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Identification of Post-mitotic Oligodendrocytes Incapable of Remyelination within the Demyelinated Adult Spinal Cord

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Abstract. In order to investigate the remyelinating potential of mature oligodendrocytes in vivo, we have developed a model of demyelination in the adult rat spinal cord in which some oligodendrocytes survive demyelination. A single intraspinal injection of complement proteins plus antibodies to galactocerebroside (the major myelin sphingolipid) resulted in demyelination followed by oligodendrocyte remyelination. Remyelination was absent when the spinal cord was exposed to 40 Grays of x-irradiation prior to demyelination, a procedure that kills dividing cells. Quantitative Rip immunohistochemical analysis revealed a similar density of surviving oligodendrocytes in x-irradiated and nonirradiated lesions 3 days after demyelination. Rip and bromodeoxyuridine double immunohistochemical analysis of demyelinated lesions indicated that Rip+ oligodendrocytes did not divide as an acute response to demyelination. Oligodendrocytes were also identified by Rip immunostaining and electron microscopy at late time points (3 weeks) within x-irradiated areas of demyelination. These oligodendrocytes extended processes that engaged axons, and on occasion formed myelin membranes, but did not lay down new myelin sheaths. These studies demonstrate that (a) oligodendrocytes that survive within a region of demyelination are not induced to divide in the presence of demyelinated axons, and (b) fully-differentiated oligodendrocytes are therefore postmitotic and do not contribute to remyelination in the adult CNS.

Key Words: CNS; Demyelination; Galactocerebroside (GalC); Multiple sclerosis; Myelin sheath; Remyelination; X-irradiation.

INTRODUCTION

The potential for remyelination of the adult central nervous system (CNS) is well established (1). However, exactly what cell population gives rise to the remyelinating cells is still controversial. The identification of oligodendrocytes in early multiple sclerosis lesions, as well as in areas of active demyelination (2–5), has led to the suggestion that remyelination of MS lesions may be carried out by surviving mature oligodendrocytes (6, 7). Several studies of experimental demyelination have indicated that remyelination-competent cells are generated by mitosis (8–11). However, it remains unclear whether remyelinating oligodendrocytes can arise from dedifferentiation and/or proliferation of mature oligodendrocytes, or are generated solely from proliferation and differentiation of glial progenitor cells. Clearly, elucidation of the proliferative ability and remyelination competence of mature oligodendrocytes is central to the development of strategies to treat demyelinated pathologies.

In order to investigate (a) whether mature oligodendrocytes are induced to divide in the presence of denuded axons and (b) the remyelinating potential of differentiated oligodendrocytes, we developed a model of demyelination in the dorsal column of the adult rat spinal cord in which some oligodendrocyte cell bodies survived within the region of demyelination. Surviving oligodendrocytes were identified with Rip antibodies, which are specific for differentiated CNS oligodendrocytes (12, 13). X-irradiation was then employed to eliminate dividing cells, and to thus examine the ability of these surviving, differentiated oligodendrocytes to divide and/or remyelinate in the presence of denuded axons. Electron microscopic and immunohistochemical data indicate that such cells are postmitotic and do not contribute to remyelination.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats aged 12 weeks were used in all experiments (n = 94). Prior to surgery, animals were anesthetized with a fentanyl citrate/fluanisone combination ("Hypnorm," Janssen Pharmaceuticals).

Immunological Demyelination

After laminectomy of the first lumbar vertebra and stabilization of the spinal column, direct injection into the exposed dorsal funiculus was performed using a silicon-coated glass micropipette attached to a 10 ul Hamilton syringe mounted on a micromanipulator. Injections consisted of polyclonal anti-galactocerebroside antibody (Chemicon International Inc., Temecula, California # AB 142) at a dilution of 1:2 with 33% guinea pig complement (Harlan SeraLab # 0006) in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Control injections consisted of anti-galactocerebroside antibody only (n = 2), guinea pig complement only (n = 2), phosphate-buffered saline only (n = 2), or anti-galactocerebroside antibody plus heat-inactivated guinea pig complement (by exposure to 50° Celsius for 30 minutes; n = 2) diluted to the above concentrations with 0.1M PBS, pH 7.4. Animals received a total volume of 4 ul injected over approximately 30 seconds.

