

Anti-myelin-associated glycoprotein antibodies alter neurofilament spacing

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Summary

Axon calibre is crucial to efficient impulse transmission in the peripheral nervous system. Neurofilament numbers determine gross axonal diameter, but intra-axonal distribution depends on the phosphorylation status of neurofilament sidearms. Myelin-associated glycoprotein (MAG) has been implicated in the signalling cascade controlling neurofilament phosphorylation and hence in the control of axon calibre. In an electron microscopic morphometric study we measured nearest neighbour neurofilament distances (NNND) in the axons of sural nerves from patients with anti-MAG paraproteinaemic neuropathies and compared these with normal human sural nerves and those from patients with Guillain-Barré syndrome or chronic inflammatory demyelinating

polyradiculoneuropathy. Axon calibre was similar in all groups. In normal human sural nerves, axonal NNND was correlated with axonal diameter ($r = 0.56$). In diseased axons this correlation did not exist. The NNND was significantly reduced in demyelinated axons (30.5 ± 2.2 nm) and those with widely spaced myelin (28.9 ± 1.3 nm) from patients with anti-MAG antibodies compared with normal axons from normal patients (39.8 ± 3.2 nm) or those with demyelinating neuropathy (35.8 ± 4.6 nm). This reinforces the hypothesis that MAG is involved in the control of neurofilament spacing through sidearm phosphorylation and demonstrates a MAG-mediated pathogenic effect of the anti-MAG antibody in peripheral nerves.

Keywords: anti-MAG antibody; demyelinating neuropathy; myelin-associated glycoprotein; neurofilaments

Abbreviations: CMT1a = Charcot–Marie–Tooth disease type 1a; MAG = myelin-associated glycoprotein; MBP = myelin basic protein; NNND = nearest neighbour neurofilament distance; PDPN = paraproteinaemic demyelinating peripheral neuropathy; PMP22 = peripheral myelin protein 22; WSM = widely spaced myelin

Introduction

Axon calibre is crucially important to the electrical properties of the neuron, as it is a primary determinant of conduction velocity (Arbuthnott *et al.*, 1980). An optimal relationship also exists between axon calibre and myelin thickness (Waxman, 1980). The intra- and extra-axonal signals that orchestrate these morphological features are still under investigation (Witt and Brady, 2000). The overall number of cytoskeletal neurofilaments is determined by the balance between their delivery and removal (Hoffman *et al.*, 1984) and is the major determinant of axon calibre. Since each axon has an essentially uniform transport rate, this mechanism does not explain the observed differences in neurofilament packing density and axon calibre at nodes of Ranvier, Schmidt–Lanterman incisures and dorsal root ganglion stem processes

(Hsieh *et al.*, 1994). Local modulation of neurofilament packing by phosphorylation of neurofilament sidearms may explain these differences, and evidence is accumulating that extra-axonal signals from ensheathing glia contribute to the neurofilament phosphorylation status.

Myelin-associated glycoprotein (MAG) has been proposed as a Schwann cell-based molecule that influences neurofilament phosphorylation and spacing. One of the sialic acid binding immunoglobulin-like lectins, or Siglecs (Collins *et al.*, 1997), MAG is invoked in axon–Schwann cell signalling and adhesion primarily because of its expression/location on the adaxonal Schwann cell membrane (Sternberger *et al.*, 1979; Martini, 2001). The absence of MAG in a MAG-null mutant mouse is correlated with

reduced axon calibre, decreased nearest neighbour neurofilament distances (NNNDs) and reduced neurofilament phosphorylation (Yin *et al.*, 1998). In contrast, mice lacking the myelin proteins P0 or myelin basic protein (MBP) (*shiverer*) or both have normal axon calibres (Martini *et al.*, 1995). These and other complementary animal studies support a role for MAG in neurofilament phosphorylation, but few data are available on neurofilament spacing and its modification in humans, in either health or disease (Sahenk and Chen, 1998; Sahenk, 1999).

