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REVIEW ARTICLE

Role of Immune Cells in Animal Models for Inherited Peripheral Neuropathies

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Abstract

Mice expressing half of the normal dose of protein zero (P0+/– mice) or completely deficient gap-junction protein connexin 32–/– mice mimic demyelinating forms of inherited neuropathies, such as Charcot-Marie-Tooth (CMT) neuropathies type 1B and CMT type 1X, respectively. In both models, an almost normal myelin formation is observed during the first months of life, followed by a slowly progressing demyelinating neuropathy. In both models, there is a substantial increase of CD8+ T-lymphocytes and macrophages within the demyelinating nerves. Recently, this has also been observed in mice mildly overexpressing human peripheral myelin protein 22 kD mimicking the most common form of CMT, CMT type 1A. In all demyelinating models, the macrophages show close contacts with intact myelin sheaths or demyelinated axons, suggesting an active role of these cells in myelin degeneration. Additionally, fibroblast-like cells contact macrophages, suggesting a functional role of fibroblast-like cells in macrophage activation. By cross-breeding P0+/– and gap-junction protein connexin 32–/– mice with immunodeficient recombination activating gene-1-deficient mutants, a substantial alleviation of the demyelinating phenotype was observed. Similarly, cross-breeding of P0+/– mice with mutants with a defect in macrophage activation led to an alleviated phenotype as well. These findings demonstrate that the immune system is involved in the pathogenesis of demyelinating neuropathies. In contrast, in P0–/– mice, which display a compromised myelin compaction and axonal loss from onset, immune cells appear to have a neuroprotective effect because cross-breeding with recombination activating gene-1 mutants leads to an aggravation of axonopathic changes. In the present review, we discuss the influence of the immune system on inherited de- and dysmyelination regarding disease mechanisms and possible clinical implications.

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Index Entries: Myelin; axonopathy; demyelination; myelin phagocytosis; lymphocytes; macrophages; endoneurial fibroblasts.

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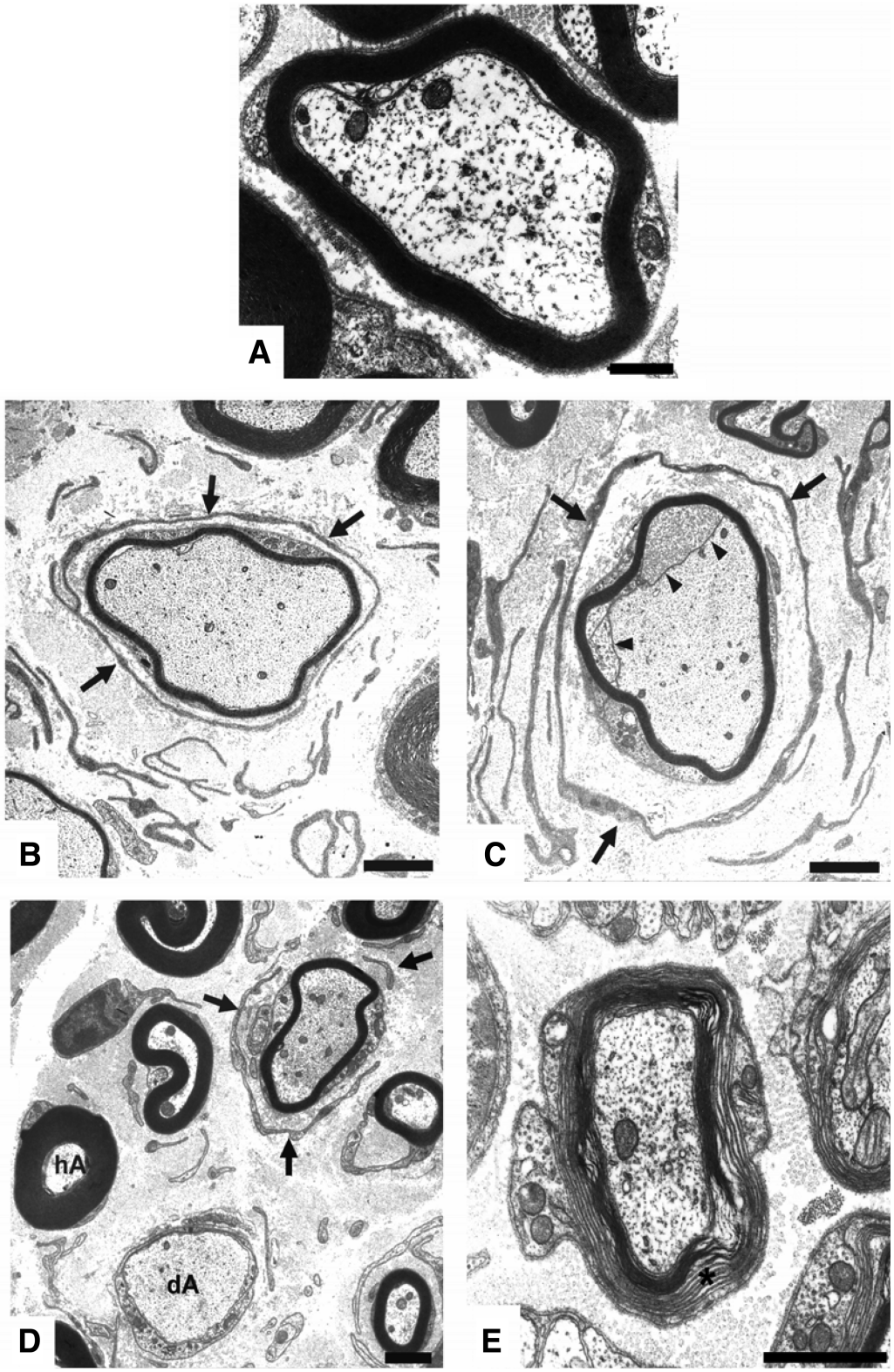
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Introduction

