Heterozygous P0 Knockout Mice Develop a Peripheral Neuropathy that Resembles Chronic Inflammatory Demyelinating Polynévropathy (CIDP)

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Abstract. Demyelinating peripheral neuropathies are clinically divided into inherited and acquired types. Inherited demyelinating neuropathies are caused by mutations in genes expressed by myelinating Schwann cells, whereas acquired ones, including chronic inflammatory demyelinating polyneuropathy (CIDP), are probably caused by autoimmune mechanisms. We find that heterozygous P0 knockout (P0+/−) mice develop a neuropathy that resembles CIDP. By one year of age, P0+/− mice develop severe, asymmetric slowing of motor nerves, with temporal dispersion or conduction block, which are features of acquired demyelinating neuropathies including CIDP. Moreover morphological analysis of affected nerves reveals severe and selective demyelination of motor fibers, focal regions of demyelination, and inflammatory cells. These data suggest that immune-mediated mechanisms may contribute to the pathogenesis of the neuropathy in P0+/− mice.

Key Words: Axon–Schwann cell interactions; Charcot-Marie-Tooth disease; CMT; Conduction block; Demyelination; Myelin; Schwann cells.

INTRODUCTION

Demyelinating peripheral neuropathies are clinically divided into inherited and acquired types. Inherited demyelinating neuropathies, or Charcot-Marie-Tooth (CMT) disease, are chronic neuropathies caused by mutations in genes expressed by myelinating Schwann cells. Mutations in peripheral myelin protein 22 kD (PMP22), protein zero (P0), and connexin32 cause CMT1A, CMT1B, and CMTX, respectively (1, 2). Acquired demyelinating neuropathies are thought to be caused by autoimmune mechanisms and are clinically separated further by their onset and associated findings (3). Acute inflammatory demyelinating neuropathies, or Guillain-Barre syndrome (GBS), can be distinguished from chronic inflammatory demyelinating polyneuropathy (CIDP), which is the most common chronic acquired demyelinating neuropathy.

Chronic inflammatory demyelinating polyneuropathy predominantly affects adults between 40 and 60 years of age (4–7). The manifestations of the disease vary, but in most patients, the legs are more severely affected than the arms and motor dysfunction is more severe than sensory dysfunction. The major pathological findings are demyelination and axonal loss. Demyelination is most evident in proximal areas, especially in the nerve roots, and axonal loss is most pronounced distally. In addition, nerve biopsies frequently show endoneurial and subperineurial edema, and occasionally infiltration of the epineurium and endoneurium by lymphocytes and macrophages, including phagocytosis of intact-appearing myelin by macrophages (8–11). These pathological changes bear similarities to those described in both GBS and experimental allergic neuritis (an animal model of GBS), and are thus thought to mediate demyelination.

The immune mechanisms that lead to demyelination in CIDP are not well established. Passive transfer of serum from patients with CIDP to animals has not reproduced the disease, and anti-peripheral nerve antibodies have not been detected in most patients (12–15). These findings indicate that auto-antibodies do not mediate demyelination, and thus implicate cell-mediated immunity. The role for cell-mediated demyelination rests on the pathological changes in affected nerves, especially the presence of lymphocytes and myelin phagocytosis by macrophages, and the response of patients to immunosuppressive therapy. Nearly all patients respond at least initially to corticosteroids, a known modulator of cellular immunity, although recovery is usually incomplete (4, 6, 7). These observations are consistent with an autoimmune basis for demyelination in CIDP but the details of the pathogenesis have not yet been established.

In order to investigate the molecular mechanisms of demyelination and to devise new treatment strategies, an animal model of CIDP would be desirable. We report here that mice lacking one of the two P0 alleles by homologous recombination (P0+/−) have the clinical, electrophysiological, and morphological features of CIDP. The neuropathy in P0+/− mice has an adult onset, and asymmetric, multi-focal slowing with temporal dispersion or conduction block by electrophysiology. Morphological

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evaluation demonstrates a demyelinating neuropathy predominantly affecting motor fibers, regions of demyelination associated with endoneurial edema and macrophages, and accumulations of lymphocytes around motor roots. The multifocal nature of the demyelination and the inflammatory features are atypical of CMT, and raise the possibility that immune-mediated mechanisms contribute to the pathogenesis of demyelination in these animals.

