EAE model, myelin oligodendrocyte glycoprotein (MOG)-specific antibody was introduced to demyelinate axons. Experiments were designed that introduced a novel antigen-specific therapy based on filamentous phage that displays the antigenic determinant of interest. The presentation of the phages to the EAE mice reduced anti-MOG antibodies in the brain, and thereby prevented demyelination. There was also a decrease in inflammation in the CNS of these mice. These results show that delivery of MOG via filamentous phages can delete MOG autoantibody levels or could be stimulating other immune mechanisms to improve clinical indicators and effects of the demyelinating disease (5).

It is apparent that the reverse approach of preventing demyelination could conceivably prove successful as a therapeutic strategy for treating MS. Using various drugs, manipulating endogenous molecules, and targeting specific parts of the immune system are all methods that are currently being explored. The research presented that focuses on the immune system is particularly important in showing that targeting antibodies as opposed to targeting cells of the immune system (which current therapies usually employ) might be a more effective method when looking to find therapeutic methods that address the autoimmune characteristics of MS. Further research in these methods of inhibiting demyelination is anticipated to deliver effective therapies in the future.

Conclusions and Next Steps

The discovery that MS disease progression is not only due to demyelination but also to the axonal damage incurred due to lack of remyelination has indeed sparked a new direction in MS research. While these methods of promoting remyelination and preventing demyelination are important advancements in MS research, it is important to remember the fact that the models that are used are artificially induced to demyelinate. Therefore, we must consider whether or not demyelination in these models is similar enough to the demyelination seen in MS patients for these studies to be relevant. In terms of inhibiting demyelination, it could be possible that the models used have dif-
ferent properties that might allow vari-
ous mechanisms to allow inhibition of
demyelination that would not work in
MS patients. Especially in cuprizone
and lysolecithin-induced demyelinated
models, it seems as though these mecha-

nisms of demyelination are vastly dif-
ferent than the mechanisms that cause
MS. To further validate the studies that
have been done, it must be shown that
the method of demyelination is less
important than the simple fact that
demyelination is occurring. This way,
these methods of inhibiting demy-
elination will be more likely relevant
in the clinical context. Alternatively,
discovering a model that does not re-
myelinate and achieving the same ex-
perimental results would be an effec-
tive way of proving clinical relevance.

In analyzing the research on pro-
moting remyelination, it seems pos-
sible that remyelination may be a diffi-
cult therapy to implement because new
myelin sheaths that are produced could
again be susceptible to autoimmune at-
tack, just as they were before. Although
remyelination would certainly slow the
disease progression and perhaps only
temporarily renew lost neuronal func-
tion, methods of ensuring that auto-
immune attack does not occur again
should be researched. In shifting the
focus of MS research from immuno-
suppressant methods to other aspects
of the disease, such as promoting remy-
elination, the autoimmune facet of this
disorder should not be forgotten. Con-
tinuing to find methods of suppressing
the MS autoimmune response should be
coupled with research on promoting
remyelination or preventing demyelina-
tion. In this manner, combination ther-
apies should be developed to tackle all
aspects of the disease simultaneously.

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