Hereditary neuropathies are a clinically and genetically heterogeneous group of diseases of the peripheral nervous system, which are mediated by mutations in presently more than 20 identified genes (Suter and Scherer, 2003; Shy et al., 2005). The predominantly demyelinating forms are designated Charcot-Marie-Tooth type 1 (CMT1) neuropathies and comprise the most frequent disorders among the inherited neuropathies (Young and Suter, 2001; Shy et al., 2002; Young and Suter, 2003). The most common culprit genes related to these disorders are three myelin genes (Suter and Scherer, 2003; Martini and Toyka, 2004), encoding for peripheral myelin protein 22 kD (PMP22) (Lupski and Chance, 2005), myelin protein zero (P0) (Shy, 2005) and connexin 32 (also designated gap-junction protein connexin 32 [GJB1]) (Scherer and Kleopa, 2005). Clinically, the disorders are defined by symmetrical distal muscle weakness, muscle atrophy, and sensory impairment (Shy et al., 2005). A mostly symmetrical reduction of nerve conduction velocities below 38 m/s is another typical feature of CMT1. Pathology is characterized by a distinct loss of myelin and myelinated axons, accompanied by an overproduction of crescent-shaped Schwann cells forming abundant onion bulbs (Shy et al., 2005) reflecting most probably various cycles of de- and remyelination, including Schwann cell proliferation (Low, 1977). All these features are slowly progressive and usually result in profound disability after varying time periods.

More severe and early onset forms of inherited neuropathies have been designated Dejerine-Sottas-syndrome or congenital hypomyelinating neuropathies. The typical features of these severe disabling disorders are abnormal myelination beginning from early childhood and the evidence of very low nerve conduction velocities usually below 10 m/s. These disorders are often caused by certain mutations in myelin-related genes, such as P0, PMP22, and the transcription factor early growth response-2 gene (Warner and Lupski, 2005)—a homologue to the rat krox-20 gene, which is relevant for myelination (Topilko et al., 1994). Interestingly, among the major CMT1 culprit genes, GJB1 has so far not been reported to cause severe forms of inherited neuropathies.

During the last years, our group has demonstrated that in two established mouse models for CMT1 disorders, immune cells fulfill pathogenetic functions (Martini, 2005). Unexpectedly, in a model for the severe Dejerine-Sottas syndrome, immune cells appear to play a neuroprotective role of still unknown mechanism (Berghoff et al., 2005).

In this review, we summarize our recent findings in models for CMT1 and in a dysmyelinating mutant regarding the involvement of the immune system into the primarily genetically mediated neuropathies.

