

## PREMYELINATING OLIGODENDROCYTES IN CHRONIC LESIONS OF MULTIPLE SCLEROSIS

ANSI CHANG, M.D., WALLACE W. TOURTELLOTTE, M.D., PH.D., RICHARD RUDICK, M.D., AND BRUCE D. TRAPP, PH.D.

**ABSTRACT**

**Background** Multiple sclerosis is an inflammatory disease of the central nervous system that destroys myelin, oligodendrocytes, and axons. Since most of the lesions of multiple sclerosis are not remyelinated, enhancement of remyelination is a possible therapeutic strategy that could perhaps be achieved with the transplantation of oligodendrocyte-producing cells into the lesions. We investigated the frequency distribution and configuration of oligodendrocytes in chronic lesions of multiple sclerosis to determine whether these factors limit remyelination.

**Methods** Forty-eight chronic lesions obtained at autopsy from 10 patients with multiple sclerosis were examined immunocytochemically for oligodendrocytes and oligodendrocyte progenitor cells. Using confocal microscopy, we examined the three-dimensional relations between axons and the processes of premyelinating oligodendrocytes.

**Results** Thirty-four of the 48 chronic lesions of multiple sclerosis contained oligodendrocytes with multiple extended processes that associated with demyelinated axons but failed to myelinate them. These axons were dystrophic and contained multiple swellings. In some regions, the densities of premyelinating oligodendrocytes (25 per square millimeter of tissue) were similar to those in the developing rodent brain (23 per square millimeter). In the patients with disease of long duration (more than 20 years), there were fewer lesions with premyelinating oligodendrocytes ( $P < 0.001$ ).

**Conclusions** Premyelinating oligodendrocytes are present in chronic lesions of multiple sclerosis, so remyelination is not limited by an absence of oligodendrocyte progenitors or their failure to generate oligodendrocytes. Our findings suggest that in the chronic lesions of multiple sclerosis, the axons are not receptive for remyelination. Understanding the cellular interactions between premyelinating oligodendrocytes, axons, and the microenvironment of lesions of multiple sclerosis may lead to effective strategies for enhancing remyelination. (N Engl J Med 2002;346:165-73.)

Copyright © 2002 Massachusetts Medical Society.

**M**ULTIPLE sclerosis is an inflammatory disease of the central nervous system that destroys myelin, the insulation that surrounds axons. Oligodendrocytes (the cells that produce myelin) and nerve fibers are also destroyed.<sup>1-4</sup> Most patients with multiple sclerosis have an initial relapsing–remitting course for 5 to 15 years that then takes a secondary progressive course of irreversible neurologic disability.<sup>5</sup> Relapses result from inflammation and demyelination, whereas restoration of nerve conduction and remission is accompanied by resolution of inflammation, redistribution of sodium channels on demyelinated axons, and remyelination.<sup>6,7</sup>

Demyelination is not always permanent in multiple sclerosis. Remyelination during early stages of the disease process has been documented by histologic analysis of tissue specimens from both biopsy and postmortem examination.<sup>8-11</sup> Most chronic lesions of multiple sclerosis, however, are not remyelinated. Remyelination requires generation of new oligodendrocytes.<sup>12</sup> Oligodendrocyte progenitor cells, identified by the expression of the platelet-derived growth factor receptor  $\alpha$  and the sulfated proteoglycan NG2, have been characterized in developing brain,<sup>13,14</sup> normal adult human brain,<sup>15-17</sup> and chronic lesions of multiple sclerosis.<sup>16,18,19</sup> Isolated progenitor cells can give rise to oligodendrocytes in vitro.<sup>20-23</sup> During development of the rodent brain, oligodendrocyte progenitor cells differentiate into premyelinating oligodendrocytes that radially extend multiple processes positive for myelin proteins that do not immediately myelinate axons.<sup>24</sup> These premyelinating oligodendrocytes have a limited life span (approximately three days) and either myelinate axons or die by programmed cell death.<sup>24,25</sup> Premyelinating oligodendrocytes are not detected in white matter in the brains of normal adult rodents or humans.

Transplantation of oligodendrocyte-producing cells into lesions of multiple sclerosis is being considered as a therapeutic strategy to enhance remyelination.<sup>26</sup> It remains to be determined, however, whether oli-

From the Department of Neurosciences, Lerner Research Institute (A.C., B.D.T.), and the Mellen Center for Multiple Sclerosis (R.R.), Cleveland Clinic Foundation, Cleveland; and the Department of Neurology, West Los Angeles Veterans Affairs Medical Center, Los Angeles (W.W.T.). Address reprint requests to Dr. Trapp at the Department of Neurosciences, NC30, Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195, or at trappb@ccf.org.

godendrocyte progenitors or production or differentiation of oligodendrocytes limits remyelination in chronic lesions of multiple sclerosis. Despite detection of cells with phenotypic characteristics of oligodendrocyte progenitor cells in chronic lesions of multiple sclerosis,<sup>16,18,19</sup> the potential for these cells to produce oligodendrocytes has not been demonstrated. This report describes oligodendrocytes in chronic lesions of multiple sclerosis that extend multiple processes that associate with but fail to myelinate axons.

## METHODS

### Tissue

The brains from 10 deceased patients with multiple sclerosis were investigated. Six brains were obtained from patients who had been followed at the Cleveland Clinic Foundation. These brains were sliced (1 cm thick) and fixed in 4 percent paraformaldehyde. Lesions were removed, cryoprotected, and sectioned (30  $\mu$ m thick) on a freezing-sliding microtome. Fresh-frozen brain slices from four other deceased patients with multiple sclerosis were obtained from the Multiple Sclerosis Human Neurospecimen Bank in Los Angeles and were simultaneously thawed and fixed in 4 percent paraformaldehyde and processed as described above. Clinical data for the patients are summarized in Table 1.