**MATERIALS AND METHODS**

**Generation of P₀ +/- Mutant Mice**

Heterozygous P₀ knockout (P₀ +/-) mice, originally obtained from Dr Philippe Soriano (16), were used. Genotypes were established by Southern analysis of genomic DNA isolated from tail clips, using a 457 bp HindIII-XbaI fragment of the P₀ gene (which is 5' to the homologous recombination site) as a probe. The cDNA was 32P-labeled with specific activities of 2.5 x 10⁶ cpm/μg, using primer extension from random hexamers (Primgene kit; Promega) according to the manufacturer's instructions. The P₀ allele containing the targeted integration of the neo construct gives a 3.2 kb band, whereas the normal P₀ allele gives a 2.1 kb band. P₀ +/- mice were also distinguished from homozygous P₀ -/- mice by their clinical phenotype, as P₀ +/- mice typically develop overt signs of neuropathy by 1 month of age (16).

**Electrophysiological Characterization of Neuropathy in P₀ +/- Mice**

Electrophysiological recordings were performed using a Dantec model 2000 machine. Mice were initially anesthetized with inhaled 4% halothane, then maintained with a combination of 3% chloral hydrate i.p. and inhaled 1% halothane. Body temperature was maintained throughout the recording at 36 to 37°C with a rectal probe servo-coupled to a radiant heat source. The compound muscle action potential (CMAP) was recorded with a pair of subdermal stainless steel electrodes positioned over the intrinsic foot muscles and proximal interphalangeal joints, respectively. Each sciatic nerve or its posterior tibial branch was stimulated subdermally using a pair of stainless steel, near-nervle electrodes in a distal to proximal sequence at the (1) lateral malleolus, (2) popliteal fossa, (3) sciatic notch, and (4) L4 spinal root (at the L4 neural foramina). A stainless steel, subdermal ground electrode was positioned between stimulation and recording sites. The amplitude, latency, and duration of evoked CMAPs were recorded. To determine the motor conduction velocities of the proximal, middle, and distal segments of the sciatic nerve, we subtracted the latencies from each site of stimulation, and measured distances between stimulation sites.

\[
\text{Proximal conduction velocity} = \text{latency from L4 spinal root - latency from sciatic notch} \\
\text{Middle conduction velocity} = \text{latency from sciatic notch - latency from popliteal fossa} \\
\text{Distal conduction velocity} = \text{latency from popliteal fossa - latency from lateral malleolus}
\]

To document dispersion and/or conduction block, the amplitudes and durations of CMAPs at each site were compared. If the proximally-evoked CMAP had a different waveform and was prolonged, it was considered to be temporally dispersed (17). If the amplitude of the proximally-evoked CMAP was less than one-half of that of the distally-evoked CMAP, it would normally be considered to demonstrate conduction block (17). However, it was technically difficult to measure the CMAP in affected P₀ +/- mice, as higher stimulating currents were required to evoke CMAPs than in control animals. The higher currents caused a wandering baseline, making precise calculations of the CMAP area difficult. Since the distinction between temporal dispersion and conduction block depends upon the CMAP area, we were not able to distinguish between the two in our studies. Our results are thus presented as demonstrating dispersion or conduction block without attempting to distinguish between them.

After nerve conduction measurements, electromyography (EMG) was performed using a concentric bipolar needle electrode at gains of 20 and 50 μV/division. The presence of spontaneous activity including fibrillation potentials, positive waves, and complex repetitive discharges was assessed in intrinsic foot muscles, tibialis anterior, gastrocnemius, gluteus muscles, as well as lumbar and thoracic paraspinal muscles. The morphology and recruitment of voluntary motor unit potentials in the paraspinal and intercostal muscles during spontaneous respiration was also analyzed.