Models for Inherited Demyelination (CMT1)

Mice Heterozygously Deficient for P0

The myelin formation is almost normal during the first 4 mo in P0+/– mutants, which makes them a suitable model for a demyelinating disease. Then a progressive demyelinating neuropathy is detectable in motor, but not in sensory nerves (Martini et al., 1995; Martini, 1997; Shy et al., 1997; Samsam et al., 2002). Typical pathological features are demyelinated axons, remarkable thin myelin sheaths reflecting incomplete remyelination, and supernumerary Schwann cells reminiscent of onion bulbs (Fig. 1). In line with the demyelinating phenotype electrophysiology reveals prolonged F-wave latencies of the compound muscle action potential (Martini et al., 1995). Thus, P0+/– mutants can be regarded as an animal model for mild forms of human CMT1B (Martini et al., 1995; Martini, 2005).

Fig. 1. *(Opposite page)* Electron microscopy of mutant myelin sheathes. Conventional transmission electron microscopy of peripheral nerves **(A, C, D)** and ventral roots **(B, E)** of adult wild-type mice **(A)**, P0+/– mice **(B)**, gap-junction (GJ) B1–/– mice **(C)**, transgenic mice mildly overexpressing PMP22 **(D)**, and P0–/– mice **(E)**. Note abundant supernumerary Schwann cells (arrows in **B–D**) and reduced myelin thickness in all demyelinating mutans **(B–D)**. Typical "genotype-specific" features are enlarged periaxonal collars in GJB1–/– mice (arrowheads in **C**) and hypermyelinated axons in PMP22 transgenic mice (hA in **D**). In the dysmyelinating P0–/– mutant, myelin compaction is defective from the outset (asterisk in **E**). dA; demyelinated axon. **(A)** is adopted from Suter and Martini (2005); **(D)** from Kobsar et al. (2005); **(E)** from Samsam et al. (2003). Bar = 0.5 μm **(A)**, 1 μm (**B, C,** and **E**), 2 μm **(D)**.

