

Schwann Cell Invasion of Ventral Spinal Cord: The Effect of Irradiation on Astrocyte Barriers

TERRY J. SIMS, PHD, M. BARBAROS DURGUN, MD, AND SHIRLEY A. GILMORE, PHD

Abstract. This study examines a radiation-induced invasion and spread of Schwann cells into ventral gray regions of the lumbar spinal cord. The prevalence of these cells within the gray matter and the time course of their appearance in the ventral spinal cord is quite different from the pattern of Schwann cell development in dorsal spinal cord reported previously. The focus is on 2 possible pathways, each involving astrocytic barriers, by which Schwann cells access the ventral gray matter. The first of these is the glia limitans covering the ventral surface of the spinal cord and the possibility that its integrity has been disrupted by the exposure to x-rays. Comparisons of the glia limitans, including its thickness, between irradiated and nonirradiated rats revealed that exposure to radiation did not result in any morphologically discernible alterations. The second barrier examined was the astrocytic covering of blood vessels. In irradiated animals the astrocyte processes that normally surround blood vessels were missing in some instances, and Schwann cells were observed at these sites. The difference between the dorsal and ventral occurrence of Schwann cells is that, whereas Schwann cells primarily follow axons, specifically dorsal root axons, to access the dorsal spinal cord, it appears that the presence of Schwann cells in the ventral portion of the spinal cord where their location is primarily in the gray matter is associated with the vasculature.

Key Words: Astrocytes; Blood vessels; Glia limitans; Schwann cell; Spinal cord; x-irradiation.

INTRODUCTION

Following irradiation of the neonatal rat lumbar spinal cord, reductions occur in the normal glial cell populations in the irradiated region. The radiation-induced loss of oligodendrocytes delays central myelin formation (1, 2) and the reduced astrocyte population results in a loss of the barrier properties of the glia limitans (GL) on the dorsal surface of the spinal cord (3). Subsequently, Schwann cells invade, proliferate, and myelinate axons in the dorsal funiculi within the irradiated region by 3 weeks following irradiation (4, 5). Schwann cells also invade the dorsal spinal cord by routes other than the dorsal roots. For example, Schwann cells have been observed in situations where they appear to be migrating into the cord through gaps in the GL at sites distant from the dorsal root entry zone (6). Additionally, isolated aggregates of Schwann cells have been reported in close association to blood vessels and well separated from other Schwann cell-occupied regions (7).

The radiation-induced depletion of the macroglial population is not restricted to the dorsal portion of the irradiated region of spinal cord; this depletion and an accompanying state of hypomyelination occur also in the ventral white matter (1, 2). Unlike the dorsal white matter, however, axons in the ventral white matter are not subsequently myelinated by Schwann cells. This differential pattern of development of intraspinal Schwann cells raises the possibility that the GL, the astrocyte-de-

rived limiting membrane of the central nervous system (CNS), was differentially affected on the dorsal and ventral surfaces. Electron microscopic examination of the GL (3) revealed that significant alterations, including development of discontinuities in the GL, occurred dorsally but not ventrally during the 10-day period immediately following irradiation. The absence of marked alterations in the ventral GL, along with the absence of intraspinal Schwann cells ventrally, were considered to be related. With lengthening of the postirradiation interval to periods longer than 2 months, however, Schwann cells made their appearance in the ventral spinal cord (8). A striking difference was observed in these animals examined at intervals of 30 days to 7 months following irradiation in that the ventrally located intraspinal Schwann cells occurred principally in the gray matter (8). Only rarely, as pointed out in that study, did it appear that the ventrally situated Schwann cells were extensions from Schwann cell aggregates developing earlier in the dorsal cord. In fact, examination of serially sectioned spinal cords revealed that the ventrally located aggregates of intraspinal Schwann cells were generally very small and were separated from those located dorsally. These same examinations also revealed that some Schwann cell aggregates, both dorsally and ventrally, were similar in that they were associated with blood vessels (8). In instances in which Schwann cells were observed in the ventral white matter, they were usually noted to be associated with blood vessels. Given these observations, a logical question is whether later changes, i.e. beyond one month following irradiation, occur in astrocyte barriers in the ventral portion of the irradiated region. In the present study, 2 astrocytic barriers were examined: the GL covering the ventral surface of the cord and the astrocytic coverings of blood vessels. Some authors consider the perivascular astrocyte processes to be part of the GL and to form a

From the Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, Arkansas (TJS, SAG) and the Department of Anatomy, Hacettepe University Faculty of Medicine, Ankara, Turkey (MBD).

Correspondence to: Terry J. Sims, Ph.D, Department of Anatomy, #510 University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205.

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covering that is continuous with that on the surface of the CNS (9). Within the present study, however, the term GL refers to the astrocyte processes on the surface of the spinal cord, whereas the processes surrounding a blood vessel are referred to as perivascular astrocyte processes. Both of these astrocyte-derived barriers were examined in irradiated animals and in nonirradiated littermate controls between 40 and 100 days postirradiation, the interval during which Schwann cells become evident in ventral spinal cord (8).

