Human monoclonal antibodies reactive to oligodendrocytes promote remyelination in a model of multiple sclerosis

Arthur E. Warrington*,†, Kunihiko Asakura‡, Allan J. Bieber*,†, Bogoljub Ciric†, Virginia Van Keulen*, Srini V. Kaveri¶, Robert A. Kyle†, Larry R. Pease†, and Moses Rodriguez*‡**

Departments of *Neurology, †Immunology, and ‡Hematology, Mayo Medical and Graduate Schools, Rochester, MN 55905; §Institut National de la Santé et de la Recherche Médicale (INSERM) U430, Paris, France; and †Department of Neurology, Nagoya University School of Medicine, Nagoya, 466-8550 Japan

Edited by John B. Robbins, National Institutes of Health, Bethesda, MD, and approved April 11, 2000 (received for review December 27, 1999)

Promoting remyelination, a major goal of an effective treatment for demyelinating diseases, has the potential to protect vulnerable axons, increase conduction velocity, and improve neurologic deficits. Strategies to promote remyelination have focused on transplanting oligodendrocytes (OLs) or recruiting endogenous myelinating cells with trophic factors. Ig-based therapies, routinely used to treat a variety of neurological and autoimmune diseases, underlie our approach to enhance remyelination. We isolated two human mAbs directed against OL surface antigens that promoted significant remyelination in a virus-mediated model of multiple sclerosis. Four additional OL-binding human mAbs did not promote remyelination. Both human mAbs were as effective as human i.v. Ig, a treatment shown to have efficacy in multiple sclerosis, and bound to the surface of human OLs suggesting a direct effect of the mAbs on the cells responsible for myelination. Alternatively, targeting human mAbs to areas of central nervous system (CNS) pathology may facilitate the opsonization of myelin debris, allowing repair to proceed. Human mAbs were isolated from the sera of individuals with a form of monoclonal gammopathy. These individuals carry a high level of monoclonal protein in their blood without detriment, lending support to the belief that administration of these mAbs as a therapy would be safe. Our results are (i) consistent with the hypothesis that CNS-reactive mAbs, part of the normal Ig repertoire in humans, may help repair and protect the CNS from pathogenic immune injury, and (ii) further challenge the premise that Abs that bind OLs are necessarily pathogenic.

Enhancement of remyelination and protection from axonal injury are important therapeutic goals in the treatment of inflammatory demyelinating central nervous system (CNS) disorders such as multiple sclerosis (MS). Remyelination in MS plaques can occur, but is limited (1, 2) even though oligodendrocyte (OL) progenitors are present in the adult (3, 4). A number of therapeutic strategies to promote remyelination have been tested in experimental animals. Transplantation of OLs (5) or their progenitors (6) into demyelinated tissue produces new myelin. Transplanted OL progenitors also can remyelinate demyelinated lesions in the adult CNS (7) and migrate toward an area of damage when placed in close proximity to the lesion (8). Unresolved issues remain concerning the survival of transplanted OL progenitors in the intact adult CNS and their ability to target to areas of myelin pathology (9). However, if CNS lesions are surgically approachable and axons are still intact, transplantation of glial cells may be a viable therapy for improving functional performance (10).

The in vitro administration of growth or trophic factors induces the expansion of OL progenitors (11, 12) or promotes mature OLs to redifferentiate and subsequently reinitiate a program of myelination (13, 14). The in vivo administration of trophic factors via genetically engineered fibroblasts to the injured CNS promotes axonal sprouting and OL proliferation (15). Obstacles to in vivo trophic factor therapy remain, specifically determining the biologically relevant local factor concentration and the potential pleiotropic roles of most trophic factors administered in high concentrations.

As an alternative, our laboratory proposes to repair CNS pathology and enhance endogenous remyelination by using CNS-binding Igs (16), building on a natural reparative response that already may be up-regulated after demyelination. Ig therapy can be rapidly adapted and tested as a treatment for human demyelinating disease (17, 18). The premise of our approach is that cells capable of remyelination—and the factors necessary to sustain their growth and differentiation—are present in the demyelinated CNS, but their capacity to produce myelin is limited. The emerging heterogeneity of pathology and OL sparing within the MS population (19) suggests that in practice the treatment of human demyelinating disease may require combinations of several therapeutic approaches based on an individual’s requirements.

