Slowly Progressive Axonal Degeneration in a Rat Model of Chronic, Nonimmune-Mediated Demyelination

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INTRODUCTION

Axon degeneration in a variety of neurologic disorders results in chronic disability. In multiple sclerosis (MS), central nervous system (CNS) axonal loss occurs throughout the disease course and has been correlated with disability scores (1–3). A large number of toxic and inflammatory substances cause disruption of axonal transport, cytoskeletal structure, and energy production, all of which ultimately contribute to axon destruction (4, 5). However, in addition, the failure of trophic support networks in the nervous system may also lead to slowly progressive axon “dropout” (6).

Specifically, oligodendrocytes and myelin seem to have axonotrophic roles within the CNS. Long-term axonal survival seems to be dependent on specific biochemical and homeostatic functions of oligodendrocytes, such as peroxisomal activity and microRNA processing (7, 8). Soluble factors produced by oligodendrocytes may also influence axon survival and phenotype (9, 10). There is increasing evidence suggesting that myelin (which is produced by oligodendrocytes and ensheaths axons, and thereby facilitates rapid saltatory conduction) is required for the long-term maintenance of axons. Studies of myelin mutants have provided important information concerning the contribution of specific myelin components to axon stability. For example, mice deficient in myelin proteolipid protein (PLP) and its splice isoform DM20 show late-onset axonal degeneration despite appropriate levels of compact myelin (11). Myelin mutants also develop axon transport abnormalities, thereby demonstrating the importance of myelin to axonal function (12). Similarly, other myelin components seem crucial in long-term survival of axons (13, 14). In these studies, however, axons are ensheathed by myelin that lacks specific components; the extent to which axons may degenerate after removal of myelin that contains all of the major myelin proteins is not clear.

A model of nonimmune-mediated oligodendrocyte injury in animals with myelin that contains all of the major myelin proteins would address the question of whether chronic oligodendrocyte dysfunction and myelin loss per se cause axon degeneration. The taiep rat mutant is characterized by abnormal microtubule accumulation within oligodendrocytes. In this rat model, there is early formation of thin CNS myelin sheaths, but this is followed by progressive myelin loss (15, 16). The axons can remain demyelinated for over a year, making the taiep rat uniquely different from the other...
FIGURE 1. Progressive demyelination in the taeip rat: (A–D) Proteolipid protein (PLP) immunohistochemistry in sections of cerebellum from wild type (A, B) and taeip rats (C, D) at 3 months (A, C) and 12 months (B, D). (E–G) Electron micrographs of taeip optic nerve at 1 month (E), 6 months (F), and 12 months (G). Scale bar D = 1000 μm for A–D; bar in G = 2 μm for E–G.
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SMI32: cerebellum                dorsal columns

3 month wt

A  B

3 month taiep

C  D

SMI32: cerebellum                dorsal columns

12 month wt

E  F

12 month taiep

G  H

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myelin mutants. In contrast to the knockout mice noted above, taiep myelin contains all the major myelin proteins, although myelin-associated glycoprotein (MAG) and myelin basic protein (MBP) messenger RNA (mRNA) are reduced, suggesting that the myelin is not entirely biochemically normal (17, 18). Demyelination in taiep rats has been proposed to result from the effect that accumulating microtubules have on the transport of certain myelin proteins or their mRNAs (18, 19). The precise genetic defect in the taiep rat is unknown, however, but it has been mapped to chromosome 9 (20). There is no immune-mediated component to the pathologic loss of myelin in taiep rats and the myelin loss seems to be selective, especially on small-caliber axons; thus, it is a useful tool to study oligodendrocyte-axonal interactions. This is especially relevant to MS in which small-diameter axons preferentially degenerate (21), as they also do in certain experimental autoimmune encephalomyelitis models (22).

In this study, we characterize age-related axonal pathology in the taiep rat in several CNS regions. We show initial early degenerative changes of myelinated axons in the optic nerve. This is followed by further evidence of progressive axonal pathology, characterized by accumulation of amyloid precursor protein (APP), dephosphorylation of neurofilaments, axonal spheroid formation, and loss of optic nerve axons; these changes accumulate as the degree of myelin loss progresses. These results support the hypothesis that long-term oligodendrocyte dysfunction and demyelination may lead to axonal degeneration in the absence of inflammation.

