Immunoglobulin-mediated CNS repair

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Our view of the immune system continues to evolve from a system dedicated primarily to defense against pathogens to a system that monitors the integrity of the organism and aids in repair following damage. Repair following injury to the central nervous system (CNS) is facilitated by both cellular and humoral components of the immune system. Transfer of macrophages or T cells activated against CNS antigens promote axon regrowth and protect axons from further damage. Animals immunized with spinal cord antigens and subsequently challenged with demyelination or transection of the spinal cord demonstrate better repair than animals without prior immunization. In both experimental systems, antibodies are the biologically active immune component. Human mAbs reactive to oligodendrocytes that arise in the absence of neurologic injury promote remyelination. These data support the hypothesis that B-cell clones producing mAbs reactive to CNS epitopes are a normal part of the human antibody repertoire. They challenge the assertion that an immune response to CNS antigens is pathogenic. Treatment with CNS-reactive human mAbs following CNS disease may facilitate CNS regeneration. (J Allergy Clin Immunol 2001;108:S121-5.)

Key words: *IVIG, remyelination, demyelination, immunoglobulin, oligodendrocyte, Theiler's virus, multiple sclerosis, autoimmune response*

Multiple sclerosis (MS), the most common inflammatory disease of the central nervous system (CNS) in humans, is characterized by damage to oligodendrocytes (OLs) and myelin, with subsequent demyelination and axonal loss. Areas of ongoing disease contain an active inflammatory response—prominent tissue infiltration of T cells and macrophages. Treatment for MS has focused on reducing the inflammatory response, traditionally considered detrimental. This approach, however, is being reexamined in light of evidence that 1) inflammation does not correlate with clinical progression of the disease[1] and 2) upregulating specific components of the immune response can be beneficial to brain and spinal cord repair in CNS disease models.

Abbreviations used

CNS:	Central nervous system
IVIG:	Intravenous immunoglobulin
MS:	Multiple sclerosis
OL:	Oligodendrocyte
SCH:	Spinal cord homogenate
TMEV:	Theiler's murine encephalomyelitis virus

ENDOGENOUS REPAIR FOLLOWING CNS INJURY

Remyelination within MS lesions can occur. It can be significant in acute lesions, but limited in chronic plaques.[2] The presence of shadow plaques—large areas of thinly myelinated axons—indicates that entire lesions can remyelinate. Studies of animal models involving toxin or virus-mediated demyelination demonstrate that spontaneous remyelination following damage to the CNS can be complete within several weeks. In MS, limited remyelination is the norm even though OL progenitors—the cells likely responsible for remyelination—are present in large numbers within and adjacent to brain lesions.[3] The inability of the mature human brain and spinal cord to completely repair itself may be due to a combination of several factors: (1) a CNS environment that inhibits axon extension and remyelination; (2) a change in the way neurons react to their environment as they mature; (3) the formation of a glial scar that acts as a physical barrier to repair; and (4) a consequence of the immune response.

A growing body of literature describes in vivo models in which immune system cells and their soluble molecules can function in repairing and protecting the CNS from pathologic damage. Cohen and Schwartz[4,5] proposed that the immune privilege of the CNS is beneficial to the healthy organism and that following injury, the limited CNS-based immune response also limits the potential for repair. When the blood/brain barrier is opened following damage, however, an uncontrolled autoimmune response may ensue, masking the benefits of a CNS-directed immune response. Whether an immune response to CNS autoantigens can be controlled, manipulated, and used to repair the CNS is under investigation. These data soon may be exploited for the design of specific therapeutics for human CNS disease and trauma.

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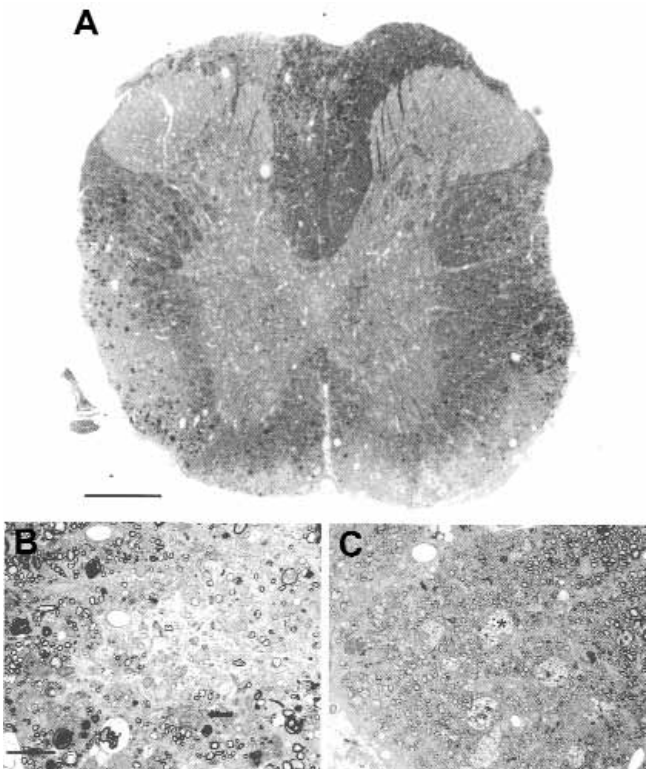