We have used a virus-mediated model of demyelination to develop Ig-based therapy. When Thélier’s murine encephalomyelitis virus (TMEV) is inoculated intracerebrally into susceptible strains of mice, TMEV induces immune-mediated progressive CNS demyelination clinically and pathologically similar to MS (20). The efficacy of therapies in human MS closely parallel those observed in the TMEV model (21), making this an important platform for the design of clinical trials. A mouse mAb raised against spinal cord homogenate, designated SCH94.03, enhances remyelination in the TMEV model (22). SCH94.03 is a polyreactive, mouse IgM mAb that binds to the surface of OLs (23). SCH94.03 also enhances the rate of spontaneous CNS remyelination after lysolecithin-induced demyelination (24) and decreases relapse in experimental autoimmune encephalomyelitis (25). Additional OL-binding mouse IgMs mAbs, several of which are routine markers for the OL lineage, also promote CNS remyelination (26).

Because mouse IgM mAbs promote remyelination, we hypothesized that polyclonal human IgM would be a more effective treatment of demyelinating disease than human i.v. Ig (IV Ig), an established therapy for immune-mediated disorders (27). Treatment of chronically TMEV-infected mice with polyclonal human IgM resulted in enhanced remyelination when compared with IV Ig.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: CNS, central nervous system; IV Ig, human i.v. Ig; MS, multiple sclerosis; OL, oligodendrocyte, SC, Schwann cell; sHIgM, serum-derived human monoclonal IgM; TMEV, Thélier’s murine encephalomyelitis virus.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF212992 and AF212993).

1A.E.W. and K.A. contributed equally to this work.

**To whom reprint requests should be addressed at: Department of Immunology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. E-mail: rodriguez.moses@mayo.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.
Two human IgM mAbs also were identified, using an antigen-independent strategy, which promote remyelination to an equivalent or greater degree than polyclonal human IgM. We suggest that human remyelination-promoting mAbs may be an easily implemented, effective therapy for human demyelinating disease. Human mAbs are readily applicable to clinical trials, can be produced free of infectious agents, and may alleviate the national shortage and high cost of IVIg. An effective human mAb that promotes remyelination also may simplify the investigation for the mechanism of action of immunomodulatory therapies.

Materials and Methods

Human Antibodies and Their Isolation. Normal human IgG purified from the pooled plasma of more than 2,500 healthy donors was obtained from S. V. K. (28). The purity of IgG was more than 90% as confirmed by SDS/PAGE. Pooled human IgG from healthy donors designated clinically as IVIg was from Miles.

Human serum samples were obtained from the dysproteinemia clinic under the direction of R.A.K. and chosen solely by the presence of an Ig clonal peak of greater than 20 mg/ml. Sera were from 102 patients with a wide variety of conditions characterized by a monoclonal IgG or IgM spike in the serum, including Waldenstrom’s macroglobulinemia, multiple myeloma, lymphoma, and monoclonal gammopathy of undetermined significance. Sera were dialyzed against water, and the precipitates were collected by centrifugation (14,000 rpm/30 min) and dissolved in PBS. Solutions were centrifuged and chromatographed on Superose-6 column (Amersham Pharmacia). IgM fractions were pooled and analyzed by SDS/PAGE. Concentrations were determined by gel staining with Sypro Orange (Molecular Probes) densitometry. IgM solutions were sterile filtered and cryopreserved.

