Natural autoreactive monoclonal IgM antibodies have demonstrated potential as therapeutic agents for central nervous system (CNS) disease. These antibodies bind surface antigens on specific CNS cells, activating intracellular repair-promoting signals. IgM antibodies that bind to surface antigens on oligodendrocytes enhanced remyelination in animal models of multiple sclerosis. IgM antibodies that bind to neurons stimulate neurite outgrowth and prevent neuron apoptosis. The neuron-binding IgM antibodies may have utility in CNS axon- or neuron-damaging diseases, such as amyotrophic lateral sclerosis, stroke, spinal cord injury, or secondary progressive multiple sclerosis. Recombinant remyelination-promoting IgM antibodies have been generated for formal toxicology studies and, after Food and Drug Administration approval, a phase 1 clinical trial. Natural autoreactive monoclonal antibodies directed against CNS cells represent novel therapeutic molecules to induce repair of the nervous system.

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Table. Properties of CNS-Reactive Signaling Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CNS Surface Membrane Target</th>
<th>CNS Signals</th>
<th>Repair</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2B5 MlgM</td>
<td>c-Series gangliosides</td>
<td>Ca(^{2+})</td>
<td>Remyelination</td>
</tr>
<tr>
<td>HNK-1 MlgM</td>
<td>Sulfated glucuronosyl</td>
<td>Ca(^{2+})</td>
<td>Remyelination</td>
</tr>
<tr>
<td></td>
<td>Glycosphingolipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O1 MlgM</td>
<td>GalC</td>
<td>Ca(^{2+})</td>
<td>Remyelination</td>
</tr>
<tr>
<td>O4 MlgM</td>
<td>Sulfatide (GalC-sulfate)</td>
<td>Ca(^{2+}); P-MAPK, Xcaspase-3, P-SFKs</td>
<td>Remyelination</td>
</tr>
<tr>
<td>SCH94.03 MlgM</td>
<td>Myelin basic protein</td>
<td>Ca(^{2+})</td>
<td>Remyelination</td>
</tr>
<tr>
<td>SCH79.08 MlgM</td>
<td>Myelin</td>
<td>Ca(^{2+}); P-MAPK, Xcaspase-3, P-SFKs</td>
<td>Remyelination</td>
</tr>
<tr>
<td>rHlgM22</td>
<td>Myelin</td>
<td>Ca(^{2+}); P-MAPK, Xcaspase-3, P-SFKs</td>
<td>Remyelination</td>
</tr>
<tr>
<td>sHlgM46</td>
<td>Myelin</td>
<td>Ca(^{2+})</td>
<td>Remyelination</td>
</tr>
<tr>
<td>rHlgM12</td>
<td>Neuronal membrane</td>
<td>Ca(^{2+}); Xcaspase-3</td>
<td>Neuroprotection, neurite outgrowth</td>
</tr>
<tr>
<td>HlgM42</td>
<td>Neuronal membrane</td>
<td>Ca(^{2+}); Xcaspase-3</td>
<td>Neuroprotection, neurite outgrowth</td>
</tr>
<tr>
<td>sHlgM39 (control)</td>
<td>None</td>
<td>No</td>
<td>None</td>
</tr>
</tbody>
</table>

Abbreviations: Ca\(^{2+}\), calcium influx; CNS, central nervous system; GalC, galactosylcerebroside; HlgM, human IgM; MlgM, mouse IgM; P-MAPK, mitogen-activated protein kinase phosphorylation; P-SFKs, Src family kinases phosphorylation; r, recombinant; s, serum; control, isotype control; Xcaspase-3, block caspase-3 activation.