FIG 1. White matter pathology within the spinal cord of TMEV-infected mice. **A,** Light photomicrograph of a thoracic level spinal cord section from an SJL/J mouse chronically infected with TMEV. White matter at the periphery stains darker than the lighter central gray matter. Demyelinated lesions are present at the extreme periphery of the dorsal, ventral, and lateral columns. **B,** Higher magnification light photomicrograph of a chronic demyelinated lesion. A few infiltrating inflammatory cells (*black arrow*), signs of active disease, are present along with demyelinated axons. Limited OL remyelination is present. **C,** An example of ongoing OL remyelination. Multiple macrophages ingesting myelin debris are present amid remyelinated axons. Remyelinated axons are distinguished by relatively thin myelin sheaths in relation to axon diameter. Normally myelinated axons are present in the upper right. Scale bar is 100 μmm in **A** and 20 μmm in **B** and **C**.

MODULATION OF THE IMMUNE RESPONSE TO AFFECT CNS REPAIR

Manipulating both the cellular and humoral components of the immune system can promote CNS repair. Macrophages activated in vitro to peripheral or CNS antigens were administered to rats that had been subjected to transection of the optic nerve or spinal cord. Animals receiving the autoantigen-activated macrophages demonstrated more nerve regrowth and recovery of function than their control littermates.[4] The CD4+ T cells raised to myelin basic protein, a dominant CNS autoantigen, were administered to rats that were subjected to a crush injury to the optic nerve or spinal cord. Both autoimmune and control T cells targeted to the crush injury sites. The animals receiving the autoimmune-activated T cells presented with enhanced nerve protection and repair, however.[5] Similar autoimmune cell-based strategies currently are in limited clinical trial in Israel.

Humoral components of the immune system, primarily antibodies to nervous system epitopes, can be either pathogenic[6] or beneficial.[7] In an attempt to exacerbate the disease course observed in a virus-induced demyelination model, Rodriguez et al[8] administered spinal cord homogenate (SCH) to mice chronically infected with Theiler's murine encephalomyelitis virus (TMEV). The white matter pathology resulting from TMEV infection in susceptible mouse strains is immune-mediated and results in chronic progressive demyelination identical to an MS lesion. Depending on their specific genetic background, animals demonstrate a wide range of disease phenotypes. In the SJL mouse strain, demyelination occurs within 30 days of infection. Animals develop an immune response against persistent virus in the white matter, leading to progressive disability and axonal damage terminating with eventual paralysis. Spontaneous remyelination is minimal, providing a model for the study of strategies to promote remyelination. The TMEV model offers advantages over the dominant model of autoimmune demyelination in rodents, experimental autoimmune encephalomyelitis. Similar to MS, TMEV-induced demyelination progresses through the interaction of both autoimmune and environ-

