Nonmyelinating Schwann Cell Involvement With Well-Preserved Unmyelinated Axons in Charcot-Marie-Tooth Disease Type 1A

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Abstract

Electron microscopic examination was performed to compare morphologic changes of nonmyelinating Schwann cells and unmyelinated axons in patients with Charcot-Marie-Tooth disease type 1A (CMT1A) with peripheral myelin protein 22 duplication (n = 27) and normal control individuals (n = 14). Complete transverse sural nerve cross-sections were obtained in 16 patients and the total number of axons and Schwann cells in each cross-section was estimated. In patients with CMT1A, the number of myelinated axons was significantly decreased, whereas unmyelinated axons were well-preserved and did not show any marked changes. The numbers of nuclei, subunits, and profiles of nonmyelinating Schwann cells were all increased significantly in patients with CMT1A, whereas the numbers of axons per unmyelinated axon-containing subunit were significantly decreased. Schwann cell subunits consisted of layers of flattened cytoplasmic profiles wrapped around unmyelinated axons in the patient with CMT1A. The numbers of nonmyelinating Schwann cell profiles were increased and the numbers of axons per unmyelinated axon-containing subunit were reduced even in young patients with well-preserved myelinated fibers. In conclusion, there is marked alteration of the population and morphology of nonmyelinating Schwann cells and unmyelinated fibers in CMT1A.

Key Words: Axon-Schwann cell interactions, Axonal damage, CMT1A, Neuropathy, Nonmyelinating Schwann cells, Unmyelinated fibers.

INTRODUCTION

Charcot-Marie-Tooth disease type 1A (CMT1A) is an inherited demyelinating disorder of the peripheral nervous system. It is most frequently caused by overexpression of the gene encoding peripheral myelin protein 22 (PMP22) due to duplication of a 1.5-Mb region on chromosome 17p11.2 (1). Because PMP22 is one of the major constituents of myelin, its role in Schwann cells associated with myelinated fibers (myelinating Schwann cells) has been investigated extensively (2–5). On histologic examination, the predominant feature of CMT1A is demyelination that is accompanied by onion-bulb formation due to hyperplasia of myelinating Schwann cells (6). Because axon-Schwann cell interactions are important for the development, function, and maintenance of peripheral nerves, demyelination is thought to induce axonal damage that results in the loss of myelinated axons in CMT1A (3–5, 7–9), although the precise mechanism of axonal degeneration is not well understood. Because axonal loss is the major factor leading to a poor functional prognosis for patients with CMT1A (6, 8, 9), it is clinically important. Previous studies concerning CMT1A have not focused on the changes of Schwann cells associated with unmyelinated fibers (nonmyelinating Schwann cells). Therefore, an understanding of the pathology of unmyelinated fibers in CMT1A is lacking. Because observation of unmyelinated fibers in CMT1A has not been based on detailed morphometric assessment (which has already been performed for myelinated fibers), it is not clear whether nonmyelinating Schwann cells, unmyelinated axons, and their interactions are affected in patients with this disease.

In the present study, we quantitatively assessed the nonmyelinating Schwann cells and unmyelinated axons in patients with CMT1A. We demonstrate that nonmyelinating Schwann cells are abnormal in CMT1A, probably owing to an intrinsic genetic defect, but found that these abnormalities did not have a substantial effect on unmyelinated axons. These findings strongly suggest that axon-Schwann cell interactions are regulated differently between myelinated and unmyelinated fibers in CMT1A.

MATERIALS AND METHODS

Patients

Twenty-seven proband CMT1A patients with 17p11.2 duplication from 27 families were investigated in this study: 14 male and 13 female patients between 2 and 74 years of age (mean ± SD: 44.0 ± 18.4 years) (Table). The PMP22
duplication was detected by Southern analysis as described previously (9–11). In some cases, fluorescence in situ hybridization was also used. Control tissue was obtained from 14 autopsy specimens from patients who died of non-neurologic diseases (male/female ratio: 4:10; age range: 5–76 years; mean ± SD: 45.4 ± 26.0 years).