**Light and Electron Microscopic Characterization of Neuropathy in P₀ +/- Mice**

Mice were deeply anesthetized with chloral hydrate, then perfused with 0.9% NaCl followed by 3% glutaraldehyde in 0.1 M phosphate buffer. The spinal cord and roots, as well as sciatic and femoral nerves, were removed and placed in fresh fixative. The sciatic nerves were divided into 3 to 4 segments, corresponding to proximal, middle, and distal segments of the nerve. For electron microscopy and teased fibers, tissues were fixed overnight at 4°C in glutaraldehyde, then post-fixed in 1% OsO₄ in 0.1 M phosphate buffer, dehydrated, and embedded in epon. Semi-thin sections were stained with toluidine blue; thin sections were stained with lead citrate and uranyl acetate. The teased fibers, osmiﬁed nerves were placed in 66% glycerol followed by 100% glycerol for at least 24 hours (h) at room temperature. Single fibers were separated using fine forceps and a dissecting microscope, mounted in glycerin, and photographed with differential interference optics.

**RESULTS**

Asymmetric, Multifocal Slowling in P₀ +/- Sciatic Nerve

As previously reported by Martini and colleagues (18, 19), we found that P₀ +/- mice are clinically normal until approximately 5 months of age, when they begin to develop a "waddling" gait. The gait disorder progresses for the next 6 to 8 months, then stabilizes, although the severity varies between individuals. While these behavioral data are consistent with the idea that P₀ +/- animals have an age-dependent, progressive peripheral neuropathy, we directly assessed the peripheral nerves in P₀ +/- animals with standard clinical electrophysiological measurements. We analyzed the amplitude and morphology of the motor
F-wave latencies were prolonged in the \( P_{0+/-} \) mice. Electromyography demonstrated acute denervation and chronic partial denervation of muscle, characterized by fibrillations, positive sharp waves, complex repetitive discharges, and large polyphasic motor units, in all 12-month-old animals (data not shown). These electrophysiological changes are consistent with an age-dependent, progressive demyelinating peripheral neuropathy with axonal loss.

The slowing of the proximal, middle, and distal MNCV were all more statistically significant in 12-month-old \( P_{0+/-} \) mice than in normal 9-month-old mice (\( P < 0.01 \) level using either the Mann Whitney test or the one-tailed Student t test modified according to Welch) (21). The slowing of the MNCV in younger animals did not reach statistical significance, but the sample size was limited. Beyond the significant slowing of the MNCV in 12-month-old \( P_{0+/-} \) mice, there was striking variability within and between individual \( P_{0+/-} \) animals. For example, the MNCV of the middle segment of the right sciatic nerve in animal #9 was 46 m/s, whereas the MNCV of the proximal left sciatic nerve was less than one-half that value. Similarly, the F-wave latencies varied by as much as 3 ms (mouse #10, right side and mouse #8, left side): no such variability was seen in control animals. Asymmetrical slowing is often found in acquired demyelinating neuropathies of humans, but is unusual in inherited demyelinating neuropathies (20, 22). These findings suggest that the degree of demyelination in \( P_{0+/-} \) mice is not uniform, which we have investigated by systematically examining the PNS of these animals, described below.

Age-dependent Neuropathy in \( P_{0+/-} \) Mice

To determine the onset, progression, and pathogenesis of peripheral neuropathy in \( P_{0+/-} \) mice, we undertook a systematic morphological evaluation of the peripheral nervous system, including the two 3-month-old (Table 1; #4 and #5), one 5-month-old, and three 12-month-old (Table 1; #6, #7, and #8) animals that had been studied electrophysiologically. The left and right sciatic nerves were removed from the ankle to the spinal nerve, and the left and right femoral nerves were taken proximal to their bifurcation into a motor and a sensory branch. The left and right L4 and L5 motor and sensory roots, which collectively form the sciatic nerves, were also collected. As controls, the same nerves were taken from one 3-month-old and two 9-month-old wild-type animals. The nerves were embedded in epoxy and examined by light and electron microscopy.