OL Cell Culture and Immunocytochemistry. Cerebral hemispheres from P0-P2 Holtzman Sprague–Dawley rats were prepared for mixed primary glial cell culture as described (29) and grown for 9 days in vitro. Rat OL progenitors were isolated as described (30). Adult human OLs were prepared from temporal lobe biopsies obtained from patients undergoing therapeutic resection for intractable epilepsy. Tissue did not contain the epileptic focus and was of normal cytoarchitecture when examined by the Department of Surgical Pathology, Mayo Clinic. Adult glial cells were isolated as described (31) and seeded onto polystyrene (Sigma) and laminin (Life Technologies, Grand Island, NY)-coated plastic mult wells (Becton Dickenson) or glass coverslips (Fisher Scientific) in a defined media of DMEM/F12 supplemented with biotin (0.01 μg/ml), triiodothyronine (15 nM), 0.5% BSA (all from Sigma), N2, 1% pen/strep (both from Life Technologies) and recombinant human platelet-derived growth factor AA (R & D Systems). Cell surface staining was done at 4°C for 12 min on unfixed cells after blocking with Hepes-buffered Earle’s balanced salt solution (E/H) with 5% BSA. All human Abs were used at 10 μg/ml. Intracellular staining for myelin basic protein using polyclonal mouse antiserum (Boehringer Mannheim) was done at room temperature after fixation with 4% paraformaldehyde and permeabilization for 5 min with 0.05% saponin. Primary Abs were detected by using fluorescently conjugated secondary Abs (Jackson ImmunoResearch). Cell monolayers were mounted in 90% glycerin/PBS with 2.5% 1,4-diazabicyclo[2.2.2]octane to prevent fading (37) and 0.1 μg/ml bisbenzimide (both from Sigma) and viewed with an Olympus Provis epifluorescent microscope equipped with a SPOT digital camera (Diagnostic Instruments, Sterling Heights, MI).

Virus and Animals. The Daniel’s strain of TMEV was used for these experiments and was prepared as described (32). Female SJL/J mice from the Jackson Laboratories were used after 1-week acclimation. Mice 4–6 weeks of age were injected intracerebrally with 2 × 10⁵ plaque-forming units of TMEV in 10 μl volume, resulting in greater than 98% incidence of chronic viral infection. Animals used in this study were 5–8 months postinfection and received a single i.p. injection of Ig or PBS. Dosages were 1.0 mg of IVIg or human polyclonal IgM or 0.5 mg of the human mAbs. Animals were killed 5 weeks after Ab treatment for morphologic assessment, chosen because studies in toxic models of demyelination indicate that CNS remyelination is almost complete by this time (33). Spinal cord sections embedded in plastic were cut by a centralized microscopy facility and returned to the laboratory marked with a numerical code. In this way slides are graded for remyelination in a blinded manner.

Western Blotting. Purified TMEV (34) was separated by SDS/PAGE and proteins were transferred to nitrocellulose. After blocking with Tris-buffered saline containing 5% nonfat dry milk and 0.05% Tween 20 for 2 h at room temperature the membrane was incubated with human Igs (10 μg/ml) or rabbit polyclonal anti-TMEV Ab (1:2,000) for 4 h. Bound Igs were detected with biotinylated goat anti-human mAbs or biotinylated goat anti-rabbit mAbs (both from Jackson ImmunoResearch) and alkaline phosphatase-conjugated streptavidin using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Kirkegaard & Perry Laboratories).

Quantitation of Spinal Cord Demyelination/Remyelination. We have developed methods to quantify the amount of spinal cord demyelination, remyelination, and atrophy in susceptible mice by using plastic-embedded cross sections stained with 4% paraphenylenediamine to visualize myelin (ref. 35, Fig. 1A). To obtain a representative sampling of the entire spinal cord, 1-μm thick cross sections were cut from every third serial 1-mm block, generating 10–12 cross sections that represent the whole spinal cord. From each cross section the area of white matter, white matter pathology, OL remyelination, and Schwann cell (SC) remyelination were calculated by using a Zeiss interactive digital analysis system (ZIDAS) and camera lucida attached to a Zeiss photomicroscope. White matter was outlined at a magnification of ×40. The areas of white matter pathology, defined as regions of white matter with demyelination or remyelination, then were traced at a magnification of ×200. Quantifications of white matter pathology of control and experimental mice were done in a blinded manner.