MATERIALS AND METHODS

Tissue Preparation for Immunohistochemistry

Taiep rats (23) were obtained from breedings between homozygous animals in a colony maintained at the University of Wisconsin-Madison. Methods for animal husbandry and sacrifices in this study were approved by the University’s Animal Care and Use Committee. Taiep and wt control rats aged 10 days, 3 months, 12 months, and 18 months were deeply anesthetized with sodium pentobarbital, perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 mol/L Sorensen phosphate buffer (pH 7.4). Animals were decapitated; brains and spinal cords were removed; tissue was fixed overnight (ON) in 4% paraformaldehyde, and snap-frozen in dry ice powder. Sections cut at 6 μm on a cryostat were mounted on glass slides and kept at −80°C until use.

Light and Electron Microscopy

Rats were anesthetized deeply with pentobarbital intraperitoneally and heparinized intracardially before the perfusion. The animals were perfused with lactated Ringer’s solution through the left ventricle, followed by modified the Karnovsky fixative (24). Brain, optic nerve, and spinal cord were dissected 1 hour after the perfusion and maintained refrigerated in the Karnovsky fixative. These samples were postfixed in osmium tetroxide, dehydrated in a series of graded alcohols, and embedded in Epon. For light microscopic evaluation, silver gray ultrathin sections of the intracranial optic nerve and thoracic spinal cord were cut with a diamond knife and mounted on formvar-coated copper grids, stained with uranyl acetate and lead citrate, and examined with a Phillips 410 electron microscope (Phillips/FEI Corporation, Eindhoven, Holland).

Immunohistochemistry

Primary antibodies and titers used were the following: mouse anti–nonphosphorylated neurofilament antibody (SMI-32, 1:100,000; Covance, Berkeley, CA), anti-PLP (1:100; Boehringer, Ingelheim, Germany), anti-complement receptor type 3 antibody (MRC OX-42, 1:500; Serotec, Raleigh, NC) as a marker for microglia/macrophage, and anti-αβ T-cell receptor antibody (clone R73, 1:5000; BD Pharmingen, San Diego, CA).

Endogenous peroxidase activity in sections was quenched with 3% H2O2 in PBS-T for 10 minutes. The slides-mounted sections were first incubated with the primary antibodies diluted with PBS Tween 20 containing 2% normal donkey serum ON, for 1 hour with biotinylated antimouse immunoglobulin G (IgG, diluted 1:100; Jackson Immuno Research, West Grove, PA), and 2% normal donkey serum in PBS-T, and for 30 minutes with the avidin-biotin peroxidase complex diluted 1:200 with PBS-T (Vector Laboratories, Burlingame, CA). Sections were washed 3 times with PBS between the procedures described above. Peroxidase activity was visualized with 0.02% 3,3’-diaminobenzidine (Pierce, Rockford, IL), and 0.0045% H2O2 in 50 mmol/L Tris-HCl buffer (pH 7.6). For APP immunolabeling, antigen retrieval was performed by incubating the slides in 10 mmol/L sodium citrate (pH 8.5) for 30 minutes at 80°C before staining.

For T-cell staining, the rats were perfusion-fixed with 2% paraformaldehyde and 0.2% picric acid in 0.1 mol/L SPB (pH 7.4). The cerebellum was removed, postfixed in the same fixative for 1 hour, cryoprotected in 15% sucrose in 0.1 mol/L SPB ON, and snap-frozen in dry ice powder. Sections cut at 6 μm on a cryostat were mounted on glass slides and kept at −80°C until use.

