Heterogeneity of Pathogenesis in Multiple Sclerosis: Implications for Promotion of Remyelination

M. Mateo Paz Soldan¹ and Moses Rodriguez¹–³

¹Program in Molecular Neuroscience and Departments of ²Neurology and ³Immunology, Mayo Medical and Graduate Schools, Rochester, Minnesota

Enhancing myelin repair remains an important therapeutic goal in primary demyelinating diseases of the central nervous system (CNS) such as multiple sclerosis (MS). The emerging heterogeneity of pathology within MS lesions, and differential oligodendrocyte survival in particular, suggests that therapeutic strategies may need to be tailored to an individual patient’s requirements. A number of therapeutic strategies have been proposed to enhance myelin repair in the CNS: cell transplantation, growth factor therapy, and antibody therapy, but each proposed therapy has different implications with respect to pathogenetic mechanisms of demyelination. Of these, antibody therapy is the most amenable to immediate application in patients—but a combination of therapeutic approaches may be required in practice.

Multiple sclerosis (MS) is one of the most common disabling neurologic diseases of young adults. Rational treatment depends upon a thorough understanding of both disease etiology and pathogenesis. Investigation into the pathology of the human disease and the use of a growing number of animal models has begun to reveal striking complexity and heterogeneity of pathogenetic mechanisms. The interplay of several susceptibility genes is involved and environmental factors may also be required to trigger the disease process. An emerging understanding of pathogenetic heterogeneity and oligodendrocyte sparing in demyelinated lesions will likely enable a more refined classification of MS subgroups. This refined classification will provide for the possibility of more differentiated therapeutic approaches.

Pathologic Heterogeneity in MS

The pathologic hallmark of MS is focal destruction of the myelin sheath. During the course of the disease, inflammatory demyelinating lesions are widely spread throughout the central nervous system (CNS) with a predilection for optic nerves, periventricular white matter, brain stem, and spinal cord. Although it is generally accepted that the immune system contributes to pathogenesis in MS, it is unclear what components of the inflammatory response may play a causative role or simply result from the disease process. Investigators have emphasized pathology of the lesion in classifying the disease and in attempts to ascribe a pathogenetic mechanism. However, variation of pathologic findings from patient to patient has made ascribing a single mechanism difficult. A further problem is that most information on MS pathology is based on autopsy material.

Pathologic analysis of a small number of cases with active lesions has revealed many different structural and immunologic features, suggesting heterogeneity in the demyelinating process. Activated macrophages [1] and microglial cells [2] have been observed in association with demyelinating activity of lesions. Cytotoxic cytokines may also play a role. Tumor necrosis factor has been found in association with astrocytes throughout the lesion and with foamy macrophages in the center of active lesions [3]. Lymphotoxin was identified in association with CD3⁺ lymphocytes and Leu-M5⁺ microglial cells at the lesion edge [4]. (Leu-M5 is an adhesion molecule of activated microglia.) There is also evidence for involvement of reactive oxygen or nitrogen species [5, 6], specific demyelinating antibodies [7, 8], and complement components [9]. In other cases, signs of dystrophy in the most distal processes of oligodendrocytes [10] or decreased expression of myelin proteins (e.g., as myelin-associated glycoprotein) [11] were noted.

A recent pathologic study that utilized a large clinical series of active lesions further characterized the heterogeneous nature of the MS lesion [12]. Lucchinetti et al. [12] determined four patterns of disease into which all lesions fit: All had inflammatory infiltrates by T lymphocytes and macrophages in common but segregated on the basis of plaque geography, distribution of myelin protein loss, evidence of immunoglobulin and complement deposition, and oligodendrocyte death. The first two patterns (I and II) primarily showed sharply demarcated perivascular demyelination with loss of all myelin proteins apparently occurring simultaneously. Sparing of oligodendrocytes in active plaques and repopulation of inactive plaques with high numbers of oligodendrocytes was observed. Pattern II was distinguished from all others by pronounced immunoglobulin and complement (C9neo) reactivity associated with degenerating myelin at the active plaque edge and with myelin degradation.
products within macrophages. Patterns III and IV showed demyelination that was not centered on veins and had ill-defined lesion borders. A nearly complete loss of oligodendrocytes in both active and inactive plaques was observed. Immunoglobulin and complement reactivity was absent. Pattern III was distinguished by a preferential loss of myelin-associated glycoprotein that was greater than that seen with the other myelin proteins.