A recombinant version of sHlgM22, rHlgM22, was engineered by cloning the antibody variable region amino acid sequence into an expression vector. The antibody rHlgM22 promoted remyelination in Thellier’s virus infection-induced model of multiple sclerosis equal to the HIgM22 promoted myelin repair in Theiler’s virus infection-induced model of multiple sclerosis equal to the HIgM22 promoted myelin repair in Theiler’s virus infection-induced model of multiple sclerosis equal to the HIgM22 promoted myelin repair in Theiler’s virus infection-induced model of multiple sclerosis equal to the HIgM22 promoted myelin repair in Theiler’s virus infection-induced model of multiple sclerosis. A dendritic cell–binding antibody binding antibodies (sHIgM12 and sHIgM42) stimulated additional human IgM antibodies for testing in other models of multiple sclerosis prior to phase 1 clinical trials. Our development of additional human antibodies from basic science to clinical therapies.

We successfully used the same strategy to identify additional human IgM antibodies for testing in other models of neurologic injury and disease. Two neuron-binding antibodies (sHlgM12 and sHlgM42) stimulated neurite extension. A dendritic cell–binding antibody (B7DC XAb) mediated melanoma tumor clearance from lungs. Several ß-amyloid (Aß1–40 and Aß1–42)–binding human antibodies will be tested in animal models of Alzheimer disease. This strategy for identifying human antibodies that directly signal cells has the potential to generate therapeutic antibodies for a broad range of human diseases.

**SPECIFIC ANTIBODY-GLYCOLIPID-PROTEIN INTERACTIONS MEDIATE rHlgM22 BINDING TO BOTH MYELIN AND OLIGODENDROCYTE MEMBRANE SURFACE**

Several mouse IgM antibodies, including A2B5, O1, O4, HNK-1, SCH79.08, and SCH94.03, bind oligodendrocytes and promote remyelination in mouse models of multiple sclerosis (Table). A2B5, O1, and O4 bind to surface glycolipid antigens on less differentiated oligodendrocytes. HNK-1, SCH79.08, SCH94.03, and rHlgM22 bind to antigens on the surface of relatively mature oligodendrocytes and myelin. We hypothesized that antibody-mediated remyelination required binding to oligodendrocyte membrane glycolipids.

Binding IgM antibodies to CNS tissue from glycolipid knockout mice demonstrates that the molecules bound by rHlgM22 in CNS myelin depend on components of the glycolipid synthesis pathway. Binding of rHlgM22 to the plasma membrane requires the presence of a substrate of cerebroside sulfotransferase (Cst). Normally, the cerebroside galactosyltransferase (Cgt) enzyme converts ceramide to galactosylcerebroside in oligodendrocytes. Then the enzyme Cst converts galactosylcerebroside to sulfatide. Immunofluorescence of live CNS tissue slices demonstrates the strong affinity of rHlgM22 for densely myelinated axons in wild-type mice (Figure 1). rHlgM22 and antibodies against myelin oligodendrocyte glycoprotein, expressed on mature myelin, bound to densely myelinated fiber tracts and to individual myelinated axons in the cerebellum. In contrast, rHlgM22 affinity for white matter tracts was abolished in CNS tissue from mice lacking sulfatide (Cst\(^{-/-}\)). Similarly, O4 antibody, which labels sulfatide, was absent in Cst\(^{-/-}\) mice. In addition, rHlgM22 binding was not detected in other sulfatide-expressing tissues, including peripheral nervous system myelin and Schwann cells. These data support the hypothesis that rHlgM22 binding depends on 1 or more Cst-sulfated antigens present exclusively on the surface myelin of oligodendrocytes. rHlgM22 may target sulfatide or a number of sulfated antigens within the CNS, including glucosylcerebroside sulfate, lactosylceramid-3-sulfate, seminolipid, bis-sulphoganglio tetraosylceramide, and bis-sulphoganglio triaosylceramide. The data support the hypothesis that rHlgM22 recognizes a complex on oligodendrocytes dependent on 1 or more sulfated antigens at the myelin plasma membrane.