MATERIALS AND METHODS

Litters of Charles River CD rats were used in this study. On the third postnatal day, 6 pups were x-irradiated with a single dose of 40 Gray and the remaining littermates served as controls. The irradiated area was restricted to a 5 mm length of lumbosacral spinal cord by a lead shield. A Philips Contact Therapy Apparatus was used to administer the radiation under the following conditions: 50 kVP; 2 mA; filter added, 0.25 mm Al; HVL, 0.16 mm Al. Details regarding the irradiation procedure have been published elsewhere (10, 11). Spinal cords from both irradiated and nonirradiated animals of 5 different age groups were examined at 43, 63, 83, 103 and 123 days of age. Those in the 43, 63 and 103 day groups were examined by both light and electron microscopy. The remaining 2 groups, 83 and 123 days, were examined by light microscopy only. Each group consisted of 3 irradiated and 2 nonirradiated, littermate control rats. At the designated age the animals were deeply anesthetized by injection of an overdose of chloral hydrate and were perfused through the heart with a fixative composed of 2% paraformaldehyde and 2% glutaraldehyde in 0.12 M Sorensen's buffer at pH 7.4. Following the perfusion, a 4 mm length of spinal cord containing the irradiated area, or its equivalent region in nonirradiated controls, was removed and stored overnight in fixative at 4°C. On the following day the spinal cords were cut into 1mm slabs in the transverse plane and post-fixed in 2% OsO₄ solution for 1.5 hours at 4°C. The tissue was then dehydrated, infiltrated with Poly/Bed 812 plastic (Polyscience, Inc.), and cast in blocks for sectioning on a Sorvall MT6000 ultramicrotome. Thick (1 μ) sections from all groups were examined and photographed using a Nikon photomicroscope. Blocks for ultrastructural examination were trimmed, and thin sections were cut and placed on grids. Thin sections were contrasted with uranyl acetate and lead citrate, and examined and photographed on a JEOL 100CX electron microscope.

Assessments of the GL: For each of the spinal cords evaluated ultrastructurally, thin sections from at least 3 different blocks containing the ventral GL from 1 hemisection of the cord (Fig. 1) were examined and photographed at a principal magnification of 4,800 times. The photographs were then printed at a total magnification of 12,240 times and assembled into a montage depicting the full extent of the GL. The entire length was first examined for evidence of breaks or interruptions. The thickness of the GL was then measured at 2.54 cm intervals along each photographic montage to determine if the earlier exposure to radiation resulted in any discernible thinning. The thickness of the GL was measured by starting at the outermost

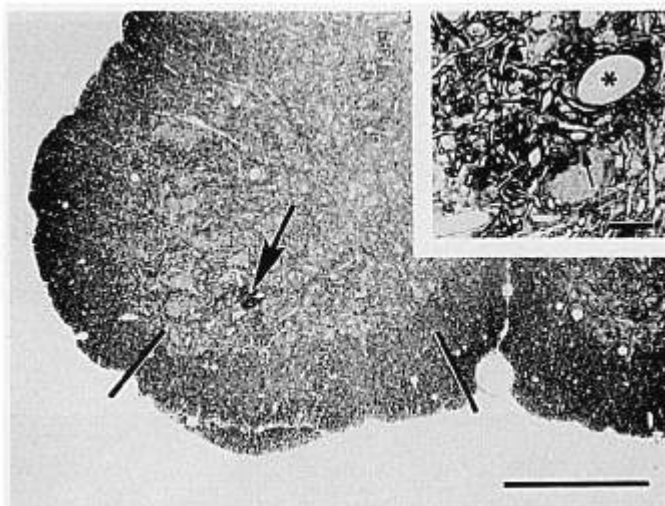


Fig. 1. Low power view of the ventral portion of a spinal cord hemisection from an animal 100 days following irradiation. The region of ventral glia limitans examined in this study is located between the 2 lines. A small cluster of Schwann cells is located adjacent to a blood vessel (arrow) in the gray matter. Scale bar = 1.0 mm Inset: Higher magnification of area indicated by arrow showing axons (small arrows) myelinated by Schwann cells adjacent to a small blood vessel (*). Thick (1 μm) plastic section stained with toluidine blue. Inset scale bar = 0.1 mm.

astrocyte surface adjacent to the basal lamina and ending where the internal surface of the GL encountered a process or cell body of a cell type other than astrocytic. If the measurement site was immediately adjacent to a blood vessel or a ventral root axon penetrating the GL or tracked along a radially oriented astrocyte process for a distance greater than 15 microns, it was not included in the assessment.

RESULTS

General Characteristics of Ventral Funiculi

Measurements of the thickness of the GL were made along the ventral surface of the spinal cord between the lines indicated in Figure 1. The spinal cord section illustrated in Figure 1 is from an irradiated animal 103 days old. In general, irradiated lumbosacral spinal cords were smaller in circumference than those from nonirradiated animals of the same age. This size difference was due in part to a persistent state of hypomyelination which is evident at the light microscopic level by the lack of the usual, clear distinction between white and gray matter at all ages.