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FIGURE 3. Axonal transport changes in the taiep rat: (A–F) Amyloid precursor protein (APP) immunohistochemistry in cerebellum of 3-month-old wild type (wt) (A, B) and taiep (D, E) rats; dorsal columns of spinal cord from 3-month-old wt (C) and taiep (F) rats. (G–L) APP immunohistochemistry in cerebellum of 12-month-old wt (G, H) and taiep (J, K) rats; dorsal columns of spinal cord from 12-month-old wt (I) and taiep (L) rats. Scale bar in J = 1000 μm for A, D, G, J; bar in K = 200 μm for B, E, H, K; bar in F = 50 μm for C, F; bar in L = 200 μm for I, L.
Whole AMVs were washed after fixation and permeabilized ON in 1% Triton X at RT. Nonspecific binding was blocked by incubating in 3% bovine serum albumin with 3% donkey serum in PBS (pH 7.4) for 2 hours before ON incubation with the primary antibody solution (mouse anti-nonphosphorylated neurofilament antibody [SMI-32] or rabbit anti-PLP) at 4°C. After further washing, the AMVs were incubated with fluorescein isothiocyanate-conjugated anti-mouse IgG and Tetramethyl Rhodamine Iso-Thiocyanate (TRITC)-conjugated anti-rabbit IgG antibodies (both 1:100) at RT for 2 hours. Anterior medullary velums were then washed and mounted on coverslips and viewed using fluorescence microscopy.

**Optic Nerve Analyses**

Age-matched control and *taiep* rats (1, 3, 6, 9, and 14 months old) were perfused through the heart with PBS and then with 4% paraformaldehyde in 0.14 mol/L SPB. Optic nerves with attached chiasms were carefully excised, cryoprotected with 30% sucrose in PBS, and transected 3 mm anterior to the chiasm to present a standardized site for acquisition of 8-μm cross sections of the nerves, thereby ensuring that similar regions were analyzed for all conditions. Sections were then incubated sequentially in 1) blocking solution (PBS with 5% normal goat serum, 2% bovine serum albumin, 0.1% Triton X-100, and 0.02% sodium azide) 15 minutes, at RT; 2) primary mouse monoclonal antibodies to phosphorylated neurofilament (SMI-31, 1:20,000; Covance) and nonphosphorylated neurofilament (SMI-32, 1:20,000) in blocking solution ON at 4°C; 3) goat antimouse IgG-biotin (1:1000; Sigma, St Louis, MO); 4) PBS; 5) avidin-HRP (1:1000; Sigma); 6) PBS; and 7) heavy metal–enhanced 3,3′-diaminobenzidine.

**Image Acquisition and Analysis**

For quantification of optic nerve axons, sections were examined with a Nikon E800 microscope (Nikon USA, Melville, NY) equipped with a 100× oil objective; digital images were captured with a CoolSNAP HQ camera (Photometrics, Tucson, AZ) using Meta-Vue software (Molecular Devices, Downingtown, PA). Axons were quantified within preselected fields (500 μm²) and counted using MetaMorph software (Molecular Devices). The total cross-sectional area for each optic nerve was measured from digital images captured at 10× after manual circumferential outlining. The total number of axons per optic nerve was calculated by multiplying the centrally sampled axon density by the total cross-sectional area for

![FIGURE 4. Axonal spheroid formation in the *taiep* rat: (A) SMI-32 staining of spheroid in 12-month-old *taiep* cerebellum. (B) Electron micrograph of spheroid in 12-month-old *taiep* optic nerve (lower). Scale bars = 5 μm. (C) Counts of spheroids per cerebellar section in wild type (wt) and *taiep* rats at 3 and 12 months; 3 sections per animal were taken through the same region of cerebellum; 5 animals per condition. *** p < 0.001.](http://jnen.oxfordjournals.org/)

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the same optic nerve. Statistical comparisons between control and taiep groups were performed with 2-sample t test using Microsoft Excel software (Microsoft Corp, Redmond, WA).

Axon Quantification in Optic Nerves
Semithin optic nerve sections from 2-month-old wt (n = 2), 2-month-old taiep (n = 3), 6-month-old wt (n = 2), and 6-month-old taiep (n = 2) rats were stained with methylene blue and examined with a Nikon E800 light microscope (Nikon) equipped with a CoolSNAP HQ camera (Photometrics). Digital images were acquired using MetaVue software (Molecular Devices), and the cross-sectional nerve areas were determined with IPLab software (Scanalytics, Rockville, MD).

Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM-1011 electron microscope (JEOL USA, Peabody, MA) operating at 80 kV. Images were acquired at 10,000× magnification with an AMT-TR-111...

FIGURE 5. Axonal changes in the anterior medullary velum of the taiep rat: (A, B) Nonphosphorylated neurofilament labeling (green) and myelin proteolipid protein (PLP) labeling (red) of anterior medullary velum (AMV) of wild type (A) and taiep rat at 12 months (B). (C) Double labeling of PLP (red) and nonphosphorylated neurofilament (SMI-32; green) of AMV of a taiep rat at 12 months showing myelinated axons negative for SMI-32 (bold arrow), small-demyelinated axons staining for SMI-32 (arrowheads) and axons undergoing demyelination (thin arrow). Scale bars = 5 μm.
digital camera (Advanced Microscopy Techniques, Danvers, MA) from 6 to 9 randomly selected fields for each nerve. The number of axons within 2 nonoverlapping 100-μm² regions in each field was quantified, and axons were classified as myelinated, unmyelinated, or degenerating. Total numbers of axons were calculated for each optic nerve using the cross-sectional areas obtained from the semithin sections. Statistical comparisons between wt and taiep groups were performed with 2-sample t test using Microsoft Excel software.

RESULTS

Nonphosphorylated Neurofilament Staining in taiep and wt Rats

Initial experiments confirmed the progressive loss of myelin within CNS white matter tracts. At 12 months, there was a marked loss of myelin in cerebellum, gracile fasciculus of the spinal cord, and optic nerve of wt CNS compared with wt CNS (Figs. 1, A–D). Electron microscopy (EM) examination confirmed the progressive nature of the demyelination (Figs. 1, E–G). Immunohistochemistry using SMI-32 antibody revealed immunoreactivity in Purkinje cells and white matter tracts of the taiep cerebellum, but there was no staining in the white matter wt rats (Fig. 2). No SMI-32 staining was seen in cerebellar white matter of 10-day-old taiep rats (not shown), but staining was observed in cerebellar white matter of 3-month-old taiep rats (Fig. 2C). There was also occasional spheroid formation seen at this stage. Axonal SMI-32 staining was prominent in 12-month-old taiep rats (Fig. 2G); at 18 months, there was characteristic spheroid formation.

Additional areas of wt and taiep rat CNS, including spinal cord and corpus callosum, were also examined. There were no differences in SMI-32 staining between areas 10-day-old wt and taiep rats. There was no SMI-32 staining in wt animal white matter at 3, 12, and 24 months, whereas there was extensive staining of dorsal columns of the spinal cord in taiep rats at these ages (Figs. 2D, H). There were similar SMI-32 staining patterns in the taiep corpus callosum (not shown).

Axonal Degeneration and Demyelination

Amyloid precursor protein staining was not seen in taiep or wt rats at 10 days (not shown). In wt rats at 3 (Figs. 3, A–C) and 12 months (Figs. 3, G–I), there was no APP immunolabeling. Likewise, no APP immunostaining was observed in these areas in wt rats at 18 months (not shown). At 3 months, taiep rats exhibited diffuse staining within cerebellar and dorsal column white matter tracts (Figs. 3, D–F), there was greater staining at 12 months, which was accompanied by APP-containing aggregates (Figs. 3, J–L).

Axonal Spheroids

Accumulation of APP in a granular pattern was observed at the light microscopic level in presumed axonal spheroids of the taiep cerebellum at 12 months (Fig. 3K). SMI-32+ spherical accumulations were occasionally observed in the cerebellum and corpus callosum of 3-month-old taiep rats and were more numerous in these regions at 12 and 24 months in taiep rats (Figs. 2G and 4A, C). By EM, spheroids in optic nerves of taiep rats exhibited accumulation of filamentous structures and mitochondria (Fig. 4B). Scattered spheroids were also seen by EM in the taiep spinal cord (not shown).