Patients with paraproteinaemic demyelinating peripheral neuropathy (PDPN) and an IgM paraprotein commonly demonstrate IgM antibody reactivity to MAG, especially its HNK-1 epitope. This epitope is also displayed on P0, peripheral myelin protein 22 (PMP22), the sulphated glycolipids SGPG (sulfoglucuronosyl paragloboside) and SGLPG (sulfoglucuronosyl lactosaminyl paragloboside) and other peripheral nerve adhesion molecules, any or all of which could play a role in the pathogenesis of PDPN. The IgM has been shown to bind at MAG-containing sites in peripheral nerve (Takatsu *et al.*, 1985; Hays *et al.*, 1988) where it might interfere with the function of MAG.

We compared NNND in the sural nerves from patients with anti-MAG PDPN with the NNND in sural nerve from patients with acquired and genetic demyelinating neuropathies and from normal subjects. Our hypothesis was that binding of anti-MAG antibody might disturb the function of MAG. If MAG were involved in influencing neurofilament spacing, this spacing would be disturbed more in anti-MAG PDPN than in other demyelinating neuropathies. This would not only have implications for the elucidation of the signalling pathways, which control axon calibre, but also for the pathogenesis of PDPN.

Material and methods

Patients and specimens

We selected sural nerve biopsy specimens from our library. They were from seven normal patients (one normal volunteer, three patients biopsied for diagnostic purposes with normal sural nerve specimens and three healthy males at autopsy shortly after death), five patients with a demyelinating neuropathy (two acute inflammatory demyelinating polyradiculoneuropathy and three chronic inflammatory demyelinating polyradiculoneuropathy, one with an IgG paraprotein without any detected antibody activity), six patients with IgM PDPN and anti-MAG activity and three patients with Charcot-Marie-Tooth disease type 1a (CMT1a) having a PMP22 gene duplication. Patients with demyelinating neuropathy were diagnosed by usual clinical criteria (Hadden *et al.*, 1998) (Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force, 1991). Other causes of neuropathy were excluded. Serum anti-MAG antibody activity was confirmed by complement fixation assay against whole human sciatic nerve homogenate (Hughes and

Stedronska, 1973) and confirmatory western blotting as previously described (Yeung *et al.*, 1991). PMP22 duplication confirmation was carried out by standard methods used in routine patient diagnosis (Yau *et al.*, 1996).

Biopsy specimens were fixed in 2.5% glutaraldehyde in Sorensen's buffer, post-fixed in 1% OsO₄ and embedded in epoxy resin. For electron microscopy, 70–80 nm sections were cut on to 200 mesh copper grids, double stained with uranyl acetate and lead citrate and examined with a Hitachi H600 electron microscope.

Imaging and classification of fibres

Fibre profiles in cross section, not near to a node or Schmidt-Lanterman incisure, were selected at random on the electron microscopy grid. Micrographs were taken at low magnification ($\times 4000$ – $12\,000$ for fibre classification) and high magnification ($\times 100\,000$ for neurofilament analysis). Profiles were classified as normal, demyelinating or with widely spaced myelin (WSM) according to the following criteria.

(i) Widely spaced myelin: two or more wraps of myelin with a regularly separated intraperiod line and an intact major dense line, occurring at the outer or inner aspect of the myelin sheath or throughout its thickness. Exclusions: loosened myelin (uncompacted myelin—separation of major dense line with or without separation of intraperiod line).

(ii) Demyelinating: demyelinated fibres included those that were actively demyelinating, demyelinated and/or had evidence of remyelination. Demyelinating fibres included those with and without macrophage-associated demyelination and/or that had evidence of active myelin degeneration where the fibres appeared intact. Remyelinated fibres were those with additional, supernumerary Schwann cell processes ('onion bulbs') or with a G-ratio [axon diameter / (axon + myelin diameter)] >0.8 (i.e. with thin myelin). Abnormalities of myelin other than WSM above were counted as demyelinating.

(iii) Normal: normal looking axons with normally lamellated myelin sheath (note: one wrap of myelin internally or externally may be loose) without evidence of remyelination.