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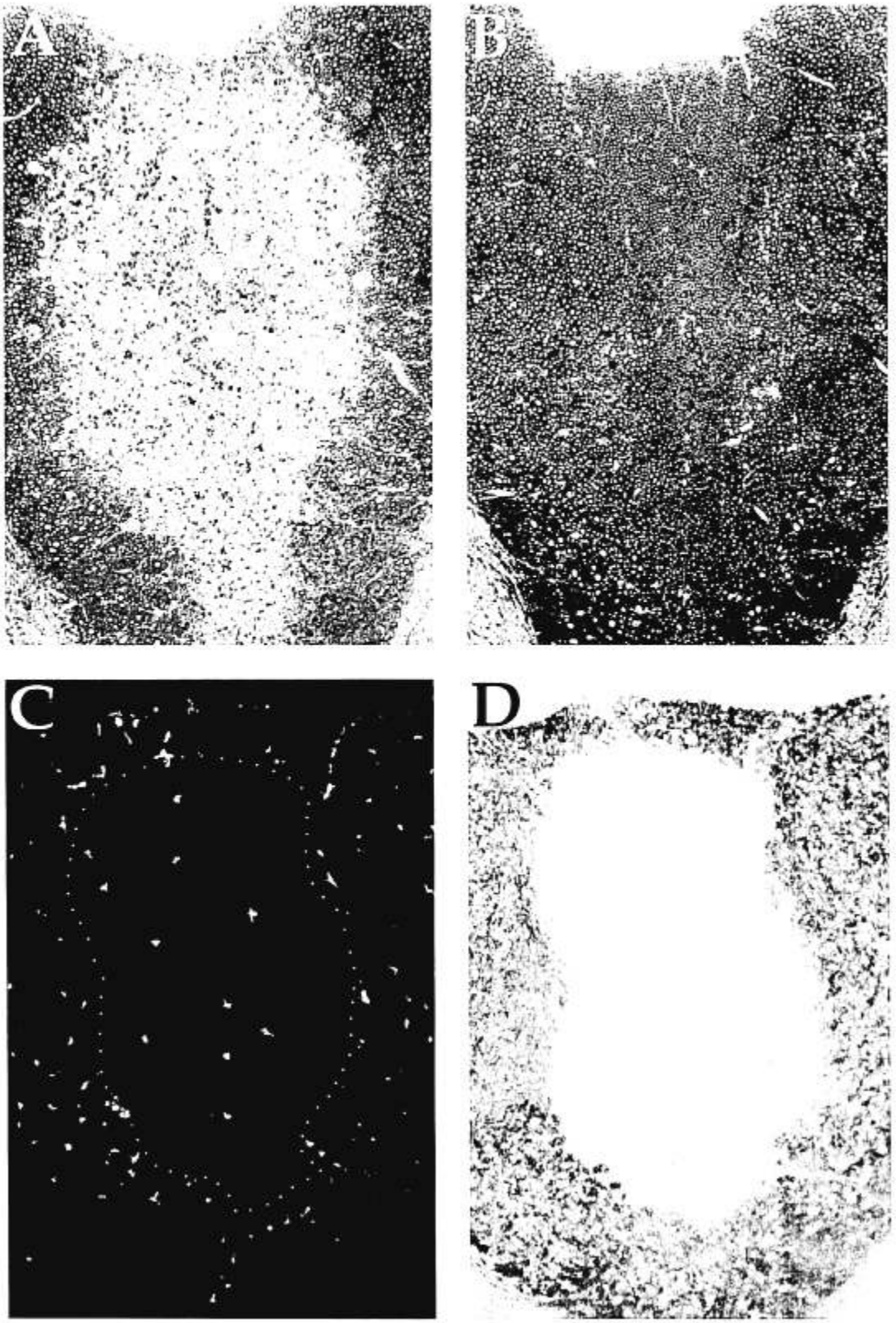

Fig. 1. Intraspinal injection of anti-GalC antibodies plus serum complement proteins results in a well-defined region of demyelination within the dorsal column of the adult rat spinal cord. A: Toluidine-blue staining of the dorsal column 7 days after the onset of demyelination; note that the area of demyelination is free from necrosis. B: Toluidine-blue staining of the dorsal column 7 days after injection of serum complement proteins alone; note that myelin is not perturbed. Demyelination was also

X-irradiation

In some experiments animals were x-irradiated 3 days prior to the onset of demyelination to kill dividing cells. The x-irradiation protocol has been described in detail elsewhere (14). Briefly, anaesthetized animals were placed in lateral recumbence and the spine between T13 and L4 was exposed to a single dose of 40 Grays of x-irradiation using a Pentak radiotherapy machine.

Toluidine Blue Staining and Electron Microscopy

Demyelinated animals were killed under pentobarbitone anesthesia at 5 hours (n = 2), 1 day (n = 3), 2 days (n = 4), 3 days (n = 3), 7 days (n = 9), 10 days (n = 4), 14 days (n = 4), 3 weeks (n = 4) and 4 weeks (n = 6) following the onset of demyelination by aortic perfusion with 4% glutaraldehyde in 0.1M phosphate buffer (PB) pH 7.4. X-irradiated plus demyelinated animals were killed at 3 days (n = 6) and 3 weeks (n = 6) following the onset of demyelination in a similar manner. The lesion-containing length of spinal cord was cut into 1 mm transverse blocks and processed so as to preserve the cranio-caudal sequence and orientation. The tissue blocks were rinsed in 0.1 M phosphate buffer pH 7.4 for 30 minutes, postfixed in 2% OsO_4, dehydrated in ascending alcohols, and embedded in TAAB resin. Thin sections (1 μm) were cut from each block, stained with alkaline Toluidine blue, and examined by light microscopy. For electron microscopic analysis, blocks were trimmed and then cut at 100 nm, mounted on copper grids, stained with uranyl acetate and lead citrate, and viewed under a Hitachi EM 600 electron microscope at 75 kV.

Histochemical Staining

Demyelinated animals were killed under pentobarbitone anesthesia at 1 day (n = 4), 3 days (n = 6), 14 days (n = 4) and 2 months (n = 2) following the onset of demyelination by aortic perfusion with 4% paraformaldehyde in 0.1 M PB pH 7.4. X-irradiated plus demyelinated animals were killed at 3 days (n = 6) and 3 weeks (n = 4) following the onset of demyelination in a similar manner. Five unoperated control animals were killed in a similar manner. The lesion-containing length of spinal cord was cut into 1-mm coronal blocks and processed so as to preserve the cranio-caudal sequence and orientation. Dissected tissue was postfixed for 24 hours, rinsed, then immersed in a 25% sucrose solution for a further 24 hours. Antigens were localized on 10-micron cryostat-sectioned tissue using standard immunohistological methodology. Four sections, each successively 50 microns apart, were analyzed for each tissue block. Standard immunohistochemical controls (e.g. omission of primary and/or secondary antibodies) were processed alongside tissue sections from experimental and control animals.