Patient 1 died from respiratory failure accompanying severe brainstem inflammation in the setting of relapsing multiple sclerosis. All the other patients died of medical complications from severe, debilitating multiple sclerosis, as listed in Table 1. Two patients received disease-modifying therapy in the year before death. Patient 1

was treated with cyclophosphamide and methylprednisolone one month before death; Patient 7 was receiving interferon beta treatment at the time of death. On the basis of the distribution of myelin protein and the staining for major-histocompatibility-complex (MHC) class II molecules, 52 lesions were identified and classified as active (4), chronic active (5), and chronic inactive (43), as described previously.<sup>4</sup>

### Immunocytochemical Analysis

Free-floating sections (30  $\mu$ m thick) were microwaved in 10 mM citric acid buffer (pH 6.0) twice for 5 minutes, incubated in 1 percent hydrogen peroxide and 10 percent Triton X-100 in phosphate-buffered saline for 30 minutes, and immunostained by the avidin-biotin complex procedure with diaminobenzidine, as described previously.<sup>24</sup> Sections were incubated in proteolipid-protein antibodies for five days at 4°C. Sections stained for NG2 were not microwaved; they were pretreated with 0.3 percent Triton X-100 and immunostained with the use of the tyramide signal-amplification method (PerkinElmer Life Sciences, Boston), as previously described.<sup>16</sup> Sections for double labeling were pretreated as above and incubated with two primary antibodies for five days and with fluorescein-conjugated and biotinylated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, Pa.) for one hour.

### Antibodies

Sections were immunostained with the following antibodies: rat anti-proteolipid protein (Agmed, Bedford, Mass.), mouse anti-myelin oligodendrocyte glycoprotein (a gift from Dr. Minnetta Gardiner, University of Iowa, Iowa City), mouse anti-human NG2 (clone 9.2.27, Pharmingen, San Diego, Calif.), mouse anti-human MHC class II (Dako, Glostrup, Denmark), mouse anti-nonphos-

TABLE 1. CHARACTERISTICS OF PATIENTS AND LESIONS STUDIED.

PATIENT No.	AGE (YR)/SEX	TYPE OF MULTIPLE SCLEROSIS	DURATION OF DISEASE	EDSS SCORE*	CAUSE OF DEATH	No. OF LESIONS ANALYZED	No. OF LESIONS WITH PREMYELINATING OLIGODENDROCYTES
1	43/M	Relapsing-remitting	8 mo	6.0	Respiratory failure	3	3
2	60/M	Primary progressive	3 yr	7.5	Urosepsis	3	3
3	60/M	Secondary progressive	9 yr	8.0	Pneumonia	2	2
4	59/M	Secondary progressive	14 yr	8.5	Pneumonia	2	2
5	43/F	Secondary progressive	15 yr	8.0	Cardiac arrest	4	3
6	54/M	Secondary progressive	15 yr	9.5	Pneumonia	14	13
7	57/F	Primary progressive	15 yr	6.5	Pulmonary embolus	3	2
8	45/M	Secondary progressive	23 yr	8.0	Urosepsis	7	4
9	61/F	Secondary progressive	35 yr	9.5	Pneumonia	7	1
10	69/F	Secondary progressive	44 yr	9.5	Urosepsis	3	1
Total						48	34

\*EDSS denotes Expanded Disability Status Scale (possible range, 1 to 10, with a higher score indicating a greater degree of disability).

phorylated neurofilament (SMI32, Sternberger Monoclonals, Baltimore), mouse anti-phosphorylated neurofilament (SMI31, Sternberger Monoclonals), and rabbit antineurofilament (Serotec, Raleigh, N.C.).

### Confocal Microscopy

Sections were examined with a laser scanning confocal microscope (Leica Microsystems, Exton, Pa.). Antibody combinations included proteolipid protein plus neurofilament and myelin oligodendrocyte glycoprotein plus neurofilament. Laser intensity was adjusted to eliminate "bleed-through." The images presented are stacks of 18 to 36 optical sections that were scanned synchronously.

### Quantification of Premyelinating Oligodendrocytes

Premyelinating oligodendrocytes in lesions of multiple sclerosis were identified as cells positive for proteolipid protein with multiple processes that did not contact myelin internodes. To determine the size of the demyelinated area, sections stained with proteolipid protein antibodies were scanned (ScanMaker 4, Microtek Lab, Redondo Beach, Calif.); the borders of the lesions were outlined on a digital image, and the demyelinated area was measured with software from the National Institutes of Health (NIH Image). The density of premyelinating oligodendrocytes was determined in the total demyelinated area in 34 lesions, in 30 lesions enriched in premyelinating oligodendrocytes, and in developing rat brain (postnatal day 14). Areas of lesions of multiple sclerosis containing more than six premyelinating oligodendrocytes per 0.38 mm<sup>2</sup> were calculated separately and classified as enriched in premyelinating oligodendrocytes; this density was chosen because it is similar to that found in areas of the developing rodent brain. The density of premyelinating oligodendrocytes in developing rat brain was determined by counting 15 regions of unmyelinated cerebral cortex. Densities of premyelinating oligodendrocytes were compared by Student's *t*-test. The relation between the percentage of lesions with premyelinating oligodendrocytes and the duration of disease, the age of the patient at the time of death, the type of disease, and the score on the Expanded Disability Status Scale (EDSS; possible range, 0 to 10, with a higher score indicating a greater degree of disability)<sup>27</sup> was determined by the Pearson correlation coefficient.