**Histopathologic Study**

Sural nerve biopsy was performed as described previously (12–14). Specimens were fixed in 2.5% glutaraldehyde in 0.125 M cacodylate buffer (pH 7.4) and embedded in epoxy resin for light and electron microscopic study. Semithin sections were stained with toluidine blue for morphometric assessment with light microscopy. Densities of myelinated axons, onion bulbs, and axonal sprouting were assessed using a computer-assisted image analyzer (Luzex FS; Nikon, Tokyo, Japan) as described previously (9, 12, 13, 15). For electron microscopic studies, epoxy resin-embedded specimens were cut into ultrathin transverse sections and stained with uranyl acetate and lead citrate. For morphometric assessment, electron microscopic photographs were taken at a magnification of 4,000× in a random fashion to cover the area of ultrathin sections (12, 13, 16). These electron micrographs were enlarged to 6,500×. Ultrathin sections were obtained from at least 3 fascicles at the same plane, and the total area analyzed in electron micrographs from each nerve was at least 0.03 mm².

**Identification of Myelinating and Nonmyelinating Schwann Cells**

In patients with CMT1A, myelinating Schwann cells often proliferate to form onion bulbs with concentric arrangement of their processes as shown in Fig. 1A (6, 9, 17). At a magnification of 4,000×, it is difficult to assess the overall arrangement of Schwann cell complexes, including onion bulbs, because these structures are relatively large. Therefore, observation was also done at a magnification of 2,000× to differentiate Schwann cells associated with unmyelinated fibers (i.e. nonmyelinating Schwann cells) from those forming onion bulbs (i.e. myelinating Schwann cells). Groups of Schwann cell subunits with concentric arrangement of their processes but no central myelinated axon were counted as myelinating Schwann cells, because it was considered that a myelinated axon had previously been present at the center to form a true onion bulb (6, 17). In patients with severe loss of myelinated axons, groups of Schwann cells are not consistently associated with the axons and sometimes show only a minor degree of concentric arrangement as evidence of their origin from onion bulbs (6). Therefore, we also regarded such groups of Schwann cells as myelinating Schwann cells even if they only showed a partial concentric arrangement.

**Assessment of Unmyelinated Axon Density**

Unmyelinated axons were distinguished from Schwann cell cytoplasmic profiles by their round or oval shape, a lighter appearance than Schwann cell cytoplasm, and often a higher incidence of microtubules (18, 19). The presence of mesaxon-like structures and the greater density of the axolemma compared with the Schwann cell membrane were also used to identify unmyelinated axons (18, 20). Although unmyelinated axons were detected in association with Schwann cells forming onion bulbs (6), these axons were not assessed in the present study because it could not be determined whether such axons were derived from regenerating myelinated fibers or represented cross-innervation from unmyelinated fibers (21). Disproportionately large unmyelinated axons (>3 μm in diameter were also not counted because these fibers were considered to have originally been myelinated and to have undergone demyelination (21, 22). Furthermore, unmyelinated axons in Schwann cell subunits that previously contained myelinated axons (i.e. bands of Büngner) were not counted because they might have been sprouts from regenerating myelinated fibers (18, 23). Unmyelinated axons that took part in the formation of regenerating clusters of myelinated axons were also not counted for the same reason (21). The bands of Büngner were distinguished from subunits of nonmyelinating Schwann cells by the following morphologic criteria according to previous reports (18, 21, 24): 1) they had a larger diameter (3–8 μm) than subunits formerly containing unmyelinated axons; 2) their profiles were larger than those of the subunits of non-myelinating Schwann cells; 3) they contained remnants of myelin and lamellated inclusions that suggested the occurrence of degeneration; and 4) they had a more irregular shape and the basement membrane was folded. However, irregularly shaped Schwann cell subunits are frequently seen among the nonmyelinating Schwann cells of patients with CMT1A. Therefore, the latter criterion was not adopted for this study.

**Assessment of Neurofilaments**

To evaluate the axonal cytoskeleton of myelinated and unmyelinated fibers, we measured the density of neurofilaments (5, 25). Numbers of neurofilaments were counted in systematically sampled squares of an overlying transparency placed upon the electron microscopic photographs at a magnification of 50,000× (13). At least 30 axons were...
examined to calculate the mean density of neurofilaments in each case for both myelinated and unmyelinated fibers.