The percent area of spinal cord white matter pathology per mouse was obtained by dividing the total area of white matter pathology by the total area of white matter pathology in a given animal. The percent area of remyelination was obtained by dividing the area of OL or SC remyelination by the total area of white matter pathology. Repeated measures of white matter pathology and extensive myelin repair revealed comparable values differing only by 1.5%. To determine the validity of using 10 cross sections as a representation of the remyelination throughout the spinal cord, a comparison was performed by using 10 cross sections versus all 32 cross sections of a single chronically infected mouse. Assessing 10 cross sections resulted in a percent area remyelination value of 47.7%, whereas the data from all 32 cross sections resulted in a
value of 40.0%. Either value would have indicated significant remyelination in our assay.

**Results**

**IVIg and Polyclonal Human IgM Promote CNS Remyelination in TMEV-Infected Mice.** Clinical studies in MS indicate that IVIg may be partially effective in stabilizing the disease course (18, 36, 37). To determine whether IVIg could promote remyelination in the TMEV model of MS, chronically infected mice were treated with a single i.p. injection of 1 mg of IVIg. A single dose was administered to avoid evoking an immune response to the foreign Ig. The total dose of human Ig was approximately 0.05 g/kg body weight, one-quarter the total dose used for IVIg treatment (18). Additional mice were treated with a single 1-mg bolus of polyclonal human IgM. Upon examination of the spinal cords, the percent area of OL remyelination in mice receiving either IVIg or polyclonal human IgM (14.15% and 23.19%, respectively) was significantly higher than the spontaneous OL remyelination observed in the PBS-treated group (6.74%, \( P < 0.05 \) for IgG, \( P < 0.01 \) for IgM). There were no statistically significant differences in the areas of white matter or the areas of white matter pathology between either treatment group or the PBS control group. The data describes two independent experiments treating groups of seven and nine mice...
with IVlg and groups of seven and 10 mice treated with polyclonal human IgM. The final values in Table 1 include only those animals that contained at least 5% white matter pathology.

Treatment with polyclonal human IgM resulted in more OL remyelination than that observed in mice treated with IVlg (*P = 0.05, Fig. 2 A and B). Approximately one-quarter of the total area of myelin pathology was remyelinated in mice treated with polyclonal human IgM, representing thousands of ensheathed axons. On average, 1 mm² within confluent remyelinated areas of pathology (Fig. 2B) corresponded to 46,000 to 125,000 remyelinated axons. Therefore, the CNS remyelination after human IgG treatment was extensive. Few inflammatory cells or macrophages were present. In contrast, in mice treated with PBS, areas of myelin pathology contained few remyelinated axons (Fig. 2H). Signs of active myelin destruction, such as myelin whirls, inflammatory cells, and macrophages were present.

As an additional, faster method to judge the effectiveness of a treatment to promote remyelination the 10 spinal cord sections representative of an animal were examined for the presence of areas of white matter pathology that demonstrated nearly complete repair. We defined complete repair as an area of white matter pathology with nearly confluent remyelinated axons and no inflammatory cells or macrophages present (as in Fig. 2 B, F, and G), a very rare event in spontaneous remyelination. At least one area of complete repair was observed in four of 10 animals treated with IVlg and in 10 of 14 animals treated with polyclonal human IgM. We concluded that both IVlg and polyclonal human IgM promote remyelination compared with PBS treatment and that polyclonal human IgM is superior to IVlg in the ability to promote CNS remyelination.

Human mAbs That Bind to OLS Promote CNS Remyelination in TMEV-Infected Mice. All of the previously identified mouse mAbs that promote CNS remyelination bind to OLS (23, 26). To screen human mAbs for testing in the TMEV model, human mAbs were tested for the ability to bind to the surface of rat OLS in unfixed mixed primary glial culture. Primary cultures established from neonatal rat brain contain OLS at varying stages of differentiation at 9 days in vitro (38). Our sources of human mAbs were serum-derived human monoclonal IgMs (sHIgMs) and serum-derived human monoclonal IgGs (sHIgGs). None of 50 sHIgGs bound to unfixed rat OLS, but six of 52 sHIgMs bound to the surface of rat OLS colabeled with the antisulfatide mAb, O4 (39).

