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 dedifferentiate 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 Theiler'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 IgMκ 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 IgMκ 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 (IVIg), 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 IVIg.

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

Abbreviations: CNS, central nervous system; IVIg, human i.v. IG; MS, multiple sclerosis; OL, oligodendrocyte, SC, Schwann cell; sHIgM, serum-derived human monoclonal IgM; TMEV, Theiler's murine encephalomyelitis virus.

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

‡A.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.

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 IgM purified from the pooled plasma of more than 2,500 healthy donors was obtained from S. V. K. (28). The purity of IgM 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 polyornithine (Sigma) and laminin (Life Technologies, Grand Island, NY)-coated plastic multiwells (Becton Dickenson) or glass coverslips (Fisher Scientific) in a defined media of DMEM/F12 supplemented with biotin (0.01 μg/ml), tri-iodotyronine (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 antisera (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 2×10^5 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. 1*A*). 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 ×100. Regions of white matter pathology often contained macrophage infiltration, inflammation, and little or no paraphenylenediamine stain (Fig. 2 *C*, *D*, and *H*). The sum of the areas of pathology containing primary demyelination with or without remyelination was determined as a measure of total demyelination.

The areas of remyelination, either OL or SC, were traced at a magnification of ×250. OLs can remyelinate multiple axon fibers, and thus, OL remyelination results in densely packed, yet thin, myelin sheaths compared with spared myelinated axons. SCs can remyelinate only a single axon fiber, resulting in thicker myelin sheaths and increased space between axon fibers compared to OL remyelination. SC bodies and nuclei can be observed adjacent to the axons they have remyelinated. Total areas were calculated for each mouse by summing all of the areas traced from each of 10–12 spinal cord sections per mouse.

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 sampled. The percent area of remyelination per mouse 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. Assaying 10 cross sections resulted in a percent area remyelination value of 47.7%, whereas the data from all 32 cross sections resulted in a