Oligodendrocytes express a repertoire of integrins during specific stages of development. Oligodendrocyte myelination correlates with the expression of laminin receptors \(\alpha 6\beta 1\) and vitronectin/fibronectin receptors \(\alpha v\beta 1, \alpha v\beta 3, \alpha v\beta 5,\) and \(\alpha v\beta 8\). Recent studies in collaboration with Jens Watzlawik, PhD, and Richard Pagano, PhD, at Mayo Clinic demonstrate a colocalization of rHlgM22 with \(\beta\) integrins on the plasma membrane of mature oligodendrocytes. We propose that sulfated molecules and \(\beta\) integrin facilitate specific rHlgM22 binding to myelin and oligodendrocytes. In addition, the pentameric structure of the IgM molecule is necessary for remyelination and may be critical to cross-linking these antigens on the oligodendrocyte surface and inducing intracellular repair signals.
IDENTIFICATION OF NEURON-BINDING ANTIBodies FOR CNS PROTECTION AND REPAIR

Two neuron-binding human IgM antibodies, sHIgM12 and sHIgM42, were identified using the strategy described earlier.10 These antibodies supported in vitro CNS neurite extension equal to the potent neurite stimulatory molecule laminin. Both IgM antibodies bound to the surface of many types of cultured neurons but not to the surface of mature oligodendrocytes. Both IgM antibodies stimulated neurite extension in the presence of CNS myelin, which normally inhibits outgrowth. These 2 IgM antibodies are novel agents to promote neurite extension and are being tested in models of CNS disease where destructive of axons and neurons.

ANTIBODY-MEDIATED MEMBRANE REARRANGEMENT INITIATES SIGNALING

We propose that natural autoreactive antibodies activate endogenous cellular mechanisms to protect CNS neurons and oligodendrocytes. Remyelination-promoting IgM antibodies bind to the surface of oligodendrocytes. However, not all IgM antibodies that bind to oligodendrocytes promote remyelination. Oligodendrocyte binding did not perfectly predict the remyelinating potential of an IgM antibody in vivo. Therefore, IgM-mediated repair requires other mechanisms besides strict binding. Specific signaling events are also required. Antibody-mediated signaling in oligodendrocytes is mediated through membrane rearrangement and microdomain signaling.2 Recent studies have demonstrated that mouse IgM antibody O4 and rHlgM22 bound to the surface of un-fixed primary oligodendrocytes diffusely at 4°F. However, allowing membrane rearrangement at 15°F resulted in small punctate structures indicative of signaling microdomain clustering. sHIgM42 under the same conditions exhibited a similar punctate membrane pattern on neurons (Figure 2). Coincident with IgM-mediated membrane clustering on oligodendrocytes was the rapid activation of intracellular mitogen-activated protein kinases. The phosphorylation of several signaling proteins increased within 1 minute and persisted for 15 minutes in primary oligodendrocytes treated with rHlgM22.

REPARATIVE IgM ANTIBODIES INDUCE RAPID CALCIUM INFLUX AND INHIBIT APOPTOSIS

The in vivo remyelination-promoting ability of an antibody correlated with its ability to induce transient calcium influx in oligodendrocytes in culture.3,9 rHlgM22 binding to the oligodendrocyte plasma membrane induced signaling cascades that downregulated caspase-3 activation to prevent apoptosis. In addition, rHlgM22 altered gene expression on calcium influx through CNQX-sensitive AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) channels in oligodendrocytes. The neuron-binding antibodies sHIgM12 and sHIgM42 demonstrated robust rescue of cultured neurons from hydrogen peroxide–induced apoptosis.

ANTIBODY-MEDIATED ACTIVATION OF CNS PROTECTION AND REPAIR

Strong parallels in character exist between remyelination-promoting IgM antibodies rHlgM22 and O4 and the neurite outgrowth–promoting IgM antibodies sHIgM42 and sHIgM12. This suggests a common membrane-rearrangement mechanism that recruits signaling mol-
ecules into clustered microdomains and ultimately leads to specific cell responses in vitro and in vivo (Figure 3).

We propose this class of autoreactive antibodies activates the cellular process of CNS protection and repair through direct signaling cascades. Although each antibody reacts to unique cell-specific antigens, binding to the appropriate cells activates the target cell in a conserved manner. Defining the common signaling components regulating changes in specific cells may lead to an understanding of the underlying mechanism of antibody-induced repair and result in the design of better strategies to promote remyelination and protect axons.

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REFERENCES