Comparisons of the Glia Limitans

Astrocyte processes forming the ventral GL of the normal spinal cords contained filaments, rough endoplasmic reticulum and mitochondria, giving the processes a moderately dense appearance (Fig. 2). Frequently, the processes in both nonirradiated and irradiated animals contained large mitochondria (Figs. 2, 3). In general, the

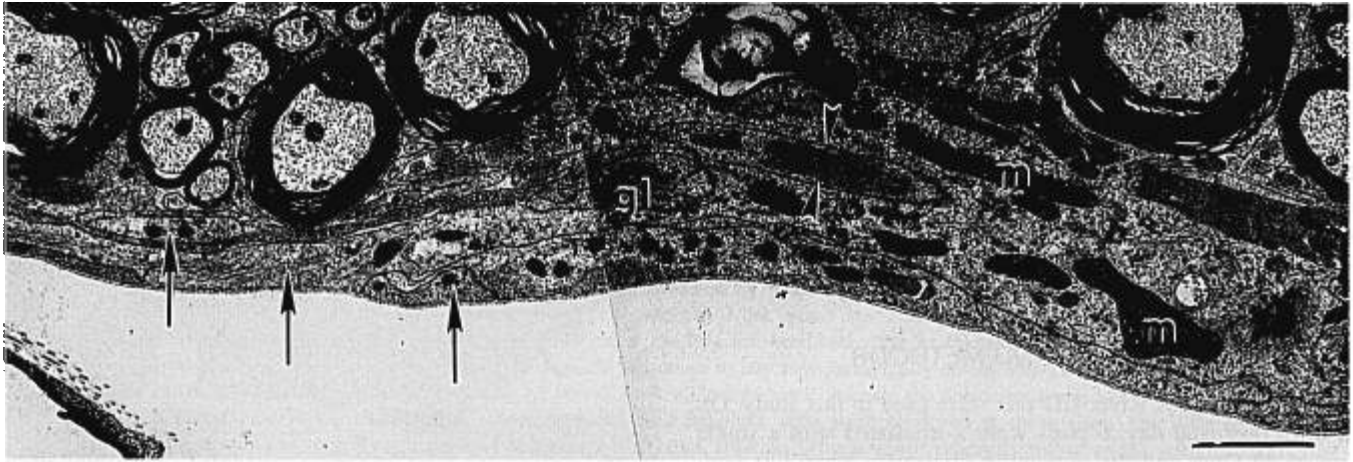


Fig. 2. An electron micrograph showing the ventral glia limitans from a 43-day-old nonirradiated rat. The glia limitans (gl) is composed of layers of astrocyte processes (arrows) of varying thickness and generally of moderate cytoplasmic density. Bundles of filaments (arrowheads) and large mitochondria (m) are a common feature of the astrocyte processes throughout the length examined. Scale bar = 2.0 μ m.



Fig. 3. An electron micrograph of the ventral glia limitans and white matter from a 63-day-old irradiated spinal cord. The glia limitans (gl) is intact and is composed of layers of astrocyte processes as observed in the nonirradiated animal (Fig. 2). The astrocyte processes (arrows) vary in thickness and in cytoplasmic density. Subpial astrocytes (A) were routinely observed. Bundles of filaments (arrowheads) and large mitochondria (m) were common occurrences in all astrocyte processes throughout the length of irradiated cord surface examined. Scale bar = 2.0 μ m.

thickness of the GL did not appear to be affected by radiation over the time intervals studied. The thickness of the ventral GL was quite variable along the length measured, irrespective of the age of the animal or whether nonirradiated or irradiated. This variability is evident in the ranges of thickness reported in Table and is reflected in the standard deviations from the mean for each group. An analysis of variance (ANOVA) of all of these thickness values confirmed that there was no significant difference as to treatment (irradiated vs. nonirradiated) or age (43, 63 or 103 days). In all the sections examined the GL had no gaps or interruptions other than for the normal passage of blood vessels between extra- and intra-spinal locations or of axons between the central and the

peripheral nervous systems. In all instances the surface of the GL was covered by a basal lamina. Light microscopic examinations of thick plastic sections from the animals in the 83- and 123-day-old groups were consistent with the 43, 63, and 103 day groups in that there was no detectable difference in the glia limitans between irradiated and control animals.

Analysis of Perivascular Astrocyte Processes

Blood vessels within the ventral portion of nonirradiated spinal cords from all age groups were covered by astrocyte processes. The thickness and number of processes tended to be greatest on the larger caliber arteries and extremely variable on capillaries. Smooth muscle

TABLE 1
Thickness Measurements of the Glia Limitans

	43-days-old		63-days-old		103-days-old	
	Normal	X-irradiated*	Normal	X-irradiated*	Normal	X-irradiated*
Glia limitans thickness-mean (μm)	$1.71 \pm 1.63\ddagger$	1.61 ± 1.51	1.59 ± 1.53	1.94 ± 1.43	1.75 ± 1.50	1.45 ± 1.50
Glia limitans thickness-range (μm)	0.16 to 13.36	0.08 to 9.51	0.08 to 9.43	0.08 to 9.10	0.08 to 9.92	0.08 to 10.21
Number of sites measured	319	700	728	1059	782	1100
Number of animals in group	2	3	2	3	2	3

* All animals irradiated at 3 days of age.