Neurofilament spacing and axon calibre

The NNND (\pm standard deviation) was measured for each axon as described previously (Hsieh *et al.*, 1994). Mean axon calibres of the selected axons were obtained from the low power images as described previously (Yin *et al.*, 1998). Total Schwann cell area within the basement membrane and the area of compact myelin were measured by similar techniques.

Statistical analysis

Axon calibres were unimodally distributed in all groups and not significantly skewed. Comparisons were made by

Table 1 Patient groups, axon calibre and NNND

	No. patients	Age [mean (range)]	Sex (M : F)	Axon type	Number of axons	Mean (SD) axon calibre (μm)	Mean (SD) NNND (nm)
Normal	7	36 (12–55)	4: 3	Normal	36	5.31 (0.58)	39.8 (\pm 3.2)
				Demyelinated WSM	0	–	–
PDPN	6	62 (42–79)	4: 2	Normal	25	4.30 (0.64)	32.9 (\pm 2.1)
				Demyelinated WSM	37	4.11 (0.67)	30.5 (\pm 2.2)*
				WSM	23	3.91 (0.40)	28.9 (\pm 1.3)*†
Other demyelinating neuropathy	5	49 (23–71)	2: 3	Normal	26	4.63 (0.76)	35.8 (\pm 4.6)
				Demyelinated WSM	17	4.46 (1.17)	31.4 (\pm 3.1)
CMT1a	3	30 (19–40)	3: 0	Normal	0	–	–
				Demyelinated WSM	17	4.77 (1.24)	33.4 (\pm 2.3)‡
				WSM	0	–	–

* $P < 0.01$, Dunn's *post hoc* test on Kruskal–Wallis test across all groups. † $P < 0.05$, Dunn's *post hoc* test on Kruskal–Wallis test PDPN-normal versus PDPN demyelinated versus PDPN-WSM. ‡ $P = 0.033$, Mann–Whitney *U*-test two-way, CMT1a versus normal axons from normal subjects.

ANOVA (analysis of variance) with the Tukey *post hoc* test for individual group significance.

The NNND within each patient was Gaussian. Inter-patient NNNDs were analysed with non-parametric statistics (Kruskal–Wallis test with Dunn's *post hoc* test where appropriate). The hypothesized two-way comparisons of NNND were undertaken with the Mann–Whitney *U*-test.

For both axonal calibre and NNND the arithmetic means of values within a patient (the most conservative statistic, using N as the number of patients) were used to compare neuropathy groups.

Correlations and linear regressions with inter-group analyses and Pearson correlation coefficients (r) were calculated. All probability values (P) are two-tailed.

Analyses were carried out using Excel (Microsoft, Redmond, Wash., USA), GraphPad Prism (Graph Pad Software Inc., San Diego, Calif., USA) and the Statistical Analysis System program (SAS Institute, Cary, NC, USA).

Results

A total of 184 axon cross sections from all patients were identified and photographed (Table 1). Fibres with WSM were found only in biopsies from patients with PDPN. In the CMT1a specimens all axons were associated with onion bulbs and neither normal nor WSM fibres were found. Three fibres (one from each of three patients) in the normal patient group had features of demyelination and were excluded from further analysis. There was no significant difference in NNND or axon calibre between the normal axons of normal biopsied and autopsied nerves. A mean of 627 (range 109–1648) neurofilaments were sampled from each axon profile.

Axon calibre is not significantly different

There was a non-significant trend ($P = 0.08$) for axons (normal and diseased) from the disease groups to be smaller than normal axons from normal subjects, and a trend for abnormal axons within diseased nerves to be smaller than normal axons from the same diseased nerves (see Table 1). Since these differences are not statistically significant and are insufficient on their own to explain any differences in NNND, direct comparison of NNND between the patient groups as identified in our original hypothesis was undertaken.

NNND is reduced in PDPN axons

The NNND of diseased axons from patients with anti-MAG PDPN was less than that of normal subjects. The NNND of inflammatory neuropathy and CMT1a subjects was intermediate between that of normal and anti-MAG PDPN groups (Fig. 1). There was a statistically significant difference in NNND when all axons from all the groups were compared ($P = 0.001$) (Table 1). This difference was accounted for by differences in NNND between normal axons (mean distance 39.8 nm) and the PDPN demyelinated (mean distance 30.5 nm, $P < 0.01$) and WSM (mean distance 28.9 nm, $P < 0.01$) axons. No other comparisons made significant contributions.

