Normal Basal Laminas Are Realized on Dystrophic Schwann Cells in Dystrophic ↔ Shiverer Chimera Nerves

ALAN C. PETERSON and GARTH M. BRAY
Department of Neurology and Neurosurgery, McGill University, The Neuroscience Unit, Montreal General Hospital Research Institute, Montreal, Quebec Canada H3G 1A4

ABSTRACT   Multiple discontinuities are observed in the basal laminas of Schwann cells in mature dystrophic mice. To explore the pathogenesis of this abnormality we have exploited a dystrophic ↔ shiverer mouse chimera preparation in which both the basal lamina phenotype and the genotype of myelin-forming Schwann cells can be determined. If the basal lamina abnormality were to arise from an intrinsic deficiency of the dystrophic Schwann cell itself, only those Schwann cells of dystrophic genotype could express the mutant phenotype, whereas the coexisting population of shiverer Schwann cells should express typically normal basal laminas. No such distinction was observed; rather both dystrophic and shiverer Schwann cells were found to express relatively normal basal laminas and two pathogenetic mechanisms remain theoretical possibilities. The dystrophic Schwann cell population may be intrinsically defective but also may be rescued by obtaining the normal product of the *dy* locus synthesized by the coexisting shiverer cells. Alternatively, an extra Schwann cell deficiency existing within dystrophic mice may be normalized by shiverer cells and the normal intrinsic potential of both dystrophic and shiverer Schwann cells can then be realized. Regardless of the exact mechanism underlying these findings, some extracellularly mediated influence, emanating in vivo from shiverer cells, is capable of ameliorating the basal lamina deficiency typically expressed by dystrophic Schwann cells.

In the peripheral nervous system of mature dystrophic mice, two striking morphological abnormalities can be recognized. In certain spinal roots and cranial nerves, Schwann cells fail to separate and ensheath axons so that groups of closely apposed naked axons, normally seen only during early development, persist (1, 2). In the remainder of the dystrophic peripheral nervous system, relatively normal axon–Schwann cell relationships are achieved (3), but the basal laminas covering both myelinated and unmyelinated fibers have multiple small gaps (4).

Both of these morphological abnormalities are phenotypic deficiencies specific to Schwann cells; the presence of naked axons results from a failure of the Schwann cell population to separate and ensheath the axons during primary development, and abnormal basal lamina has been observed only on the surface of Schwann cells. Various experimental manipulations of the peripheral nervous system in dystrophic mice have indicated that both of these "Schwann cell" deficiencies can be modified during regeneration (5, 6) and in tissue cultures (7, 8). Nevertheless, it is not yet known what cell type

or types are primarily responsible for the pathology observed in vivo. Although the in vitro experiments have indicated that endoneurial fibroblasts exert an important influence on the development of basal laminas by dystrophic Schwann cells, either the Schwann cell itself, a defect expressed exclusively by some extra Schwann cell type, or a more complex interaction between both Schwann cells and some extra glial element sharing the same intrinsic deficiency may be involved.

In an attempt to dissect this pathogenetic sequence, we have examined the basal lamina expressed within the peripheral nerves of chimeras. If the basal lamina abnormalities on Schwann cells in dystrophic mice were entirely a consequence of an intrinsic defect expressed by the Schwann cell itself, we would predict that within these chimera nerves only those Schwann cells of dystrophic genotype could express patchy basal laminas. However, if some component extrinsic to the Schwann cell itself were involved in the pathogenesis of this basal lamina abnormality, no such direct correlation would be expected.

In dystrophic mice, Schwann cells of myelinated nerve fibers express P1 myelin basic protein (MBP)[1] (9), whereas, in shiverer mice, the MBP structural gene is abnormal (10), and shiverer Schwann cells, although otherwise affected only with subtle ultrastructural abnormalities (11, 12), contain no detectable P1 MBP (13). MBP is detectable with immunocytochemical techniques, and therefore the genotype of every myelinated Schwann cell within dystrophic ↔ shiverer chimera nerves can be established. Moreover, this procedure can be performed on sections of nerves embedded in plastic, and adjacent ultrathin sections permit an ultrastructural evaluation of the basal lamina phenotype expressed on both the dystrophic (P1 positive) and shiverer (P1 negative) Schwann cells coexisting within the same chimera nerve. Thus, the object of the experiment reported here was to determine whether or not the abnormalities expressed by Schwann cells in dystrophic mice would be reproduced in dystrophic ↔ shiverer chimeras, and if the mutant or normal phenotype of each Schwann cell would correlate directly with its genotype.

MATERIALS AND METHODS

Mice: The dystrophic colony was maintained by $dy^{2J}/dy^{2J} \times dy^{2J}/+$ matings or by $dy^{2J}/dy^{2J} \times dy^{2J}/dy^{2J}$ matings. The dy^{2J} allele is maintained on the C57BL/6J background.

The shiverer colony was founded by $shi/+$ mice generously provided by Dr. T. Bird, Veteran's Administration Hospital, Seattle, WA. These mice were derived from multiple crosses between SWV, the strain in which the shiverer mutation occurred, and ICR. Since 1979, this stock has been maintained as a closed colony in the animal facility of the Montreal General Hospital Research Institute. The colony of shiverer mice was maintained by heterozygote ($shi/+$) matings and by $shi/+ \times shi/shi$ matings (usually successful if the male was ~3 mo old).