† Standard deviation.

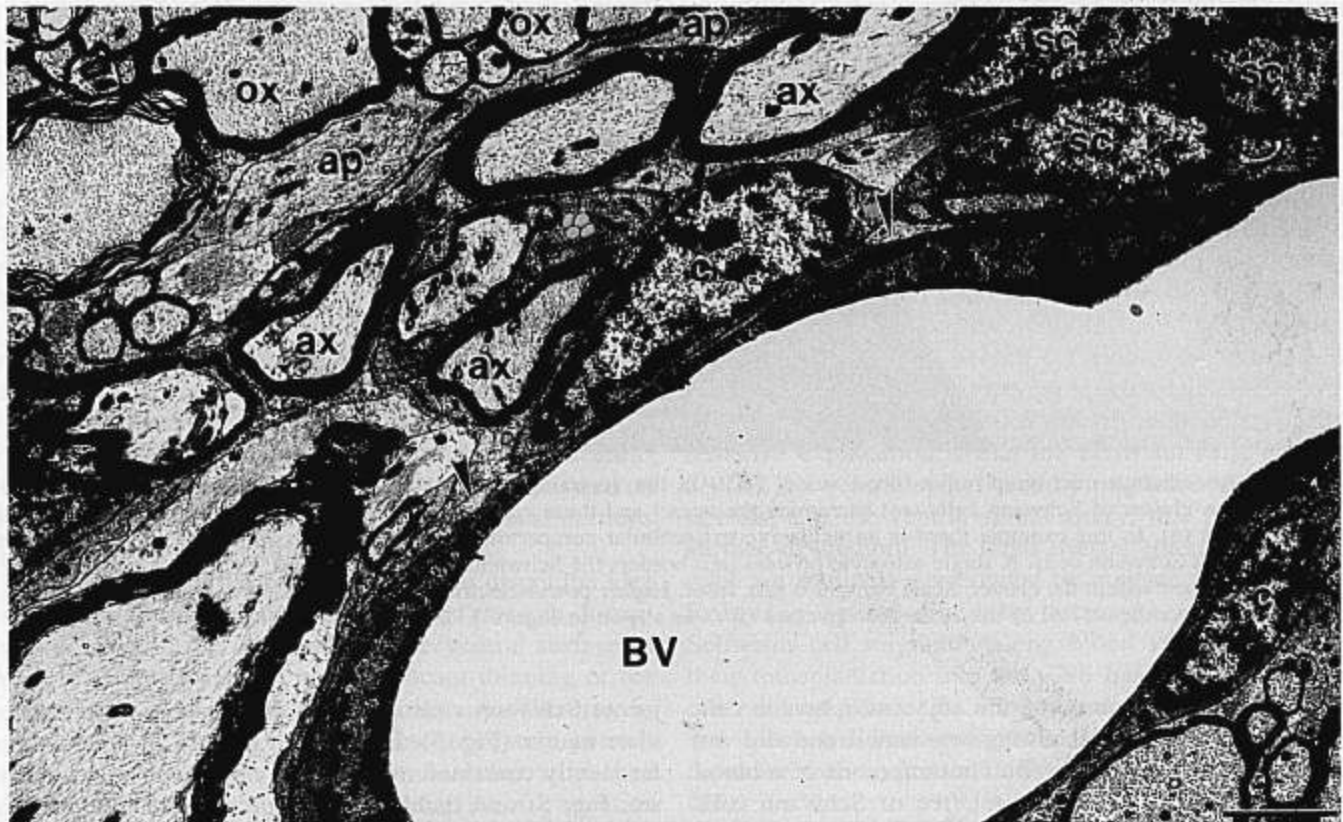


Fig. 4. An electron micrograph of a small perivascular cluster of Schwann cells (sc) and Schwann cell-myelinated axons (ax) are in close proximity to a blood vessel (BV), which is only partially surrounded by astrocyte processes (arrowheads). Note that astrocyte processes (ap) are also positioned between Schwann cell-myelinated axons and oligodendrocyte-myelinated axons (ox). Other cells (c) adjacent to the blood vessel are probably immature Schwann cells but lack distinguishing morphological features that would allow these cells to be identified with certainty. Scale bar = 2.0 μm .

cells were observed in association with arteries and arterioles, and in several instances axon terminals appeared to be in direct contact with these cells. Schwann cells, however, were not observed in association with these axon terminals or any other structure within the ventral portion of the spinal cord.