The mouse anti-Rip antibody (a gift from B. Friedman) was used at a dilution of 1:1000 in 0.5% goat serum in PB. For the purposes of accurate oligodendrocyte quantification, triton and hydrogen peroxide incubations were omitted from the Rip immunohistochemical protocol to eliminate myelin immunoreactivity. The secondary antibody was a goat anti-mouse biotinylated immunoglobulin (SeraLab) diluted 1:200 in 0.5% goat serum in PB. Rip antigens were localized with a streptavidin-TRITC complex (SeraLab) diluted 1:200 in PB. BrdU was localized using a BrdU detection kit (Amersham) with a diaminobenzidine chromagen prior to Rip immunostaining for double labeling.

After immunostaining, all sections were incubated at room temperature in Hoechst solution (1:5000 in PBS) for 10 minutes, rinsed, and coverslipped. Only those immunopositive cells with a clearly Hoechst stained nucleus were scored.

Areas of demyelination were delineated using cryostat-sectioned tissue (10 microns) incubated with solochrome cyanine stain for myelin. Serial sections to those used for immunohistochemical staining were incubated at room temperature in a solochrome cyanine solution for 10 minutes. Sections were then rinsed, differentiated with a 10% ferrous oxide solution for 5 minutes, rinsed again and coverslipped. Morphometric analysis of lesion size in solochrome cyanine–stained sections was conducted on a Seescan imaging system.

Bromodeoxyuridine Incorporation

In some experiments (see Results) bromodeoxyuridine (BrdU; Sigma) in 50% ethanol/PBS was introduced into the peritoneal cavity at 250 mg/kg body weight, beginning 48 hours after the onset of demyelination (n = 4). Animals were given booster injections of BrdU (250 mg/kg) into the peritoneal cavity every 6 hours, and killed under anesthesia 72 hours after the onset of demyelination. In each of 4 BrdU-treated animals, 3 adjacent 1 mm tissue blocks were taken from the center of the lesion. Two blocks from each animal were sectioned and double-stained for Rip and BrdU. One block from each animal was sectioned and stained for Rip and BrdU in a serial fashion to control for nonspecific binding or antigen masking in the double immunostained tissue.

RESULTS

Intraspinal Injection of GalC Antibodies Plus Complement Induces Demyelination: In all animals, a well-defined region of myelin disruption was evident within the dorsal columns following intraspinal injection of anti-galactocerebroside (GalC) antibodies plus serum complement proteins (Fig. 1*A*). The size of the lesion was maximal at the point of injection, and was progressively smaller in cranial and caudal tissue blocks. The average lesion size at the point of injection was very consistent, with the exception of the 3-day nonirradiated demyelinated group, which exhibited larger lesions (Fig. 2). The cranio-caudal extent of the lesions varied from 8 to 10

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absent following intraspinal injection of anti-GalC antibodies alone, PBS vehicle alone, or anti-GalC antibodies plus heat-inactivated serum complement. C: Rip immunostaining of a demyelinated lesion 3 days after the onset of demyelination; note the Rip-immunopositive cells within the region of demyelination. D: Solochrome cyanine staining for myelin of a 10-micron serial tissue section to C delineating the extent of the demyelinated area. ×200.

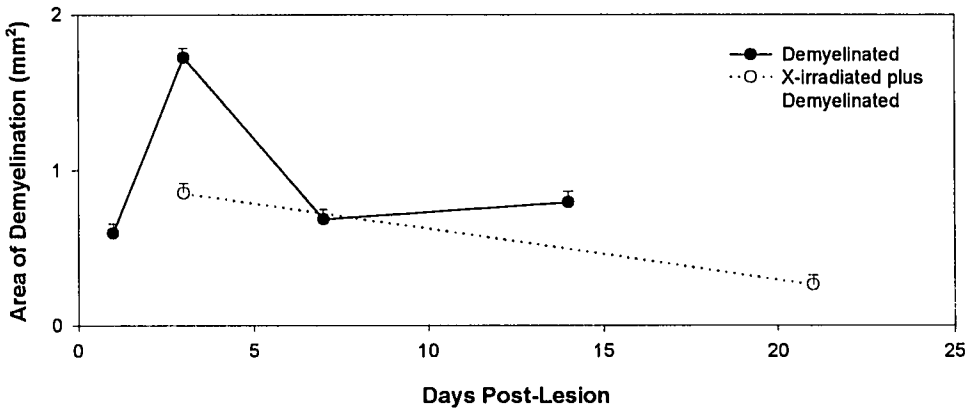

Fig. 2. Mean cross-sectional area of demyelination at early and late time points in demyelinated and x-irradiated plus demyelinated spinal cords (n = 4 for each).

mm. Light and electron microscopic examination of resin-embedded experimental and control tissue sections indicated that axonal integrity was preserved at all time points within lesions as indicated by the absence of Wallerian degeneration cranial and caudal to the area of demyelination.

Control injections consisted of either (a) anti-GalC antibodies alone, (b) guinea pig complement alone, (c) phosphate-buffered saline alone, or (d) anti-GalC antibodies plus heat-inactivated guinea pig complement (by exposure of the complement to 50° Celsius for 30 minutes). Light microscopic examination of resin-embedded toluidine blue–stained tissue sections 7 days following the onset of demyelination indicated that control solutions did not induce demyelination or Wallerian degeneration (Fig. 1B).