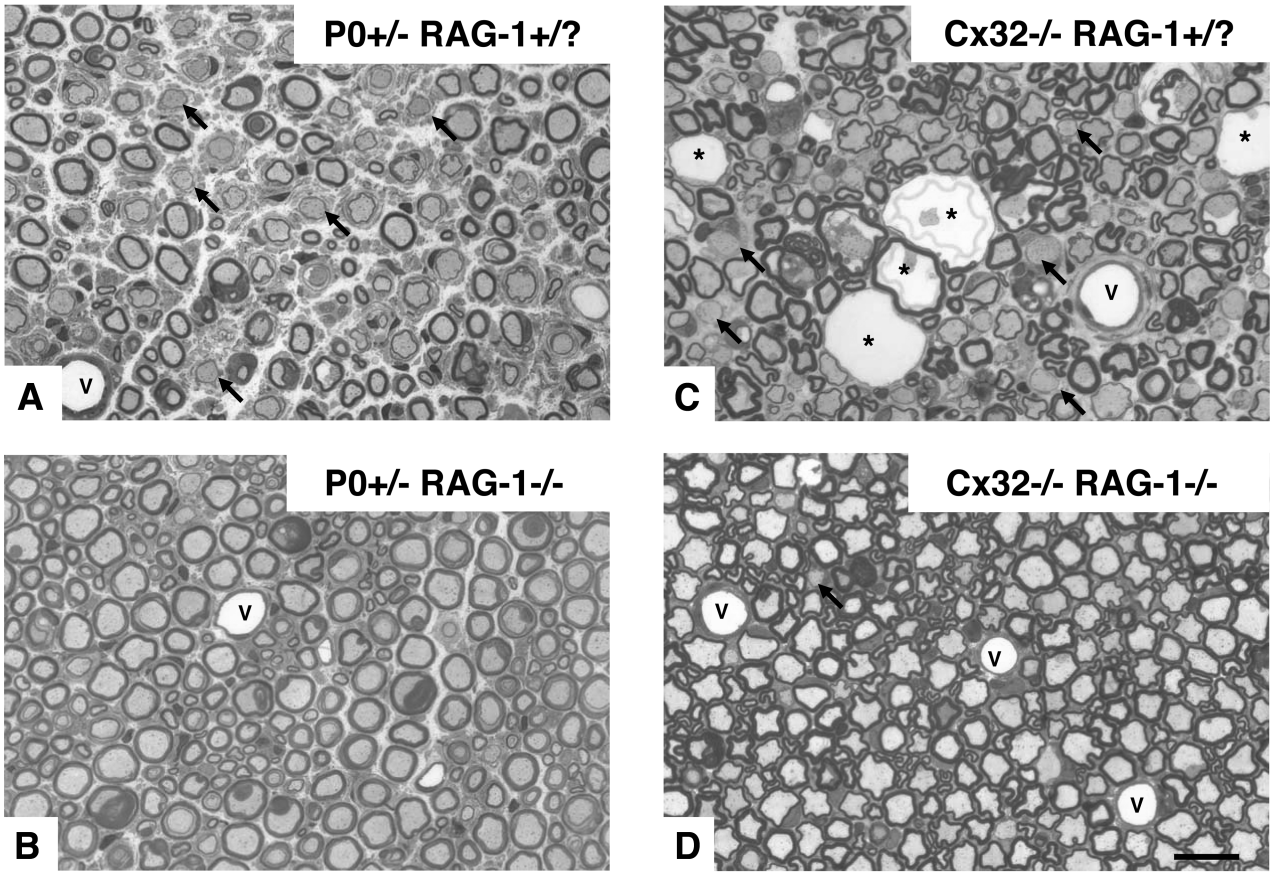


Fig. 2. RAG-1 deficiency alleviates inherited myelinopathy and axonopathy. Semithin sections of femoral quadriceps nerves **(A, B)** and of ventral roots **(C, D)** of P0+/– **(A, B)** and GJB1–/– mice **(C, D)** with intact (RAG-1+/?; **A, C**) and deficient (RAG-1–/–; **B, D**) immune system. Note that the pathological alterations are much more mild in immune-deficient myelin mutants. Some demyelinated axons are indicated by arrows; asterisks mark periaxonal vacuoles as axonopathic changes in GJB1 mutants. V, blood vessel. Bar in **D** (for **A–D**): 20 µm.

A striking and initially unexpected feature was the presence of CD8+ T-lymphocytes and macrophages within the endoneurium of these mutants. This feature was first described by Shy and colleagues (Shy et al., 1997). The number of these immune cells increased with time and progressing demyelinating neuropathy (Shy et al., 1997; Schmid et al., 2000). In order to investigate the functional role of these cells, the myelin mutants were cross-bred with mice deficient in mature T- and B-lymphocytes, i.e., recombination-activating gene (RAG)-1-deficient mice (Mombaerts et al., 1992).

Histologically, the double mutants showed less severe myelin degeneration in the absence of lymphocytes (Fig. 2). This improvement in myelin maintenance was reflected by an amelioration of nerve conduction properties (Schmid et al., 2000). In addition, the rescue of the demyelinating neuropathy by introduction of the RAG-1-deficiency was experimentally reversible, as P0+/–/RAG-1–/– double mutants showed an aggravated phenotype when reconstituted with bone marrow from immune-competent wild-type mice (Mäurer et al., 2001). Together with the observation that P0+/– mice deficient in the T-lymphocyte receptor-α show a similar amelioration in the demyelinating phenotype as P0+/–/RAG-1–/– double mutants (Schmid et al., 2000), the reconstitution experiments proved that it is rather the absence of T-lymphocytes than the RAG-1-deficiency *per se* that ameliorated demyelination in P0+/– mice.

The role of macrophages in inherited demyelination was also investigated in the mutants. Electron