In the irradiated groups blood vessels within the ventral portions of the spinal cords were, in general, covered by astrocyte processes, although in some instances the astrocyte covering was either absent or incomplete. When

this occurred, clusters of Schwann cells were observed in proximity to these vessels, and these vessels were usually located in the gray matter (Inset, Fig. 1). These perivascular Schwann cells were observed adjacent to endothelial cells with no intervening astrocyte processes (Figs. 4, 5). Schwann cells were readily identified by the thickness of the myelin sheath and by the presence of a basal lamina around the cell and its processes. The endothelial cells and the perivascular Schwann cells were separated by a basal lamina which sometimes fused with

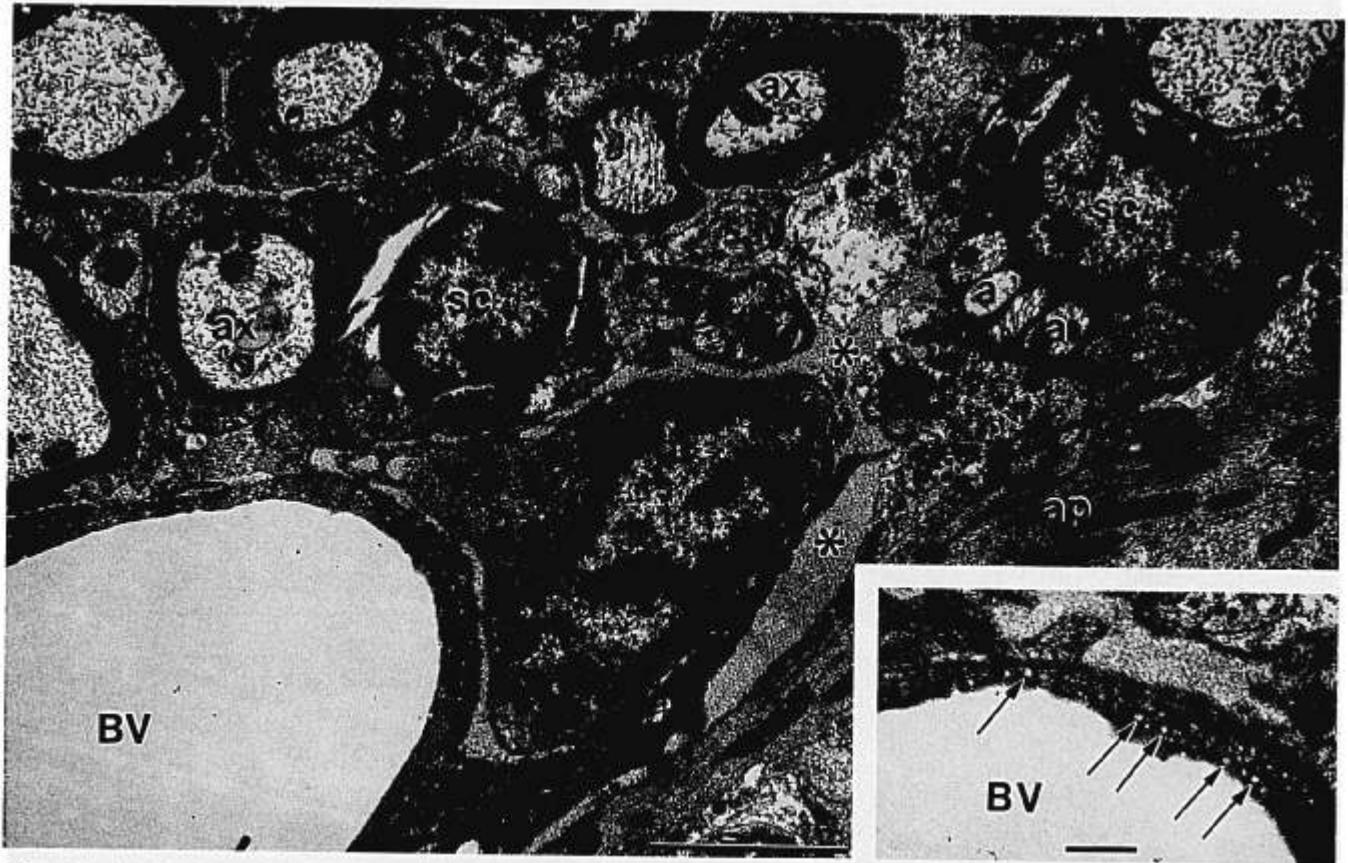


Fig. 5. An electron micrograph of a blood vessel (BV) in the ventral gray matter of a 103-day-old irradiated animal. A relatively large cluster of Schwann cells (sc) surrounds the vessel and these cells myelinate axons (ax) or ensheath the smaller diameter axons (a). In this example there is an extensive extracellular compartment (*) which surrounds both the blood vessel and the adjacent Schwann cells. A single astrocyte process (ap) borders the Schwann cell cluster in this region, but such processes are rarely observed within the cluster. Scale bar = 2.0 μm . Inset. Higher power electron micrograph depicting many clear vesicles (arrows) in an endothelial cell of the same blood vessel (BV) as shown in Figure 5. Inset scale bar = 0.25 μm .