$dy^{2J}/dy^{2J} \leftrightarrow shi/shi$ chimeras were produced by techniques previously described (14). Embryos derived from $dy^{2J}/dy^{2J} \times dy^{2J}/dy^{2J}$ and $shi/shi \times shi/+$ matings were aggregated at the eight-cell stage, cultured for 1 d, and surgically transplanted to the uterus of pseudopregnant females to complete in utero development. Seven chimeras were produced; coat color mosaicism and strain-specific isozymes of glucosephosphate isomerase demonstrated the presence of both cell lines in each of these mice. However, as the embryos obtained from the shiverer stock were derived from $shi/shi \times shi/+$ matings, only one-half of these chimeras were expected to contain shi/shi cells, whereas the remainder should contain $shi/+$ cells. To establish the actual genotype of the chimeras we relied upon the immunocytochemical demonstration of two clearly demarcated classes of myelinated Schwann cells (MBP positive and MBP negative) within their peripheral nerves. Of the six chimeras analyzed, three were determined to be $dy^{2J}/dy^{2J} \leftrightarrow shi/shi$. These mice, identified as chimeras A, B, and C, were killed at 12 mo (chimeras A and B) and at 4 mo (chimera C). The presence of some myelin sheaths with an interdemiate density of reaction product, and the failure to detect any P1-negative cells in the nerves of the three others was taken as evidence that their genotype was $dy^{2J}/dy^{2J} \leftrightarrow shi/+$.

Only one of the seven chimeras produced for this study expressed gross behavioral abnormalities similar to a shiverer homozygote. It died at 9 mo of age and was not analyzed. Of the remaining chimeras, only 1 (chimera A) revealed any indication of a manifesting disease by a slightly asymmetrical flexion assumed by the hind limbs when it was suspended by the tail.

Sample Preparations: The chimeras were anesthetized with sodium pentobarbital (M.T.C. Pharmaceuticals, Hamilton, Canada) and systemically perfused with 1.5% glutaraldehyde and 1% paraformaldehyde in a 0.2 M phosphate buffer at pH 7.4. Spinal roots and sciatic nerves were recovered within 1 h and immersed in cold (4°C) 2% osmium tetroxide in a 0.1 M phosphate buffer (pH 7.6) for an additional 18–24 h. Each nerve was cut into segments 5–10 mm long and embedded in epoxy resin (Epon) by standard procedures. All materials were supplied by J. B. Electron Microscopy Services Inc., Dorval, Canada.

Light Microscopy: 0.5-μm-thick cross sections were cut with glass knives on a Reichert ultramicrotome (C. Reichert, Wien, Austria) and stained with toluidine blue (Fisher Scientific Co. Ltd., St. Foy, Canada). Sections from at least one block from each nerve were examined by light microscopy, particularly for the presence of ensheathment defects.

---

[1] *Abbreviation used in this paper:* MBP, myelin basic protein.

Immunocytochemistry: The primary antiserum used throughout this study was raised in rabbits against the 18,500-mol-wt species of bovine central nervous system MBP and cross-reacts with all four bovine central nervous system MBP species as well as the 18,500-mol-wt P1 MBP of rodent peripheral nervous system. The antisera was generously provided by Dr. P. Braun, Department of Biochemistry, McGill University. A full description of the immunocytochemical techniques used including the results of all control experiments has been presented elsewhere (15). In brief, 2-μm-thick sections were cut and mounted on 22-mm square cover slips and dried at 60°C for a minimum of 4 h. To prepare the sections for immunocytochemistry, the Epon was removed by immersing the coverslips in sodium ethoxide, diluted 1:3 with absolute ethanol, for 10–30 min. The coverslips were then washed in absolute ethanol (four times for 2 min), immersed in 0.1% H_2O_2 for 5 min at room temperature, and washed (three times for 10 min) with 0.5 M Tris HCL, pH 7.6. The coverslips were then drained, and each section was covered first with 100 μl of 3% normal goat serum (Sternberger-Meyer, Gaithersberg, MD) in 0.5 M Tris HCL, pH 7.6, and incubated for 30 min at room temperature followed by 50 μl of P1 antiserum (1:1000) dilution, 0.5 M Tris HCL, pH 7.6, containing 1% NGS) and incubated in a humidified chamber at 4°C overnight (16). Peroxidase-antiperoxidase immunocytochemical detection of bound rabbit antibody was then performed by standard procedures (17) and the sections were subsequently viewed with a Leitz Orthoplan microscope (E. Leitz [Canada] Ltd., Midland, Canada).

Electron Microscopy: Uultrathin sections from the blocks containing chimera nerves were mounted on copper grids, stained with lead citrate and uranyl acetate, and viewed with a Siemens 201 electron microscope (Siemens, Berlin, Federal Republic of Germany). Typically, each block was serially sectioned in the following sequence: one 0.5-μm section for light microscopy, two 2-μm sections for immunocytochemical detection of P1-positive Schwann cells, and 5–10 ultrathin sections for ultrastructural analysis. A demonstration of the experimental approach is presented in Fig. 1.

Basal Lamina Analysis: The basal laminas of P1-positive and P1-negative Schwann cells were analyzed by identifying the same myelinated fibers in both semithin cross sections of nerve processed for P1 immunocytochemistry and in adjacent ultrathin sections examined by electron microscopy. Each fiber showing adequate ultrastructural myelin preservation was assigned a number that was recorded directly on a light micrograph of the same fiber in the adjacent immunocytochemical preparation.

The electron micrographs were printed at ×50,000 for spinal root samples and at ×30,000 for sciatic nerve samples. As revealed by light microscopy, both grossly normal nerves and others with morphological abnormalities (i.e., naked axons) were included in the sample.