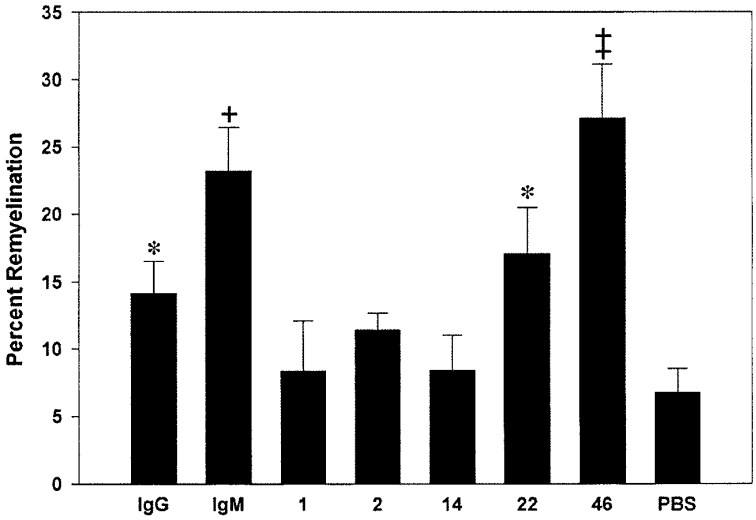

FIG 2. Treatment with human antibodies promotes remyelination in spinal cord lesions. Comparison of the percent remyelination measured in chronically TMEV-infected mice following treatment with IVIG (*IgG*), polyclonal human IgM, human mAbs 1, 2, 14, 22, and 46, and phosphate buffered saline (*PBS*). Human mAbs 14, 22, and 46 bind to human OLs in culture; human mAbs 1 and 2 do not bind. Morphology was performed 5 weeks following intraperitoneal injection of human antibodies. Values represent the mean ± SEM the area of CNS remyelination as a percent of the total area of white matter pathology. Statistical analysis comparing mice treated with human antibodies to mice treated with PBS was performed using one way ANOVA and Student *t* test. Such analysis revealed: * $P < .05$; + $P < .01$; and ‡ $P < .001$. Comparison of mice treated with polyclonal human IgG to other treatments revealed polyclonal human IgM $P = .05$ and sHIgM 46 $P < .05$. All other comparisons were not statistically significant. There was no statistical difference between the CNS-type remyelination between polyclonal human IgM, sHIgM 22, and sHIgM 46. There was no statistical difference in the total area of white matter pathology or the area of myelin pathology in the various treatment groups. Number of mice in each treatment group was IgG (10), IgM (14), human mAb 1 (4), human mAb 2 (4), human mAb 14 (7), human mAb 22 (8), human mAb 46 (5), and PBS (7). For a detailed description of the methods for quantitation of white matter area, myelin pathology, and remyelination, see reference 15.

mental factors. Effective therapy in experimental autoimmune encephalomyelitis has had limited predictive value for MS clinical trial success.[9]

Surprisingly, immunizing chronically infected mice with spinal cord antigens promoted spinal cord remyelination rather than increased demyelination (Fig 1) and accelerated clinical disease. Immunoglobulin reactive to SCH was the active component that promoted remyelination. Further experiments demonstrated that passive transfer of either antiserum or purified Ig from uninfected syngeneic animals immunized with SCH promoted remyelination in mice chronically infected with TMEV. Antibody-mediated remyelination was accompanied by a decrease in the number of inflammatory infiltrating cells at lesion sites. This demonstration of a beneficial role for an autoimmune response contrasts with the classical view that autoantibodies play a pathogenic role in demyelinating disease.

Monoclonal antibodies (mAbs) that promoted remyelination in the Theiler's model subsequently were isolated from SJL mice immunized with SCH.[10] The protypical remyelination-promoting mAb, designated SCH94.03, binds to the surface of OLs, is polyreactive, and of the IgM isotype. Additional mouse mAbs that promoted remyelination also were identified based on their binding to the surface of OLs, many of them established markers of the OL lineage, and all of them IgMs.[11]

Huang et al[12] used a similar approach to study antibody-mediated repair of spinal cord axon injury. Mice were preimmunized with SCH prior to spinal cord hemisection. Upon examination 3 weeks after injury, mice immunized with SCH had far more axons extending through the lesion than controls. The researchers proposed that a polyclonal immune response to white-matter antigens blocked many of the inhibitory epitopes within the mature CNS that normally prevent axon extension. Because identical immunization protocols and mouse strains were used in the TMEV/remyelination and spinal cord injury studies, the polyclonal antibodies that promote remyelination and axon extension may be similar.

Axons in the mature CNS are surrounded by an environment replete with molecules that block neurite extension. Many proteoglycans of the extracellular matrix, as well as proteins of myelin and OLs, such as myelin-associated glycoprotein[13] and Nogo,[14] inhibit neurite extension. The mABs raised against Nogo allowed axons to grow on normally nonpermissive myelin in vitro and in vivo. More axons grew further in animals preimmunized with SCH than in animals treated with monoclonal antiNogo, indicating that multiple inhibitory epitopes need to be blocked for significant axonal regrowth in vivo. Our laboratory's experience with identifying mAbs that promote remyelination has led us to identify mAbs

that bind to and promote neurite outgrowth in vitro. We hypothesize that the immune response to SCH also could upregulate certain mAbs that actively promote axon regrowth in addition to blocking inhibitory molecules.

HUMAN ANTIBODIES CAN PROMOTE CNS REPAIR IN ENCEPHALOMYELITIS

Intravenous immunoglobulin (IVIG), also referred to as polyclonal human immunoglobulin, is used widely for the treatment of autoimmune neurological disorders.[15] IVIG therapy reduced the relapse rate and stabilized the disease course in several small clinical trials for MS.[16] The time course between treatment with IVIG and the observed clinical effects are consistent with the period required for remyelination. Because of these data, we tested human IVIG for the ability to promote remyelination in the TMEV model.[17] We also tested a preparation of human polyclonal IgM that was effective in protecting against autoimmune uveitis in rats, because all of the mouse mAbs that promote remyelination are IgMs.[18]

Mice chronically infected with TMEV were treated with a single intraperitoneal injection of 1 mg of polyclonal human IgG or IgM. The total dose of human Ig was far smaller than that used for IVIG treatment of neurologic disorders in humans.