## RESULTS

### Premyelinating Oligodendrocytes in Chronic Lesions of Multiple Sclerosis

The clinical history and number of lesions studied from each patient are shown in Table 1. Forty-eight chronic lesions of multiple sclerosis from 10 deceased patients with multiple sclerosis were examined for oligodendrocytes. Chronic lesions of multiple sclerosis were identified by the presence of demyelination and a low density of cells positive for MHC class II molecules (insets in Fig. 1A). Premyelinating oligodendrocytes were detected in 34 lesions (71 percent). These premyelinating oligodendrocytes were not evenly distributed within the lesions and tended to occur in groups (Fig. 1A). The general morphologic appearance of the cells varied, depending on location. The perikarya of the premyelinating oligodendrocytes were often detected in the axon-free subventricular zone (Fig. 1B and 1C). These cells asymmetrically extended multiple processes positive for proteolipid protein into the area of demyelinated axons. Most premyelinating oligodendrocytes were clustered through-

out the lesions (inset in Fig. 1D) and radially extended multiple processes positive for proteolipid protein that were oriented parallel to demyelinated axons (Fig. 1D).

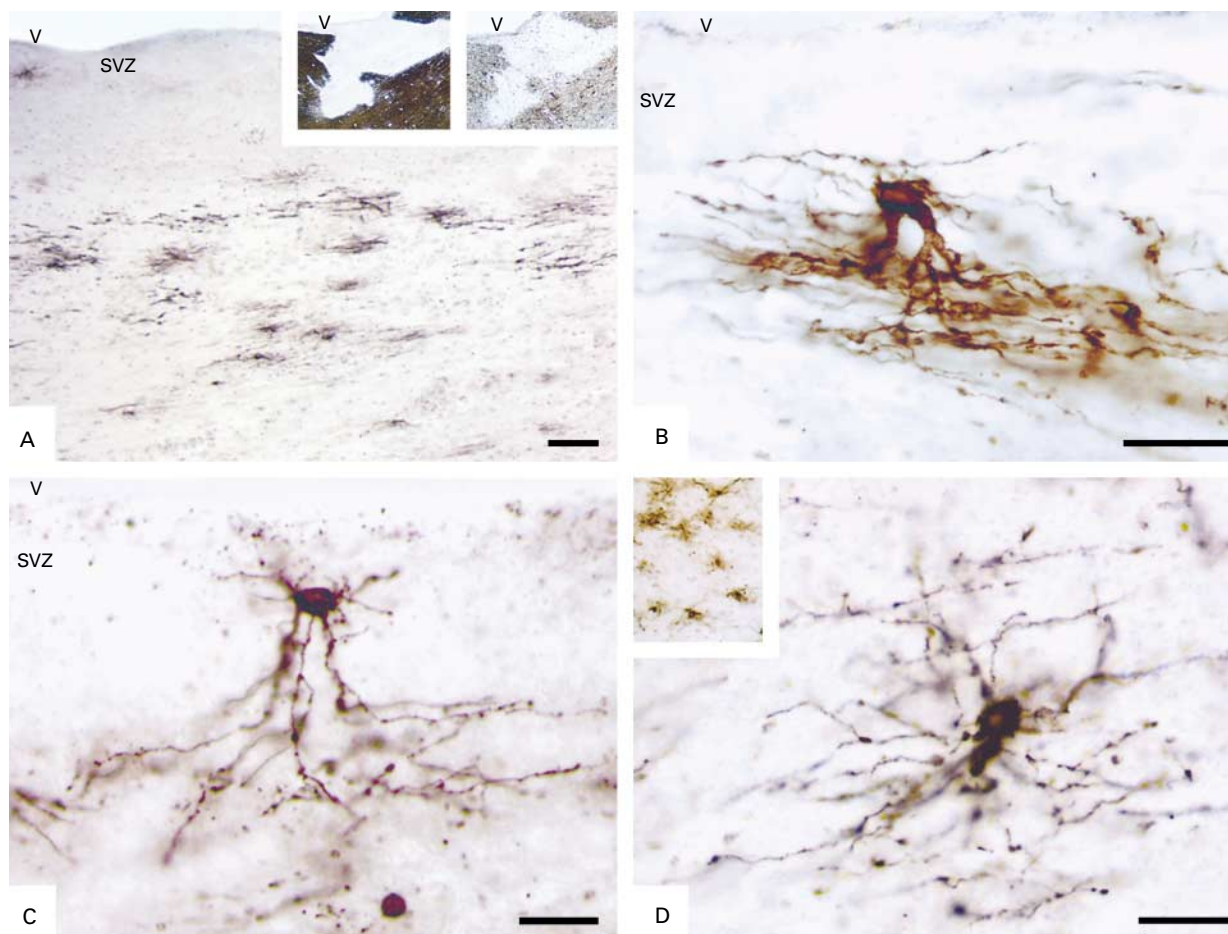
In the 34 chronic lesions with premyelinating oligodendrocytes, the average density of premyelinating oligodendrocytes was 2 per square millimeter (Fig. 2). However, in 30 lesion areas enriched in premyelinating oligodendrocytes, the density of oligodendrocytes (25 per square millimeter) was similar to that found in developing rat brain (23 per square millimeter) (Fig. 2). Thirteen of the 30 areas were located close to the subventricular zone. In the 34 lesions analyzed, premyelinating oligodendrocytes were enriched in approximately 7 percent of the total area of the lesions.

Fourteen of the 48 chronic lesions studied did not contain detectable premyelinating oligodendrocytes (Table 1). Eleven lesions without premyelinating oligodendrocytes came from the three patients with the longest duration of disease (23, 35, and 44 years), whereas only 1 of 14 lesions from Patient 6 was negative for premyelinating oligodendrocytes. The percentage of lesions with premyelinating oligodendrocytes varied inversely with the duration of disease (Pearson correlation coefficient,  $-0.90$ ; 95 percent confidence interval,  $-0.62$  to  $-0.98$ ;  $P < 0.001$ ). The age of the patient at the time of death, the type of disease, and the EDSS score did not correlate with the percentage of lesions with premyelinating oligodendrocytes ( $P > 0.05$ ).