Basal lamina measurements on each cell included the length of plasma membrane observable, the total length of the basal lamina corresponding to the plasma membrane, and the number and size of the gaps present in the basal lamina. Only those areas of the microphaph in which plasma membrane was clearly observable, typically corresponding to regions of the cell in which myelin lamellae were also clearly demarcated, were included in the measurements. After defining these parameters on each myelinated fiber surface in the cross-section electron micrographs, the actual measurements were obtained with a Zeiss IBAS image analyzer (Carl Zeiss, Oberkochen, Federal Republic of Germany). The micrographs were placed directly on a digitizing tablet and the appropriate contours were traced with a magnetic pen. The genotype of the Schwann cells represented in the electron micrographs was unknown to the individual measuring these parameters.

Basal Lamina Control Measurements: To assess the potential effects of both the particular fixation protocol adopted for P1 immunocytochemistry, and the chimera preparation itself, one dy^{2J}/dy^{2J} and one shi/shi mouse as well as one $+/+ \leftrightarrow shi/shi$ chimera were similarly prepared and examined. From the chimera nerves, basal lamina measurements were obtained from 104 $+/+$ and 105 shi/shi Schwann cells identified by their P1 MBP phenotype. From the dystrophic mouse, 102 Schwann cells were similarly analyzed. All results compared in this study were analyzed for statistical significance by using the Mann–Whitney U test.

RESULTS

Light Microscopy

Cross sections from the sciatic nerves and the midportion of all available ventral and dorsal roots were examined. The one chimera (A) with suspected behavioral deficiencies had small bundles of naked axons in both left and right L4 ventral roots. Although all other nerves were generally unremarkable, an occasional isolated axon without appropriate ensheathment was also found in several spinal roots. Thus, the
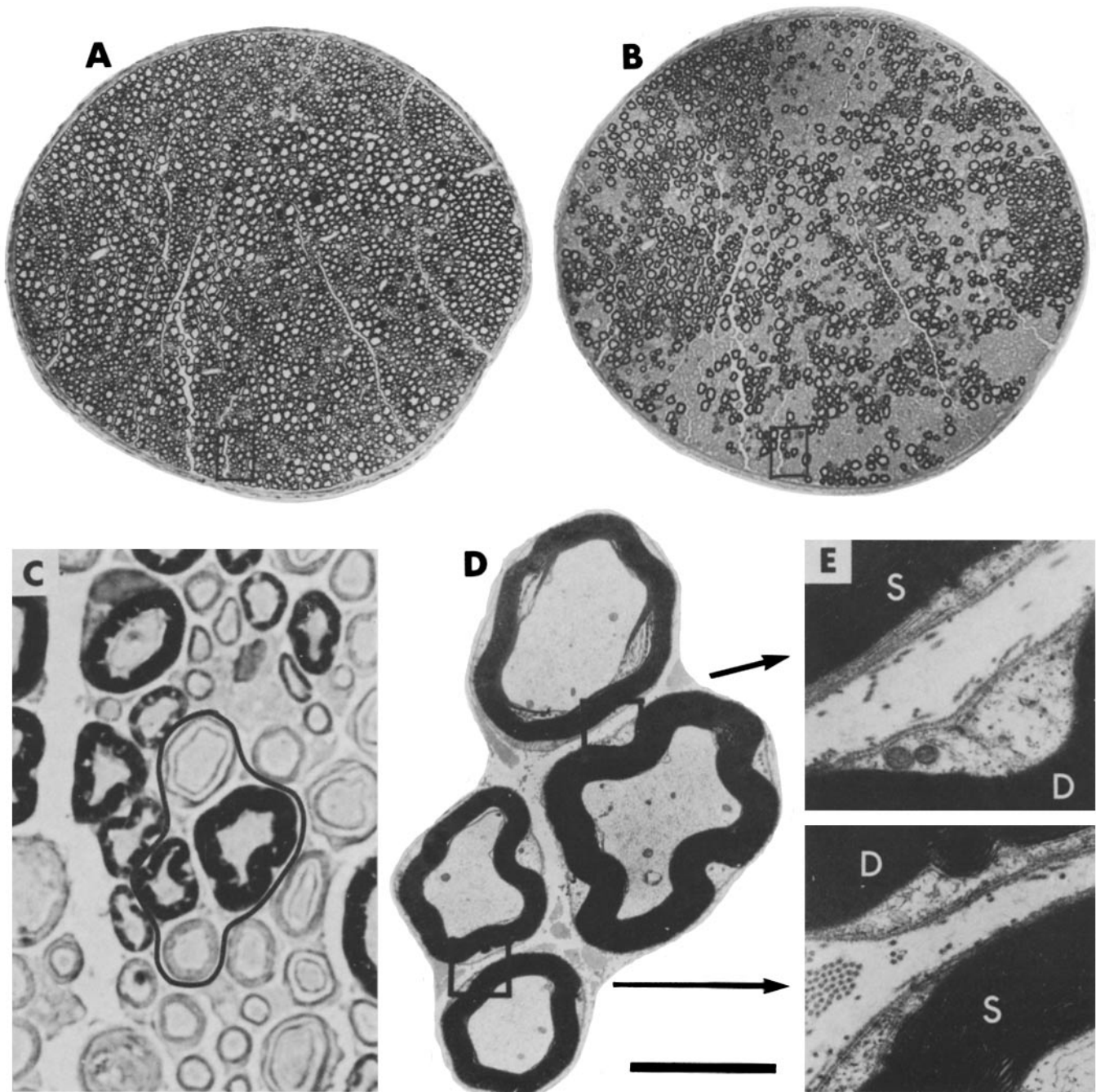
FIGURE 1   Demonstration of experimental approach and typical results. Serial semithin cross sections of a dystrophic ↔ shiverer sciatic nerve are analyzed by light microscopy for structure using toluidine blue stain (A) and for Schwann cell genotype by P1 MBP immunocytochemistry (B). Myelin-forming cells derived from the dystrophic cell line express P1 MBP and bind antibody detected by peroxidase-antiperoxidase immunocytochemistry. Shiverer Schwann cells do not express P1 MBP and remain unstained. Thin sections obtained from the immediately adjacent block face are viewed by electron microscopy; cells identified in both the immunocytochemical preparation (C also outlined in A and B) and in electron micrographs (D) and photographed at high magnification (E) for subsequent analysis of basal lamina. Partial profiles of the two dystrophic (indicated on myelin profiles with D) and the two shiverer (S) Schwann cells demonstrate intact basal laminas. Calibration bar (lower right of plate) represents: 200 μm (A and B); 12.0 μm (C); 5.4 μm (D); and 1.0 μm (E).

