Remyelination by Oligodendrocytes Stimulated by Antiserum to Spinal Cord

Moses Rodriguez, M.D., Vanda A. Lennon, M.D., Ph.D., Etty N. Benveniste, Ph.D., and Jean E. Merrill, Ph.D.

Abstract. The new synthesis of myelin and the proliferation of oligodendrocytes was stimulated by serum from syngeneic mice immunized with homogenized spinal cord (SCH). Treatment with this antiserum produced a 10-fold increase in the area of remyelination in spinal cords that had become demyelinated previously as a result of infection by Thielер’s murine encephalomyelitis virus. Inflammation was decreased in regions of white matter that showed remyelination. Oligodendrocytes exposed to anti-SCH in vitro incorporated three to five times more [H]thymidine than resting cells did and expressed more myelin basic protein in their cytoplasm, suggesting stimulation of myelogenesis. Thus, there is a factor present in anti-SCH antiserum that stimulates central nervous system-type remyelination. This finding may provide clues for the therapy of patients with demyelinating disorders such as multiple sclerosis.

Key Words: Demyelination; Microscopy, electron; Multiple sclerosis; Regeneration; Remyelination; Thielер’s virus.

INTRODUCTION

The full improvement of neurologic function after demyelinating diseases of the central nervous system (CNS) appears to be prevented by the relative failure of axons to be remyelinated (1). In contrast, axons in the peripheral nervous system (PNS) are readily remyelinated by Schwann cells after Wallerian degeneration and in primary demyelinating conditions. The picornavirus, Thielер’s murine encephalomyelitis virus (TMEV), causes a primary demyelination in the CNS that probably is the result of immune-mediated destruction (2–7). The virus infection results in pathologic abnormalities similar to those of multiple sclerosis, thus providing an ideal model for study of factors that control demyelination and remyelination.

Spontaneous remyelination by oligodendrocytes after inoculation of animals with TMEV is a rare finding (4–6). Studies by Dal Canto and Lipton and experiments in our laboratory, which used the Daniel (DA) strain of TMEV in SJL/J mice, have demonstrated incomplete CNS-type remyelination by oligodendrocytes but, during the later phases of disease, previously demyelinated lesions showed remyelination by Schwann cells (4–6). This is in contrast to other studies by Dal Canto and Barbano (8) with the WW strain of TMEV in C3H-1 mice; these studies demonstrated remyelination within the CNS by both oligodendrocytes and Schwann cells. Previous experiments by Lang and co-workers (9) showed that, in mice infected persistently

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TABLE 1
Summary of Morphometric Analysis of Demyelination and Remyelination after Treatment with Various Sera

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>PBS/IFA</th>
<th>SCH/IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of white matter, mm²</td>
<td>11.1 ± 1.2</td>
<td>10.2 ± 1.2</td>
<td>10.6 ± 1.1</td>
</tr>
<tr>
<td>Demyelinating lesions, number</td>
<td>23.0 ± 8.0</td>
<td>23.0 ± 9.0</td>
<td>29.0 ± 4.0</td>
</tr>
<tr>
<td>Area of demyelination, mm²</td>
<td>2.17 ± 1.0</td>
<td>1.64 ± 1.1</td>
<td>2.07 ± 0.53</td>
</tr>
<tr>
<td>Demyelination area/white matter area, %</td>
<td>19.3</td>
<td>16.9</td>
<td>19.4</td>
</tr>
<tr>
<td>CNS-type remyelination:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesions, number</td>
<td>2.3 ± 2.0</td>
<td>4.0 ± 3.5</td>
<td>14.7 ± 8.7</td>
</tr>
<tr>
<td>Area, mm²</td>
<td>0.07 ± 0.1</td>
<td>0.13 ± 0.11</td>
<td>0.80 ± 0.50</td>
</tr>
<tr>
<td>CNS-type remyelination/demyelination, %</td>
<td>3.2</td>
<td>7.9</td>
<td>38.0</td>
</tr>
<tr>
<td>PNS-type remyelination:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesions, number</td>
<td>2.0 ± 2.0</td>
<td>2.0 ± 1.8</td>
<td>1.4 ± 1.5</td>
</tr>
<tr>
<td>Area, mm²</td>
<td>0.11 ± 0.2</td>
<td>0.21 ± 0.09</td>
<td>0.06 ± 0.09</td>
</tr>
<tr>
<td>PNS-type remyelination/demyelination, %</td>
<td>5.1</td>
<td>12.8</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Means (±SD) based on examination of ten spinal cord sections from seven mice in each group treated with pooled sera from mice inoculated with phosphate-buffered saline (PBS), PBS plus incomplete Freund’s adjuvant (IFA), or homogenized spinal cord (SCH) plus IFA. Slides were examined without examiner bias and analyses were performed after all slides had been studied. Underline indicates significant difference (p < 0.001) between animals treated with SCH antisera and animals treated with control sera (PBS or PBS/IFA).