### Oligodendrocyte Progenitor Cells in Chronic Lesions of Multiple Sclerosis

Cells expressing the sulfated proteoglycan NG2 may be oligodendrocyte progenitor cells in lesions of multiple sclerosis.<sup>16</sup> The distribution of NG2-positive cells was investigated in sections cut adjacent to sections stained with proteolipid protein antibodies. Subventricular areas of the lesions with premyelinating oligodendrocytes contained stellate NG2 cells (Fig. 3A) that had an appearance similar to that of NG2 cells in brains from deceased patients without neurologic disease. As in these brains, many of the NG2-positive cells projected processes to blood vessels that were also NG2-positive. Other regions of the lesions of multiple sclerosis enriched in premyelinating oligodendrocytes contained elongated (Fig. 3A) or stellate (Fig. 3B) NG2 cells.

Stellate NG2 cells were detected in lesions without premyelinating oligodendrocytes. The density of these cells, however, was less than that in nonlesion areas of the same sections. In addition, these NG2 cells had fewer, shorter, and thicker processes than those in nonlesion areas (Fig. 3C). Elongated NG2 cells were not detected in lesions without premyelinating oligodendrocytes.



**Figure 1.** Premyelinating Oligodendrocytes in Chronic Lesions of Multiple Sclerosis.

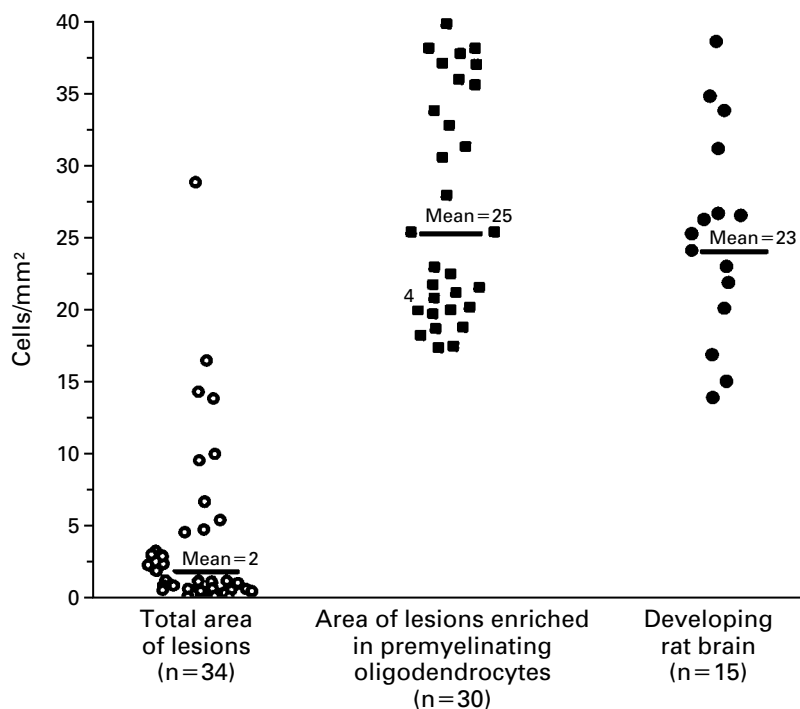
Chronic demyelinated lesions of multiple sclerosis were identified by a lack of myelin (left inset in Panel A) and a low density of cells positive for MHC class II molecules within the lesion (right inset in Panel A). Premyelinating oligodendrocytes were often detected in clusters (Panel A) just beneath the subventricular zone (SVZ). Some perikarya of premyelinating oligodendrocytes were located within the axon-free subventricular zone (Panels B and C) and extended processes that were oriented parallel to the demyelinated axons. Clusters of premyelinating oligodendrocytes were detected within lesions (inset in Panel D). These cells radially extended multiple processes that were oriented parallel to demyelinated axons (Panel D). All premyelinating oligodendrocytes were stained with proteolipid-protein antibodies. V denotes ventricle. The scale bar in Panel A represents 100  $\mu\text{m}$ ; the scale bars in Panels B, C, and D represent 30  $\mu\text{m}$ .

#### Processes of Premyelinating Oligodendrocytes Associated with Axons

The orientation of processes of premyelinating oligodendrocytes (Fig. 1) suggests that they physically associate with demyelinated axons. This possibility was investigated by determining the three-dimensional relation between processes of premyelinating oligodendrocytes and axons in confocal images of sections immunostained with proteolipid protein and neurofilament antibodies. Figure 4A shows a premyelinating oligodendrocyte located in the axon-free subventric-

ular zone. This cell extended processes positive for proteolipid protein into the zone of demyelinated axons. When these processes reached the demyelinated axons, many assumed a parallel orientation with individual axons (Fig. 4A). Three-dimensional analysis of rotated images indicated that many processes longitudinally spiraled around axons (Fig. 4B). Radial ensheathment of axons, however, was rare.

During normal development of the rodent brain, myelin oligodendrocyte glycoprotein is considered a marker of mature or myelinating oligodendrocytes<sup>28</sup>



**Figure 2.** Scatter Plot of the Density of Premyelinating Oligodendrocytes in the Total Areas of Chronic Lesions, in Lesion Areas Enriched in Premyelinating Oligodendrocytes, and in Rat Cerebral Cortex (Postnatal Day 14).

The density of premyelinating oligodendrocytes was significantly enriched in approximately 7 percent of the total area of chronic lesions ( $P < 0.001$ ). These areas contained densities of premyelinating oligodendrocytes similar to those in developing rat brain.