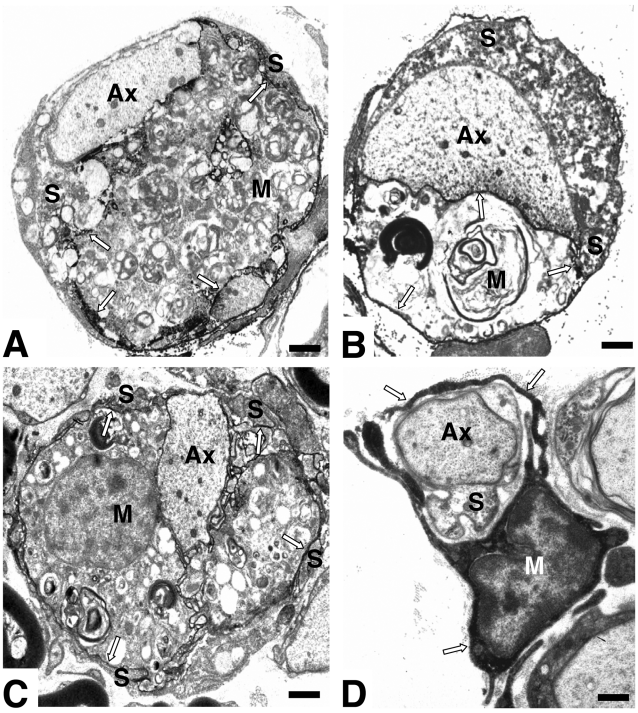


Fig. 3. Spatial myelin–macrophage relationship in different myelin mutants. Immunoelectron microscopic identification of macrophages using the specific antibodies to F4/80 in the PNS of adult P0+/– mice **(A)**, GJB1–/– mice **(B)**, transgenic mice mildly overexpressing PMP22 **(C)** and P0–/– mice **(D)**. Note that in the demyelinating mutants **(A–C)**, the association between demyelinated axons (Ax) and F4/80+ macrophages (M; arrows mark electron-dense F4/80-immunoreactivity) is similar among the different genotypes. In the dysmyelinating mutant **(D)**, the processes of a F4/80+ macrophage "embrace" a dysmyelinated axon without penetrating the Schwann cell basal lamina. S; Schwann cells. **(B)** is adopted from Kobsar et al. (2002); **(C)** from Kobsar et al. (2005); **(D)** from Berghoff et al. (2005). Bar = 1 μm **(A, B, D)**; 2 μm **(C)**.

microscopy and immunoelectron microscopy revealed that, in the P0+/– mutants, some macrophages had entered the endoneurial tubes either contacting still morphologically intact myelin or demyelinated axons (Fig. 3). This apposition of macrophages with endoneurial tubes was highly suggestive for an involvement in degeneration and resembled macrophage-myelin interaction in Guillain-Barré-Syndrome (Ballin and Thomas, 1969; Lampert, 1969; Ho et al., 1998). In addition, this feature is also seen in other demyelinating mutants, such as GJB1-deficient mice and PMP22 overexpressors (Fig. 3). This is why we cross-bred the P0+/– mutants with spontaneous mutants deficient in the macrophage-colony-stimulating factor (M-CSF) hence displaying impaired macrophage activation. In the P0+/– double mutants lacking M-CSF, the numbers of macrophages did not increase within in the demyelinating nerves. The demyelinating phenotype was less severe than in the P0+/– single mutants demonstrating that macrophages are functionally involved in genetic demyelination (Fig. 4) (Carenini et al., 2001).

We also investigated the origin of macrophages in the P0+/– mutants. Two scenarios are conceivable; namely that elevated levels of macrophages could either be explained by acutely infiltrating cells or owing to local proliferation and activation of resident macrophages (Mäurer et al., 2003). To resolve this question, we performed bone marrow transplantation experiments using bone marrow from transgenic mice expressing green fluorescent protein in all somatic cells. As a result, we found that both resident but also recently immigrated macrophages contribute to the elevated macrophage numbers in demyelinating nerves. Interestingly, both cells appear to proliferate *in situ*. Thus, local signaling in the nerve rather than extrinsic signals are responsible for the activation of macrophages within the nerves of the mutants.

In order to initiate an approach to characterize putative macrophage-related signals, we performed real-time polymerase chain reaction (PCR) with extracts from wild-type and P0+/– mutant nerves using probes for various cytokines and chemokines. Strikingly, monocyte chemoattractant protein (MCP)-1 RNA was already upregulated at approx 3 mo (i.e., before robust macrophage infiltration takes place). By contrast, the proinflammatory cytokines tumor necrosis factor-α and interleukin-6 were not elevated. Further studies are underway to characterize the functional role of MCP-1 and the signaling pathways that mediate MCP-1 upregulation in the P0+/– mutants (Fischer, Kleinschnitz, Berghoff, Weishaupt, Troppmair and Martini unpublished).

When characterizing the phenotype of bone marrow-derived cells within the peripheral nerves of the mutants, we unexpectedly identified green fluorescent protein+ cells that fulfilled morphological and immunohistochemical criteria of