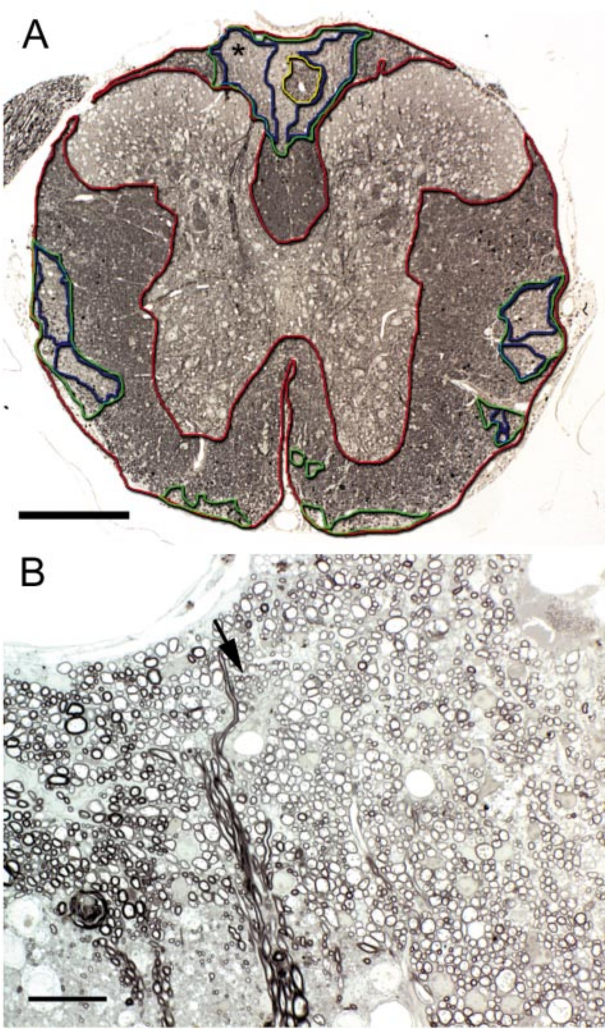

Fig. 1. The methodology used to quantify white matter, white matter pathology, and remyelination in the spinal cords of TMEV-infected mice. Light photomicrograph of a thoracic level spinal cord section from an SJL/J mouse chronically infected with TMEV and treated with polyclonal human IgM (*A*). White matter at the periphery stains darker than the lighter central gray matter. The area of total white matter is traced (indicated by the red outlines), at a magnification of ×40. Then at a magnification of ×100 the areas of white matter pathology are traced (indicated by the green outlines). In this example, the areas of white matter pathology appear as lighter areas at the periphery of the section. Finally, at a magnification of ×250 the areas of OL remyelination (indicated by the blue outlines) and SC remyelination (indicated by the yellow outline) are traced. OL remyelination is characterized by thin myelin sheaths in relation to axon diameter. The percent area of white matter pathology is calculated by dividing the area in green by the area in red × 100. The percent area of OL remyelination is calculated by dividing the area in blue by the area in green × 100. Ten spinal cord cross sections are traced for each animal considered and the areas are combined to calculate a score for that animal. Generally, 7–8 animals are treated in each experimental group to allow for deaths and animals that did not contain at least 5% total white matter pathology. Usually 4–5 treated animals met the criteria for inclusion into the final data set. A high magnification field of the dorsal column white matter (*B*, from the area indicated by * in *A*) demonstrates significant OL remyelination (arrow). (Scale bars are 250 μm in *A* and 20 μm in *B*.)

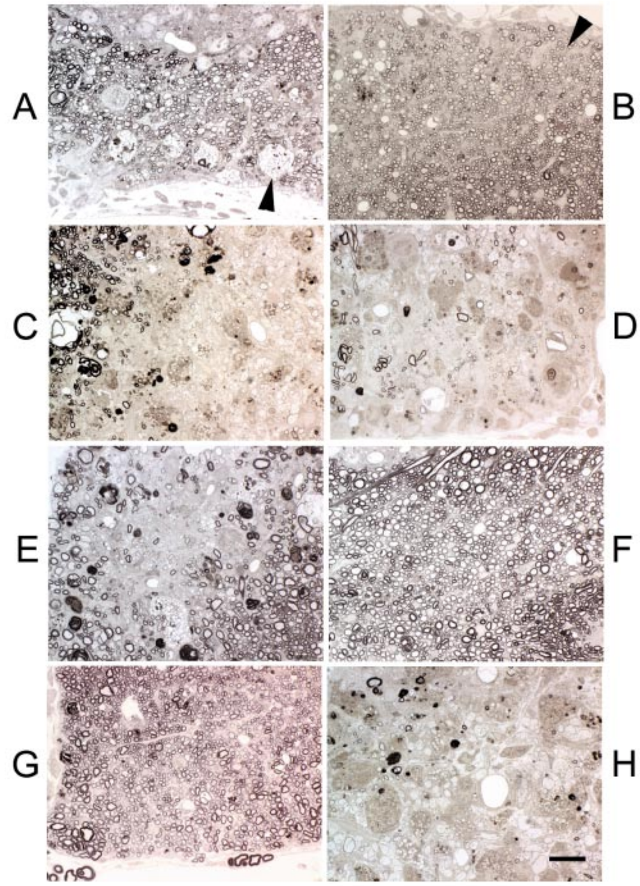

Fig. 2. After treatment with human Abs, chronically TMEV-infected mice demonstrate significant OL remyelination. Light photomicrographs of representative areas of spinal cord white matter pathology of different treatment groups. Treatment with IVIg resulted in significant OL remyelination (*A*). Almost complete OL remyelination, characterized by densely packed thin myelin sheaths in relation to axon diameter (*B*, arrowhead), was observed in sections from the spinal cords of mice after treatment with polyclonal human IgM (*B*) and human mAbs sHIgM 22 (*F*) and sHIgM46 (*G*). In contrast, after treatment with human mAbs sHIgM1 (*C*), sHIgM2 (*D*), sHIgM14 (*E*), or PBS (*H*) mice demonstrated white matter pathology without significant OL remyelination. Infiltrating inflammatory cells, macrophages ingesting myelin debris (*A*, arrowhead), and signs of active myelin destruction were also evident. Spinal cord cross sections in four of eight animals treated with sHIgM22 and five of five animals treated with sHIgM46 contained at least one area of nearly confluent OL remyelination, a rare event indicating significant tissue repair. In contrast, the 10 spinal cord cross sections from each mouse treated with sHIgM1, sHIgM2, sHIgM14, or PBS contained none. (Scale bar is 20 μm.)

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 (Table 1, 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

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

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

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 IVIg 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 CNS-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 mm^2. 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.

with IVIg 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 IVIg ($P = 0.05$, 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 mm^2 within confluently remyelinated areas of pathology (Fig. 2*B*) corresponded to 46,000 to 125,000 remyelinated axons. Therefore, the CNS remyelination after human Ig 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. 2*H*). 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 IVIg and in 10 of 14 animals treated with polyclonal human IgM. We concluded that both IVIg and polyclonal human IgM promote remyelination compared with PBS treatment and that polyclonal human IgM is superior to IVIg 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 sera-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 mm^2. This corresponded to values of 0.0–6.92 percent area of SC remyelination as a function of white matter pathology. There were no statistical differences in the percent area of SC remyelination between any treatment group.