**Morphometry for Nonmyelinating Schwann Cells**

A conglomerate of Schwann cell processes with or without unmyelinated axons, which were enclosed by a continuous loop of basal lamina, was designated as a “Schwann cell subunit” as illustrated in Figure 1 (21). For Schwann cell subunits, only those of nonmyelinating Schwann cells were assessed. The small, isolated Schwann cell projections made up of a single profile unassociated with an axon, which are considered to be an outgrowth from Schwann cells and end blindly in the endoneurium, were counted separately as single protrusions as demonstrated in Figure 1 (18).

The number of Schwann cell profiles per axon in the unmyelinated axon-containing Schwann cell subunits was counted as illustrated in Figure 1 (21). At least 100 randomly selected subunits were assessed to determine the mean number of profiles for each case. The magnification used for these measurements was 10,000×. When the boundary of each cytoplasmic process of a Schwann cell was not clear, these subunits were reexamined at a magnification of 25,000×.

**Assessment of the Total Population of Axons and Schwann Cells Per Sural Nerve Cross-Section**

Because the endoneurial area is often enlarged in patients with CMT1A, we evaluated the total number of axons and Schwann cells per complete cross-section of the sural nerve as well as their densities. The total endoneurial area was assessed using an image analyzer (Luzex FS) when complete transverse sections of the sural nerve could be obtained, and the total numbers of axons and Schwann cells were estimated. To determine the total population per sural nerve cross-section, the density was multiplied by the total endoneurial area from which the subperineurial space (devoid of nerve fibers) was subtracted. We obtained complete transverse sections of the sural nerve in 16 of 27 patients, and we analyzed the total population per cross-section in these patients. Among the 14 normal control individuals we assessed total numbers per complete cross-section of the sural nerve in 11 cases. These normal control individuals were selected to match the mean age of the patients with...
CMT1A (48.2 ± 17.5 years for CMT1A, 48.5 ± 27.8 years for normal control individuals).

**Statistical Analyses**

Quantitative data were presented as the mean ± SD. Statistical analyses were performed using the Mann-Whitney U test or Pearson’s correlation coefficient analysis as appropriate. p < 0.05 was considered to indicate significance.

**RESULTS**

Morphometric indices, including the densities, from light and electron microscopic observations are listed in Supplemental Table 1. Based on these indices, the numbers of axons, Schwann cell nuclei, and Schwann cell subunits were analyzed using complete cross-sections of the sural nerve.

**Endoneurial Area, Myelinated Axons, and Unmyelinated Axons**

The fascicles were enlarged to varying degrees in the patients with CMT1A and the average total endoneurial area was almost 3 times larger than that in normal individuals (2.890 ± 1.056 vs 0.991 ± 0.141 mm²) (Supplemental Table 1).

The number of myelinated axons per cross-section of the sural nerve was significantly reduced in the patients with CMT1A compared with that in normal individuals (4,051 ± 2,453 vs 7,997 ± 1,449; p < 0.001; Fig. 2A), and axonal sprouting of myelinated fibers was observed in most cases (116 ± 85/mm²) (Supplemental Table 1). In some of the middle-aged to elderly patients, regenerating fibers were conspicuous, and there was a marked increase in the number of myelinated axons. Therefore, there was no significant age-associated change in the number of myelinated axons per cross-section of the sural nerve (Fig. 2B), although the density of myelinated axons showed a significant reduction with increasing age (r = −0.658, p < 0.001), as reported previously (17).

Accumulation of organelles in myelinated axons and remnants of myelin sheath were found in some cases, suggesting that damage to myelinated axons occurs in CMT1A (Fig. 3A, B). The neurofilament density of myelinated axons was significantly increased in CMT1A patients compared with normal individuals (142.2 ± 31.7 vs 113.5 ± 15.0 filaments/µm²; p < 0.01) (Figs. 3C, D, 4A), indicating the occurrence of axonal atrophy of myelinated fibers (25).