Schwann cell basal lamina phenotypes expressed by shiverer and dystrophic Schwann cells within both grossly normal and abnormal roots in the same chimera were compared along with the basal lamina phenotype expressed by Schwann cells existing in the other chimeras whose nerves appeared normal at this level of analysis.

Immunocytochemistry

A total of 59 nerves (roots and sciatic) from three dy^{2J}/dy^{2J} ↔ shi/shi chimeras have been examined for the proportion and distribution of P1-positive and negative Schwann cells. Although the proportion of each type was found to vary

considerably between nerves within the same chimera and between the same nerves in different chimeras, all nerves examined were mosaic. This circumstances permitted a comparison of the basal lamina phenotypes expressed in nerves dominated by each genotype of Schwann cell.

A full description the Schwann cell mosaicism and the ensheathment properties of the nerves of these chimeras has been submitted for publication elsewhere.

Basal Lamina Measurements

The ultrastructural appearance of the basal laminas realized by Schwann cells in dystrophic and shiverer mice is presented in Fig. 2. Schwann cells within the spinal roots (L4V and L4D) and sciatic nerve of the dystrophic mouse have typical discontinuities of their basal laminas whereas the basal lamina in Schwann cells from shiverer mice is intact, demonstrating that the fixation protocol for optimal immunocytochemical detection of MBP P1 did not affect the visualization of the dystrophic abnormality. Within the dystrophic mouse, the average basal lamina coverage on Schwann cells in spinal roots was ~70% (65–73% depending upon the parameters averaged) whereas in sciatic nerve, the deficiency was relatively less pronounced with 84% of the plasma membranes covered (Table I). Within the mosaic nerves of the control +/+ ↔ shi/shi chimera, both shi/shi and +/+ Schwann cells expressed ultrastructurally normal basal laminas. Although the occasional discontinuity in basal laminas was observed, particularly in spinal root samples, the average plasma membrane coverage was 98.6% for both genotypes of Schwann cells. Thus, neither the shiverer mutant nor the chimera preparation itself appears to affect the normal realization of Schwann cell basal lamina.

Among the 3 dy^{2J}/dy^{2J} ↔ shi/shi chimeras, no obvious differences in the ultrastructural appearance of basal lamina on the dy^{2J}/dy^{2J} and shi/shi cells were apparent; typical

results from a sciatic nerve sample are included in Fig. 1 and from a spinal root sample in Fig. 3. Both genotypes of Schwann cell appeared to express morphologically normal basal lamina. This visual impression is confirmed by the quantitative results (Table I). No significant differences in basal lamina measurements were found in all possible comparisons of the total Schwann cell populations sampled in each of chimeras A, B, and C and the control chimera. However, all chimera results were significantly greater than the results obtained from the dystrophic mouse.

To determine if any differences existed between the dystrophic and shiverer Schwann cells existing within single chimera nerves, a statistical comparison between the results obtained for the two types of cells in each nerve was performed. In all but one case, these dystrophic vs. shiverer comparisons were not significant. In the right L4V of chimera A, a significant difference was observed, but in this case the shiverer population expressed the lower mean basal lamina coverage.

To ascertain if the basal lamina phenotype expressed by Schwann cells in roots containing naked axons differed from that expressed in morphologically normal roots, the combined samples from the left and right L4V roots of chimera A were compared in all possible combinations with the L4V root samples of chimera B, C, and control. The dystrophic Schwann cells in the L4V roots of chimera A did not differ from either the normal or shiverer cells in the L4V ventral roots of the control chimera. However, all other comparisons were significantly different. Despite the observations that the dystrophic Schwann cells in chimera A express a significantly improved basal lamina phenotype, this result could indicate that the extent of that amelioration may not be total in roots expressing other features of the dystrophic neuropathy.