Vesiculation and disintegration of myelin sheaths was evident 5 hours after intraspinal injection of anti-GalC antibodies plus complement. Few completely demyelinated axons were present 24 hours after injection; myelin sheaths were separated from axons and were in various degrees of disintegration. Completely demyelinated axons were evident at 2 days and 3 days among myelin debris. A complete and well-defined region of demyelination was evident 7 days after injection (Fig. 1*A*). From this time point onwards, myelin debris was found only within macrophages. Thin myelin sheaths could be detected at 10 days and 14 days following injection, indicating the commencement of oligodendrocyte remyelination. Remyelination of all axons was evident at 3 weeks and 4 weeks following injection, where myelin sheaths were characteristically thin compared to unlesioned tissue.

Oligodendrocytes survive demyelination and do not divide: Electron microscopic analysis at 5 hours, 1 day, 2 days and 3 days following injection revealed the presence of cells with a mature oligodendrocyte morphology within lesions. The presence of oligodendrocytes within lesions was confirmed and quantified by immunostaining using Rip antibodies (Fig. 1*C*) that are specific for differentiated CNS oligodendrocytes and myelin (12, 13). The Rip immunohistochemical protocol used here was modified so as to eliminate myelin immunoreactivity and resulted in intense staining of oligodendrocyte cell bodies, which allowed for accurate quantification. For consistency, analysis was restricted to the center 1 mm block of the lesions and the area of demyelination was delineated with the aid of solochrome cyanine–stained 10-micron serial tissue sections (Fig. 1D). Rip+ oligodendrocytes were randomly dispersed within lesions and the average number of Rip+ cells within 10-micron transverse sections of lesions was 4.75 at 1 day, 9.83 at 3 days and 23.5 at 2 weeks following the onset of demyelination (see Fig. 3). The higher number of Rip+ cells at 3 days following the onset of demyelination was associated with a larger average lesion size (Fig. 2); however, the density of Rip+ cells (number of cells divided by lesion area in mm^2) was similar to that at 1 day (Fig. 3). The average Rip+ cell density within areas of demyelination (see Fig. 3) was 48% of the average Rip+ cell density in control animals at one day, 52% at 3 days, 172% at 2 weeks, and 134% at 2 months following the onset of demyelination. The similar density of Rip+ cells 1 and 3 days following demyelination suggests that oligodendrocytes within the lesion that survive the demyelinating process do not dedifferentiate as an acute response to demyelination.

The proliferative ability of oligodendrocytes within lesions was assessed with bromodeoxyuridine (BrdU) and Rip double immunohistochemistry 3 days after the onset of demyelination, following 24 hours exposure to BrdU. Although Rip+, BrdU-cells, and Rip-, BrdU+ cells were present within the area of demyelination as well as in the surrounding normal white matter, no Rip+, BrdU+ cells were identified in either the lesion or the surrounding myelinated dorsal column (Fig. 4). The lack of BrdU labeling indicates that oligodendrocytes do not divide as an acute response to demyelination.

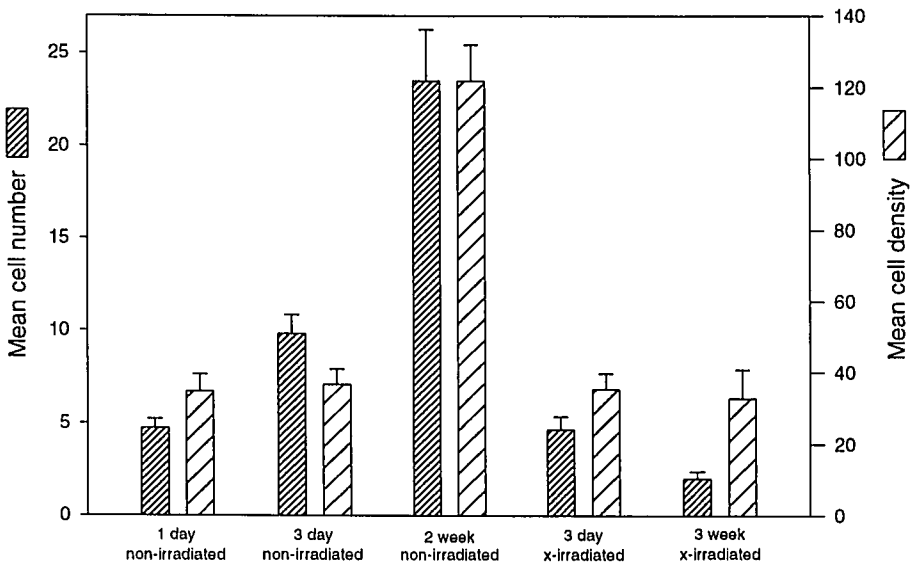

Fig. 3. Rip-immunopositive cell numbers and cell densities within areas of demyelination at early and late time points in transverse sections of demyelinated and x-irradiated plus demyelinated lesions. The density of Rip immunopositive cells at 3 days in x-irradiated and nonirradiated areas of demyelination is not statistically different (t = 0.163), indicating that surviving oligodendrocytes do not divide as an acute response to demyelination (oligodendrocyte mitosis would have resulted in cell death in x-irradiated animals, and a subsequent decrease in cell density).

Oligodendrocytes survive x-irradiation plus demyelination and do not divide: Electron microscopic analysis of 3-week lesions revealed a small number of cells with oligodendrocyte morphology within the well-defined region of demyelination (Fig. 5A). The presence of oligodendrocytes within lesions was confirmed and quantified by immunostaining using Rip antibodies (Fig. 6). Quantification of Rip+ cells indicated that the number of cells at 3 days was similar to the number of cells at 1 day in nonirradiated lesions, and secondly, that the density of cells at 3 days in x-irradiated lesions was similar to that found at 1 and 3 days in the nonirradiated lesions (Fig. 3). These findings suggest that Rip+ cells were not dividing (dividing cells exposed to 40 Grays of x-irradiation die, and thus if Rip+ cells were dividing between 1 and 3 days, the number and density of Rip+ cells in x-irradiated tissue would be reduced).