The six OL-binding sHIgMs were used to treat TMEV-infected mice. Groups of five animals each received a single injection of 0.5 mg of human mAb. The average percent area of OL remyelination after treatment with sHIgM22 and sHIgM46 (Fig. 2 F and G) both were significantly above the background levels attributable to spontaneous remyelination. The other four OL-binding sHIgMs promoted remyelination at levels comparable to or below the level observed after treatment with PBS. A second set of animals were treated with sHIgM22, sHIgM46, or PBS to confirm the initial observations. sHIgM14 also was repeated as an example of a human mAb that bound to OLS, but did not promote remyelination. The combined data are presented in Table 1. Only animals that contained at least 5% total white matter pathology were included in statistical analysis.

The highest percent area of OL remyelination was observed in animals treated with sHIgM46 (27.1%), followed by animals treated with sHIgM22 (17.1%). The percent area of remyelination after treatment with sHIgM14 (8.41%) was similar to that observed after treatment with PBS (6.74%). To test whether any sHIgM, irrespective of antigen specificity, could promote remyelination we studied two mAbs in vivo that demonstrated no immunoreactivity to OLS in mixed primary culture, sHIgM1 and sHIgM2 (Fig. 2 C and D). The percent area of remyelination after treatment with sHIgM1 (8.3%) and sHIgM2 (11.4%) were not significantly different from the sHIgM14 or PBS treatment groups. In all groups the areas of white matter and areas of white matter pathology were not statistically different. Compared with the remyelination observed in the PBS-treated group, the percent area of remyelination after treatment with sHIgM46 or sHIgM22 resulted in *P values of <0.001 and <0.05, respectively. The area of peripheral nervous system-type SC remyelination ranged within treatment groups from 0 to 0.08 mm². This corresponded to 0.0 to 6.92 percent area of peripheral nervous system-type SC remyelination as a function of myelin pathology. There was no statistical difference in the area of myelin pathology in the various treatment groups compared to PBS or in the peripheral nervous system-type SC remyelination between groups.

**Table 1. CNS remyelination in mice after treatment with human Abs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Area of white matter, mm²</th>
<th>Area of myelin pathology, mm²</th>
<th>Area of CNS type remyelination, mm²</th>
<th>Area of CNS-type remyelination, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVlg</td>
<td>10</td>
<td>8.60 ± 0.52</td>
<td>0.86 ± 0.10</td>
<td>0.13 ± 0.02</td>
<td>14.15 ± 2.38*</td>
</tr>
<tr>
<td>Human IgM</td>
<td>14</td>
<td>9.70 ± 0.43</td>
<td>1.21 ± 0.21</td>
<td>0.24 ± 0.04</td>
<td>23.19 ± 3.26</td>
</tr>
<tr>
<td>sHIgM 1</td>
<td>4</td>
<td>9.34 ± 1.93</td>
<td>0.68 ± 0.07</td>
<td>0.03 ± 0.01</td>
<td>8.35 ± 3.73</td>
</tr>
<tr>
<td>sHIgM 2</td>
<td>4</td>
<td>8.78 ± 0.70</td>
<td>0.87 ± 0.12</td>
<td>0.10 ± 0.01</td>
<td>11.37 ± 1.30</td>
</tr>
<tr>
<td>sHIgM 14</td>
<td>7</td>
<td>11.01 ± 0.60</td>
<td>1.13 ± 0.18</td>
<td>0.08 ± 0.03</td>
<td>8.41 ± 2.59</td>
</tr>
<tr>
<td>sHIgM 22</td>
<td>8</td>
<td>10.55 ± 0.41</td>
<td>1.16 ± 0.22</td>
<td>0.19 ± 0.05</td>
<td>17.06 ± 3.42*</td>
</tr>
<tr>
<td>sHIgM 46</td>
<td>5</td>
<td>9.44 ± 0.36</td>
<td>0.66 ± 0.06</td>
<td>0.18 ± 0.04</td>
<td>27.12 ± 4.01*</td>
</tr>
<tr>
<td>PBS</td>
<td>7</td>
<td>9.78 ± 0.60</td>
<td>1.20 ± 0.22</td>
<td>0.06 ± 0.02</td>
<td>6.74 ± 1.80</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM. One-way ANOVA and t test were used to compare the percent area of CNS-type remyelination in mice treated with human antibodies to mice treated with PBS. Such analysis revealed *P < 0.05; †P < 0.01, ‡P < 0.001. Comparison of mice treated with IVlg to other treatments revealed polyclonal human IgM P = 0.05, sHIgM 46 P = 0.05. All other comparisons were not statistically significant. There was no difference in the percent type remyelination between polyclonal human IgM, sHIgM 22, and sHIgM 46. Area of peripheral nervous system-type SC remyelination ranged from 0 to 0.08 mm². This corresponded to 0.0 to 6.92 percent area of peripheral nervous system-type SC remyelination as a function of myelin pathology. There was no statistical difference in the area of myelin pathology in the various treatment groups compared to PBS or in the peripheral nervous system-type SC remyelination between groups.
sHlgM2, sHlgM14, or PBS contained a single area of complete repair.