with the DA strain of TMEV, a CNS-type of remyelination could be promoted by treatment with an emulsion of incomplete Freund’s adjuvant (IFA) containing homogenized spinal cord (SCH) or myelin basic protein (MBP) with galactocerebroside. We tested the hypothesis that serum factors stimulate myelinogenesis after myelin injury. Here we present evidence that a factor in the serum of uninfected mice immunized with SCH in IFA can stimulate oligodendrocytes to proliferate in vitro and to synthesize new myelin in vivo.

MATERIALS AND METHODS

Demyelination was induced in female SJL/J mice, ages four to six weeks (wk) (The Jackson Laboratory, Bar Harbor, ME), by standard intracerebral inoculation with 2 × 10³ plaque-forming units of DA strain TMEV (10–13) given in 10 μl. Evidence that this demyelinating process is immune-mediated has been published (14–19). Antiserum pools for passive transfer were made by injecting groups of 20–30 donor syngeneic SJL/J mice, ages six to eight wk, subcutaneously in the flanks with 1 mg doses of SCH/IFA, phosphate-buffered saline (PBS) and IFA (1:1), or PBS only, twice weekly for four wk and then monthly for one year. Serum was collected weekly after the eighth injection, pooled by treatment group, and stored at −70°C. Mice infected chronically with TMEV (six to nine months after inoculation) were assigned randomly to groups for treatment with one of the serum pools, 0.5 ml intraperitoneally, twice weekly for five wk.

Mice were anesthetized with 0.2 mg of pentobarbital, exsanguinated by intracardiac puncture, and killed by intracardiac perfusion with phosphate-buffered 2.5% glutaraldehyde (pH 7.2). Spinal cords were dissected carefully from the bony canal, omb to conus medullaris, and the entire cord was sectioned precisely into coronal blocks 1 mm thick. Every third block was postfixed in 1% osmium tetroxide for two hours (h) and embedded in Araldite. Cross sections (1 μm) from each block were stained with 4% p-phenylenediamine. Selected areas were trimmed and prepared for electron microscopy.

<table>
<thead>
<tr>
<th>Animal</th>
<th>CNS-type remyelination</th>
<th>PNS-type remyelination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Area, mm²</td>
</tr>
<tr>
<td>Serum from PBS-treated mice</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0.074</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.086</td>
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<td>7</td>
<td>9</td>
<td>0.359</td>
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<td>Serum from PBS/IFA-treated mice</td>
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</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.104</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.097</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.149</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>0.252</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>0.331</td>
</tr>
<tr>
<td>Serum from SCH/IFA-treated mice</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0.433</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>0.361</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.747</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>1.286</td>
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<tr>
<td>6</td>
<td>23</td>
<td>1.636</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>1.116</td>
</tr>
</tbody>
</table>

Number and area of remyelinating lesions were measured in seven animals in each group. Number and area of CNS-remyelinated lesions were significantly greater (p < 0.05 and p < 0.001) in mice treated with anti-SCH antiserum than in mice treated with control sera.

A detailed morphometric analysis was performed on 1 μm spinal cord cross sections from seven mice in each group with a Zeiss interactive digital analysis system and camera lucida attached to a Zeiss Photomicroscope.

To test the antiserum pools for their capacity to induce proliferation of oligodendrocytes in vitro (20), primary glial cell cultures were established from neonatal rat cerebra (20). By immunofluorescence the cultured cells were 85% to 92% positive for galactocerebroside, a myelin-associated galactosphingolipid that is a surface marker of oligodendrocytes (21, 22). Between 5% and 7% of the cells had cytoplasmic reactivity for glial fibrillary acidic protein (GFAP), a marker of astrocytes (22), and 4% to 7% were positive for esterase, a marker of macrophages and microglia. Diluted serum samples (0.1 ml) were added to triplicate cultures of oligodendrocytes seeded (2 × 10⁴ cells per well) in Falcon Microtest plates. Three days later [³H]thymidine (1 μCi/ml) was added, and the cells were harvested 17 h afterward.