Upon examination of spinal cord histopathology 5 weeks after antibody treatment, the extent of CNS remyelination in mice receiving either polyclonal human IgG or IgM was significantly higher than spontaneous remyelination observed in a group treated with saline (Fig 2). Although polyclonal human IgG and IgM enhanced remyelination, IgM was more potent. These antibody preparations clearly differed in their ability to bind to CNS antigens. Polyclonal human IgG bound to slices of unfixed rodent CNS in a weak nonspecific pattern, whereas polyclonal human IgM bound intensely to white matter. Polyclonal IgM also bound to the surface of mature myelin basic protein-positive rat OLs in culture. Polyclonal human IgG did not bind to the surface of mature OLs, even at concentrations as high as 200 μg/mL. Because IVIG contains little IgM, IgM-induced remyelination may proceed via a mechanism distinct from that of IVIG. While the activity of polyclonal IgM may depend upon its reactivity with myelin and OL antigens, IVIG may function on the basis of its immunomodulatory activity. This possibility has clear relevance to the use of antibodies as therapy. Combining antibodies that function via immunomodulation with those that function by direct binding to OLs may act synergistically to enhance remyelination efficiency.

Operating under the premise that humans also synthesize natural autoantibodies that can promote remyelination, we screened sera from patients with a variety of monoclonal gammopathies for mAbs that bound to rat and human OLs in culture. None of 50 sera with an IgG monoclonal peak bound to OLs, but 6 of 52 sera with an IgM peak did. The OL-binding mAbs were then tested for the ability to promote remyelination in the TMEV model.[17] Two of the 6 OL-binding human mAbs tested (No. 22 and No. 46, Fig 2) resulted in significantly more remyelination than the saline-treated control group. Four of the human mAbs that bound to OLs in culture did not promote remyelination, indicating that the mere ability to bind to the surface of human OLs is not predictive of an mAb's ability to stimulate repair and that specificity of ligand binding likely is involved.

MECHANISM OF IMMUNE-BASED THERAPIES FOR NEUROLOGIC DAMAGE

Several immunomodulatory mechanisms have been proposed to account for IVIG's action[15]: (1) the neutralization of circulating autoantibodies through an anti-idiotype network; (2) the masking of self antigens or MHC class I molecules; (3) the blocking of Fc receptors; and (4) the modulating of cytokine production.

We have proposed 2 hypotheses suggesting how mAbs may promote remyelination. The first is a direct mechanism, where mAbs bind to ligands on the surface of cells of the OL lineage. The second is an indirect mechanism where mAbs bind to OL and myelin epitopes at lesion sites, which triggers other resident CNS cells to some activity that drives remyelination. In support of the direct hypothesis, evidence shows that binding an mAb to the surface of a cell can alter its biology by affecting membrane microdomains, initiating calcium flux, or changing the level of protein phosphorylation.[19,20] In support of the indirect hypothesis, IVIG can stimulate the movement of macrophages into injured nerves and enhance the phagocytosis of myelin debris.[21] IVIG has no detectable effect on the proliferation, migration, or differentiation of OL progenitors in culture, but it does protect OLs from complement lysis.[22] Because the mAbs that enhance remyelination react with multiple distinct antigens expressed on OLs and myelin, it is unlikely that they function directly to enhance myelin production through a common receptor. These mAbs may target to demyelinated lesions to enhance the clearance of cellular debris, facilitating the normal process of spontaneous CNS remyelination.

SUMMARY

Methods to augment the natural autoimmunity to CNS antigens without inducing a chronic pathologic autoimmune response may provide a novel treatment for brain and spinal cord disease. Evidence suggests that a reactivity to CNS antigens may predominate in the limited natural autoimmunity present in the absence of an open blood-brain barrier. For example, myelin-reactive T cells can be isolated both from healthy individuals and MS patients, and monoclonal antibodies reactive to CNS antigens are common in individuals free of neurological deficit.

The administration of human mAbs may be a safe, controllable, and definable therapy. The potential synergistic effects of combining immunomodulatory human antibody preparations with human mAbs that promote remyelination or axon extension are exciting areas of

research yet to be explored. Preimmunization of humans with CNS antigens, even when limited to at-risk populations, is not a feasible approach to control demyelinating disease. The genetic diversity of humans makes such a tactic risky and unpredictable. Targeted delivery of recombinant human mAbs to injury site is a feasible goal in an attempt to protect axons from permanent damage and preserve neurologic function.

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