**Human mAbs, but Not Polyclonal Human Igs, Bind to Human OLs.** If human mAbs are to be a potential therapy to promote remyelination in humans, a reactivity to surface antigens on human OLs may prove important in targeting to areas of human CNS pathology. Therefore, we determined whether human remyelination-promoting mAbs could bind to OLs obtained from the adult human brain. Human glial cell cultures were established from adult temporal lobe biopsies and immuno-labeled with human mAbs at several time points in culture.

Three of the six sHlgMs that bound to the surface of OLs in our initial screen, also bound to human OLs. At 1 week in culture morphologically immature sulfatide-positive human OLs labeled with sHlgM14 and sHlgM46, but not with sHlgM22. By 3 weeks in culture, morphologically complex sulfatide-positive human OLs colabeled with sHlgM14, sHlgM22, and sHlgM46 (Fig. 3 A–C). By 4 weeks in culture, virtually all myelin basic protein-positive human OLs also bound sHlgM22 and sHlgM46, but the binding of sHlgM14 was greatly reduced (data not shown).

Neither IVlg nor polyclonal human IgM bound to the surface of human OLs in culture at any time tested. However, polyclonal human IgM bound strongly to white matter tracts and a variety of neuronal populations when incubated with fresh unfixed slices of rodent CNS. IVlg was completely negative in this binding assay (data not shown). SHlgM22 and SHlgM46, both of which promoted remyelination, and sHlgM14, which did not promote remyelination, also bound to the surface of myelin basic protein-positive rat OLs (data not shown).

We concluded that an affinity for OL antigens may be necessary, but is not sufficient for a human mAb to promote remyelination. The fact that both human mAbs that promote significant remyelination also bind to mature differentiated human OLs underscores the possible requirement for mAbs to be directed against surviving adult OLs for in vivo function.

To exclude the possibility that human Igs or mAbs promoted remyelination by neutralizing virus, each preparation was tested for reactivity to purified TMEV antigens by Western blotting (34). None of the human Ab preparations reacted with TMEV proteins; however, rabbit polyclonal Ig raised against TMEV reacted strongly to four virus capsid proteins (data not shown).

Peripheral B cells were obtained from the individual from which sHlgM22 was identified. The light and heavy chain variable domain sequences of sHlgM22 were determined. The sHlgM22 light chain variable region (GenBank accession no. AF212992) belongs to the \( \alpha \) subgroup I of the human light chain variable regions. The sHlgM22 heavy chain variable region (GenBank accession no. AF212993) belongs to subgroup III of the human heavy chain variable regions. There were significant differences between the sHlgM22 variable domains and the closest known human germ-line variable domain sequence (40). Insufficient blood or sera was available from the individual from which sHlgM46 was identified for variable region sequence analysis.