**Fig. 1.** Electron micrographs of ultrathin spinal cord sections stained with uranyl acetate and lead citrate. A. From TMEV-infected mouse (six months post-infection) that was treated with pooled sera from normal syngeneic mice inoculated repeatedly with phosphate-buffered...
saline. Multiple demyelinated axons with abortive CNS-type remyelination are characterized by abnormally thin myelin sheaths around previously demyelinated axons (arrowheads). The axons are normal in appearance, indicative of a primary demyelination. Macrophage (m) containing myelin debris is at lower left. ×6,500. B. From TMEV-infected mouse (seven months post-infection) that was treated with antiserum to SCH. Virtually all of the nearly 100 axons in the picture have evidence of new myelin formation. Three oligodendrocytes (o) in the field are identified by their dark cytoplasm, large nuclei, and intimate association with new myelin sheaths. At a higher magnification, some oligodendrocytes had redundant myelin surrounding the perikaryon, indicative of cellular hyperactivity. Morphometric analyses (Table 1) of spinal cords of mice treated with anti-SCH showed that the numbers and areas of CNS-type remyelinated lesions like this were significantly greater (p < 0.001) than in mice treated with control serum. ×6,000.
Fig. 2. Extensive CNS-type remyelination within a previously demyelinated lesion in the spinal cord of a mouse treated with anti-SCH serum and persistently infected with TMEV. Remyelination is identified morphologically by abnormally thin myelin sheaths surrounding each axon. Arrowheads outline the periphery (meninges) of the spinal cord. Note normally myelinated peripheral nerve axons (PN) demonstrating the specificity of demyelination only within the CNS. At the edge of the remyelinated area are numerous CNS axons with normally thick myelin sheaths that never underwent demyelination. × 500.
RESULTS

In vivo Studies

The mean numbers of demyelinating lesions and the mean areas of demyelination per mouse were similar in the three groups (Tables 1, 2). Demyelination was present in 16% to 19% of spinal cord white matter areas examined (Fig. 1A). Some CNS-type remyelination was found in the groups that received serum from mice inoculated with PBS or PBS/IFA. The mean number of CNS-type remyelinating lesions in the control groups was two to four per animal; the mean area was small (Table 1). In contrast, CNS-type remyelination was prominent in animals that received serum from mice hyperimmunized with SCH/IFA: on average, 14 remyelinated lesions per animal and a mean area of CNS-type remyelination six to 11 times greater than in control groups.

Approximately 38% of the originally demyelinated areas in mice treated with anti-SCH had widespread evidence of marked CNS-type remyelination (Fig. 2). By electron microscopy almost every large-diameter axon in the field had morphologic evidence of new myelin formation—namely, disproportionately thin myelin sheaths (Fig. 1B). In addition, oligodendrocytes were numerous and tended to be clustered in groups in remyelinated areas. In several oligodendrocytes the cytoplasm was surrounded by layers of myelin sheaths, a phenomenon described previously in CNS regeneration. Some oligodendrocytes appeared to be myelinating multiple axons (Fig. 3A), and there were instances of long cytoplasmic extensions making contact with new myelin sheaths. The presence of numerous microtubules in the cytoplasm of some oligodendrocytes suggested that the myelin-producing cells were in a state of hyperactivity. Inflammatory cells were less numerous in remyelinating areas, and of particular interest was the presence of large astrocytes with abundant filaments. This finding suggests that the astroglial response characterizing the reparative phase of CNS demyelination does not interfere with remyelination.

Evidence of PNS-type remyelination of CNS axons was encountered in all three groups of serum recipients (Tables 1, 2; Fig. 3B). This type of remyelination was identifiable by the thickness of the myelin sheath, the one-to-one relationship of Schwann cells to axons, the presence of a basement membrane around newly myelinated axons, and an abundant collagen matrix nearby. Areas of PNS-type remyelination predominated in the periphery of the spinal cord, frequently in direct association with the root entry zone (6). In all cases, the subpial glial limiting membrane was disrupted. This presumably allowed Schwann cells to penetrate into the CNS. Remyelination of the PNS type was often localized to areas of maximal inflammatory infiltration, suggesting that inflammatory cells may have destroyed the glial limiting membrane or triggered migration and proliferation of Schwann cells in the CNS, or both. The presence of collagen matrix in areas of PNS-type remyelination suggests that Schwann cell migration into the spinal cord may depend on interaction with collagen (23, 24).