The right L4 dorsal root of chimera A had the highest proportion (89%) of genotypically dystrophic Schwann cells. Whereas the basal lamina measurements obtained from this
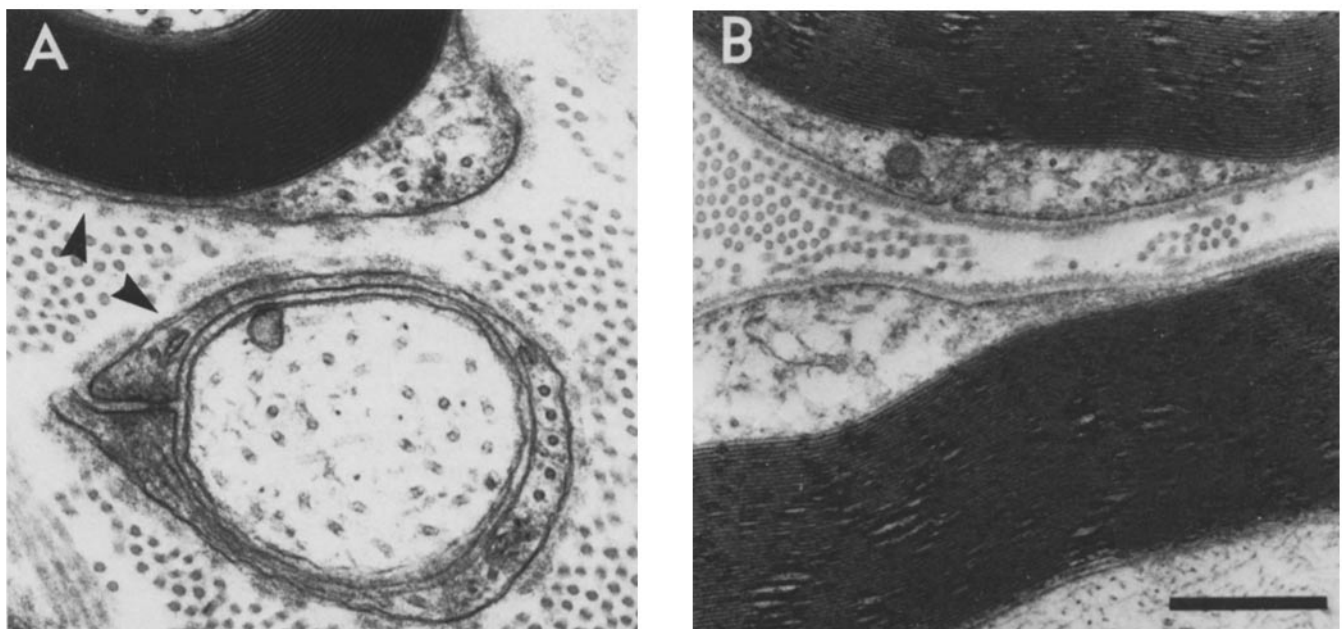

FIGURE 2 (A) Typical Schwann cell abnormalities expressed in dystrophic mice. Multiple gaps in Schwann cell basal lamina (examples indicated by arrows) are observed on both myelinated and unmyelinated fibers. (B) Schwann cells in shiverer mice express intact basal laminas. The MBP-deficient myelin in shiverer Schwann cells consistently reveals multiple small interlamellar gaps (12). Bar, 0.4 μm.

TABLE I
Schwann Cell Lamina Measurements

Schwann cell source			Distance/cell in μm (X̄ ± SD)			Total distance/cell type in μm		
Nerve	Geno-type	No.	Plasma membrane	Basal lam-ina gaps	% Coverage	Plasma membrane	Basal lamina gaps	% Coverage
C57BL/6 dy²ᴶ/dy²ᴶ								
Sci(L)	Dy.	46	4.2 ± 1.7	0.7 ± 0.5	84.8 ± 8.5	191.1	30.0	84.4%
L4V(L)	Dy.	26	6.2 ± 2.3	1.7 ± 1.9	74.8 ± 25.3	161.3	44.0	72.7%
L4D(L)	Dy.	30	4.0 ± 1.4	1.4 ± 0.7	64.5 ± 14.3	121.3	42.0	65.4%
					X̄ Dy = 76.1 ± 18.0	Σ dy = 473.7	116.0	75.5%
±/± ↔ shi/shi								
Sci(L)	+	40	5.2 ± 1.5	0.3 ± 0.1	99.4 ± 1.0	209.4	1.4	99.3%
	Shi.	40	5.8 ± 1.7	0.3 ± 0.1	99.5 ± 0.8	230.0	1.3	99.4%
L4V(R)	+	31	4.9 ± 1.5	0.1 ± 0.1	97.5 ± 2.2	153.1	3.8	97.5%
	Shi.	32	5.7 ± 1.4	0.1 ± 0.1	97.8 ± 2.1	183.5	4.0	97.8%
L4D(R)	+	33	4.2 ± 1.6	0.1 ± 0.7	98.7 ± 1.4	139.6	2.0	98.6%
	Shi.	33	4.0 ± 1.4	0.1 ± 0.1	98.3 ± 2.0	130.4	2.2	98.3%
					X̄ + = 98.6 ± 1.8	Σ + = 502.2	7.2	98.6%
					X̄ shi. = 98.6 ± 1.8	Σ shi. = 543.9	7.5	98.6%
Chimera A dy²ᴶ/dy²ᴶ ↔ shi/shi								
Sci(L)	Dy.	26	6.6 ± 2.2	0.2 ± 0.3	96.9 ± 6.0	170.5	4.4	97.4%
	Shi.	25	7.2 ± 3.1	0.2 ± 0.3	96.6 ± 6.1	179.3	4.9	97.3%
L4V(L)	Dy.	51	5.8 ± 2.7	0.2 ± 0.2	95.9 ± 3.2	296.7	12.3	95.9%
	Shi.	48	5.5 ± 5.2	0.3 ± 0.4	93.4 ± 6.7	263.6	15.0	94.3%
L4V(R)	Dy.	30	5.3 ± 1.2	0.1 ± 0.1	97.9 ± 2.0	158.6	3.2	98.0%
	Shi.	32	4.8 ± 1.4	0.3 ± 0.3	93.8 ± 5.8	154.0	9.3	94.0%
L4D(L)	Dy.	33	4.2 ± 1.8	0.1 ± 0.2	97.0 ± 3.4	138.7	4.2	97.0%
	Shi.	30	4.7 ± 1.7	0.1 ± 0.1	97.2 ± 2.3	139.5	3.6	97.4%
L4D(R)	Dy.	33	4.8 ± 1.8	0.1 ± 0.1	97.6 ± 3.2	159.7	3.5	97.8%
	Shi.	50	4.9 ± 1.4	0.1 ± 0.2	97.8 ± 2.6	245.9	5.8	97.6%
					X̄ Dy. = 96.9 ± 3.6	Σ Dy. = 924.2	27.6	97.0%
					X̄ Shi. = 95.7 ± 5.3	Σ Shi. = 982.3	38.6	96.1%
Chimera B dy²ᴶ/dy²ᴶ ↔ shi/shi								
Sci(L)	Dy.	22	7.9 ± 4.0	0.0 ± 0.0	100.0 ± 0.0	173.6	0.0	100.0%
	Shi.	28	5.8 ± 3.4	0.0 ± 0.0	100.0 ± 0.2	162.2	0.1	99.9%
L4V(L)	Dy.	29	5.3 ± 1.8	0.0 ± 0.1	99.4 ± 1.5	153.6	1.0	99.4%
	Shi.	35	5.4 ± 1.9	0.1 ± 0.5	99.6 ± 1.1	190.9	0.7	99.6%
L4D(L)	Dy.	36	6.0 ± 1.7	0.0 ± 0.0	99.8 ± 0.5	216.4	0.4	99.8%
	Shi.	40	5.7 ± 1.9	0.0 ± 0.6	99.8 ± 0.8	227.2	0.7	99.7%
					X̄ Dy. = 99.7 ± 0.9	Σ Dy. = 543.6	1.4	99.7%
					X̄ Shi. = 99.9 ± 0.8	Σ Shi. = 580.3	1.4	99.8%
Chimera C dy²ᴶ/dy²ᴶ ↔ shi/shi								
Sci(L)	Dy.	31	5.4 ± 1.9	0.2 ± 0.1	99.6 ± 1.1	168.3	0.7	99.6%
	Shi.	31	5.4 ± 1.7	0.0 ± 0.1	99.6 ± 1.2	168.7	0.6	99.6%
L4V(L)	Dy.	34	6.5 ± 1.8	0.0 ± 0.1	99.6 ± 1.0	219.8	1.0	99.5%
	Shi.	32	6.3 ± 1.8	0.1 ± 0.1	99.0 ± 1.9	200.8	2.3	98.9%
L4D(L)	Dy.	21	6.2 ± 2.7	0.0 ± 0.0	100.0 ± 0.0	131.1	0.0	100.0%
	Shi.	31	4.9 ± 2.4	0.1 ± 0.1	99.6 ± 1.2	151.6	0.5	99.7%
					X̄ Dy. = 99.7 ± 0.9	Σ Dy. = 519.2	1.7	99.7%
					X̄ Shi. = 99.4 ± 1.5	Σ Shi. = 521.1	3.4	99.3%
					Grand total A+B+C	Σ Dy. = 1987.0	30.7	98.5%
						Σ Shi. = 2083.6	43.4	97.9%