the basal lamina surrounding the adjacent Schwann cell. When the Schwann cell cluster was small and did not completely surround the entire circumference of a blood vessel, the portion of the vessel free of Schwann cells was contacted by astrocytic processes (Fig. 4). Many of the perivascular Schwann cells either myelinated (Figs. 4, 5) or ensheathed axons (Fig. 5) in close proximity to the blood vessel. A few cells in perivascular locations in the irradiated cords were suspected of being immature Schwann cells but could not be identified conclusively due to the lack of distinguishing features. Usually, the long axis of these cells ran parallel to the course of adjacent blood vessels, and the cytoplasmic density and pattern of nuclear chromatin was similar to that in nearby ventral Schwann cells (Fig. 4). In general, astrocyte processes were not prevalent in areas of gray matter occupied by large clusters of Schwann cells, although astrocyte processes were routinely observed in close proximity to these clusters (Fig. 4). In instances where Schwann cell clusters were relatively large, the perivascular space was continuous with extracellular space surrounding ad-

acent Schwann cells and was filled with a light particulate matrix (Fig. 5). Endothelial cells in these locations frequently contained more clear cytoplasmic vesicles (Inset, Fig. 5) and included more omega-like indentations on their surfaces than endothelial cells in regions not occupied by Schwann cells. In all cases where the inter-endothelial junctions were clearly observed, they were in the form of *zonula occludentes*.

DISCUSSION

Irradiation of the immature rat lumbar spinal cord results in a reduction in both macroglial (astrocytes and oligodendrocytes) and microglial cell populations in the irradiated region. While the consequences of the loss of microglia (12) have yet to be determined, many of the changes due to the reduction in macroglia have been characterized within the irradiated spinal cord (7, 13). Following a postirradiation interval of approximately 2 weeks, Schwann cells begin to invade the dorsal funiculi. The earliest appearance of intraspinal Schwann cells occurs principally in the region of the dorsal root entry

zone, suggesting an association with centrally projecting processes of dorsal root axons (5, 8, 14). Schwann cells also enter the spinal cord by migrating directly through radiation-induced gaps in the GL covering the dorsal funiculi (3, 6) or in association with blood vessels penetrating from the surface of the dorsal cord (See Fig. 10 in ref. 13). In general, the entry of Schwann cells into the dorsal spinal cord clearly involves the loss of normal astrocyte barriers (3). With increasing postirradiation intervals, the Schwann cells spread throughout the dorsal funiculi and invade the dorsal gray matter, especially along the course of primary afferents. In these locations Schwann cells either myelinate or ensheath axons that have not acquired central myelin due to the radiation-induced loss of oligodendrocytes (4, 6, 15).

The spread of Schwann cells in the dorsal portions of the spinal cord is considerably advanced within 30 days following irradiation and by 45 days proliferation has essentially ceased (5). During this time the ventral spinal cord remains virtually free of Schwann cells. At intervals longer than 2 months following irradiation, Schwann cell aggregates appear in ventral portions of the spinal cord in approximately 40% of the irradiated rats and occur predominantly in the gray matter (8). When Schwann cells were observed in the ventral white matter, they did not appear at the light microscopic level to be associated with axons that form the ventral roots (8). Further ultrastructural examinations in the present study confirmed an absence of relationships between the intraspinal portions of motor axons and ventral Schwann cells.

The results from the present study also dispel the idea that Schwann cells migrate into the cord through disruptions or gaps in the GL covering the ventral surface of the cord. Neither gaps in nor significant thinning of the ventral GL was observed at short intervals following irradiation (3) or during the later post-irradiation periods examined in the current study. It is clear that at no time in this study were Schwann cells observed in a situation that would suggest their migration directly across the GL on the ventral cord surface, as has been described on the dorsal surface of the cord (3).

The lack of evidence correlating the Schwann cells with axons forming the ventral spinal nerve roots or with disruptions in the glia limitans, in contrast to the association between these cells and the vasculature, support the idea that the presence of intraspinal Schwann cells in the ventral gray matter is, indeed, in some way related to the blood vessels. Studies of human spinal cords have also revealed the presence of intraspinal Schwann cells, described under the term "schwannosis" (16) or as aberrant peripheral nerve bundles (17). In both of these extensive studies, it is interesting to note that the preponderance of these cells occurred in association with intramedullary branches of the anterior spinal artery. The locations of the Schwann cell aggregates occurring in an-

imals examined in the present study, as in the earlier one from this laboratory (8), also correlate with the intraspinal distribution of branches of the ventral spinal artery in the rat.