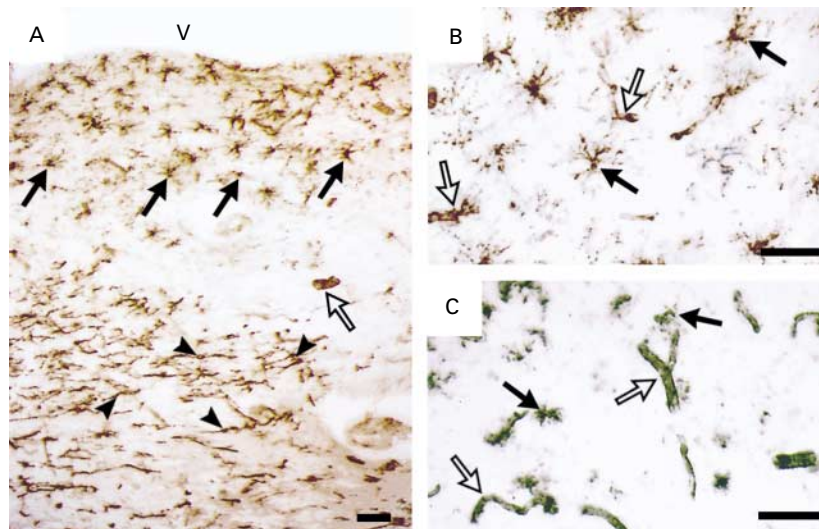
and has not been detected in premyelinating oligodendrocytes (unpublished data). Many premyelinating oligodendrocytes in chronic lesions of multiple sclerosis, however, were stained by myelin oligodendrocyte glycoprotein antibodies (Fig. 4C). Perikarya of premyelinating oligodendrocytes located within lesions radially extended processes that also associated with individual axons (Fig. 4D). Some processes extended considerable distances before associating with axons, and many axons close to oligodendrocyte-cell bodies were not ensheathed by oligodendrocyte processes. Dying premyelinating oligodendrocytes, which were characterized by fragmented processes, condensed perinuclear cytoplasm, and nuclei stained for proteolipid protein (inset in Fig. 4D), were also detected in chronic lesions of multiple sclerosis.

#### Oligodendrocytes in Remyelinating Lesions

Previous studies identified abundant oligodendrocytes in some subacute lesions of multiple sclerosis.<sup>11,29</sup> These oligodendrocytes extended a few short pro-

cesses, but they did not myelinate axons. Four acute lesions with abundant macrophages that were positive for MHC class II molecules contained regions with abundant, small, round oligodendrocytes with few or no processes (data not shown). Premyelinating oligodendrocytes with multiple radially oriented processes were not detected in these acute lesions. Remyelination was evident, however, at the edge of many chronic lesions. The relation between remyelinating oligodendrocytes and axons was investigated by confocal microscopy in these partially remyelinated lesions or “shadow plaques.”

Remyelinating oligodendrocytes had relatively large perikarya positive for proteolipid protein and extended processes to short myelin internodes (Fig. 5A). The number of processes was often matched to the number of internodes, particularly in remyelinating oligodendrocytes that formed longer internodes (Fig. 5B). In areas of shadow plaque with more remyelination and longer internodes, oligodendrocyte perikarya were smaller and less intensely stained by proteolip-



**Figure 3.** Oligodendrocyte Progenitor Cells in Chronic Lesions of Multiple Sclerosis.

Stellate NG2-positive cells lined the ventricle (area above solid arrows in Panel A) of chronic lesions of multiple sclerosis. Deeper within the lesion, elongated (arrowheads in Panel A) or stellate (solid arrows in Panel B) NG2-positive cells were present. In lesions without premyelinating oligodendrocytes, stellate NG2-positive cells (solid arrows in Panel C) were detected at a lower density than lesions with premyelinating oligodendrocytes, and many had shorter and fewer processes. NG2 antibodies also stained blood vessels in the central nervous system (open arrows in Panels A, B, and C). V denotes ventricle; the scale bars represent 100  $\mu\text{m}$ .

id-protein antibodies (Fig. 5C) than in premyelinating oligodendrocytes (Fig. 1B, 1C, 1D, and 4A) or in early remyelinating oligodendrocytes (Fig. 5A and 5B). Oligodendrocyte processes extending to myelin internodes were not always detected within shadow plaques. Premyelinating oligodendrocytes were not detected in shadow plaques of the chronic lesions of multiple sclerosis that we analyzed.

The morphologic appearance of axons in chronic lesions containing premyelinating oligodendrocytes and remyelinating oligodendrocytes differed. Chronic lesions with premyelinating oligodendrocytes contained axons (Fig. 4A, 4B, and 4C) with multiple swellings, marked variations in diameter, and a more tortuous course. In contrast, remyelinated axons (Fig. 5B and 5C) were straight, with relatively consistent diameters. The edge of shadow plaques often contained a combination of dystrophic and healthy-appearing axons (Fig. 5A). In general, the majority of remyelinated internodes surrounded the healthy-appearing axons.

#### DISCUSSION

In the central nervous system of patients with multiple sclerosis, remyelination may be able to restore

rapid nerve conduction and protect demyelinated axons from degeneration. To develop strategies for successful remyelination, one needs to identify why remyelination fails. We detected premyelinating oligodendrocytes in 34 of 48 chronic lesions of multiple sclerosis, which establishes that some other factors limit remyelination of such lesions. The premyelinating oligodendrocytes physically associated with axons and were abundant in patients with clinical disease of 1 to 15 years' duration. This provides an extended window of opportunity to deliver remyelinating therapeutic agents that target premyelinating oligodendrocytes, the microenvironment of chronic lesions of multiple sclerosis, or both.