Comparing the percent area of OL remyelination observed after treatment with either human polyclonal or monoclonal preparations revealed that sHIgM46 was statistically superior to IVIg ($P < 0.05$), but not to polyclonal human IgM. The percent area of OL remyelination observed after treatment with sHIgM22 was no different from that after treatment with IVIg, polyclonal human IgM, or sHIgM46.

When examined for areas of white matter pathology with complete repair at least one area was observed in four of eight animals treated with sHIgM22 and in five of five animals treated with sHIgM46. In contrast, none of the animals treated with sHIgM1,

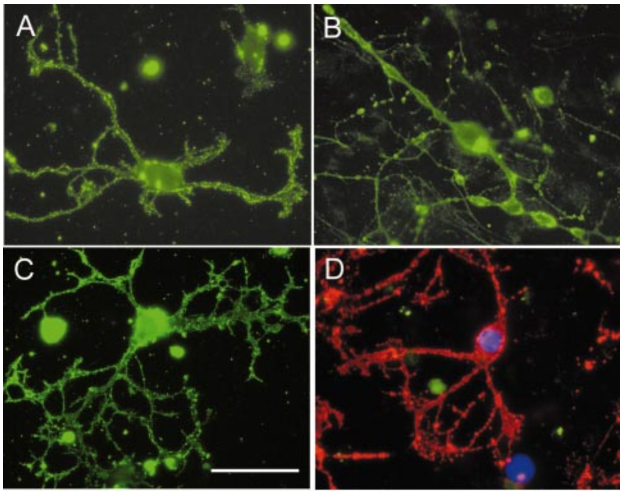

Fig. 3. Human mAbs isolated for their ability to bind to rat OLs also bind to the surface of human OLs in culture. sHIgM14 (*A*), which did not promote remyelination, and sHIgM22 (*B*) and sHIgM46 (*C*), which did promote remyelination, bound to the perikaryon and elaborate process and membrane extensions of sulfatide-positive human OLs maintained in culture for 3 weeks. sHIgM2 (*D*, green channel) is an example of a human mAb that did not bind to sulfatide positive (*D*, red channel) human OLs. Nuclei are labeled blue. IVIg, polyclonal human IgM, and human mAbs sHIgM1 and sHIgM2 did not bind to the surface of human OLs at any time point examined. (Scale bar is 25 μm.)

sHIgM2, sHIgM14, 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 sHIgMs 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 sHIgM14 and sHIgM46, but not with sHIgM22. By 3 weeks in culture, morphologically complex sulfatide-positive human OLs colabeled with sHIgM14, sHIgM22, and sHIgM46 (Fig. 3 *A–C*). By 4 weeks in culture, virtually all myelin basic protein-positive human OLs also bound sHIgM22 and sHIgM46, but the binding of sHIgM14 was greatly reduced (data not shown).

Neither IVIg 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. IVIg was completely negative in this binding assay (data not shown). SHIgM22 and sHIgM46, both of which promoted remyelination, and sHIgM14, 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 sHIgM22 was identified. The light and heavy chain variable domain sequences of sHIgM22 were determined. The sHIgM22 light chain variable region (GenBank accession no. AF212992) belongs to the λ subgroup I of the human light chain variable regions. The sHIgM22 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 sHIgM22 variable domains and the closest known human germ-line variable domain sequence (40). Insufficient blood or sera was available from the individual from which sHIgM46 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 IVIg. In addition, we identified two human monoclonal IgMs that consistently enhanced remyelination. Both mAbs were isolated from the sera of patients with Waldenstrom'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 IVIg and polyclonal human IgM promoted remyelination neither bound to rat or 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 IVIg had no affect on the differentiation, migration, or proliferation of OL progenitors in culture; however, the binding of IVIg to OL progenitors was not assessed. The lack of affinity of IVIg to OLs likely explains the lack of any discernible affect on OL progenitors. Nevertheless, the fact that IVIg 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 IVIg, 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 polyvalent 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 appear to bind to lipids (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 Igs 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 completely predictive of an Ab's ability to promote remyelination. In fact, one human mAb tested in chronically TMEV-infected mice appears to suppress remyelination below the level of spontaneous remyelination, suggesting that certain OL-binding human mAbs can inhibit remyelination *in vivo* or may exacerbate demyelination. This is consistent with the observation that specific mAbs reactive to OL antigens (i.e., myelin oligodendrocyte glycoprotein, ref. 51) enhance demyelination in experimental autoimmune encephalomyelitis (52). Ultimately, proof of an Ab's remyelinating potential and lack of pathogenicity requires *in vivo* testing.