Nearly all myelinated fibers looked normal in the 3- and 5-month-old \( P_{0+/-} \) mice, except for an occasional fiber that had a myelin sheath that was inappropriately thin for the axonal caliber. At 12 months, however, many...
TABLE 1
Electrophysiological Studies of $P_{y}^{+/-}$ Mice

<table>
<thead>
<tr>
<th>#</th>
<th>$P_{y}$ genotype</th>
<th>Age (months)</th>
<th>Side</th>
<th>Nerve Conduction</th>
<th>DML (ms)</th>
<th>F-lat (ms)</th>
<th>CMAP (mV)</th>
<th>Cond block/dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>$+/+$</td>
<td>6</td>
<td>R</td>
<td>Prox 37.5</td>
<td>0.88</td>
<td>NP</td>
<td>7.5</td>
<td>None</td>
</tr>
<tr>
<td>2.</td>
<td>$+/+$</td>
<td>9</td>
<td>R</td>
<td>Mid 37.5</td>
<td>0.96</td>
<td>NP</td>
<td>8.5</td>
<td>None</td>
</tr>
<tr>
<td>3.</td>
<td>$+/+$</td>
<td>9</td>
<td>R</td>
<td>Dist 39.1</td>
<td>0.84</td>
<td>4.24</td>
<td>7.4</td>
<td>None</td>
</tr>
<tr>
<td>4.</td>
<td>$+/-$</td>
<td>3</td>
<td>R</td>
<td>Prox 42.8</td>
<td>0.80</td>
<td>4.48</td>
<td>3.2</td>
<td>None</td>
</tr>
<tr>
<td>5.</td>
<td>$+/-$</td>
<td>3</td>
<td>L</td>
<td>Mid 43.7</td>
<td>0.84</td>
<td>5.4</td>
<td>10.2</td>
<td>None</td>
</tr>
<tr>
<td>6.</td>
<td>$+/-$</td>
<td>6</td>
<td>R</td>
<td>Dist 39.1</td>
<td>0.96</td>
<td>5.8</td>
<td>7.4</td>
<td>None</td>
</tr>
<tr>
<td>7.</td>
<td>$+/-$</td>
<td>12</td>
<td>R</td>
<td>Prox 45.8</td>
<td>1.04</td>
<td>6.7</td>
<td>9.2</td>
<td>None</td>
</tr>
<tr>
<td>8.</td>
<td>$+/-$</td>
<td>12</td>
<td>L</td>
<td>Mid 38.4</td>
<td>1.12</td>
<td>6.0</td>
<td>2.16</td>
<td>None</td>
</tr>
<tr>
<td>9.</td>
<td>$+/-$</td>
<td>12</td>
<td>L</td>
<td>Dist 33.3</td>
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<td>8.4</td>
<td>1.62</td>
<td>None</td>
</tr>
<tr>
<td>10.</td>
<td>$+/-$</td>
<td>12</td>
<td>R</td>
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<td>6.8</td>
<td>1.28</td>
<td>None</td>
</tr>
<tr>
<td>11.</td>
<td>$+/-$</td>
<td>12</td>
<td>R</td>
<td>Mid 23.0</td>
<td>1.56</td>
<td>6.7</td>
<td>1.64</td>
<td>None</td>
</tr>
<tr>
<td>12.</td>
<td>$+/-$</td>
<td>20</td>
<td>R</td>
<td>Dist 25.4</td>
<td>1.40</td>
<td>4.4</td>
<td>2.0</td>
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</tr>
</tbody>
</table>

Abbreviations: Prox. mid and dist = proximal, middle and distal stimulation points (see text for details); NP = not performed.

Fig. 2. Demyelination in 12-month-old $P_{y}^{+/-}$ mice. These micrographs are from a transverse section of a femoral nerve; the motor branch is the upper fascicle in (A); an enlargement is shown in (B). The sensory branch is the lower fascicle in (A); an enlargement is shown in (C). Note that demyelinated (large arrow) and remyelinated (small arrow) axons are more numerous in the motor branch. Scale bar: 10 μm.
myelin sheaths were abnormally folded or split. Other myelin sheaths were inappropriately thin for the axonal caliber, indicating that these fibers had been demyelinated and remyelinated. Some of these remyelinated axons were partly surrounded by Schwann cell processes, so-called "onion bulbs," which are hallmarks of demyelination and remyelination (23). Finally, some relatively large axons (>2 μm in diameter), which would be expected to have myelin sheaths, were demyelinated. At 20 months, all of these pathological changes were even more pronounced (Fig. 2). There was also evidence of axonal loss, as there were Schwann cells that were not associated with any axons as well as empty basal laminae (data not shown). These findings demonstrate that there is age-related demyelination and remyelination as well as axonal loss in the peripheral nerves of P<sub>0</sub>+/− mice.