The differentiation of oligodendrocytes in chronic lesions of multiple sclerosis appears to recapitulate the premyelinating stage identified during the development of the rodent brain.<sup>24</sup> The positive correlation between the distribution of NG2 cells and premyelinating oligodendrocytes suggests that NG2 cells are a source of these premyelinating oligodendrocytes. It remains to be determined whether premyelinating oligodendrocytes are generated from a stem or progenitor cell that repopulates the lesion or from the NG2 cells in or near the lesion. In either event,

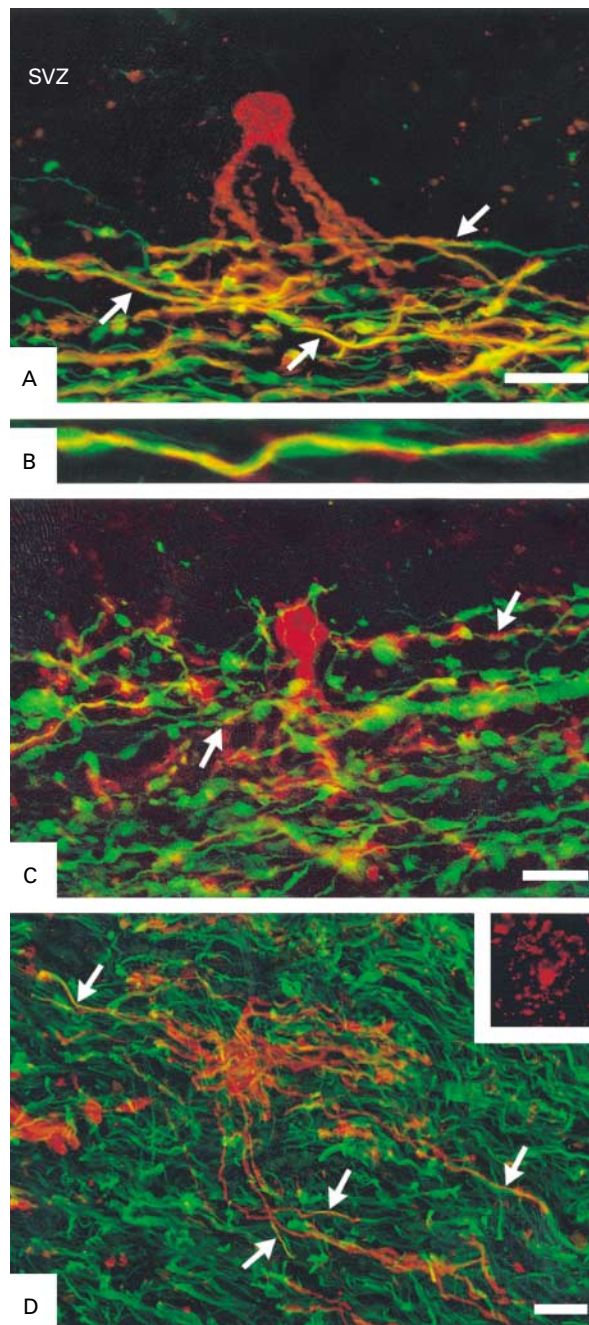
the detection of premyelinating oligodendrocytes unequivocally establishes the presence of oligodendrocyte progenitor cells in chronic lesions of multiple sclerosis. All patients examined had lesions with premyelinating oligodendrocytes (Table 1). It remains to be determined, however, whether proposed variations in the pathogenesis of multiple sclerosis<sup>30</sup> will affect the number of premyelinating oligodendrocytes in chronic lesions. Fourteen chronic lesions were negative for premyelinating oligodendrocytes. Eleven of these lesions were from patients with disease of more than 20 years' duration, suggesting that chronic lesions eventually lose the ability to maintain or produce new oligodendrocytes.

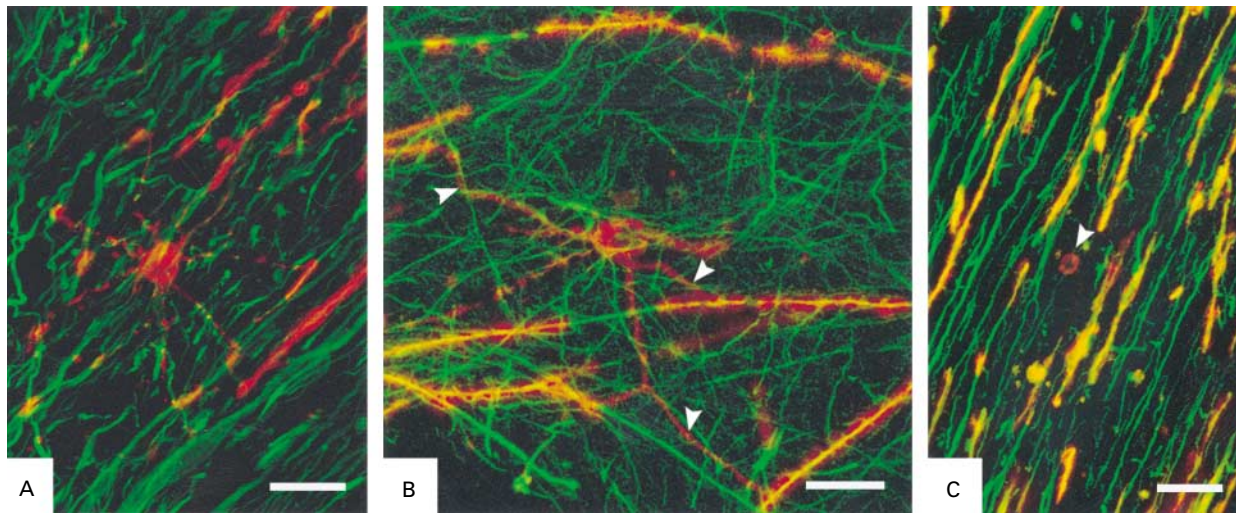
We confirmed previous reports<sup>11,31-33</sup> describing abundant, small, round oligodendrocytes that were positive for myelin oligodendrocyte glycoprotein in acute lesions of multiple sclerosis. Although premyelinating oligodendrocytes were not detected immunocytochemically in previous studies, cells positive for proteolipid protein messenger RNA were detected by in situ hybridization in tissue sections of chronic lesions of multiple sclerosis<sup>11,31,32</sup> and are likely to represent, at least in part, the premyelinating oligodendrocytes identified in the present report. Optimal immunocytochemical detection of premyelinating oligodendrocytes in developing brain<sup>24</sup> or lesions of multiple sclerosis (Fig. 1, 4, and 5) requires microwave pretreatment and five days of incubation with primary antibody. In addition, resolution of elaborate processes, the morphologic hallmark of premyelinating oligodendrocytes, is best achieved in sections of 30  $\mu\text{m}$  or thicker. As in other studies, we have not detected premyelinating oligodendrocytes with more conventional staining procedures.