The number of unmyelinated axons per cross-section of the sural nerve from patients with CMT1A did not differ from that in normal individuals (30,069 ± 6,478 vs 30,700 ± 4,328; Fig. 2C). Regeneration of unmyelinated fibers as indicated by clusters of small unmyelinated axons (23, 26) was not conspicuous, even in the older patients. Ballooning of axons, accumulation of specific organelles, and other morphologic abnormalities suggesting damage to unmyelinated

**FIGURE 2.** Number of myelinated axons (A) and unmyelinated axons (C) per complete cross-section of the sural nerve and correlation with the age at biopsy (B, D) in 16 patients with Charcot-Marie-Tooth disease type 1A (CMT1A) and 11 normal control individuals. Error bars represent the standard deviation. Compared with normal individuals, patients with CMT1A had a significantly smaller number of myelinated axons (p < 0.001; A), but had well-preserved unmyelinated axons (C). Neither the number of myelinated axons (B) nor unmyelinated axons (D) showed a correlation with the age at biopsy.
axons were not apparent in any of the patients. Furthermore, no significant age-associated change in the number of unmyelinated axons was noted (Fig. 2D). In contrast to myelinated axons, the neurofilament density of unmyelinated axons was not altered (73.1 ± 12.7 vs 71.2 ± 12.9 filaments/μm²) (Figs. 4B, 5A).

**FIGURE 3.** (A–D) Representative electron microscopic photographs suggesting myelinated axonal damage in patients with Charcot-Marie-Tooth disease type 1A (CMT1A). To compare axonal cytoskeletons, a representative electron microscopic photograph from a normal control case is presented (E). Accumulation of organelles in a myelinated axon (A) and a remnant of the myelin sheath in the center of an onion bulb (B) are found. Neurofilament densities are increased in patients with CMT1A (C, D) compared with a normal individual shown in E. Scale bars = (A) 1 μm; (B) 0.5 μm; (C–E) 0.2 μm.

**FIGURE 4.** Neurofilament density of myelinated axons (A) and unmyelinated axons (B) in 27 patients with Charcot-Marie-Tooth disease type 1A (CMT1A) and 14 normal control individuals. Error bars represent the SD. Compared with normal individuals, patients with CMT1A had a significantly increased neurofilament density in their myelinated axons (p < 0.01) (A), whereas unmyelinated axons showed no difference (B).

**FIGURE 5.** The comparison of electron microscopic photographs of unmyelinated axons from a patient with Charcot-Marie-Tooth disease type 1A (CMT1A) (A) and a normal control individual (B). The neurofilament density was not increased in CMT1A patients. Scale bars = 0.5 μm.
Myelinating and Nonmyelinating Schwann Cell Populations

The numbers of nuclei of myelinating Schwann cells in complete cross-sections of the sural nerve ranged widely from 987 to 8,395 and were significantly increased compared with that in normal individuals (3,996 ± 2,150 vs 478 ± 196, \( p < 0.0001 \)) (Fig. 6A), suggesting that the degree of proliferation of myelinating Schwann cells varied extensively among the patients with CMT1A. The number of nonmyelinating Schwann cell nuclei per cross-section was also quite variable, ranging from 1,586 to 7,294, and was also significantly higher than in normal individuals (4,238 ± 1,498 vs 1,395 ± 348, \( p < 0.0001 \)) (Fig. 6C). Neither the number of myelinating nor nonmyelinating Schwann cell nuclei showed a correlation with age in the patients with CMT1A (Fig. 6B, D). The number of nonmyelinating Schwann cell nuclei showed a positive correlation with the number of myelinating Schwann cell nuclei (\( r = 0.705, p < 0.01 \)) (Fig. 6E), suggesting that nonmyelinating Schwann cells also increased as myelinating Schwann cells underwent proliferation.

Schwann Cell Subunits Associated With Unmyelinated Fibers

The number of Schwann cell subunits per complete cross-section of the sural nerve was markedly increased.

![FIGURE 6](http://jnen.oxfordjournals.org/). Numbers of myelinating (A) and nonmyelinating (C) Schwann cell nuclei per complete cross-section of the sural nerve, correlation with the age at biopsy (B, D), and correlation between the number of myelinating and nonmyelinating Schwann cell nuclei (E) in 16 patients with Charcot-Marie-Tooth disease type 1A (CMT1A) and 11 normal control individuals. Error bars represent the SD. Bold lines represent regression lines. Compared with normal individuals, patients with CMT1A showed a variable, but significant, increase of both myelinating Schwann cell nuclei (\( p < 0.0001 \)) (A) and nonmyelinating Schwann cell nuclei (\( p < 0.0001 \)) (C). Neither the number of myelinating (B) nor nonmyelinating (D) Schwann cell nuclei showed a correlation with the age at biopsy in patients with CMT1A. The number of nonmyelinating Schwann cell nuclei showed a positive correlation with the number of myelinating Schwann cell nuclei (\( r = 0.705, p < 0.01 \)) (E), suggesting that the number of nonmyelinating Schwann cells increases along with the proliferation of myelinating Schwann cells.
in the patients with CMT1A compared with the normal control individuals (6.1559 ± 2.0185 vs 2.8424 ± 1.1258, p < 0.001) (Fig. 7A). Both groups showed a trend for an increase with increasing age (r = 0.714, p < 0.01 for patients with CMT1A; r = 0.913, p < 0.0001 for normal control individuals) (Fig. 7B).