NNND is significantly reduced in axons with WSM

When NNND in normal, demyelinated and WSM axons from PDPN patients was compared, the NNND was significantly reduced in the visibly diseased axons ($P = 0.033$). The difference was explained by the closer spacing of neurofilaments in axons with WSM ($P < 0.05$). The difference in

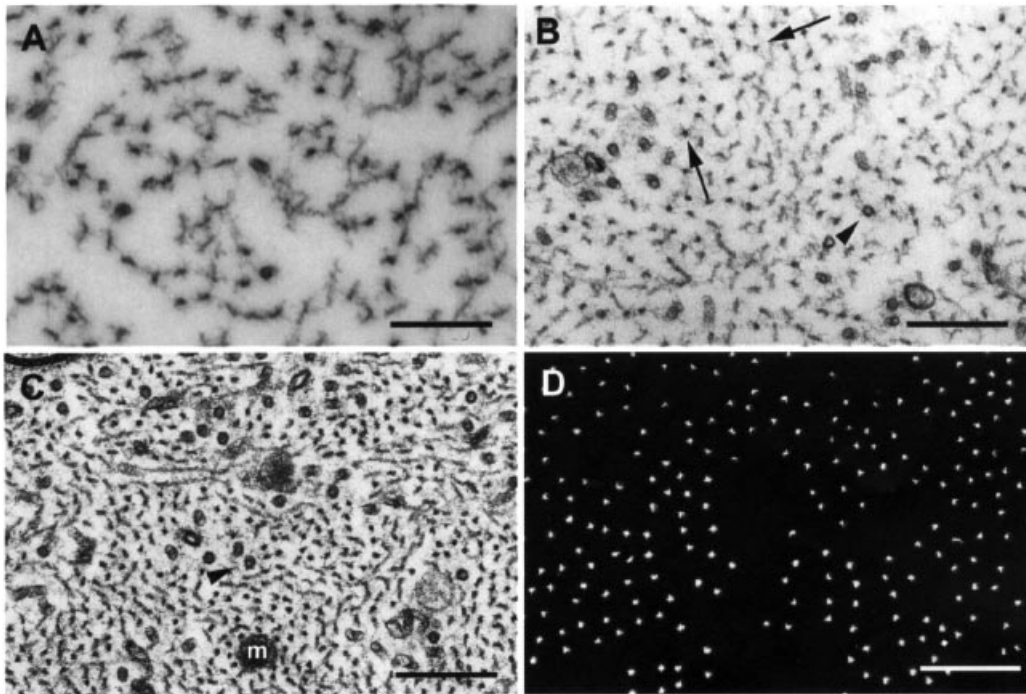


Fig. 1 Representative images from sural nerve axons of normal patients or those with inflammatory neuropathy or PDPN ($\times 100\,000$). Neurofilaments in the normal axons (**A**) are more widely spaced than those from demyelinated axons in inflammatory neuropathy (**B**) or axons with widely spaced myelin in PDPN (**C**). Neurofilaments (arrows) have visible extensions thought to be sidearms of NF-M (neurofilament medium) and NF-H (neurofilament heavy). Microtubules (arrowheads) and mitochondria (m) are also present. (**D**) Example of a light array obtained from neurofilament piercing from which neurofilament co-ordinates and NNND were calculated. Bar = 150 nm.

NNND between normal and demyelinated axons in the demyelinating neuropathy group (no anti-MAG antibody) was not significant ($P = 0.15$). In a pairwise comparison, the NNND in CMT1a axons (all diseased) was significantly closer than in axons from normal subjects ($P = 0.03$).