Although the density of Rip+ cells was similar in the 3-day and 3-week lesions made in x-irradiated tissue, the number of Rip+ cells in the area of demyelination as well as the lesion sizes were smaller, which could indicate that cells had been lost from the area of demyelination between 3 days and 3 weeks. However, immunohistochemical analysis of the 3 week lesions consistently showed an increased number and density of oligodendrocytes at the margin between myelinated and demyelinated tissue (see Fig. 6 and Table 1). When the number of these cells was combined with the number of cells within the lesion center, there was no significant difference between the number of Rip+ cells associated with lesions in the 3-day and 3-week animals (Table 1). Since no new cells can be added to the tissue in the x-irradiation paradigm, we concluded that cells within lesions had been redistributed by 3 weeks as a result of tissue compaction.

Our findings indicate, therefore, that some Rip+ oligodendrocytes survive within regions of demyelination induced by the injection of anti-GalC antibodies and complement and that since the number of these cells remains constant in tissue exposed to 40 Grays of x-irradiation and there is no evidence for BrdU incorporation into Rip+ cells, these cells are not induced to divide by the presence of demyelinated axons.

Surviving oligodendrocytes in x-irradiated lesions do not remyelinate: There was no evidence of remyelination in animals killed 3 weeks after x-irradiation plus demyelination (Fig. 5). Associated with some of the oligodendrocytes in the 3-week lesions were processes that separated demyelinated axons (Fig. 5). Some of these processes showed compaction of their membranes to form sheets of myelin-like membrane (Fig. 5B). These myelin-like membranes were often apposed to more than one axon. In no instances were axons surrounded by a continuous membrane spiral characteristic of myelin sheaths.

DISCUSSION

Identification of the origin of remyelinating cells is of great importance for the development of strategies to increase the extent of remyelination following demyelinating diseases in the adult CNS such as multiple sclerosis. Although the potential for remyelination of the adult CNS is well established (15–19), identification of the cell population which gives rise to remyelinating cells remains

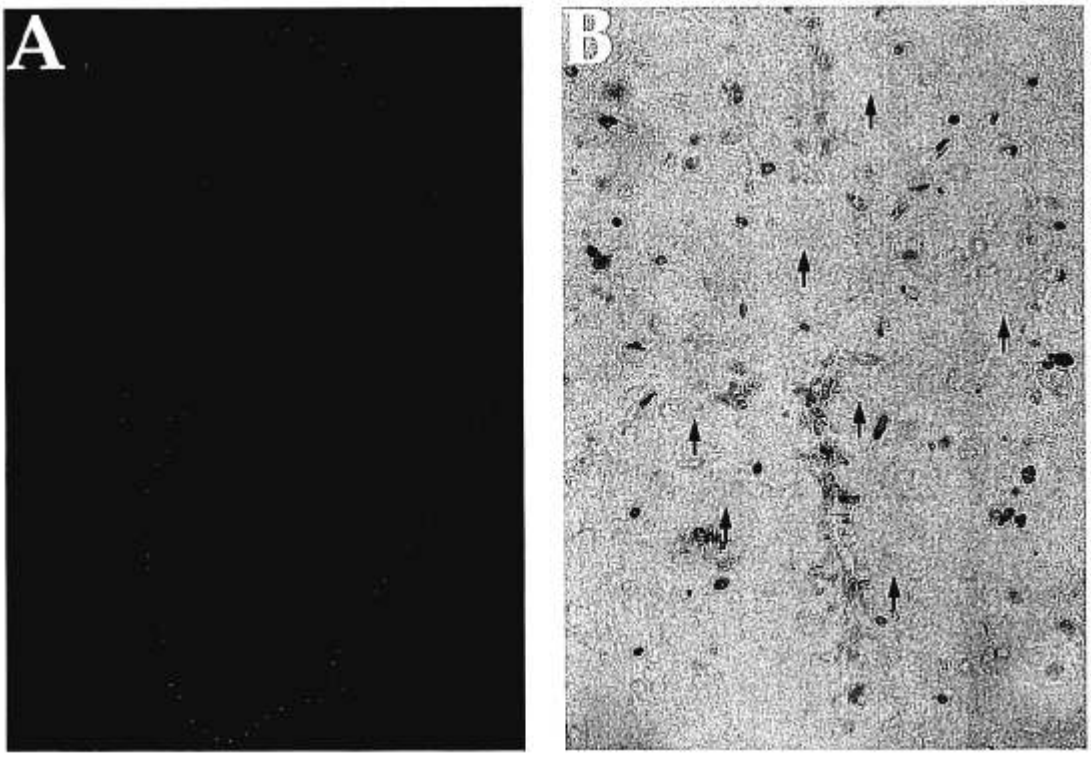

Fig. 4. Rip and bromodeoxyuridine (BrdU) double immunostaining of an area of demyelination in the dorsal column of the adult rat spinal cord in transverse section. A: Rip immunostaining 3 days after the onset of demyelination. B: BrdU immunostaining of the same section; arrows point to the location of Rip-immunopositive cells in A (lesion outline obtained from a solochrome cyanine–stained serial section). ×200.

controversial. Remyelination-competent cells may derive from differentiated oligodendrocytes or precursor cells (for reviews see 1 or 20). Our in vivo studies reported here address the role of differentiated oligodendrocytes in remyelination and demonstrate two important findings: (a) remyelination requires cell division, and (b) oligodendrocytes that survive within and around an area of demyelination do not divide in response to demyelination and appear incapable of remyelinating axons.