**Discussion**

In this series of experiments we have demonstrated that human Abs can promote CNS remyelination. More extensive remyelination was observed in the spinal cords of TMEV-infected mice after treatment with polyclonal human IgM than treatment with IVlg. In addition, we identified two human monoclonal IgMs that consistently enhanced remyelination. Both mAbs were isolated from the sera of patients with Waldenström’s macroglobulinemia (WM), a class of lymphoma characterized by the malignant clonal expansion of a single B cell at the late stage of maturation that floods the serum with a monoclonal IgM (41). The high level of these mAbs does not appear to be deleterious. In patients with WM the dominant IgM normally recognizes antigens that are recognized by the IgM repertoire present in healthy individuals (42). Our ability to readily identify and isolate OL antigen-binding, remyelination-promoting mAbs from the human population lends support to the concept that these Abs are common among the B cell repertoire and may function as modifiers in response to CNS injury. Remyelination-promoting mAbs may be produced in the sera of individuals when confronted with CNS damage.

Although both IVlg and polyclonal human IgM promoted remyelination neither bound to rat nor human OLs in culture. In contrast, both human mAbs that promoted remyelination bound to both rat and human OL surface antigens. The increased efficacy of human mAbs to promote remyelination may be because of the effective targeting to adult OLs in the area of damage. Stangel et al. (43) reported that IVlg had no effect on the differentiation, migration, or proliferation of OL progenitors in culture; however, the binding of IVlg to OL progenitors was not assessed. The lack of affinity of IVlg to OLs likely explains the lack of any discernible effect on OL progenitors. Nevertheless, the fact that IVlg does not bind to OLs implies that the mechanism of action in promoting remyelination may be distinct from that of the human mAbs.

The very same preparation of polyclonal human IgM used in this study has been demonstrated to neutralize autoantibodies (28) and alter cytokine expression in experimental autoimmune encephalomyelitis (44) and to be beneficial in a mouse model of myasthenia gravis (45). Polyclonal human IgM, but not IVlg, binds to myelinated tracts in unfixed slices of rodent brain. Neither polyclonal
preparation bound to fresh human white matter. Polyclonal human IgM may promote significant remyelination in the mouse via a combination of general immunoregulation, binding to pathogenic antibodies, and opsonization of myelin debris.

The mechanism by which Igs promote remyelination remains to be elucidated. Because many of the remyelination-promoting mAbs bind to OLs and/or myelin, it is reasonable to hypothesize a direct effect on the recognized cells. There are examples of mAbs binding to and altering the biology of OLs in culture (46–48). However, because the mAbs that promote remyelination have varying specificities (23, 26) it is unlikely that each mAb functions directly through a common antigen or receptor. A polyclonal molecule like an IgM could bring normally disparate signaling molecules into close proximity within the plasma membrane with subsequent activation (49). Because most of the remyelination-promoting mAbs bind to myelin (26), the binding of these IgMs to the cell surface could reorganize the plasma membrane and facilitate a signaling pathway. When SCH94 03 is added to mixed primary glial cultures a 2- to 3-fold increase the uptake of tritiated thymidine is observed (M.R., unpublished observations), but the exact identity of the proliferating cells remains to be determined.

Another potential mechanism by which remyelination-promoting mAbs may function is by targeting to myelin debris or damaged OLs. Binding to OLs or myelin may enhance the clearance of cellular debris from areas of damage, allowing the normal process of spontaneous CNS repair to progress. Perhaps the mechanism of action of polyclonal human IgGs is primarily through immunomodulation—via an inhibition of B cell differentiation or an alteration of cytokine expression and the anti-idiotypic network (27, 50)—whereas the action of the human mAbs is via a direct targeting to OL antigens and/or myelin. No characteristic was produced free from potential pathogen infection and can be structurally altered to augment their effectiveness and immunogenicity. In contrast to mouse mAbs or “humanized” mouse mAbs, human mAbs should result in minimal immune response and are readily applicable to human trials. Given that human mAbs promoted remyelination in chronically paralyzed animals provides hope that successful therapies can be developed for patients with long-standing disabilities.

We thank the generosity of Mr. and Mrs. E. Applebaum. This work was supported by National Institutes of Health Grant NS24180 and Acorda Therapeutics.