Several double-blind, placebo-controlled trials with IVIg have shown some efficacy in MS (18, 36, 37). Polyclonal human IgM, sHIgM22, and sHIgM46 all enhanced CNS remyelination in the TMEV model as well as IVIg, suggesting that these Abs may be as effective in MS. Human mAbs that bind to OLs may have the additional benefit of direct OL stimulation. Human mAbs can be 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.

1. Perier, O. & Gregoire, A. (1965) *Brain* **88,** 937–952.
2. Prineas, J. W. & Connel, F. (1979) *Ann. Neurol.* **5,** 22–31.
3. Wolswijk, G. & Nobel, M. (1989) *Development (Cambridge, U.K.)* **105,** 387–400.
4. Armstrong, R. C., Dorn, H. H., Kufta, C. V., Friedman, E. & Dubois-Dalcq, M. E. (1992) *J. Neurosci.* **12,** 1538–1547.
5. Gumpel, M., Baumann, N., Raoul, M. & Jaques, C. (1983) *Neurosci. Lett.* **37,** 307–312.
6. Warrington, A. E., Barbarese, E. & Pfeiffer, S. E. (1993) *J. Neurosci. Res.* **34,** 1–13.
7. Groves, A. K., Barnett, S. C., Franklin, R. J., Crang, A. J., Mayer, M., Blakemore, W. F. & Noble, M. (1993) *Nature (London)* **362,** 453–455.
8. Franklin, R. J. M., Bayley, S. A. & Blakemore, W. F. (1996) *Exp. Neurol.* **137,** 263–276.
9. O'Leary, M. T. & Blakemore, W. F. (1997) *J. Neurosci. Res.* **48,** 159–167.
10. Jeffery, N. D., Crang, A. J., O'Leary, M. T., Hodge, S. J. & Blakemore, W. F. (1999*) Eur. J. Neurosci.* **11,** 1508–1514.
11. McKinnon, R. D., Matsui, T., Dubois-Dalcq, M. & Aaronson, S. A. (1990) *Neuron* **5,** 603–614.
12. Bögler, O., Wren, D., Barnett, S. C., Land, H. & Noble, M. (1990) *Proc. Natl. Acad. Sci.* USA **87,** 6368–6372.
13. Rosano, C., Felipe-Cuervo, E. & Wood, P. M. (1999) *Glia* **27,** 189–202.
14. Bansal, R. & Pfeiffer, S. E. (1997) *J. Neurosci. Res.* **50,** 215–228.
15. McTigue, D. M., Horner, P. J., Stokes, B. T. & Gage, F. H. (1998) *J. Neurosci.* **18,** 5354–5365.
16. Miller, D. J., Asakura, K. & Rodriguez, M. (1995) *J. Neurosci. Res.* **41,** 291–296.
17. Noseworthy, J. H., O'Brien, P. C., van Engelen, B. G. & Rodriguez, M. (1994) *J. Neurol. Neurosurg. Psychiatry* **57,** Suppl., 11–14.
18. Fazekas, F., Deisenhammer, F., Strasser-Fuchs, S., Nahler, G. & Mamoli, B. (1997) *Lancet* **349,** 589–593.
19. Lucchinetti, C. F., Bruck, W., Rodriguez, M. & Lassmann, H. (1996) *Brain Pathol.* **6,** 259–274.
20. Dal Canto, M. C. & Lipton, H. L. (1997) *Am. J. Pathol.* **88,** 497–500.
21. Drescher, K. M., Rivera-Quinones, C., Lucchinetti, C. & Rodriguez M. (1998) *J. Neuroimmunol.* **88,** 111–119.
22. Miller, D. J., Sanborn, K. S., Katzmann, J. A. & Rodriguez, M. (1994) *J. Neurosci.* **14,** 6230–6238.
23. Asakura, K., Miller, D. J., Murray, K., Bansal, R., Pfeiffer, S. E. & Rodriguez, M. (1996) *J. Neurosci. Res.* **43,** 273–281.
24. Pavelko, K. D., van Engelen, B. G. & Rodriguez, M. (1998) *J. Neurosci.* **18,** 2498–2505.
25. Miller, D. J., Bright, J. J., Sriram, S. & Rodriguez, M. (1997) *J. Neuroimmunol.* **75,** 204–209.