Whether these Schwann cells arise from preexisting cells within the spinal cord or from cells that migrate into the spinal cord using the vasculature as a guide or pathway is not known. It has been postulated that Schwann cells within the human nervous system are derived from primitive multipotential mesenchymal elements within the CNS (18) or from nerves associated with the vasculature (19). Although difficult to disprove, it is unlikely that the ventral Schwann cells in the present study arise from cells residing within the spinal cord at the time of irradiation, since all indications are that exposure to 40 Gray of x-radiation at 3 days of age greatly reduces the number of glial cells within the spinal cord (7) and very markedly reduces the number of dividing cells in cultures derived from irradiated spinal cord (20). In regard to proliferating Schwann cells it has been shown that in peripheral nerves they are susceptible to radiation damage after exposure to an even smaller dose (20 Gray) of x-rays (21). Also, the present study included a diligent, extensive ultrastructural search for mature Schwann cells associated with axons supplying smooth muscles of larger blood vessels and failed to locate such cells. The alternative explanation is that the Schwann cells gain access to the ventral spinal cord by migration along blood vessels, e.g. the ventral spinal artery, that penetrate the surface of the cord. The large arteries entering the spinal cord are routinely surrounded by a space (the Virchow-Robinson space) lined by astrocyte processes (9). Schwann cell migration along blood vessels following their transplantation into the CNS has been reported by other investigators (22, 23, 24) and the Virchow-Robinson space has been recognized as a major conduit for Schwann cell migration following their transplantation (25).

The lack of astrocytic processes that normally surround blood vessels raises an interesting point. What is the status of the blood-brain barrier in Schwann cell-occupied regions of the gray matter? In all cases during this study the identifiable junctions between endothelial cells were of the *zonula occludentes* type, which are the morphological basis of restricting substance movement through the space between adjacent endothelial cells (26, 27). Observations in the present study suggest that junctions between endothelial cells do not change when surrounded by Schwann cells. This may be due to the influence of the Schwann cells or the influence of astrocytes outside of the immediate region of Schwann cell contact. In view of relationships between astrocytic perivascular end feet and vascular endothelial cells described in a recent report (28), it would be difficult to argue that endothelial cells in Schwann cell-occupied areas are totally devoid of

astrocytic contact. The increase in vesicles within endothelial cells in Schwann cell-occupied regions is indicative of increased substance transport across the vessel wall. Preliminary data indicate that, when injected intravenously, horseradish peroxidase does not extravasate into the irradiated region of spinal cord, including the Schwann cell-occupied areas. The ability of Schwann cells to maintain the integrity of the blood-brain barrier in the absence of astrocytes has been reported in another model involving the rat spinal cord (29). In that study, demyelinating lesions were induced by injection of ethidium bromide, which also causes a loss of astrocytes and a subsequent repair of the myelin deficit by Schwann cells (30). Intravenous injection of horseradish peroxidase into such animals revealed that remyelination of these areas by Schwann cells prevented the extravasation of this protein in spite of the absence of astrocytes. Thus, in 2 quite different animal models, the presence of Schwann cells appears to be effective in precluding the extravasation of large proteins.

Associated with the larger clusters of Schwann cells is a dramatic increase in extracellular space and the deposition of matrix material within this space. This matrix deposition is probably responsible for the positive staining of reticular fibers observed in Schwann cell-occupied regions at the light microscopic level (8). The effect of this alteration in the extracellular environment may be of no great consequence when restricted to white matter fiber tracts as transplanted Schwann cells have been used to restore normal conductive properties of demyelinated spinal cord axons (31). The presence of Schwann cells in the gray matter in the absence of a normal astrocyte component, however, may have a profound effect on the neuropil. Recent observations suggest that there is a marked loss of synapses in Schwann cell-occupied, ventral neuropil (32). It appears to be quite possible that Schwann cells are capable of modifying the CNS extracellular space in such a way as to render portions of gray matter a nonsupportive environment for the maintenance of synaptic contacts. If Schwann cells are to be transplanted to the CNS in order to restore conduction properties of demyelinated axons, the possible long-term effect on the neuropil should be considered as there is the potential for these cells to spread from white matter to the gray matter.