In vitro Studies

Cells exposed to anti-SCH antiserum incorporated three to five times more [³H]thymidine than resting cells did (Table 3). This level of stimulation was observed even at a serum dilution of 1:4,000. Serum from mice inoculated with PBS/IFA did not significantly affect [³H]thymidine uptake. Although these data suggest specific stimulation of oligodendrocytes, proliferation of astrocytes or microglia was not ruled out.

Fig. 3. A. Oligodendrocyte (O) in spinal cord white matter from TMEV-infected mouse treated with anti-SCH, showing remyelination of three previously demyelinated axons. A connection is visible between the oligodendrocyte and new myelin sheaths. × 19,500. B. PNS-type remyelination in spinal cord white matter of TMEV-infected mouse treated with serum
TABLE 3

<table>
<thead>
<tr>
<th>Serum dilution</th>
<th>PBS</th>
<th>PBS/IFA</th>
<th>SCH/IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50</td>
<td>1,598 ± 330</td>
<td>1,760 ± 495</td>
<td>3,348 ± 1,351</td>
</tr>
<tr>
<td>1:100</td>
<td>1,672 ± 269</td>
<td>3,074 ± 303</td>
<td>3,377 ± 638</td>
</tr>
<tr>
<td>1:200</td>
<td>1,144 ± 200</td>
<td>1,860 ± 829</td>
<td>3,886 ± 1,479</td>
</tr>
<tr>
<td>1:400</td>
<td>2,024 ± 326</td>
<td>3,050 ± 118</td>
<td>5,087 ± 1,596</td>
</tr>
<tr>
<td>1:4,000</td>
<td>2,503 ± 273</td>
<td>2,391 ± 145</td>
<td>5,348 ± 1,376</td>
</tr>
</tbody>
</table>

Data are shown, in cpm, as mean ± SD. [\(^{3}H\)]Thymidine incorporation in the presence of control medium alone was 1,912 ± 514 cpm.

Immunofluorescence testing showed that 40% to 48% of the cells exposed to anti-SCH (1:50 dilution) were doubly labeled with antibodies to galactocerebroside and MBP. Less than 10% of cells exposed to control sera were labeled doubly. These results indicate that the production of cytoplasmic MBP was accelerated in the presence of anti-SCH, or that selective proliferation of MBP-positive cells was stimulated. In either case, the net result was that, in the presence of anti-SCH, more cells had MBP in their cytoplasm, a finding that is consistent with stimulation of myelinogenesis.

DISCUSSION

These experiments using SJL/J mice inoculated with the DA strain of TMEV demonstrate that a factor present in anti-SCH antiserum stimulates CNS-type remyelination in vivo and the proliferation of oligodendrocytes in vitro. Data from mice receiving control antisera confirm the previous observations that CNS-type remyelination is sporadic and incomplete in spinal cords of animals infected persistently with this strain of virus (4, 5, 8). The results are in contrast to those found with the WW strain of TMEV in which remyelination of oligodendrocytes has been reported (8). The reason for this difference in remyelination is not apparent from our study but may relate to different biologic properties of the two strains of viruses or to the extent of host inflammatory response within the demyelination lesion. Nevertheless, the present experiments suggest that humoral factors may be important in determining the extent of remyelination after virus-induced demyelination.

Remyelination of CNS axons has been described in several experimental models of demyelination, e.g. after cerebrospinal fluid barbotage (25), intraspinal injection of lysolecithin (26), experimental autoimmune encephalomyelitis (EAE) (27–29), acute spinal cord compression (30), cuprizone toxicity (31, 32), and JHM hepatitis virus infection (33–35). The CNS-type remyelination that results from cuprizone toxicity or JHM-virus infection occurs spontaneously and is widespread; it is noteworthy that immunologic mechanisms are not implicated in myelin destruction in from mice inoculated with PBS/IFA. Note the one-to-one association between Schwann cell (S) and remyelinated CNS axon. An astrocytic process (As) with abundant glial fibrils is close to the remyelinated axons. There is also an abundance of collagen matrix (c) in the remyelinated area, and a lymphocyte (L) also is present. These findings suggest that the collagen matrix and astroglial reparative response do not interfere with PNS-type remyelination and may stimulate Schwann cell migration into the CNS. ×15,500.
these two models of demyelination. However, spontaneous remyelination is not
common in immune-mediated disorders such as chronic EAE and TMEV encepha-
alomylitis induced by the DA strain of virus but, in both disorders, remyelination
can be enhanced by systemic administration of emulsions containing myelin com-
ponents (MBP with galactocerebroside) (36, 37). Raine and Traugott (36) demon-
strated widespread remyelination and oligodendroglial proliferation in the spinal
cords of chronic EAE animals treated with myelin components; the results are similar
to the remyelination in TMEV-infected mice treated with anti-SCH antisera.