**Schwann Cell Profiles in Subunits With Unmyelinated Axons**

The number of Schwann cell profiles per axon in unmyelinated axon-containing Schwann cell subunits was markedly increased in the patients with CMT1A compared with the normal control individuals (4.41 ± 1.04 vs 2.41 ± 0.69, p < 0.0001) (Figs. 1B, 8A), suggesting that Schwann cell morphology was often abnormal in CMT1A. Most of the Schwann cell subunits consisted of layers of flattened cytoplasmic profiles wrapped around unmyelinated axons in the patients with CMT1A. An increased number of Schwann cell profiles was even observed in cases with less prominent onion-bulb formation. Not only the adult or elderly patients but also the young patients with CMT1A had highly irregular Schwann cell profiles, and the number of profiles was markedly increased even in younger patients compared with that of normal individuals (Fig. 1C). In these

**FIGURE 7.** Numbers of subunits of nonmyelinating Schwann cells per complete cross-section of the sural nerve (A) and correlation with the age at biopsy (B) in 16 patients with Charcot-Marie-Tooth disease type 1A (CMT1A) and 11 normal control individuals. Error bars represent the SD. Bold lines represent regression lines. Compared with normal individuals, the patients with CMT1A showed a significant increase in the number of subunits (p < 0.001) (A). Both patients with CMT1A and control individuals showed a tendency for the number of subunits to increase with increasing age (r = 0.714, p < 0.01 for patients with CMT1A; r = 0.913, p < 0.0001 for normal control individuals) (B).

**FIGURE 8.** Number of Schwann cell profiles per axon in unmyelinated axon-containing subunits (A) and correlation with the age at biopsy (B) in 27 patients with Charcot-Marie-Tooth disease type 1A (CMT1A) and 14 normal control individuals. (B) The results for 3 patients with the hypertrophic variant of chronic inflammatory demyelinating polyradiculoneuropathy (H-CIDP) are added as black boxes. Error bars represent the SD. Bold lines represent regression lines for the patients with CMT1A and normal control individuals. Compared with the normal control individuals, the patients with CMT1A showed a significant increase in the number of profiles (p < 0.0001) (A). The number of profiles tended to increase with age in both the patients with CMT1A and normal control individuals (r = 0.407, p < 0.05 for CMT1A; r = 0.679, p < 0.01 for normal control individuals) (B). Not only the adult/elderly but also the young patients with CMT1A showed an increase of profiles. H-CIDP is characterized by marked onion-bulb formation (32), similar to CMT1A, and was studied to evaluate the effect of proliferation of myelinating Schwann cells on nonmyelinating Schwann cells. The 3 male patients with H-CIDP (ages 47, 59, and 70 years) all showed improvement of neuropathic symptoms with steroid therapy and did not have PMP22 duplication. Specimens were processed as described in the Materials and Methods section.

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younger patients, particularly a 2-year-old subject, myelinating Schwann cells were relatively well preserved. The number of Schwann cell profiles tended to increase with age in both the patients with CMT1A and the normal control individuals ($r = 0.407$, $p < 0.05$ for patients with CMT1A; $r = 0.679$, $p < 0.01$ for normal control individuals) (Fig. 8B). Interestingly, this age-related increase was similar in both groups, suggesting that normal age-dependent changes of Schwann cell morphology also occurred in patients with CMT1A.