NNND: correlations

NNND increases slightly with axon calibre ($r = 0.56$, $P < 0.001$) and with the G-ratio (Friede and Samorajski, 1967) ($r = 0.34$, $P < 0.05$), but not with myelin thickness ($P = 0.75$) (see Fig. 2). Comparison of normal rat axon data collected by Hsieh *et al.* (1994) with our data demonstrated that the relationship between NNND and axon calibre was stronger than in humans in the rat ($P = 0.015$) (Fig. 3). The correlation between NNND and axon calibre in the normal PDPN axons is described by a shallower but still significant correlation (Fig. 4). In the demyelinated and WSM fibres this correlation was not apparent either overall or in any disease group. The NNND of CMT1a axons remained significantly correlated to axon calibre ($P < 0.001$); although the slope of the correlation was not significantly different from that of normal axons from normal subjects, the intercept of the slope was significantly reduced ($P < 0.0001$).

Discussion

Our results show that patients with anti-MAG IgM PDPN have significantly reduced axonal NNND compared with normal controls. The effect is more prominent than can be explained by demyelination alone. In mice, MAG has been implicated in the control of neurofilament spacing through the regulation of neurofilament sidearm phosphorylation. The presumed effect of anti-MAG antibody in this study suggests that MAG may play a similar role in the control of neurofilament phosphorylation and spacing in human nerve. In reaching these conclusions we have been careful in choosing conservative statistical techniques and we are confident that our results are valid.

Axon calibre is crucial in the efficient functioning of the whole nervous system. It is controlled at several incompletely understood levels (Martini, 2001). Neurofilaments (class IV intermediate filaments) are formed from the associated trimers of neurofilament heavy, medium and light (NF-H, NF-M and NF-L) subunits arranged in regularly spaced longitudinal bundles (Shaw, 1991). The average overall axon calibre is predominantly determined by transport of neurofilaments both into and away from the axon ((Parhad *et al.*, 1987). However local reductions in axon calibre associated with reduced NNND are seen especially at the nodes of Ranvier, the Schmidt–Lanterman incisures and the dorsal

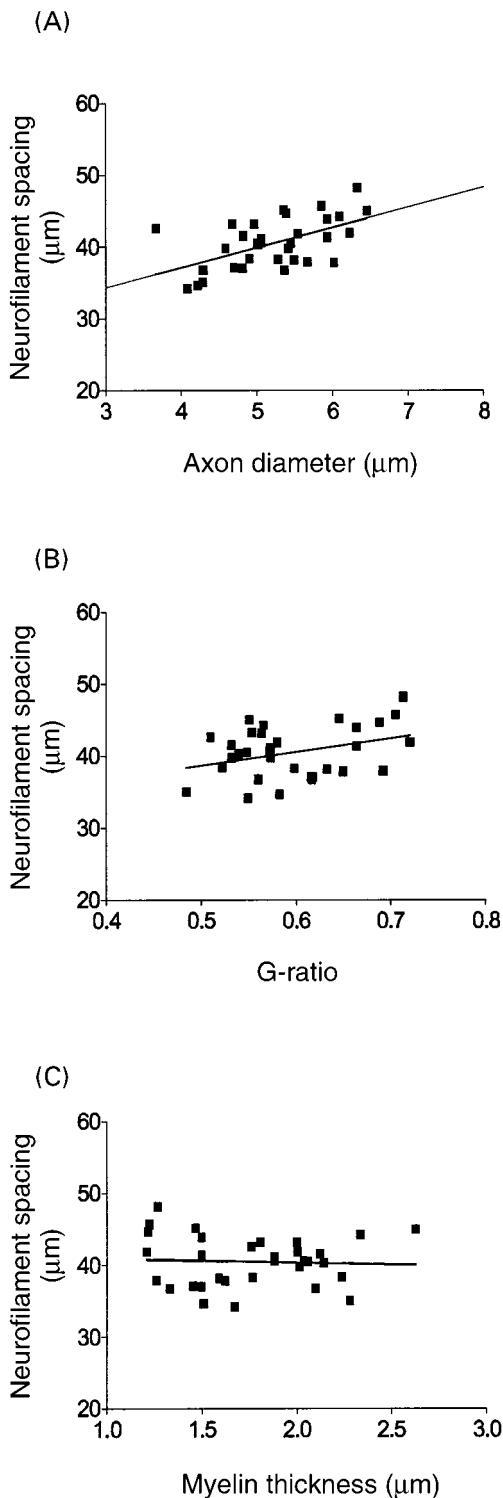


Fig. 2 Correlations of mean NNND with axon calibre (A), G-ratio (B) and myelin thickness (C) for 36 normal axons from seven normal subjects. Note that significant correlations exist for axon calibre ($r = 0.56$, $P < 0.001$) and G-ratio ($r = 0.34$, $P = 0.05$), but no correlation exists for myelin thickness ($P = 0.75$).