We noticed that the larger fibers appeared to be preferentially demyelinated, and that the affected fibers were often found in fascicles that were devoid of unmyelinated axons. Within the sciatic nerve there is a fascicular organization, and in some places groups of large myelinated motor fibers are found together in the absence of unmyelinated axons (24). Therefore, these observations suggested that affected fibers were motor rather than sensory. To evaluate this possibility, we examined the femoral nerve of 12-month-old P<sub>0</sub>+/− animals, as the sensory branch of this nerve (the saphenous nerve) contains predominantly sensory fibers, whereas the motor branch (which innervates the quadriceps muscle) contains motor and sensory fibers. As shown in Figure 2, many myelinated fibers in the motor branch had inappropriately thin myelin sheaths and even onion bulbs, whereas these findings were uncommon in the sensory branch. We also examined the ventral and dorsal roots in the cauda equina in 12-month-old P<sub>0</sub>+/− animals. While different ventral roots were affected to varying degrees, all of the ventral roots in the caudal equina showed modest to marked demyelination and remyelination; one of the most severely affected ones is shown in Figure 3. By comparison, the amount of demyelination and remyelination in the dorsal roots was small. As in the sciatic nerve, there were abnormally folded and split myelin sheaths in both the ventral and dorsal roots.

To evaluate further the demyelination and remyelination, we also examined osmicated teased fibers from a one-year-old P<sub>0</sub>+/− animal by differential interference microscopy (Figs. 4, 5). Teased fibers from the L4 and L5 ventral roots revealed that all myelinated fibers had undergone extensive remodeling, evidenced by the great variability in the length of myelin internodes and the thickness of the myelin sheaths, even along a single fiber. There were segments of demyelinated and thinly remyelinated axons, many of which were surrounded by supernumerary Schwann cells. The myelin sheaths themselves were often abnormally folded, especially at paranodes, resembling so-called tomacula. The sciatic nerve, which contains predominately sensory fibers, was much less affected, with only a minority of fibers exhibiting the changes seen in ventral roots. These observations confirm that demyelination affects motor axons more than sensory axons, and extend our previous findings by showing that single axons are affected for long distances.

Inflammatory Features in P<sub>0</sub>+/− Nerves

In our systematic examination of the PNS, we noticed some unusual inflammatory changes in P<sub>0</sub>+/− mice. The most conspicuous example was in the middle segment of the right sciatic nerve of animal #7, a 12-month-old P<sub>0</sub>+/− mouse that had focal slowing over this segment (Table 1). In this region, nearly every myelinated axon had abnormally thin myelin sheaths (Fig. 6). As only about 25% of myelinated axons in rodent sciatic nerve are motor fibers (25), many sensory axons in this segment must also have been affected. There were a large number of macrophages containing lipid droplets (Fig. 6), and some of the endoneurial blood vessels were partially surrounded by mononuclear cells (Fig. 7A). As this segment was remarkably more affected than the more proximal (not shown) and distal (Fig. 6) segments, we conclude that it was focally demyelinated, which is the likely reason for focal slowing by electrophysiology.

To determine whether macrophages mediate demyelination, as has been reported in CIDP (8–10), we looked for macrophages "stripping" a myelin sheath surrounding a normal appearing axon. There were several examples of macrophages containing myelin debris within Schwann cell basal laminae, but in most of these cases the myelin was obviously degenerating. An example of a macrophage phagocytosing a relatively intact appearing myelin sheath is shown in Figure 7B, but even in this example, the axon is no longer surrounded by its myelin sheath. Our results are thus more consistent with the well-known phenomenon that macrophages phagocyte myelin debris (26), but they do not rule out the possibility of macrophage-mediated demyelination.