Changes in the AMV

Selective myelin deficits occur in the AMV of taiep rats (25). Therefore, we studied SMI-32 staining in the AMV. At 3 months, there was a reduction in PLP staining within the taiep versus wt AMV and the appearance of SMI-32 staining in some small fibers in the taiep AMV (data not shown). At 12 months, there was extensive loss of PLP staining concomitant with increased SMI-32 staining in taiep AMV (Fig. 5B); SMI-32 staining was not seen in wt AMV (Fig. 5A). Some fibers in the taiep AMV remained ensheathed

FIGURE 6. Lack of T-cell immunoreactivity in the taiep rat. (A–D) Immunolabeling for αβ–T-cell receptor in the cerebellum of 12-month-old wild type (wt) (A, C) and taiep (B) rats. The inset in C is a high-power magnification of the T cell in C. Scale bars = (A–C) 50 μm; (C inset) 5 μm.
with myelin and were PLP+ but SMI-32-negative (Fig. 5C). Most small-caliber axons in the 
taiep AMV lacked were PLP-negative and strongly SMI-32+. A few fibers had an inter-
mediate phenotype, with patchy SMI-32 labeling and minimal 
preservation of PLP staining (Fig. 5B).

**Immune Cell Labeling**

To examine the degree of inflammatory activity, immu-
monolabeling was performed for αβ-T-cell receptor, a label 
for most T cells. Only sporadic T lymphocytes were found in the 
cerebellum of 12-month-old wt and 
taiep rats (Fig. 6). Neither the distribution nor the number of T lymphocytes was 
affected by the 
taiep phenotype or age (data not shown).

In 12-month-old wt rats, there was a diffuse distribution of 
OX-42+ microglia throughout the entire cerebellum. Micro-
glia seemed to be activated, showing shorter and thicker pro-
cesses and dense OX-42 immunoreactivity at 24-month-old wt 
rats (Figs. 7, A–D). In contrast, microglial activation was found 
throughout the 
taiep cerebellum at 12 months, with prominent 
staining in the white matter (Figs. 7E, F). Enhanced labeling 
was persistent or slightly intensified in 18-month-old 
taiep rats 
(Figs. 7G, H).

**Optic Nerve Counts**

Optic nerves from wt and 
taiep rats were immuno-
stained for phosphorylated (SM-I31) and nonphosphorylated

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**FIGURE 7.** Microglia in the 
taiep rat. (A–H) OX-42 immunolabeling in cerebellum of wild type (wt) (12-month-old [A, B] and 
24-month-old [C, D]) and 
taiep (12-month-old [E, F] and 18-month-old [G, H]) rats. Scale bar in G = 500 μm for A, C, E, G; bar 
in H = 50 μm for B, D, F, H.

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**FIGURE 8.** Optic nerve axon counts in wild type (wt) and 
taiep rats. (A) Transverse sections of optic nerves from 1-, 3-, 6-, 9-, and 
14-month-old wt and 
taiep rats were reacted with antibodies to nonphosphorylated and phosphorylated antibodies to label all 
axons. The numbers of axons with wt optic nerves are relatively constant between 1 and 14 months, but there are more larger-
diameter axons at latter developmental ages. Taiep optic nerves exhibit a steady decrease in the number of axons with development from 1 to 14 months. (B) Quantification of total number of axons in optic nerves demonstrates a significant reduction of 
axons within 
taiep versus wt optic nerves at all ages after 1 month. There is a 59.7% (30,782 versus 76,370) loss of axons between 
1 and 14 months in 
taiep optic nerves.
thinner myelin sheaths than the wt myelinated axons. Degenerating axons continued to be present in 6-month-old taiep rats. Axon loss was marked in the 12-month-old taiep optic nerve (Fig. 10).

Optic nerves of 2- and 6-month-old wt rats exhibited similar total numbers of axons (101,400 ± 4167 and 109,297 ± 9760, respectively), whereas the total number of axons in optic nerves of taiep rats at 2 and 6 months (79,934 ± 2859 and 70,814 ± 5142, respectively) was significantly reduced compared with their age-matched cohorts (p < 0.05). There was a continuing loss of axons with increasing age.