26. Asakura, K., Miller, D. J., Pease, L. R. & Rodriguez, M. (1998) *J. Neurosci.* **18,** 7700–7708.
27. Dwyer, J. M. (1992) *N. Engl. J. Med.* **326,** 107–116.
28. Hurez, V., Kazatchkine, M. D., Vassilev, T., Ramanathan, S., Pashov, A., Basuyaux, B., de Kozak, Y., Bellon, B. & Kaveri, S. V. (1997) *Blood* **90,** 4004–4013.
29. Gard, A. L. & Pfeiffer, S. E. (1989) *Development (Cambridge, U.K.)* **106,** 119–132.
30. Asakura, K., Hunter, S. F. & Rodriguez, M. (1997) *J. Neurochem.* **68,** 2281–2290.
31. Hunter, S. F. & Bottenstein, J. E. (1991) *J. Neurosci. Res.* **28,** 574–583.
32. Rodriguez, M., Leibowitz, J. L. & Lampet, P. W. (1983) *Ann. Neurol.* **13,** 426–433.
33. Blakemore, W. F., Eames, R. A., Smith, K. J.& McDonald, W. I. (1977) *J. Neurol. Sci.* **33,** 31-43.
34. Njenga, M. K., Pavelko, K. D., Baisch, J., Lin, X., David, C., Leibowitz, J. & Rodriguez, M. (1996) *J. Virol.* **70,** 1729–1737.
35. McGavern, D. B., Murray, P. D. & Rodriguez, M. (1999) *J. Neurosci. Res.* **58,** 492–504.
36. Achiron, A., Pras, E., Gilad, R., Ziv, I., Mandel, M., Gordon, C. R., Noy, S., Sarova-Pinhas, I. & Melamed, E. (1992) *Arch. Neurol.* **49,** 1233–1236.
37. Sorensen, P. S., Wanscher, B., Jensen, C. V., Schreiber, K., Blinkenberg, M., Ravnborg, M., Kirsmeier, H., Larsen, V. A. & Lee, M. L. (1998) *Neurology* **50,** 1273–1281.
38. Pfeiffer, S. E. (1984) in *Oligodendroglia*, ed. Norton, W. T. (Plenum, New York), pp. 233–298.
39. Sommer, I. & Schachner, M. (1981) *Dev. Biol.* **83,** 311–327.
40. Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S. & Foeller, C. (1991) *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD), 5th Ed.
41. Kyle, R. A. & Garton, J. P. (1987) *Mayo Clin. Proc.* **62,** 719–731.
42. Lacroix-Desmazes, S., Mouthon, L., Pashov, A., Barreau, C., Kaveri, S. V. & Kazatchkine, M. D. (1997) *Int. Immunol.* **8,** 1175–1183.
43. Stangel, M., Compston, A. & Scolding, N. J. (1999) *J. Neuroimmunol.* **96,** 228–233.
44. Pashov, A., Bellon, B., Kaveri, S. V. & Kazatchkine, M. D. (1997) *Mult. Scler.* **3,** 153–156.
45. Vassilev, T., Yamamoto, M., Aissaoui, A., Bonnin, E., Berrih-Aknin, S., Kazatchkine, M. D. & Kaveri, S. V. (1999) *Eur. J. Immunol.* **29,** 2436–2442.
46. Bansal, R. & Pfeiffer, S. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6181–6185.
47. Dyer, C. A. & Benjamins, J. A. (1990) *J. Cell Biol.* **111,** 625–633.
48. Cohen, J. A., Williams, W. V., Geller, H. M. & Greene, M. I. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1266–1270.
49. Bansal, R., Winkler, S. & Bheddah, S. (1999) *J. Neurosci.* **19,** 7913–7924.
50. Stangel, M. Toyka, K. V. & Godl, R. (1999) *Arch. Neurol.* **56,** 661–663.
51. Lebar, R., Lubetzki, C., Vincent, C., Lombrail, P. & Boutry, J.-M. (1986) *Clin. Exp. Immunol.* **66,** 423–443.
52. Genain, C. P., Nguyen, M. H., Letvin, N. L., Pearl, R., Davis, R. L., Adelman, M., Lees, M. B., Linington, C. & Hauser, S. L. (1995) *J. Clin. Invest.* **6,** 2966–2974.

NEUROBIOLOGY