**Figure 4.** Processes of Premyelinating Oligodendrocytes Associated with Axons.

Confocal micrographs of lesions of multiple sclerosis stained with proteolipid protein antibodies (red in Panels A, B, and D), myelin oligodendrocyte glycoprotein antibodies (red in Panel C), and neurofilament antibodies (green in Panels A, B, C, and D) are shown. A premyelinating oligodendrocyte (red in Panel A) in the subventricular zone (SVZ) extended processes into the region of demyelinated axons (green in Panel A) in a chronic lesion of multiple sclerosis. Many of these processes (arrows in Panel A) spiraled around axons, as shown at higher magnification (Panel B). Some premyelinating oligodendrocytes in chronic lesions of multiple sclerosis were also positive for myelin oligodendrocyte glycoprotein and extended processes that associated with axons (arrows in Panel C). Premyelinating oligodendrocytes within the lesion (red in Panel D) radially extended processes that also contacted and longitudinally associated (arrows in Panel D) with axons (green in Panel D). Dying premyelinating oligodendrocytes had fragmented processes that were positive for proteolipid protein and condensed perinuclear cytoplasm (inset in Panel D). The scale bars represent 20  $\mu\text{m}$ .

The remyelinating potential of premyelinating oligodendrocytes in chronic lesions of multiple sclerosis will depend on the life span of the cells. Although it is impossible to determine the life spans of cells in the human brain, the detection of apoptotic premyelinating oligodendrocytes in chronic lesions of multiple sclerosis (inset in Fig. 4D) indicates that their





**Figure 5.** Remyelinating Oligodendrocytes in Chronic Lesions of Multiple Sclerosis.

Remyelinating oligodendrocytes at the edge of shadow plaques (red areas in Panel A) extended processes to very short myelin internodes. Many unmyelinated axons (green areas in Panel A) appeared dystrophic. As shown in Panel B, in demyelinated regions of brain that contained straight, healthy-appearing axons (green), oligodendrocytes (red) extended processes (arrowheads) to myelin internodes. Within shadow plaques, many but not all axons were ensheathed by myelin internodes (red in Panel C). Occasional perikarya of oligodendrocytes were positive for proteolipid protein (arrowhead in Panel C), but oligodendrocyte processes that were positive for proteolipid protein were rarely detected. Axons within shadow plaques were straight and of a consistent diameter (green in Panel C). The scale bars represent 20  $\mu\text{m}$ .

life spans are limited. If the life span is two to three days, as reported for the developing rodent central nervous system,<sup>25,34</sup> the total number of oligodendrocytes produced in chronic lesions that are years or decades old should be more than enough to remyelinate the lesions. Rodent premyelinating oligodendrocytes that are produced in the absence of axons of the optic nerve extend fewer and shorter processes than those in normal nerves<sup>35</sup> or in chronic lesions of multiple sclerosis (Fig. 1 and 4). Axons in the chronic lesions of multiple sclerosis therefore appear capable of supporting differentiation of an oligodendrocyte to a premyelinating phenotype, to association with axons, and to expression of myelin oligodendrocyte glycoprotein, but not to myelination.

Little is known about the molecular mechanisms responsible for the initiation of myelination or whether the inhibition of myelination in lesions of multiple sclerosis is due to dysregulated growth factors, the altered molecular composition of axons, or the presence of an inhibitory signal. The correlation between dystrophic axons with swellings and lesions with premyelinating oligodendrocytes but no remyelination supports the hypothesis that axonal pathologic processes limit the remyelination of chronic lesions of multiple

sclerosis. This hypothesis is also supported by data in animals<sup>36-38</sup> and the morphologic integrity of remyelinated axons in shadow plaques described here and in previous studies.<sup>37</sup> At present, there is no evidence of genetic defects specific for oligodendrocytes that would limit remyelination in patients with multiple sclerosis.

With regard to possible remyelination therapies, an important question is whether transplantation of the appropriate cell into lesions of multiple sclerosis would promote remyelination. Our data indicate that the environment of many chronic inactive lesions of multiple sclerosis supports the production of new premyelinating oligodendrocytes from endogenous cell populations and suggest that it would support such production from transplanted progenitor cells. However, it appears that the environment within chronic lesions will not provide the appropriate signals for remyelination. We speculate that failure of remyelination by premyelinating oligodendrocytes in chronic lesions of multiple sclerosis is due to an abnormal molecular composition of chronically demyelinated axons or an imbalance of growth factors that regulate myelination. The challenge is therefore to understand the interactions between premyelinating cells, axons,

and the microenvironment of the lesions better. If lesions of multiple sclerosis can be modified to promote the myelination of axons by processes of premyelinating oligodendrocytes, cell transplantation into chronic lesions could prove beneficial.

Supported by grants (PO1 NS38667 and RO1 NS35058) from the National Institutes of Health.