**DISCUSSION**

We have shown that pathologic changes and degeneration occur in brain, optic nerve, and spinal cord axons of adult taiep rats. Using IHC, we determined that neurofilament dephosphorylation occurs in taiep axons from 3 months. These changes are more pronounced as the degree of myelin loss increases and characteristic spheroids rich in dephosphorylated neurofilaments become abundant. Electron microscopy confirmed axonal spheroid formation. Amyloid precursor protein staining that indicates abnormalities of axonal transport is also increased in taiep rat white matter tracts. Immunohistochemistry of the AMV revealed neurofilament dephosphorylation of small-diameter axons that had lost myelin in taiep rats. The wt rats did not show any of these alterations. Moreover, there was a progressive quantitative loss of taiep optic nerve axons. The absence of increased T cells in the taiep rats at all ages confirms the non–immune-mediated nature of the myelin injury and implies that axons are not damaged through primary immunologic injury. The increase in microglia in white matter tracts of the cerebellum is likely to be a secondary phenomenon relating to myelin and axon degeneration. Thus, the data increase the evidence that normal oligodendrocyte function is required for long-term axonal stability and survival. The absence of a primary inflammatory event in the taiep rat suggests that primary oligodendrocyte dysfunction per se may be a key determinant of axonopathy.

Previous studies of myelination in taiep rats have demonstrated initial hypomyelination of CNS axons with reduction in mean myelin thickness at early time points (16). Myelin levels peak at 2 months, and this is followed by progressive CNS demyelination (17). Excessive accumulation of microtubules throughout the cytoplasm of taiep oligodendrocytes occurs, and demyelination is thought to result from impairment in the transport of myelin proteins or their mRNAs (19). The microtubule abnormality seems to be specific to oligodendrocytes; no accumulations of microtubules were seen in axons. Myelin loss is most severe around small-caliber axons, although large-caliber axons are also affected (25). We have previously shown that demyelination begins in

**FIGURE 10.** Electron micrograph of the optic nerve of a 12-month-old taiep rat: Most axons lack myelin; those that are myelinated are hypomyelinated. There is a profusion of astrocyte processes (a), as indicated by their shape and glial filaments, associated with a labeled astrocyte (A). The increase in astrocyte processes suggests loss of axons. The degree of gliosis makes interpretation of axonal ultrastructure difficult. Scale bar = 2 μm.

**FIGURE 9.** Electron micrographs of optic nerves from 2- and 6-month-old wild type (wt) and taiep rats. (A, B) Most axons within optic nerve of the 2-month-old wt rat (A) have myelin sheaths, whereas in the 2-month-old taiep rat (B), there are thinly myelinated axons and numerous unmyelinated axons. There are degenerating axons (arrows) in the taiep rat at this age. (C, D) Virtually all axons in the optic nerve of a 6-month-old wt rat (C) are myelinated, and there are no degenerating axons. The 6-month-old taiep rat (D) has large-caliber axons with thin myelin sheaths, unmyelinated axons and degenerating axons (arrows). Scale bar = 2 μm.
the optic nerve at 1 month and that, by 2 months, there are fewer myelinated axons (16). In the present study, axonal loss in taiep optic nerves is present even at early time points (at 2 months), but structural axonal changes progress over time. This suggests that there may be an early loss of predominantly myelinated fibers that is followed by later degeneration of demyelinated fibers. It is possible that dysmyelination of axons accounts for the loss of axons seen at 2 months. Indeed, changes in CNS myelin biochemistry have been noted in taiep rats of that age; there are significant reductions in MAG, as shown by IHC in the spinal cord (18). Although MBP levels were not abnormal (as demonstrated by Western blotting in our earlier study on taiep myelin), O’Connor et al (18) found a reduction in MBP mRNA. These combined data suggest that taiep rat myelin is abnormal and that the early degeneration of myelinated axons seen in the optic nerve is related to this biochemical defect in thinly myelinated axons. Studies of MAG-null mice have shown reductions in axonal densities within optic nerves before 2 months and disruptions in axonal ultrastructure (26, 27). The progressive accumulation of non-phosphorylated neurofilament and APP (and progressive axonal spheroid formation) at later time points in structures that are totally demyelinated suggests that chronic oligodendrocyte dysfunction and demyelination put axons at increased risk for late degeneration. Although the precise genetic mutation responsible for the taiep phenotype is unknown, the underlying pathophysiological process is accumulation of microtubules within oligodendrocytes. We hypothesize that the axonal changes are secondary to oligodendrocyte and myelin abnormalities, although we cannot completely discount the possibility that the taiep mutation itself has direct axonal effects.