The intraspinal injection of antibodies to galactocerebroside, the major sphingolipid in myelin, plus serum complement proteins resulted in focal demyelination of several segments of the rat dorsal column (Fig. 1). Similar methods have been used, with varying degrees of specificity, to demyelinate or disrupt myelination in vitro (21–25), as well as guinea pig optic nerve (26, 27), cat optic nerve (28, 29), rat spinal cord (30, 31) and chicken spinal cord (32, 33) in vivo. In the present study, demyelinating lesions were produced in which approximately 50% of oligodendrocytes survived within the area of demyelination, and therefore their contribution to remyelination could be examined. Our investigation examined two responses of these cells to the presence of demyelinated axons—(a) their ability to divide and (b) their ability to remyelinate.

Oligodendrocyte remyelination was evident 10 days following the onset of demyelination and was accompanied by an increase in both the number and density of Rip+ oligodendrocytes. Since remyelination was not observed in x-irradiated lesions, a situation where dividing cells die, one can conclude that remyelination is associated with the generation of Rip+ oligodendrocytes by cell division.

We determined the number of oligodendrocytes within the area of demyelination at different survival times in x-irradiated and nonirradiated white matter to examine whether surviving oligodendrocytes could divide either as Rip+ cells or after dedifferentiation to Rip− cells. In order to compare the number of Rip+ cells within lesions of differing size we calculated cell density. While accepting the fact that the cellular composition of an area of demyelination, with the exception of the number of axons, will vary with time and thus the area of demyelination may not be the ideal denominator for calculating cell density, we believe that the density data as presented supports rather than refutes conclusions which could be drawn simply by a qualitative analysis of our experiments. Thus, our conclusion that surviving oligodendrocytes within demyelinated lesions did not give rise to remyelinating oligodendrocytes is supported by a number

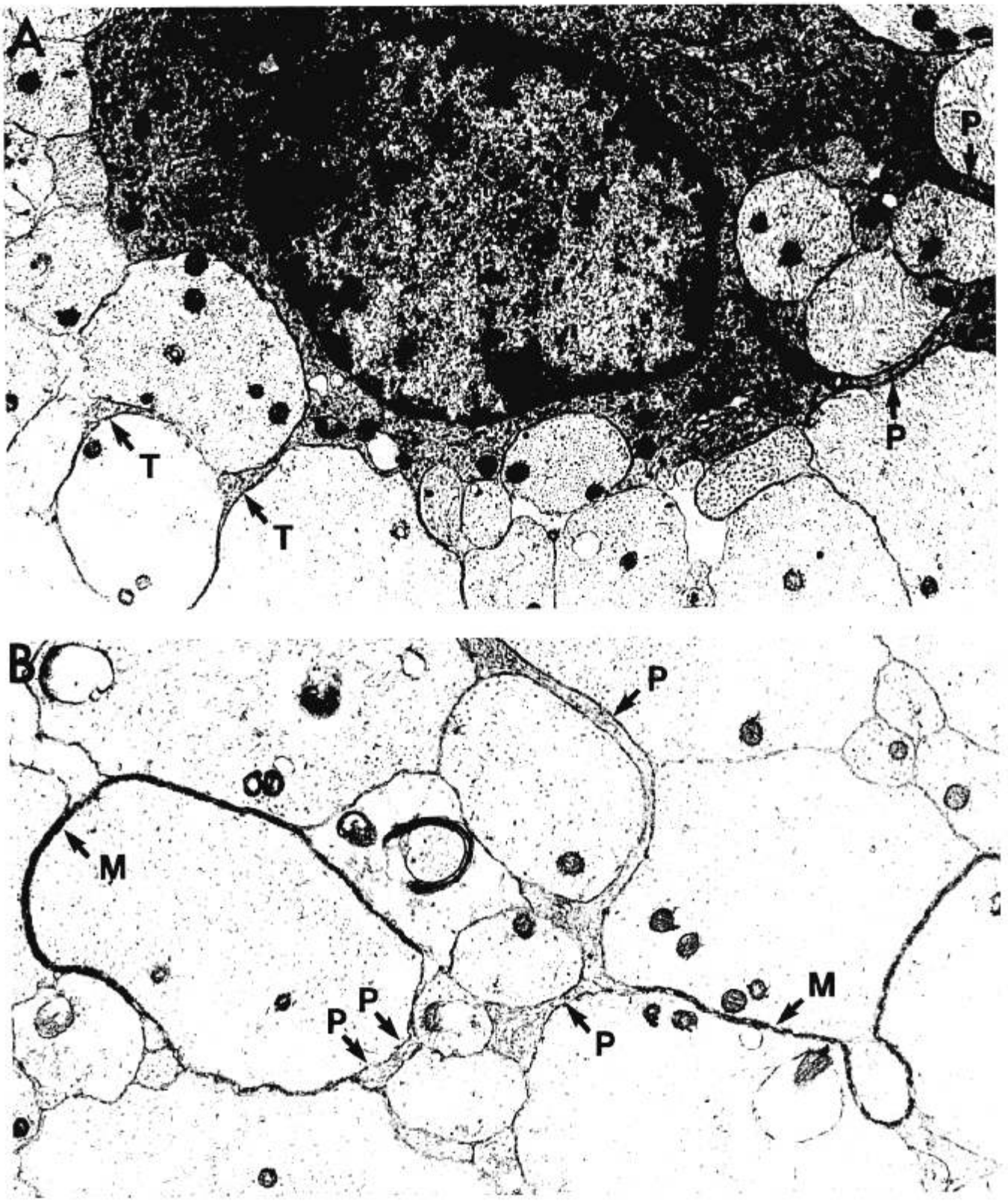

Fig. 5. Electron micrographs of 3-week-old x-irradiated demyelinated lesions in the dorsal column of the adult rat spinal cord in transverse section. A: A surviving oligodendrocyte with cellular processes (P) that engage neighboring axons. Two or more oligodendrocyte tongues (T) were often observed around a single axon, rather than the single tongue typical of normal myelination. B: Higher magnification of aberrant myelin membranes; note that the myelin membranes (M) are apposed to more than one axon, and note the absence of sheath formation. ×12,000 for A; ×24,000 for B.