Schwann cell lamina measurements obtained from Schwann cells in dystrophic (dy), normal (+) ↔ shiverer (shi) chimera and dystrophic ↔ shiverer chimera nerves. Sci = sciatic nerve; L4 = fourth lumbar root; D = dorsal; V = ventral. Left and right sides are indicated by (L) and (R) respectively.

root were deficient relative to the dorsal roots of chimera B (26% dystrophic Schwann cells) and chimera C (9% dystrophic Schwann cells), they were similar to the values obtained from the control chimera (15% +/+ cells and 85% shi/shi cells). Therefore, no convincing correlation between the proportion of dystrophic Schwann cells existing within each nerve and relative basal lamina coverage is revealed by this analysis.
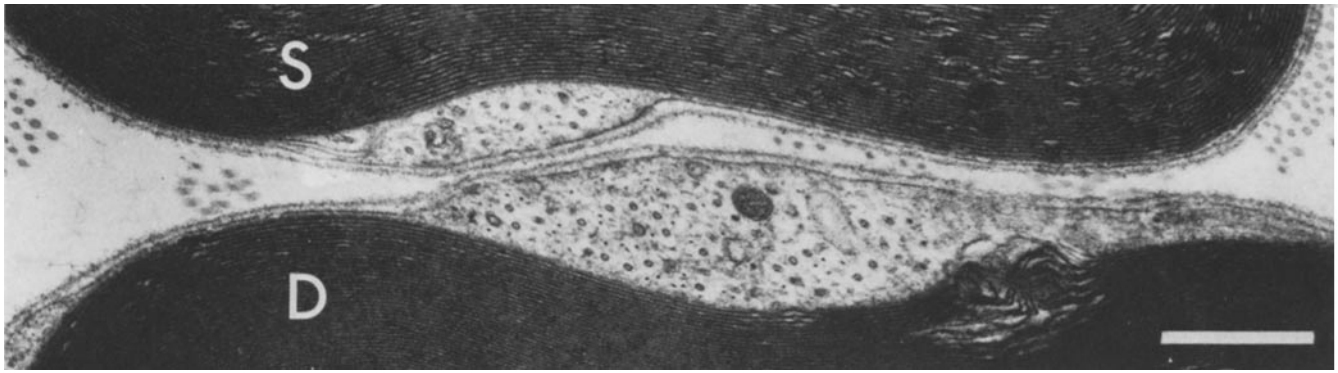
FIGURE 3   Intact basal laminae are expressed by shiverer (S) and dystrophic (D) Schwann cells in chimera spinal roots (Left L4D, chimera C). Bar, 0.4 μm.