In the spontaneous remyelinating situations of cuprizone toxicity and JHM hep-
atitis, uptake of [3H]thymidine by oligodendrocytes has been demonstrated, indi-
cating that cell division precedes myelinogenesis (32, 33). Ludwin (32) suggested
that, in the remyelinating phase of cuprizone toxicity, dividing oligodendrocytes may
be derived from immature cells appearing during the glial response. The stimulus for
oligodendrocyte division and differentiation may arise during the demyelinating
stage, from damaged myelin, inflammatory cells, or activated astroglial cells. Alter-
natively, remyelination could be the normal consequence of non-immune-mediated
demyelination but it may be blocked in immune-mediated demyelinating conditions
by factors released in inflammatory responses.

Several candidates might be suggested for the systemic humoral factor in anti-
SCH antiserum that stimulates CNS-type remyelination in vivo and oligodendroglial
proliferation and differentiation in vitro.

Immunoglobulins may be directed to an antigen common to myelin and to the
surface of oligodendrocytes that serves on the latter as a receptor for stimuli inducing
division or differentiation. Consistent with this mode of action is the finding (38)
that, when added to myelinated cultures of mouse spinal cord tissue, heated (de-
complemented) serum from animals with EAE causes oligodendroglia to develop a
profusion of cytoplasmic processes and to produce aberrant myelin. Galactocere-
broside, a cell surface component of oligodendrocytes, could be the receptor. How-
ever, this suggestion is not supported by the finding that repeated in vivo inoculation
with emulsions containing galactocerebroside and IFA does not induce the extent of
CNS-type remyelination induced by SCH in animals demyelinated by TMEV or
chronic EAE (9, 36, 37).

A stimulatory lymphokine/cytokine could be produced by T cells, B cells, mi-
croglia, or astrocytes and trigger oligodendrocytes to divide and to myelinate. For
example, an HTLV-II-infected MO-T cell line releases a factor that stimulates rat
brain oligodendrocytes to synthesize DNA and to proliferate (20), and factors released
by mitogen-stimulated T cells stimulate proliferation of mouse brain astrocytes in
vitro (39, 40). Astrocytes themselves secrete lymphokines and possibly also regulatory
molecules that control oligodendrocyte differentiation.

Remyelination stimulated by anti-SCH antiserum may have resulted from
suppression of white matter destruction. In this scheme, a suppressor factor or
immunoglobulin in the antiserum may have inhibited demyelination and inflam-
mation, which secondarily stimulated remyelination. For example, the anti-SCH
antiserum may have contained a humoral soluble promoter of antigen-specific sup-
pressor cells. This is possible because hyperimmunization with antigen in IFA prefer-
entially elicits antigen-specific suppressor cells (41).

We favor the hypothesis that the remyelination factor is contained within the
immunoglobulin fraction of anti-SCH antiserum. This would best explain the spec-
ificity of stimulating CNS-type remyelination with the anti-SCH antiserum rather
than with IFA alone. Anti-idiotypic antibodies also could be involved and would
satisfy the requirement of specificity. Possibly, more than one factor may be involved. One factor may provide specificity while others may augment the response.

The results of our experiments may have practical applications to multiple sclerosis and related human demyelinating disorders. Rare examples of spontaneous CNS-type remyelination ("shadow plaques") are found in multiple sclerosis (42), and occasional PNS-type remyelination is found in demyelinated spinal cord plaques near the root entry zone (43-46). Oligodendrocytes are infrequent at the center of chronic plaques in multiple sclerosis but they appear to proliferate at the periphery of plaques, where they are associated with abortive remyelination (47). These clinical observations indicate that new myelin formation is possible in multiple sclerosis. The remyelination that we stimulated in mice with TMEV-induced demyelination by using a systemic humoral factor may hold promise for a therapeutic application in multiple sclerosis.

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