root ganglion stem processes (Hsieh *et al.*, 1994). A repeated lysine-serine-proline (K-S-P) repeat motif in a C-terminal

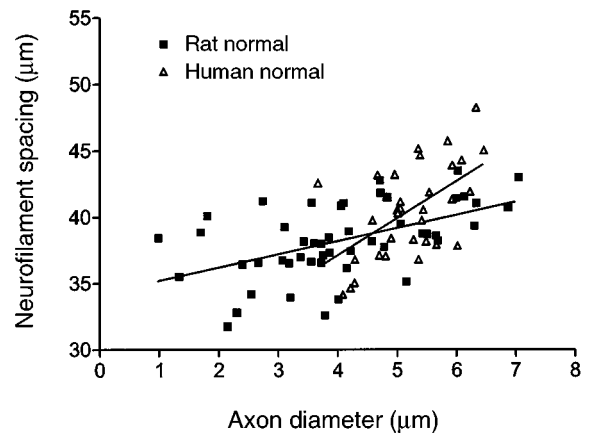


Fig. 3 Comparison of normal axonal NNND and axon calibre from human sural nerve with that from myelinated internodal axon segments from the sural nerve of Sprague-Dawley rats (see Hsieh *et al.*, 1994). Significant correlations exist in both species, but the relationship is stronger in the human sural nerve.

side-arm structure of NF-H and NF-M can be phosphorylated (Julien and Mushynski, 1983) and potentially acquire considerable negative charge (Wong *et al.*, 1995). Kinases and phosphatases known to act on the cytoskeleton (Nixon and Lewis, 1986; Shetty *et al.*, 1995) probably regulate this modification. Alterations in side-arm phosphorylation control the density and spacing of the neurofilament units and axon calibre, through retention and accumulation (Nixon *et al.*, 1994), decreased catabolism (Pant, 1988) and increased separation (Hsieh *et al.*, 1994). Regions of reduced spacing correlate with areas of decreased neurofilament phosphorylation (Hsieh *et al.*, 1994). Evidence is accumulating that extra-axonal signals, probably from Schwann cells, contribute to neurofilament phosphorylation status.

All peripheral nervous system axons are ensheathed by Schwann cell processes. Small axons remain unmyelinated, and in unmyelinated optic nerve or peripheral nerve axons, neurofilament spacing remains close (Sanchez *et al.*, 1996). The onset of myelination and the existence of a myelinating Schwann cell confer greater neurofilament spacing and hence axon calibre, than the presence of a non-myelinating cell or no Schwann cell (Windebank *et al.*, 1985; Sanchez *et al.*, 1996). In the tellurium-induced demyelination model, where the Schwann cell is lost, neurofilament spacing and axon calibres were significantly reduced. Normal spacing and axonal calibres were restored when a few wraps of normally compacted myelin had been re-established (Hsieh *et al.*, 1993). However, myelin itself does not regulate neurofilament spacing. In animals unable to form myelin because of myelin protein gene mutations, but having axons ensheathed by phenotypic myelinating cells, axon diameter was increased and the neurofilament spacing was normal (Sanchez *et al.*, 1996). In the dysmyelinating *Trembler* mouse, peripheral nerve neurofilaments had lower phosphorylation and axons were of reduced calibre (de Waegh *et al.*, 1992).