A comparison of pathologic and immunopathologic features in lesion patterns I and II with experimental autoimmune encephalomyelitis reveals close similarities to T cell–mediated or T cell plus antibody–mediated autoimmune demyelination, respectively. By contrast, lesion patterns III and IV are highly suggestive of an oligodendroglialopathy with subsequent demyelination, reminiscent of virus- or toxin-induced models of demyelination. Although patterns of demyelination were heterogeneous between patients, multiple lesions within a patient all manifested the same. This suggests that lesion patterns are not separate stages of a single pathogenetic mechanism but distinct mechanisms present in subgroups of MS patients. Understanding the pathogenetic mechanisms in demyelinating lesions has significant implications for developing and implementing appropriate therapies. In particular, understanding the relative loss or sparing of oligodendrocytes will determine what therapeutic strategies have potential efficacy in an individual MS patient.

**Therapeutic Goals for MS Treatment**

*Inhibition of pathogenesis.* Consensus that MS is immune mediated has logically led to therapies based on immunomodulation. In general these therapies are expected to reduce pathology rather than stimulate myelin repair. A multitude of conventional immunomodulatory treatments and general immunosuppressants have been tested for efficacy in MS patients [13], but none had sufficiently positive effects to warrant approval as a treatment. Currently, only interferon (IFN)-β and copolymer-1 (COP-1) are approved treatments for MS patients. A number of trials of IFN-β have shown a 30% reduction in relapse rate, decreased magnetic resonance imaging (MRI) activity, and delay to sustained clinical progression [14]. Although initially tried in MS for their antiviral effect, antiproliferative actions or reduction of major histocompatibility complex class II expression may contribute to the mechanism of clinical benefit with IFN-β. Trials of COP-1 similarly demonstrated a 29% reduction in relapse rate and decreased MRI activity [15]. For COP-1, the proposed mechanism of clinical benefit stems from its structure. COP-1 is a synthetic basic random copolymer of L-alanine, L-glutamine, L-lysine, and L-tyrosine in a molar ratio of 6.1:1.9:4.7:1.0. The structure of COP-1 is similar to that of myelin basic protein (MBP) peptides and significant cross-reactivity exists between COP-1 and MBP at both the B cell [16] and T cell [17] level. It is hypothesized that COP-1 may compete with myelin antigens for MHC binding on autoimmune cells or may activate suppressor T cells.

Limited therapeutic benefit achieved with the above-mentioned immunotherapies may relate to the apparent pathogenetic and clinical heterogeneity of MS. The proposed mechanism of action differs between IFN-β and COP-1. An improved understanding of the pathologic processes involved may allow therapies to be targeted to subgroups of MS patients that are most likely to respond. An example of this potential is illustrated by the likely pathogenetic mechanism of lesions exhibiting pattern II. The pronounced immunoglobulin and complement deposition in pattern II active lesions logically suggests that removal of autoreactive antibodies will inhibit pathogenesis. Although the mechanism has not been elucidated, therapeutic plasma exchange resulted in clinically significant improvement in a subset of patients who experienced corticosteroid unresponsive severe neurologic deficits after attacks of inflammatory demyelinating disease [18, 19]. Clearly, in order to tailor therapy for each patient, classification of pathogenetic mechanism by using noninvasive methods will be necessary.

*Enhancement of myelin repair.* There are two fundamentally different mechanisms by which tissue may repair after injury: regenerative repair and scar formation. In the CNS, scar formation predominates but remyelination in primary demyelinating diseases such as MS is a striking example of CNS regeneration [20]. Damage to the CNS induces an injury response that recruits glial cells from surrounding tissue. It is important to understand how to manipulate this glial response to favor the balance toward regenerative repair. CNS remyelination can occur after many types of pathology [10, 21, 22] but is by no means always successful. Thus, attempts to promote myelin repair have focused on stimulating or enhancing the natural process. In the context of a demyelinated lesion where remyelination has failed, it is necessary to understand what aspect of the glial injury response is nonfunctional or insufficient. This understanding will allow for development of more targeted therapies.