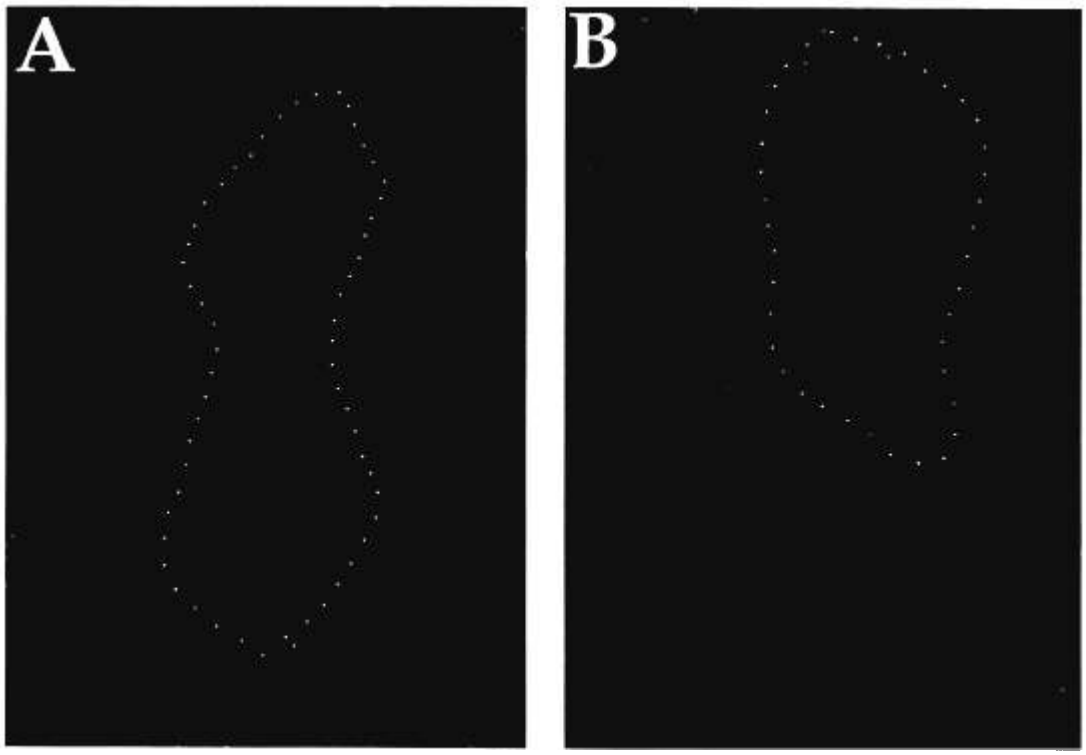

Fig. 6. Rip immunostaining of x-irradiated demyelinated areas in the dorsal column of the adult rat spinal cord in transverse section. A: Rip immunostaining of an area of demyelination 3 days after the onset of demyelination; note the Rip-immunopositive cells within the area of demyelination. B: Rip immunostaining of an area of demyelination 3 weeks after the onset of demyelination; note the relative increase in Rip-immunopositive cell density within the perimeter of the area of demyelination (outline obtained from a solochrome cyanine–stained serial section). ×200.

of observations. The similar density of cells in x-irradiated and nonirradiated lesions at 3 days (Fig. 3), together with or independent from the presence of cells within the x-irradiated lesions at 3 weeks (Table 1), exclude the possibility that surviving Rip+ cells within the lesion divided, as division would have resulted in a significant difference in cell density in the x-irradiated and nonirradiated 3-day lesions and an elimination of cells by 3 weeks in x-irradiated tissue. Furthermore, no Rip+ cells within or outside the lesions incorporated BrdU 48–72 hours after demyelination (Fig. 4), the time when remyelination-competent cells have been shown to be dividing (11). Therefore, the source of Rip+ cells associated with remyelination are Rip− cells.

Because there was no decrease in Rip+ cells between 1 and 3 days in the nonirradiated animals, and because similar numbers of Rip+ cells were associated with x-irradiated lesions at 3 days and 3 weeks, one can also exclude the possibility that Rip+ cells undergo de-differentiation and cell division. If this had happened, one would have observed a decrease in cell number between 1 day and 3 days in nonirradiated animals and a decrease in cell number between 3 days and 3 weeks in the x-irradiated animals. The lack of a decrease in Rip+ cell number in both x-irradiated and nonirradiated experimental groups also suggests that x-irradiation does not influence the ability of cells to de-differentiate. The source of remyelinating cells is therefore the oligodendrocyte progenitor cell population present in adult white matter (34). In further studies using this lesion model, we have demonstrated that many of the BrdU+ cells observed 3 days after demyelination (see Fig. 4) express the oligodendrocyte progenitor marker NG2 (35).