*We are indebted to the Cleveland Clinic Foundation Multiple Sclerosis Tissue Donation Program and the Multiple Sclerosis Human Neurospecimen Bank for providing tissue specimens; to Drs. Richard Ransohoff, Jeff Cohen, and Robert Miller for helpful comments; to Dr. Grahame Kidd for confocal-image analysis; to Renata Klinkosz for technical help; and to Victoria Pickett for editorial assistance.*

## REFERENCES

- Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N Engl J Med* 2000;343:938-52.
- Raine CS. Multiple sclerosis: immune system molecule expression in the central nervous system. *J Neuropathol Exp Neurol* 1994;53:328-37.
- Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997;120:393-9.
- Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998;338:278-85.
- Weinshenker BG, Bass B, Rice GP, et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. *Brain* 1989;112:133-46.
- Waxman SG. Demyelinating diseases — new pathological insights, new therapeutic targets. *N Engl J Med* 1998;338:323-5.
- Trapp BD, Ransohoff RM, Fisher E, Rudick RA. Neurodegeneration in multiple sclerosis: relationship to neurological disability. *Neuroscientist* 1999;5:48-57.
- Prineas JW, Connell F. Remyelination in multiple sclerosis. *Ann Neurol* 1979;5:22-31.
- Prineas JW, Kwon EE, Cho ES, Sharer LR. Continual breakdown and regeneration of myelin in progressive multiple sclerosis plaques. *Ann N Y Acad Sci* 1984;436:11-32.
- Raine CS, Wu E. Multiple sclerosis: remyelination in acute lesions. *J Neuropathol Exp Neurol* 1993;52:199-204.
- Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions: a study of 113 cases. *Brain* 1999;122:2279-95.
- Keirstead HS, Blakemore WF. Identification of post-mitotic oligodendrocytes incapable of remyelination within the demyelinated adult spinal cord. *J Neuropathol Exp Neurol* 1997;56:1191-201.
- Nishiyama A, Lin XH, Giese N, Heldin CH, Stallcup WB. Co-localization of NG2 proteoglycan and PDGF  $\alpha$ -receptor on O2A progenitor cells in the developing rat brain. *J Neurosci Res* 1996;43:299-314.
- Idem*. Interaction between NG2 proteoglycan and PDGF  $\alpha$ -receptor on O2A progenitor cells is required for optimal response to PDGF. *J Neurosci Res* 1996;43:315-30.
- Armstrong RC, Dorn HH, Kuffa CV, Friedman E, Dubois-Dalq ME. Pre-oligodendrocytes from adult human CNS. *J Neurosci* 1992;12:1538-47.
- Chang A, Nishiyama A, Peterson J, Prineas J, Trapp BD. NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. *J Neurosci* 2000;20:6404-12.
- Gogate N, Verma L, Zhou JM, et al. Plasticity in the adult human oligodendrocyte lineage. *J Neurosci* 1994;14:4571-87.
- Scolding N, Franklin R, Stevens S, Heldin C-H, Compston A, Newcombe J. Oligodendrocyte progenitors are present in the normal adult human CNS and in the lesions of multiple sclerosis. *Brain* 1998;121:2221-8.
- Wolswijk G. Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. *J Neurosci* 1998;18:601-9.
- Raff MC, Miller RH, Noble M. A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on culture medium. *Nature* 1983;303:390-6.
- Ffrench-Constant C, Raff MC. Proliferating bipotential glial progenitor cells in adult rat optic nerve. *Nature* 1986;319:499-502.
- Wolswijk G, Noble M. Identification of an adult-specific glial progenitor cell. *Development* 1989;105:387-400.
- Shi J, Marinovich A, Barres BA. Purification and characterization of adult oligodendrocyte precursor cells from the rat optic nerve. *J Neurosci* 1998;18:4627-36.
- Trapp BD, Nishiyama A, Cheng D, Macklin W. Differentiation and death of premyelinating oligodendrocytes in developing rodent brain. *J Cell Biol* 1997;137:459-68.
- Barres BA, Hart IK, Coles HSR, et al. Cell death and control of cell survival in the oligodendrocyte lineage. *Cell* 1992;70:31-46.
- Duncan ID, Grever WE, Zhang S-C. Repair of myelin disease: strategies and progress in animal models. *Mol Med Today* 1997;3:554-61.
- Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an Expanded Disability Status Scale (EDSS). *Neurology* 1983;33:1444-52.
- Pfeiffer SE, Warrington AE, Bansal R. The oligodendrocyte and its many cellular processes. *Trends Cell Biol* 1993;3:191-7.
- Prineas JW, Kwon EE, Goldenberg PZ, et al. Multiple sclerosis: oligodendrocyte proliferation and differentiation in fresh lesions. *Lab Invest* 1989;61:489-503.
- Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 2000;47:707-17.
- Ozawa K, Suchanek G, Breitschopf H, et al. Patterns of oligodendroglia pathology in multiple sclerosis. *Brain* 1994;117:1311-22.
- Brück W, Schmied M, Suchanek G, et al. Oligodendrocytes in the early course of multiple sclerosis. *Ann Neurol* 1994;35:65-73.
- Wolswijk G. Oligodendrocyte survival, loss and birth in lesions of chronic-stage multiple sclerosis. *Brain* 2000;123:105-15.
- Barres BA, Jacobson MD, Schmid R, Sendtner M, Raff MC. Does oligodendrocyte survival depend on axons? *Curr Biol* 1993;3:489-97.
- Ueda H, Levine JM, Miller RH, Trapp BD. Rat optic nerve oligodendrocytes develop in the absence of viable retinal ganglion cell axons. *J Cell Biol* 1999;146:1365-74.
- Jeffery ND, Blakemore WF. Locomotor deficits induced by experimental spinal cord demyelination are abolished by spontaneous remyelination. *Brain* 1997;120:27-37.
- Kornek B, Storch MK, Weissert R, et al. Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. *Am J Pathol* 2000;157:267-76.
- Murray PD, McGavern DB, Sathornsumetee S, Rodriguez M. Spontaneous remyelination following extensive demyelination is associated with improved neurological function in a viral model of multiple sclerosis. *Brain* 2001;124:1403-16.

Copyright © 2002 Massachusetts Medical Society.