**Remyelination Strategies**

*Cell transplantation.* The motivating premise for transplantation is that the injured CNS no longer has cells capable of affecting repair. Numerous laboratories have shown successful remyelination by transplanted oligodendrocytes in experimental animals [23–25]. This remyelination improves conduction block in the rat spinal cord [26] and is associated with improved neurologic function [27]. The capacity of glial cells to remyelinate is dependent upon many factors, but two that are particularly relevant to transplanted cells are the abilities to migrate and proliferate. With respect to migration, studies show that transplanted oligodendrocyte progenitor cells survive and remyelinate in acute lesion areas, but normal white matter is inhibitory to the migration of these cells [28]. In a multifocal
disease such as MS, it is impractical to transplant cells directly into every demyelinated lesion. However, irradiation allows progenitors to migrate [29] through nondemyelinated white matter, suggesting the adult CNS may be amenable to the proper manipulation.

**Growth factor therapy.** When growth factor treatment to promote remyelination is considered, the assumption is that the injured CNS has cells capable of recapitulating myelin but the glial response to injury does not produce factors appropriate and necessary for stimulating myelogenesis. Cytokines are one method of manipulating the glial response. With respect to myelinating glia, oligodendrocyte progenitor cells can be maintained in a proliferative state by the combination of platelet-derived growth factor (PDGF) and basic fibroblast growth factor. Both are expressed in CNS lesions [30, 31] and the PDGF antagonist tripidil inhibits myelin repair [32]. The most extensively investigated candidate in this context is insulin-like growth factor (IGF). IGF induces the synthesis of myelin-like membranes in vitro and IGF-I lessens clinical severity and up-regulates myelin protein expression in experimental autoimmune encephalomyelitis (EAE) [33]. In addition, transgenic mice overexpressing IGF-I increased myelin synthesis.

Although growth factor therapy may seem superficially attractive, there are several unresolved issues and an increasingly complex characterization of the requirement for multiple and different factors has emerged. Particular combinations of growth factors have been described through the sequential phases of oligodendrocyte proliferation, migration, differentiation, and myelination. There are also clear but incompletely explored differences between human oligodendrocyte progenitors and their better characterized rodent counterparts. While both human and rodent cells proliferate in cocultures with astrocytes, human oligodendrocyte progenitors do not respond to mitogens known to trigger proliferation in rodent cells [34, 35]. There is also the potential to trigger apoptosis in fully differentiated oligodendrocytes exposed to mitogenic growth factors [36]. Thus, it is difficult to determine what growth factors to treat with and when to administer them. When combined with the general difficulties of controlled and sustained peptide delivery to the CNS, these issues all mitigate against the implementation of growth factor therapy.

**Antibody therapy.** An alternative strategy proposed by our laboratory is to enhance endogenous remyelination by treatment with monoclonal antibodies (MAbs) [37]. This approach implies that the cells capable of remyelination and that the factors that sustain their growth or differentiation are present in demyelinated lesions, but there are mechanisms that inhibit this response and thus prevent full remyelination. Of the strategies proposed to enhance myelin repair, antibody therapy is the most amenable to immediate application in patients.

We have identified six mouse MAbs (table 1) that increase remyelination four- to 6-fold in the Theiler’s murine encephalomyelitis virus (TMEV) model [38]. More recently we identified human MAbs (table 1) that promote remyelination in the TMEV model [39]. Figure 1 shows representative remyelination. These human MAbs promote remyelination to an equal or greater degree than human intravenous immune globulin, an established therapy for immune-mediated disorders. Subsequent experiments have shown remyelination promotion in both immune and nonimmune experimental models of demyelination. Specifically, treatment with remyelination-promoting MAb is efficacious in the immune-mediated model of TMEV [38–40] and in the toxic-traumatic model of lysolecithin-induced demyelination [41]. The remyelination-promoting MAbs also reduce relapse rates and prolong relapse onset in the autoimmune model of EAE [42]. Therapeutic effectiveness in multiple experimental models indicates that the underlying mechanism is not modulation of model-specific pathogenesis but a fundamental physiologic stimulation of reparative systems. Elucidation of the mechanism by which these remyelination-promoting antibodies exert their beneficial effect is necessary for development of targeted therapies in the context of MS heterogeneity.

**Mechanism of Antibody-Promoted Remyelination**

All remyelination-promoting antibodies identified to date are of the IgM isotype. The characteristic polyreactivity of IgM antibodies presents a considerable challenge to identifying the relevant antigen and in elucidating the therapeutic mechanism. One mechanism by which the antibodies may mediate remyelination is through binding to a unique receptor on CNS cells. In this respect, antibodies can exert their influence by blocking or stimulating function. We have proposed that remyelination-promoting antibody receptor binding may mimic normal signals that induce or coordinate myelination.