It has been increasingly recognized that oligodendrocytes play a major role in providing trophic support for axons. Several specific biochemical processes occurring within oligodendrocytes seem to have axonoprotective properties. For example, mice with selective inactivation of the Pex5 gene within myelinating oligodendrocytes show progressive axonal degeneration and neuroinflammation (7). Furthermore, silencing the dicer gene, which is essential for the generation of functional microRNAs, in myelinating oligodendrocytes causes neuronal impairment in mice (8). Oligodendrocytes also produce a variety of neurotrophic factors, which is essential for the generation of functional microRNAs, in myelinating oligodendrocytes causes neuronal impairment in mice (8). Oligodendrocytes also produce a variety of neurotrophic factors, although their functional significance in vivo is not known (10, 28). Specific myelin components have been implicated in the long-term survival of axon populations. For example, mice lacking $2',3'$-cyclic nucleotide phosphodiesterase, a relatively minor component of myelin, have morphologically normal myelin, but show delayed axonal swelling and degeneration (13). Shiverer mice contain a deletion in the MBP gene, leading to the complete absence of compact CNS myelin and a phenotype of tremor and seizures with decreased life span. Central nervous system axonal changes within these animals include an increase in slow axonal transport, and microtubule density and number, and altered neurofilament assembly and phosphorylation (14, 29, 30). Mice lacking the Plp gene show late-onset axonal degeneration and swelling (11). Nonmyelinated axons within optic nerves from female Plp-null heterozygotes (which show mosaicism for Plp expression and patches of normally myelinated and non-myelinated axons) do not develop axonal swellings over the expected time course. This suggests that axons may become dependent on oligodendrocyte support after myelination has been completed. These findings imply a role for PLP in maintaining axons that have become oligodendrocyte-dependent, although the signal triggering this putative dependency remains unknown. Although myelin mutant studies demonstrate important protective properties of myelin, axons in these models are ensheathed by myelin lacking specific molecules and thus may have different survival requirements. Previous studies have also shown that experimental autoimmune encephalomyelitis and toxin-induced demyelination may be associated with axonal injury (31, 32). However, the extent to which toxins or the inflammatory milieu in these models contributes to primary axonal injury cannot be fully determined. The functional importance of remyelination with respect to axonal preservation is highlighted in the cuprizone-induced model of demyelination in which remyelination protects axons from further degeneration (33).

Diseases that are thought to be predominantly myelin disorders, such as MS, are usually accompanied by CNS axonal degeneration (1). Although inflammatory mediators induce axonal injury, there is increasing evidence to suggest that chronic axonal dropout may occur at later stages of MS in the absence of acute inflammation (34). The mechanisms of such processes are not fully understood, but lack of trophic support from surrounding myelin and glia may play an important role. Dephosphorylated neurofilaments within axons, APP accumulation, and spheroid formation are found in MS at different disease stages (1, 3). In early stages of MS, axons in acute lesions may be acutely transected; the extent of axonal injury may correlate with the degree of inflammation (specifically, CD8$^+$ and microglial numbers) (35). In contrast, chronic MS lesions are characterized by a lack of inflammation, demyelination, and a paucity of axons, suggesting that axonal attrition may occur in these lesions despite little or no further inflammation (1). Furthermore, epidemiological data have revealed a striking uniformity of disease course during progressive phases of the MS, which seems independent of inflammation once a certain threshold of disability has been reached (36, 37). Thus, noninflammatory axon degeneration related to chronic oligodendrocyte dysfunction or demyelination may be the pathologic substrate that contributes to disability by patients with secondary progressive MS. The current study highlights the need for effective protection of axons early in the disease process and implies that restoration of normal oligodendrocyte function not only may improve impulse conduction along axons but also may be axonoprotective.

REFERENCES