DISCUSSION

In the peripheral nerves of dystrophic ↔ shiverer chimeras we have observed a striking amelioration of the basal lamina defect typically expressed by Schwann cells in dystrophic mice. As these dystrophic Schwann cells have undergone their entire primary development in a chimera preparation without further experimental manipulation, this result indicates that an influence extrinsic to dystrophic Schwann cells and emanating from unspecified shiverer cells is capable of conferring an apparently normal phenotype to the dystrophic Schwann cell. This result could therefore indicate that a primary genetic defect in some other cell type is entirely responsible for the abnormalities expressed by Schwann cells in dystrophic mice. However, if the genotype of the Schwann cell is not a relevant component of the pathogenesis, shiverer Schwann cells in this chimera preparation could also respond to such an abnormal extrinsic influence and express abnormal basal laminas. Although some shiverer Schwann cells in chimera A were partially deficient in basal lamina coverage, they nonetheless expressed relatively normal basal laminas compared with those of Schwann cells in dystrophic mice. Therefore, it is not yet possible to conclude that some extra Schwann cell component is capable of imposing the basal lamina deficiency on otherwise normal Schwann cells, and it remains possible that the Schwann cell genotype is relevant in the pathogenesis of the disease. Nonetheless, our results clearly demonstrate that the in vivo phenotype of dystrophic Schwann cells can be substantially ameliorated by an unknown influence emanating from a population of nondystrophic cells simply coexisting in the same mouse.

The results of two other experimental strategies have also revealed that the basal lamina phenotype expressed by dystrophic Schwann cells can be modified. Bunge and co-workers (5, 6, 18, 19) have demonstrated that dystrophic Schwann cells, but not genotypically normal Schwann cells, express basal lamina deficiencies when grown in co-cultures of neurons and Schwann cells; the normal or dystrophic genotype of the neurons in such cultures does not affect the basal lamina phenotype. However, the addition of genotypically normal fibroblasts to such cultures leads to a marked amelioration of the defect expressed by dystrophic Schwann cells. These authors therefore proposed that the primary defect in dystrophic mice may be expressed by fibroblasts (20). However, the expression of the basal lamina abnormality on dystrophic Schwann cells in culture can also be interpreted as

evidence for an intrinsic deficiency expressed by the Schwann cell itself; that is, mutant Schwann cells express their abnormality in vitro, but that phenotype can be normalized by some influence emanating from genotypically normal fibroblasts. A marked change in the phenotype expressed by dystrophic Schwann cells is also observed in regenerated nerves within the dystrophic mouse. Not only do the axons in the spinal roots of dystrophic mice become ensheathed after nerve crush (5, 6), but such regenerated fibers express intact basal laminas (6). This result requires that changes in either the local density of some cell type (e.g., fibroblasts) or the process of regeneration per se is adequate to modify this abnormality. In particular, these experiments demonstrate that the presence of genotypically normal cells is not a prerequisite for dystrophic Schwann cells to acquire a normal phenotype in vivo. In contrast, the results of the present study demonstrate that nerves undergoing their entire primary development and maturation within chimeras and without further experimental manipulations can also provide an environment in which dystrophic Schwann cells achieve a normalized phenotype.

The present chimera studies on dystrophic Schwann cells share one technical deficiency with the previous regeneration and in vitro experiments; in each case the dystrophic or normal lamina phenotype has been established entirely on ultrastructural criteria. Thus, we are not able to deduce whether the normal appearance of basal lamina achieved by dystrophic Schwann cells does, in fact, reflect basal lamina composed of all the normal components in their appropriate proportions and whether such basal laminas actually fulfill their normal function. In general, the multiple components of basal lamina are ubiquitous to the basal lamina of all cell types but little is known about their relative proportions and organization in different tissues (21). The majority of the evidence suggests that the major basal lamina components are synthesized by the local cell (22, 23). If this hypothesis is strictly true, then the influence modifying the dystrophic Schwann cell phenotype would have to act via some pathway stimulating the dystrophic Schwann cell itself. Alternatively, the chimera results may indicate that one or more components synthesized by other cells may be incorporated into the basal laminas of dystrophic Schwann cells. Such cooperation between different cell types in the realization of fibronectin fibrils has been demonstrated in co-cultures of chick myogenic cells and rat fibroblasts. Differentially labeled, species-specific, antifibronectin antibodies label the same fibrils, indicating that fibronectin released into the medium by chick myogenic

cells can be incorporated into an extracellular matrix laid down by fibroblasts (24). If such a sharing of basal lamina components were at the base of our present observations, it would further indicate that dystrophic Schwann cells express appropriate receptors for basal lamina components and that their specific deficiency involves either a quantitative or qualitative perturbation in one or more basal lamina components themselves. In the latter circumstances, the most obvious source of such shared basal lamina components would be the shiverer Schwann cells coexisting within the same nerves. However, the postulated role for fibroblasts in the amelioration of the dystrophic Schwann cell phenotype in vitro could also be operative in the in vivo manipulations that lead to normal-appearing basal laminas; the increased numbers of dystrophic fibroblasts in regenerating nerves or the presence of functionally normal fibroblasts derived from the shiverer cell line in the chimeras could provide a missing component directly to the Schwann cell or serve to provide some product necessary for the appropriate processing of a basal lamina component. Despite these multiple possibilities, the chimera results demonstrate that dystrophic Schwann cells in vivo can achieve a morphologically normal phenotype. Regardless of the source or nature of that normalizing influence, such dystrophic Schwann cells are able to obtain or express the necessary component(s) or function by existing in the environment provided within the nerves of dystrophic ↔ shiverer chimeras.