Others have demonstrated that CNS-specific antibodies can induce physiologic changes in their target cells. Exposure of oligodendrocytes to antibodies directed against myelin/oligodendrocyte-specific protein (MOSP) results in an increase in the number and thickness of microtubular structures [43]. Elaboration of cytoskeleton is likely to be critical to immature oligodendrocytes for membrane process extension and growth of the myelin sheath. In contrast, antibody binding to galacto-

### Table 1. Major antigen target of remyelination-promoting antibodies.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antibody species</th>
<th>Major antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCH 79.08</td>
<td>Mouse</td>
<td>Unknown/MBP</td>
</tr>
<tr>
<td>SCH 94.03</td>
<td>Mouse</td>
<td>Unknown</td>
</tr>
<tr>
<td>O1</td>
<td>Mouse</td>
<td>GalC</td>
</tr>
<tr>
<td>O4</td>
<td>Mouse</td>
<td>Sulfatide</td>
</tr>
<tr>
<td>A2B5</td>
<td>Mouse</td>
<td>GQ1b</td>
</tr>
<tr>
<td>HNK-1</td>
<td>Mouse</td>
<td>MAG</td>
</tr>
<tr>
<td>hHgM22</td>
<td>Human</td>
<td>Unknown</td>
</tr>
<tr>
<td>hHgM46</td>
<td>Human</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**NOTE.** GalC, galactocerebroside; GQ1b, ganglioside GQ1b; MAG, myelin-associated glycoprotein; MBP, myelin basic protein.
Cerebroside (GalC), sulfatide [44], or myelin/oligodendrocyte glycoprotein [43] results in microtubule depolymerization. MBP domains are thought to be involved in myelin compaction, and elimination of cytoskeleton from myelin membrane lamellae is likely critical in this process.

Sulfatide and GalC may also regulate proliferation and differentiation in the oligodendroglial lineage. These galactosphingolipids, major components of oligodendrocyte membranes and myelin, are first expressed at a point when progenitors cease proliferating and commence terminal differentiation. Transgenic mice unable to synthesize these lipids due to mutation of the ceramide galactosyltransferase gene exhibit a 3-fold enhancement in the number of terminally differentiated oligodendrocytes [45]. Anti-sulfatide antibodies also block terminal differentiation of oligodendrocytes in culture [45]. Therefore, there is the potential for antibodies to directly interact with and activate CNS glia, in particular, myelin-producing oligodendrocytes.

Cytoskeletal changes are preceded by an influx of Ca\(^{2+}\) in oligodendrocytes exposed to α-GalC, α-sulfatide [44], or α-
Figure 2. Schematic illustrates potential mechanisms by which remyelination-promoting antibodies may function. It is proposed that antibodies bind to receptors on the surface of oligodendrocytes or astrocytes thereby inducing Ca\(^{2+}\) signals and subsequent physiologic effects. Direct stimulation of oligodendrocytes may induce proliferation and/or differentiation of progenitor cells or rescue injured oligodendrocytes from cell death. Direct stimulation of astrocytes may induce the release of growth factors that in turn mediate oligodendrocyte proliferation, differentiation, or cell survival.

MOSP [43]. Thus, the influx of Ca\(^{2+}\) might be critical in the regulation of oligodendrocyte structure and function. Recently we demonstrated similar glial Ca\(^{2+}\) signaling by using remyelination-promoting antibodies as ligands. Antibodies mediate a transient rise of intracellular calcium in a subpopulation of both astrocytes and oligodendrocytes. There is a significant correlation between an antibody’s ability to induce Ca\(^{2+}\) changes in vitro and enhancement of myelin repair in vivo, suggesting these two phenomena are interrelated. Figure 2 depicts three potential mechanisms by which antibody-mediated glial activation could function to promote remyelination: stimulation of mature brain oligodendrocyte progenitors, stimulation of astrocytes, and stimulation of injured or dying mature oligodendrocytes. It remains to be determined whether remyelination-promoting antibodies utilize one or several of these mechanisms.

Summary

The therapeutic approaches proposed to enhance myelin repair in MS have different implications with respect to lesion pathology. Classification of pathogenetic mechanisms in an individual patient may be necessary to provide targeted therapies. Depending upon mechanism of action, antibody therapy may provide a more approachable alternative to the as yet problematic approaches of cell transplantation or growth factor administration. Antibody therapy may also serve to enhance other approaches such as glial cell transplantation in patients who lack progenitor cells and mature oligodendrocytes.

References