Mononuclear cells infiltrating the pia mater were seen in two 12-month-old P<sub>0</sub>+/− mice (Fig. 7C, D). These cells were particularly concentrated in the region of the ventral roots, but they did not invade the glial limitans of the spinal cord. Although we could not classify the majority of these cells, some appeared to be plasma cells, as they contained abundant rough endoplasmic reticulum and had large masses of heterochromatin at the margins of the nuclear membrane. Subsequently, many of these mononuclear cells have been demonstrated to be T lymphocytes, which are likely to be a component of a cellular immune response in the nerve (Menichella et al, in preparation).
Fig. 3. Demyelination in 12-month-old P<sub>0</sub>+/+ mice. These micrographs are from transverse sections of ventral (A, C, E) and dorsal (B, D) roots of a 12-month-old P<sub>0</sub>+/+ mouse. Note that the degree of demyelination is much more extensive in the ventral root (C), and that large axons (*) are more severely affected. In Panel E, note that the neurofilaments (NF) are much more closely packed in the thinly remyelinated axon (left) than in the well-myelinated axon on the right (37). Scale bars: 10 μm (A, B), 1 μm (C, D); 0.1 μm (E).
DISCUSSION

Demyelinating Neuropathy in P0+/− Mice

P0+/− mice develop an age-dependent, demyelinating peripheral neuropathy. Unlike P0−/− mice, in which the formation of myelin is disrupted from the onset of myelination (16, 18), myelination begins normally in P0+/− mice. The neuropathy begins in adult life and is characterized electrophysiologically as a multifocal demyelinating neuropathy with axonal loss. The morphological evaluation confirms these features, and extends them by showing that the neuropathy predominately affects motor fibers. Finally, the inflammatory changes in peripheral nerves and nerve roots are unexpected for a genetic neuropathy, and raise the question of whether demyelination in P0+/− mice is immune-mediated.

We have confirmed and extended the findings of Martini and colleagues, who first reported that P0+/− mice develop an age-dependent demyelinating neuropathy (18, 19). They also noted that the saphenous nerve, a sensory nerve, was clearly less affected than muscular nerves, and we have extended this finding to the motor and sensory fibers of the ventral and dorsal roots. The chief difference in our results relates to the uniformity of demyelination: they did not report focal demyelination, whereas this was one of the most salient features of our electrophysiological and anatomical analyses. This discrepancy may simply reflect the fact that we measured the proximal, middle, and distal conduction velocities separately, which enabled us to determine that there was nonuniform slowing. We corroborated this finding by systematically examining the PNS of the animals that we had studied electrophysiologically, and determined that there was focal demyelination in at least one segment that had marked focal slowing.

The Relationship between P0+/− Mice and CMT1B

It has been suggested that P0+/− mice provide a model for CMT1B (18, 19). To date, 30 different missense and nonsense mutations have been identified in the human P0 gene, all of which cause neuropathy, although the severity of neuropathy caused by different mutations differs widely (27, 28). Some patients with P0 mutations are said to have Dejerine-Sottas disease, the traditional name for a severe demyelinating neuropathy with infantile onset. Although Dejerine-Sottas syndrome was originally thought to be recessively inherited, many of these patients have now been shown to have new, dominant mutations of either P0 or PMP22 (1, 28). The majority of P0 mutations, however, cause CMT1B, which has a later (typically childhood) onset and less severe demyelination.