REFERENCES

1. Bradley, W. G., and M. Jenkison. 1973. Abnormalities of peripheral nerves in murine muscular dystrophy. *J. Neurol. Sci.* 18:227-247.
2. Bradley, W. G., and M. Jenkison. 1975. Neural abnormalities in the dystrophic mouse. *J. Neurol. Sci.* 25:249-255.
3. Jaros, E., and W. G. Bradley. 1979. Atypical axon–Schwann cell relationships in the common peroneal nerve of the dystrophic mouse: an ultrastructural study. *Neuropathol. Appl. Neurobiol.* 5:133-147.
4. Madrid, R. E., E. Jaros, M. J. Cullen, and W. G. Bradley. 1975. Genetically determined defect of Schwann cell basement membrane in dystrophic mouse. *Nature (Lond.).* 257:319-321.
5. Stirling, C. A. 1975. Experimentally induced myelination of amyelinated axons in dystrophic mice. *Brain Res.* 87:130-135.
6. Bray, G. M., S. David, T. Carlstedt, and A. Aguayo. 1983. Effects of crush injury on the abnormalities in the spinal roots and peripheral nerves of dystrophic mice. *Muscle Nerve* 6:497-503.
7. Bunge, M. G., A. K. Williams, P. M. Wood, J. Uitto, and J. J. Jeffrey. 1980. Comparison of nerve cell and nerve cell plus Schwann cell cultures with particular emphasis on basal lamina and collagen formation. *J. Cell Biol.* 84:184-202.
8. Cornbrooks, C. J., F. Mithen, J. M. Cochran, and R. P. Bunge. 1983. Factors affecting Schwann cell basal lamina formation in cultures of dorsal root ganglia from mice with muscular dystrophy. *Dev. Brain Res.* 6:57-67.
9. Peterson, A. C., G. M. Bray, and J. Marler. 1982. Disease expression in mosaic nerves of dystrophic ↔ shiverer mouse chimaeras. *Neurosci. Abstr.* 8:1008.
10. Roach, A., K. Boylan, S. Horvath, S. Prusiner, and L. Hood. 1983. Characterization of cloned cDNA representing rat myelin basic protein: absence of expression in brain of shiverer mutant mice. *Cell.* 34:799-806.
11. Rosenbluth, J. 1980. Peripheral myelin in the mouse mutant shiverer. *J. Comp. Neurol.* 193:729-739.
12. Peterson, A. C., and G. M. Bray. 1984. Hypomyelination in the peripheral nervous system of shiverer mice and in *shiverer* ↔ normal chimaera. *J. Comp. Neurol.* 227:348-356.
13. Kirschner, O. A., and A. L. Ganser. 1980. Compact myelin exists in the absence of basic protein in the shiverer mutant mouse. *Nature (Lond.).* 283:207-210.
14. Mullen, R. J., and W. K. Whitten. 1971. Relationship of genotype and degree of chimera in coat color to sex ratios and gametogenesis in chimeric mice. *J. Exp. Zool.* 178:165-176.
15. Peterson, A. C., and J. Marler. 1983. P1 deficiency in shiverer myelin is expressed by Schwann cells in shiverer ↔ normal mouse chimaera nerves. *Neurosci. Lett.* 38:163-168.
16. Trapp, B. D., Y. Itoyama, N. D. Sternberger, R. H. Quarles, and H. de F. Webster. 1981. Immunocytochemical localization of Po protein in Golgi complex membranes and myelin of developing rat Schwann cells. *J. Cell Biol.* 90:1-6.
17. Sternberger, L. A., P. H. Harcy, J. J. Cuculis, and H. G. Meyer. 1970. The unlabelled antibody enzyme method of immunohistochemistry. *J. Histochem. Cytochem.* 18:315-333.
18. Bunge, R. P., M. B. Bunge, E. Okada, and C. J. Cornbrooks. 1980. Abnormalities expressed in cultures prepared from peripheral nerve tissues of trembler and dystrophic mice. *In* Neurological Mutations Affecting Myelination. N. Baumnann, editor. Elsevier/ North Holland Biomedical Press, Amsterdam. 433-446.
19. Cochran, M., C. Cornbrooks, F. Mithen, and R. P. Bunge. 1979. Persistence of basal lamina defect in cultures of dystrophic mouse Schwann cells in contact with normal mouse neurons. *Neurosci. Abstr.* 5:427.
20. Bunge, R. P., M. B. Bunge, A. K. Williams, and L. K. Wartels. 1982. Does the dystrophic mouse nerve lesion result from an extracellular matrix abnormality? *In* Diseases of the Motor Unit. D. Shotland, editor. John Wiley Sons, New York. 1982 pp. 23-35.
21. Laurie, G. W., C. P. LeBlond, and G. R. Martin. 1982. Localization of type IV collagen, laminin, heparin sulfate proteoglycan, and fibronectin to the basal lamina of basement membranes. *J. Cell Biol.* 95:340-344.
22. Laurie, G. W., C. P. LeBlond, I. Cournil, and G. Martin. 1980. Immunohistochemical evidence for the intracellular formation of basement membrane collagen (Type IV) in developing tissues. *J. Histochem. Cytochem.* 28:1267-1274.
23. Farquhar, M. G. 1981. The glomerular basement membrane: A selective macromolecular filter. *In* Cell Biology of the Extracellular Matrix. E. Hay, editor. Plenum Press, New York. 335-378.
24. Chiquet, M., and D. Fambrough. 1984. Extracellular matrix assembly during muscle development studied with monoclonal antibodies. *Exp. Biol. Med.* 9:87-92.