In the patients with CMT1A, the number of Schwann cell profiles per unmyelinated axon did not show any correlation with the number of either myelinating or nonmyelinating Schwann cell nuclei per sural nerve cross-section (data not shown). In contrast, the number of profiles was positively correlated with the number of Schwann cell subunits per sural nerve cross-section ($r = 0.658$, $p < 0.01$; data not shown). This result suggested that the increase in the number of profiles was closely related to the division or branching of Schwann cell cytoplasmic processes. Taken together, the findings suggested that the increase of Schwann cell profiles was not merely a consequence of Schwann cell branching of Schwann cell cytoplasmic processes. Taken together, the findings suggested that the increase of Schwann cell profiles was not merely a consequence of Schwann cell proliferation.

**Number of Unmyelinated Axons Per Schwann Cell Subunit**

The mean number of unmyelinated axons per axon-containing Schwann cell subunit was significantly decreased in the patients with CMT1A compared with the normal control individuals ($1.17 \pm 0.20$ vs $1.54 \pm 0.49$, $p < 0.001$) (Fig. 9A). Both groups showed a tendency for a decrease of axons with increasing age ($r = 0.849$, $p < 0.0001$ for patients with CMT1A; $r = -0.789$, $p < 0.001$ for normal control individuals) (Fig. 9B), suggesting that the physiologic aging process also occurred in the patients. However, the number of unmyelinated axons per Schwann cell subunit was even reduced in the young patients with well-preserved myelinated fibers.

**DISCUSSION**

In previous studies, it was not determined whether nonmyelinating Schwann cells and unmyelinated axons are affected in CMT1A. Making such an evaluation is somewhat difficult because the morphometric indices of nonmyelinating Schwann cells change with advancing age, even in normal subjects (21, 23). Also, the fascicular area of patients with CMT1A is larger than that of normal subjects, and such enlargement limits the accuracy of quantitative assessment. Therefore, we estimated morphometric indices for the total population of axons and Schwann cells in each complete cross-section of the sural nerve. We also assessed these morphometric indices in relation to the age at examination. Furthermore, we assessed the number of Schwann cell profiles per axon and the number of axons per axon-containing Schwann cell subunit, because these indices are little influenced by enlargement of the fascicular area. Based on these parameters, we clearly demonstrated abnormalities of nonmyelinating Schwann cells in CMT1A.

Myelinating Schwann cells are well known to undergo proliferation in CMT1A (6, 9, 17). We demonstrated that the number of nonmyelinating Schwann cell nuclei was also markedly increased in patients with CMT1A, suggesting that nonmyelinating Schwann cells proliferate as well in this disease. Wide variation in the extent of proliferation of both myelinating and nonmyelinating Schwann cells was seen in our patients with CMT1A, which may suggest that genetic factors other than PMP22 duplication also determine the phenotype of CMT1A, as described previously (27, 28). Previous studies have suggested that demyelinating nerve injury induces proliferation of Schwann cells associated with unmyelinated fibers in rats (29, 30). Degeneration of myelinated axons has been reported to produce signals that are mitogenic for nonmyelinating Schwann cells with intact axons (31). These observations suggest that the release of chemical mediators such as Schwann cell-proliferating mitogen may be increased by injury to myelinated fibers and may induce the proliferation of nonmyelinating Schwann...
cells. To evaluate the effect of acquired demyelination and onion-bulb formation on nonmyelinating Schwann cells, we examined 3 patients who had the hypertrophic variant of chronic inflammatory demyelinating polyradiculoneuropathy (H-CIDP) with massive onion bulbs (32). The morphometric indices are listed in Supplemental Table 2. These patients showed a marked increase in the number of myelinating Schwann cell nuclei (5,493 ± 2,040 for H-CIDP vs 3,996 ± 2,150 for CMT1A per cross-section of the sural nerve), whereas the increment of nonmyelinating Schwann cells was only slight (1,894 ± 583 for H-CIDP vs 4,238 ± 1,498 for CMT1A per cross-section), in contrast to the findings in patients with CMT1A. Although further studies are needed to determine whether the proliferation of nonmyelinating Schwann cells in CMT1A is directly linked to PMP22 duplication or to secondary effects associated with demyelination or axonal degeneration of myelinated fibers, these observations in patients with H-CIDP support the view that proliferation is not merely a consequence of such secondary effects.