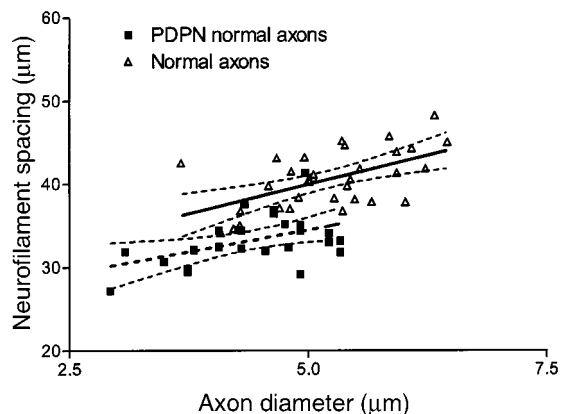


Fig. 4 Comparison of correlations of NNND with axon calibre in normal human sural nerves and in normal nerve fibres from patients with anti-MAG PDPN. Note that the correlation is less pronounced in the axons from the diseased nerves. Plotted lines are linear regression with 95% confidence intervals (curved dashed lines).

MAG was proposed as a candidate myelin membrane molecule involved in neurofilament phosphorylation signalling control.

MAG is expressed on the adaxonal Schwann cell membrane in the myelin internodes (Trapp *et al.*, 1989) and is a member of the expanding family of 'Siglecs' that have adhesion and signalling roles (Li *et al.*, 1996). The MAG-null knockout mouse has axons of reduced calibre, reduced neurofilament phosphorylation and more densely packed neurofilament than its wild-type littermates (Yin *et al.*, 1998), supporting the hypothesis that MAG is important in neurofilament phosphorylation signalling. Mice lacking either P0 or MBP (*shiverer*), or both, had normal axon calibres (Martini *et al.*, 1995). Since such knockout models have more widely reaching molecular abnormalities than altered expression of the knocked out molecule alone (Giese *et al.*, 1992), we wished to look for evidence for the involvement of MAG in an alternative model.

In 50–60% of patients with IgM PDPN the paraprotein reacts with the HNK-1 epitope found on MAG (Yeung *et al.*, 1991). HNK-1 is also found on P0, PMP22, SGPG, SGLPG and other myelin molecules. Anti-MAG IgM can be found bound to sites where MAG is localized in demyelinated nerve fibres, with or without WSM, but it is not clear that the pathogenesis of PDPN is mediated by MAG binding. In human sural nerves from patients with demyelinating neuropathies we postulated that NNND would be less than that in normal subjects because of the non-specific, inflammation-derived alteration in the axon–Schwann cell interrelationship. Our hypothesis was that in patients with an anti-MAG antibody, MAG function would be altered and phosphorylation decreased leading to a greater reduction in NNND.

In this study, the NNND was reduced in human peripheral nerve axons that had been demyelinated or otherwise

disturbed in acquired or inherited neuropathies. Although a trend for reduced NNND is evident in all the diseased axon groups, the difference was statistically significant only in the demyelinated and WSM axons of the patients with anti-MAG antibodies. The reduction in NNND was not accounted for by differences in axon calibre. Furthermore it was not merely due to demyelination. The presence or thickness of myelin does not seem to control NNND, because there was no relationship between NNND and myelin thickness or the G-ratio. Indeed, axons with WSM from PDPN patients had the greatest reductions in NNND, but myelin sheath thickness was normal (data not shown). We would suggest that interference with the signalling between axon and Schwann cell by the anti-MAG antibodies and subsequent decreased neurofilament phosphorylation could account for the greater reduction in NNND in anti-MAG PDPN patients. We can only speculate that these changes are secondary to alteration of neurofilament phosphorylation, and a further prospective study will answer this question. These findings are complementary to and comparable with those found in MAG knockout mice (Yin *et al.*, 1998).

Reduced neurofilament phosphorylation has been demonstrated in *Trembler* mice (de Waegh *et al.*, 1992). As a result, Yin *et al.* (1998) suggested that both MAG and PMP22 participate in a final common pathway controlling neurofilament phosphorylation. A reduction in phosphorylation of neurofilaments has been demonstrated in man in CMT1a (Watson *et al.*, 1994). In the CMT1a axons in this study, the correlation between NNND and axon calibre was maintained. Furthermore, the slope of that correlation, the mean axon calibre and the mean NNND are not significantly different from those of normal axons. The results do not support the hypothesis that MAG and PMP22 participate in a final common pathway. It is likely that anti-MAG antibodies bind to several HNK-1-bearing molecules, but these findings argue against anti-MAG antibodies causing their effects through binding to the HNK-1 of PMP22. The likelihood of MAG being the target is increased.