The ability of x-irradiation to inhibit the remyelinating behavior of the progenitor population enabled us to examine the remyelinating potential of the surviving oligodendrocytes within the lesion. These cells extended processes that engaged neighboring axons and on occasion generated myelin membranes, but on no occasion did we observe any evidence of myelin sheath formation. Thus, although oligodendrocytes that survive loss of their myelin sheath appear able to synthesize myelin membranes and engage axons, it would seem that they are unable to repeat a program of axon ensheathment that results in the formation of a myelin sheath.

The failure of an oligodendrocyte that has lost its myelin sheaths to re-engage in myelin sheath formation may reflect the true remyelinating potential of these cells.

TABLE 1
Rip+ Cell Numbers in X-irradiated Demyelinated Lesions at 3 days and 3 Weeks

	X-irradiation plus Demyelination					
	3 days			3 weeks		
	Cells in lesion	Cells in perimeter	Total cells	Cells in lesion	Cells in perimeter	Total cells
	5	3	8	3	4	7
	4	2	6	2	6	8
	5	3	8	2	4	6
	4	3	7	1	6	7
Average # of cells in area of demyelination	–	4.5	–	–	2	–
Average # of cells in perimeter of demyelinated area	–	2.75	–	–	5	–
Average # of total cells associated with area of demyelination	–	7.25	–	–	7	–

[1] The number of nucleated Rip-immunopositive cells within areas of demyelination and along the perimeter in 10-micron transverse sections of x-irradiated demyelinated lesions at 3 days and 3 weeks. Rip-immunopositive cell numbers are higher in the perimeter of the 3-week-old areas of demyelination due to compaction, and the number of cells associated with the areas of demyelination at this time point is not statistically different from the earlier time point (t = 0.397).

However, it is also possible that the observation we have made in this study might be a consequence of x-irradiation exposure on either the oligodendrocyte or the lesion environment. We are confident in excluding the latter, since the myelin sheath formation that results from the introduction of a wide variety of myelinogenic cells into x-irradiated lesions indicates that the environment is clearly capable of supporting myelin sheath formation (36). It is more difficult to exclude the possibility that our observations are the result of the effects of x-irradiation on the surviving oligodendrocyte. The formal proof that this is not the case could be obtained by transplanting a pure population of oligodendrocytes deprived of their myelin sheaths and that had not been exposed to x-irradiation into a demyelinating lesion. However, it has proved remarkably difficult to obtain such populations due to the difficulty of removing progenitor populations (37) and a dearth of markers that unequivocally identify a cell in vitro as one that has formed and maintained myelin sheaths. The late-expressing myelin protein MOBP may provide such a marker (38). Nevertheless, there are a number of other observations that support our interpretation. Oligodendrocyte preparations with little or no mitotic potential can be prepared from adult human brain (39) and from sheep (40). When preparations of oligodendrocytes prepared from the adult human brain are introduced into demyelinating lesions in immunosuppressed rats, myelin membranes can be observed, but no myelin sheaths are produced (41). Similarly, preparations of sheep oligodendrocytes that show no mitotic potential and form myelin sheets in vitro (40) fail to ensheath axons when added to cultures of rat dorsal root ganglion neurons (42). In both of these examples one can exclude the xenogeneic nature of the interaction per se as the reason for failure to form myelin sheaths, since there have been no cross-species transplantation experiments yet performed that have revealed a species barrier to myelination of axons of one species by myelinogenic cells of another species (36). Thus, it would appear that oligodendrocytes that have been deprived of their myelin sheaths by an isolation procedure and that are postmitotic show a restricted ability to form myelin sheaths. However, until a way can be found to prepare pure cultures of postmitotic rat oligodendrocytes uncontaminated by mitotically active cells, and these cells are introduced into an area of demyelination that has no inherent remyelinating capacity, uncertainty will remain as to whether failure of the cells to reform myelin sheaths in the present study truly reflects the remyelinating potential of oligodendrocytes that survive an episode of demyelination. What is clear from our study is that for remyelination to be successful, cells must undergo mitosis and that oligodendrocytes that are or have been associated with myelin sheaths appear postmitotic.

Our conclusion that oligodendrocytes do not divide is at variance with a number of previous studies that have suggested that differentiated oligodendrocytes undergo mitosis. Using morphological criteria and/or immunostaining to identify oligodendrocytes, a number of in vitro (42, 44) and in vivo (10, 45–47) studies suggest that differentiated oligodendrocytes can incorporate tritiated thymidine. However, due consideration must be given to the age of the experimental animals, the length of exposure of animals to tritiated thymidine, and time to sacrifice after the last tritiated thymidine application, when interpreting these studies. For example, in studies that have used very young animals (47) or experimental animals exposed to tritiated thymidine for very long periods (10), one cannot rule out the possibility that labeled oligodendrocytes did not acquire tritiated thymidine during a more undifferentiated state. In contrast, other authors conclude that proliferation in the oligodendrocyte lineage is restricted to precursors in studies of the brain of adult mice (48) and the forebrain (49) and cerebellum (50, 51) of young rats. Other studies on the glial response to trauma have demonstrated tritiated thymidine uptake by astrocytes, macrophages and monocytes, but not oligodendrocytes (52, 53).

The studies reported here clearly demonstrate that oligodendrocytes that survive within a region of demyelination in the adult rat spinal cord are not induced to divide by the presence of demyelinated axons. The radiation resistance of such cells indicates that differentiated oligodendrocytes are postmitotic and thus cannot be considered to make a significant contribution to remyelination in the CNS. Furthermore, our results indicate that these cells do not form myelin sheaths. Thus, our findings indicate that strategies to increase the extent of remyelination in demyelinating diseases should be directed towards increasing the availability, migration and remyelinating capacity of oligodendrocyte progenitor cells rather than enhancing the survival of oligodendrocytes that have lost their myelin sheaths.

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