A striking finding was the marked increase of nonmyelinating Schwann cell profiles in patients with CMT1A, which showed a complicated cross-sectional morphology. This may have been due to intrinsic genetic abnormalities that affected nonmyelinating Schwann cell morphogenesis, because Schwann cell profiles were markedly increased even in young patients, whereas myelinating Schwann cells were relatively normal in these young patients (Fig. 1C). Investigation of cultured Schwann cells with PMP22 overexpression has demonstrated that PMP22 regulates cell morphology and spreading (33). In cultures of dorsal root ganglion cells from transgenic rats overexpressing PMP22, nonmyelinated axons surrounded by 2 or 3 redundant turns of Schwann cell cytoplasmic profiles, similar to our findings, are observed more frequently than in cultures of normal cells (34). In a transgenic mouse model of CMT1A, excessive Schwann cell membrane formation around unmyelinated fibers has been reported (35). It has also been demonstrated that PMP22 is present in the plasma membrane of nonmyelinating as well as myelinating Schwann cells (36). PMP22 has been detected in a wide range of neural and nonneural tissues during murine development, suggesting that it has biologic functions other than those related to myelination (37, 38). These observations support the view that PMP22 may play an important role in regulating the development and morphology of nonmyelinating Schwann cells. It could be argued that these morphologic changes of nonmyelinating Schwann cells were secondary effects of Schwann cell proliferation. However, the number of profiles was not correlated with the extent of the increase of Schwann cell nuclei in our study. However, there was a correlation between the number of profiles and the number of Schwann cell subunits, which represents the division or branching of Schwann cell cytoplasmic processes, supporting our view that the increase of profiles was not merely a consequence of Schwann cell proliferation. Furthermore, as indicated by the black boxes in Figure 8B, there was no increase of nonmyelinating Schwann cell profiles in patients with H-CIDP with marked onion-bulb formation (Fig. 8B).

In addition, our study showed that the number of unmyelinated axons per axon-containing Schwann cell subunit was reduced in patients with CMT1A, even in young patients, compared with normal individuals. This finding may also indicate the presence of intrinsic genetic abnormalities of nonmyelinating Schwann cells in CMT1A, because it seems to be the result of excessive separation of Schwann cells that remain arrested at the promyelinating stage of differentiation without proceeding to the myelinating stage. Indeed, in transgenic rats overexpressing PMP22, Schwann cells establish a one-to-one relationship but fail to advance from the promyelinating stage to the myelinating stage (39). Considering the hypothesis that axon caliber, but not axon quality, determines whether an axon becomes myelinated or unmyelinated (40), excessive separation of Schwann cells in CMT1A may be related to impaired recognition of axon caliber by Schwann cells. An alternative hypothesis, that signals from the axon, independent of its diameter, determine whether myelination occurs, would suggest that Schwann cell recognition of axonal signals is impaired in CMT1A (41).

Another striking observation was that the number and morphology of unmyelinated axons were well preserved in our patients with CMT1A, suggesting that axonal degeneration leading to axonal loss only occurred in myelinated fibers and not in unmyelinated fibers. Therefore, the impairment of nonmyelinating Schwann cells observed in patients with CMT1A does not appear to affect unmyelinated axons. These observations strongly suggest that axon-Schwann cell interactions are regulated differently between myelinated and unmyelinated fibers. Abnormalities of PMP22 have been found to result in impairment of the cytoskeletal organization and lead to axonal damage (3, 5, 25). However, these findings were obtained in studies of myelinating Schwann cells and myelinated axons, and information concerning nonmyelinating Schwann cells and unmyelinated axons has been lacking. Recently, various molecules that mediate axon-Schwann cell interactions have been identified, among which myelin-associated glycoprotein and neuregulin-1 may be important for maintaining normal peripheral nerve structure and function (42–45). Myelin-associated glycoprotein is expressed in the myelin sheaths of the peripheral nervous system (46), and axonal damage in myelinated fibers may be associated with myelin-associated glycoprotein disruption. On the other hand, neuregulin-1 may play a role in both myelinated and unmyelinated fibers (44, 45). The molecular mechanisms underlying the interactions between nonmyelinating Schwann cells and unmyelinated axons remain to be determined. Because axonal damage and loss are major factors in the poor functional prognosis of patients with CMT1A (6, 8, 9), the different levels of axonal involvement resulting from Schwann cell impairment are important from a clinical viewpoint.

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J Neuropathol Exp Neurol • Volume 66, Number 11, November 2007