Although the neuropathy in P0+/− mice is caused, directly or indirectly, by a loss-of-function mutation of P0, it is disputed whether most cases of CMT1B are caused by loss-of-function mutation of P0. The true loss-of-function phenotype may be much milder than that in typical CMT1B patients. For example, Warner et al (28) identified 2 cousins who were heterozygous for a frameshift mutation (after Gly-74) and were thought to have a loss of function mutation because of the truncated size of the resultant protein. These patients had such a mild phenotype that they did not come to medical attention until their children were diagnosed as having Dejerine-Sottas syndrome. Similarly, Ikekami et al (27) reported 2 cousins who were both heterozygous for a Phe-64 deletion and who only came to medical attention after their children were diagnosed with Dejerine-Sottas syndrome. If these mildest cases of P0 mutations represent a pure
Fig. 5. Demyelination and remyelination in the ventral roots of a 12-month-old P_o +/- mouse. These are photomicrographs (differential interference optics) of osmicated teased fibers. Axons (a) and a supernumerary Schwann cell (arrowheads) are indicated. Panel A shows an abnormally wide node, with a 10-μm gap separating the left and right paranodes. The left paranode has an abnormally folded myelin sheath and terminates at the pair of large, apposed arrowheads, the right paranode has a very thin myelin sheath and terminates at the pair of small, apposed arrowheads. There is a supernumerary Schwann cell between the 2 paranodes. Panel B shows a demyelinated axon (with 3 supernumerary Schwann cells; arrowheads) and a myelinated axon whose Schwann cell nucleus is indicated (arrow). Scale bar: 10 μm.

loss-of-function, then the majority of PO mutations causing CMT1B probably have dominant, deleterious effects. For these reasons, P_o +/- mice appear to be a model of how a pure loss-of-function mutation causes demyelination by a dosage effect; they are not a model of CMT1B as it is typically defined (29).

Distinguishing between Inherited and Acquired Demyelinating Neuropathies

The findings of temporal dispersion of CMAPs, non-uniform slowing of conduction velocities, and focal slowing associated demyelination in P_o +/- mice raise the issue of how to distinguish inherited and acquired demyelinating neuropathies. These mice would be expected to have an inherited demyelinating neuropathy, but these features have traditionally been associated with acquired, not inherited, demyelinating neuropathies. For example, the nerve conduction velocities in individuals with CMT3 are remarkably similar both along individual nerves and between different nerves, whereas in patients with CIDP, conduction velocities typically differ along the same nerve and between different nerves (20, 22, 30). Similarly, temporal dispersion and conduction block are features of CIDP and not CMT (3, 20). In addition, nerve biopsies in CMT1 typically show uniform demyelination with little endoneurial edema or mononuclear infiltrates (31, 32). Thus, the nonuniform slowing and focal demyelination in P_o +/- mice is unexpected.

One possible explanation for the nonuniform demyelination in P_o +/- mice is that there is a superimposed, inflammatory demyelination. This possibility is potentially relevant to a subset of patients thought to have
CMT, except that they acutely worsened, then responded to corticosteroid treatment (33–37). In some cases, furthermore, nerve conductions were focally slowed and robust inflammatory changes were seen on nerve biopsy. Since most of these cases were published before genetic testing for CMT was available, it remains to be shown that these patients actually had CMT. Nevertheless, these findings raise the possibility that some patients who clinically appear to have CIDP have an underlying inherited demyelinating neuropathy that somehow triggers an inflammatory neuropathy. The neuropathy itself could trigger the inflammatory response owing to the immunogenicity of myelin constituents, as auto-antibodies against myelin have been described in rodents after peripheral nerve injury (38). Alternatively, the abnormal myelin sheaths in inherited demyelinating neuropathies may be more susceptible to an immune attack. These possibilities are under investigation in the P0 +/− mice.
Fig. 7. Inflammatory cells in aging P<sub>6</sub>+/− animals. Panel A shows a collection of mononuclear cells (m) adjacent to an endoneurial blood vessel (bv) in a 12-month-old animal. Panel B shows a macrophage penetrating a Schwann cell basal lamina (between arrowheads) and phagocytosing a degenerating myelin sheath in a 20-month-old animal. Two axonal sprouts (a) are present. Panels C and D show the ventral root entry zone of a 12-month-old animal. The astrocytic endfeet of the glial limitans are indicated (arrowheads). There are a large number of mononuclear cells, including plasma cells (p), in the pia mater adjacent to a blood vessel (bv) and a ventral rootlet (vr). Scale bars: 1 μm (A, B, D); 10 μm (C).
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