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REFERENCES

- Gilmore SA. The effects of x-irradiation on the spinal cords of neonatal rats. II. Histological observations. *J Neuropathol Exp Neurol* 1963;22:294-301
- Gilmore SA. Delayed myelination induced by x-irradiation of the neonatal rat spinal cord. *Neurology* 1966;16:749-53
- Sims TJ, Gilmore SA, Waxman SG, Klinge E. Dorsal-ventral differences in the glia limitans of the spinal cord: An ultrastructural study in developing normal and irradiated rats. *J Neuropathol Exp Neurol* 1985;44:415-29
- Gilmore SA, Duncan D. On the presence of peripheral-like nervous and connective tissue within irradiated spinal cord. *Anat Rec* 1968;160:675-90
- Gilmore SA, Sims TJ, Heard JK. Autoradiographic and ultrastructural studies of areas of spinal cord occupied by Schwann cells and Schwann cell myelin. *Brain Res* 1982;239:365-75
- Sims TJ, Gilmore SA. Interactions between intraspinal Schwann cells and the cellular constituents normally occurring in the spinal cord: An ultrastructural study in the irradiated rat. *Brain Res* 1983;276:17-30
- Gilmore SA, Sims TJ. The role of Schwann cells in the repair of glial cell deficits in the spinal cord. In: Wallace RB, Das GD, ed. *Neural transplantation and regeneration*. New York: Springer-Verlag, 1986:245-69
- Gilmore SA, Phillips NP, White P, Sims TJ. Schwann cell induction in the ventral portion of the spinal cord. *Brain Res Bull* 1993;30:339-45
- Peters A, Palay SL, Webster HD. *The fine structure of the nervous system. Neurons and their supporting cells*. New York: Oxford University Press, 1991:274-97
- Gilmore SA, Heard JK, Leiting JE. Patterns of x-radiation induced Schwann cell development in spinal cords of immature rats. *Anat Rec* 1983;205:313-19
- Heard JK, Gilmore SA. A comparison of histopathologic changes following x-irradiation of mid-thoracic and lumbosacral levels of neonatal rat spinal cord. *Anat Rec* 1985;211:198-204
- Gilmore SA, Sims TJ, Davies DL, Durgun MB. Microglial development is altered in immature spinal cord by exposure to radiation. *Int J Devl Neurosci* 1997;15:1-14
- Gilmore SA, Sims TJ. Glial-glia and glial-neuronal interfaces in radiation-induced, glia-depleted spinal cord. *J Anat* 1997;190:5-21
- Gilmore SA. Autoradiographic studies of intramedullary Schwann cells in irradiated spinal cords of immature rats. *Anat Rec* 1971;171:517-28
- Heard JK, Gilmore SA. Intramedullary Schwann cell development following x-irradiation of mid-thoracic and lumbosacral spinal cord levels in immature rats. *Anat Rec* 1980;197:85-93
- Adelman LS, Aronson SM. Intramedullary nerve fiber and Schwann cell proliferation within the spinal cord (schwannosis). *Neurology*, 1972;22:726-31
- Kamiya M, Hashizume Y. Pathological studies of aberrant peripheral nerve bundles of spinal cords. *Acta Neuropathol* 1989;79:18-22
- Feigin I, Ogata J. Schwann cells and peripheral myelin within human central nervous tissues: The mesenchymal character of Schwann cells. *J Neuropathol Exp Neurol* 1971;30:603-12
- Klintonworth GK. Axon regeneration in the human spinal cord with formation of neuromata. *J Neuropathol Exp Neurol* 1964;23:127-34
- Sims TJ, Davies DL, Gilmore SA. Glial development in primary cultures established from normal and x-irradiated neonatal spinal cord. *Glia* 1994;12:319-28
- Scaravilli SL, Myers R. X-irradiation impairs regeneration of peripheral nerve across a gap. *J Neurocytol* 1986;15:439-49
- Baron-Van Evercooren A, Clerin-Duhamel E, Boutry JM, Hauw JJ, Gumpel M. Pathways of migration of transplanted Schwann cells in the demyelinated mouse spinal cord. *J Neurosci Res* 1993;35:428-38
- Franklin RJM, Blakemore WF. Migration of Schwann cells: Requirements for Schwann cell migration within CNS environments: A viewpoint. *Int. J. Devl. Neurosci* 1993;11:641-49

24. Brook GA, Lawrence JM, Raisman G. Morphology and migration of cultured Schwann cells transplanted into the fimbria and hippocampus in adult rats. *Glia* 1993;9:292-304
25. Baron-Van Evercooren A, Avellana-Adalid V, Ben Younes-Chenoufi A, Gansmuller A, Nait-Oumesmar B, Vignais L. Cell-cell interactions during the migration of myelin-forming cells transplanted in the demyelinated spinal cord. *Glia* 1996;16:147-64
26. Reese TS, Karnovsky MJ. Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J Cell Biol* 1967;34:207-17
27. Brightman MW, Reese TS. Junctions between intimately apposed cell membranes in the vertebrate brain. *J Cell Biol* 1969;40:648-77
28. Kacem K, Lacombe P, Seylaz J, Bonvento G. Structural organization of the perivascular astrocyte end feet and their relationship with the endothelial glucose transporter: a confocal microscopy study. *Glia* 1998;23:1-10
29. Felts PA, Smith KJ. Blood-brain barrier permeability in astrocyte-free regions of the central nervous system remyelinated by Schwann cells. *Neuroscience* 1996;75:643-55
30. Blakemore WF. Invasion of Schwann cells into the spinal cord of the rat following local injections of lysolecithin. *Neuropathol Appl Neurobiol* 1976;2:21-39
31. Honmou O, Felts PA, Waxman SG, Kocsis JD. Restoration of normal conduction properties in demyelinated spinal cord axons in the adult rat by transplantation of exogenous Schwann cells. *J Neurosci* 1996;16:3199-208
32. Gilmore SA, Durgun MD, Sims TJ. Schwann cell-neuron relationships in spinal cord gray matter. *Glia* 1996;18:261-68

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