Normative data are available for neurofilament spacing in animal peripheral nerve (Berthold, 1978; Tohyama *et al.*, 1983) and axon and myelin proportions in healthy human sural nerve (Jacobs and Love, 1985; Behse, 1990). The NNND measured by us (39.8 ± 3.1 nm) in 36 axons from seven normal human sural nerves is comparable both with that at normal myelinated internodes of mouse (39.7 ± 10.7 nm; Tohyama *et al.*, 1983) and rat (40.3 ± 9.3 nm; Hsieh *et al.*, 1994) peripheral nerves. Donaghy *et al.* (1988) measured a median neurofilament spacing of ~35–40 nm in three control nerves in a study of giant axonal neuropathy. The NNND measured here also demonstrates a weak relationship between NNND and axon calibre, as seen in rats (Hsieh *et al.*, 1994) and has been implied in humans (Nukada and Dyck, 1984), but no relationship between myelin thickness and NNND exists. Much more physiological demyelination and remyelination is likely to have occurred during the lifetime of the human sural nerve

specimens than in the lifetime of a rat, possibly accounting for much of the variation in myelin thickness and lack of correlation seen in humans. Furthermore, differences between humans and rats in the slopes of correlations may be due to artefactual methodological differences in measurement caused by fixation.

Few studies have examined the spacing of peripheral nerve neurofilaments in human disease. Nukada and Dyck (1984) quantified the neurofilament densities in the axons of normal subjects and CMT1 patients and compared them with myelin spiral length (as an indicator of previous axon calibre). The mean CMT1 axon calibre was decreased, in addition to a small but statistically insignificant decrease in the neurofilament density. They interpreted this finding as reflecting axonal atrophy, possibly secondary to abnormal, decreased neurofilament transport. In the light of more recent knowledge of the molecular basis of CMT1a it is more likely that the axons are small and hyper-myelinated. Sahenk and Chen (1998) and Sahenk *et al.* (1999) grafted fascicular segments of sural nerve from normal subjects or those with PMP22 or connexin 32 mutations into the sciatic nerves of nude mice. Within the CMT1a or CMTX grafts of nerves from subjects with PMP22 or connexin 32 mutations, neurofilament densities were significantly increased. However, phylogenetic incompatibility between host and grafted specimens and changes possibly occurring as a result of trauma and regeneration may partially explain these findings.

Our findings may be functionally important. Demyelination is the primary feature of PDPN, but our study was not designed to address the mechanism of demyelination. Axonal atrophy is well described in PDPN (Mendell *et al.*, 1985) and, in the long term, axonal loss manifests as accumulated neurological deficit. Reduced NNND may disturb axon transport, impairing axon survival. Furthermore, reduced axon calibre may contribute to reducing conduction velocity. If this process progresses centripetally, the axonal shrinkage may contribute to the prominent distal slowing in the neurophysiology of PDPN (Kaku *et al.*, 1994). Longitudinal specimens were not available for this study and further directed prospective studies are required to answer this and other questions raised by the data. Demyelination may be secondary to changes in neurofilament spacing, or an additional primary effect of anti-MAG antibodies, but these data do not provide evidence to support or refute either suggestion. However, the findings generate reasonable explanations to account for much of the pathology seen in anti-MAG antibody associated neuropathy.

Acknowledgements

We wish to thank R. Skolasky (Johns Hopkins University) for statistical advice. M.P.T.L. is supported by the Brain Neurology Entry Scholarship and the Patrick Berthoud Charitable Trust, and K.A.S. is the recipient of a Johns Hopkins School of Medicine Clinician-Scientist award; this work was also supported by NIH grant NS31528.

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Received July 31, 2001. Revised October 22, 2001.
Accepted October 26, 2001