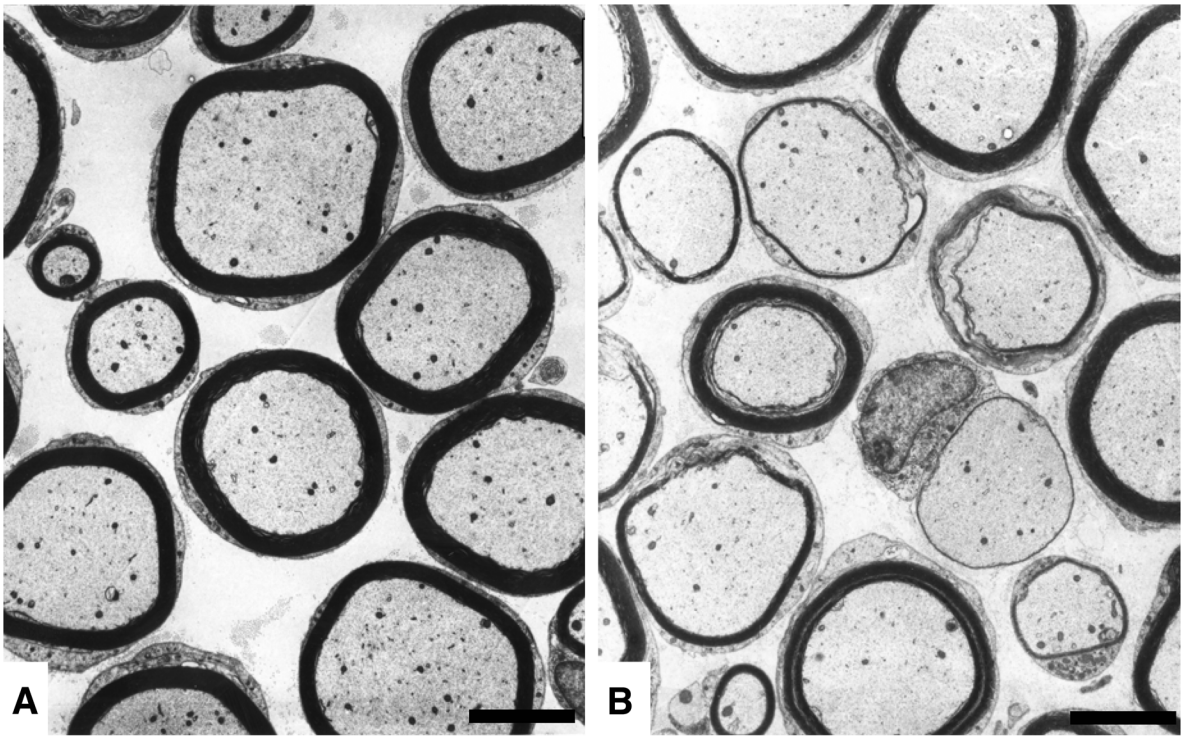


Fig. 4. Macrophages as culprit cells in a demyelinating mutant. Conventional transmission electron microscopy of ventral roots of P0+/– mice deficient in M-CSF **(A)** and with intact M-CSF expression **(B)**. Note that retarded macrophage activation leads to a substantially improved myelin maintenance **(A)**. Adopted from Carenini et al. (2001). Bar = 5 μm.

endoneurial fibroblasts. By fluorescence microscopy, these cells could be identified by their positive expression of both the fibroblast marker platelet-derived growth factor-receptor-α and the hematopoetic stem cell marker CD34. Most interestingly, these cells appear to frequently make contacts with F4/80-positive macrophages (Figs. 5 and 6). Interestingly, similar observations could be made in GJB1 and PMP22 mutants (Fig. 6), possibly reflecting a widespread phenomenon.

Mice Homo/Hemizygous-Deficient for GJB1

We addressed the question whether the involvement of immune cells in the primarily genetically mediated demyelination is unique to P0+/– mutants or may be a more widespread phenomenon. For this purpose, we selected a myelin mutant that shows a comparable demyelination pattern as P0+/– mutants (i.e., a nearly normal myelination pattern for a couple of months, followed by constantly progressing demyelination). These criteria were fulfilled by the

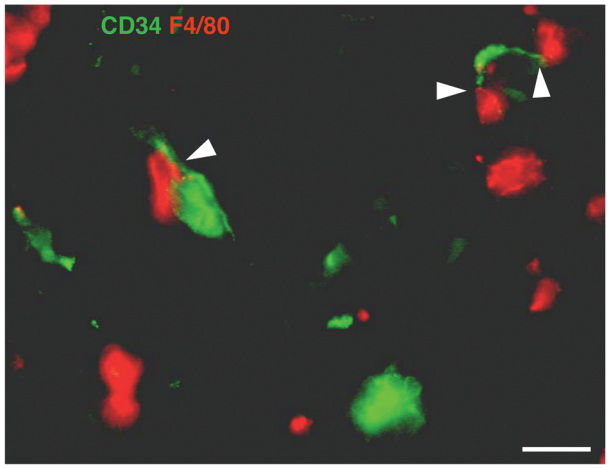
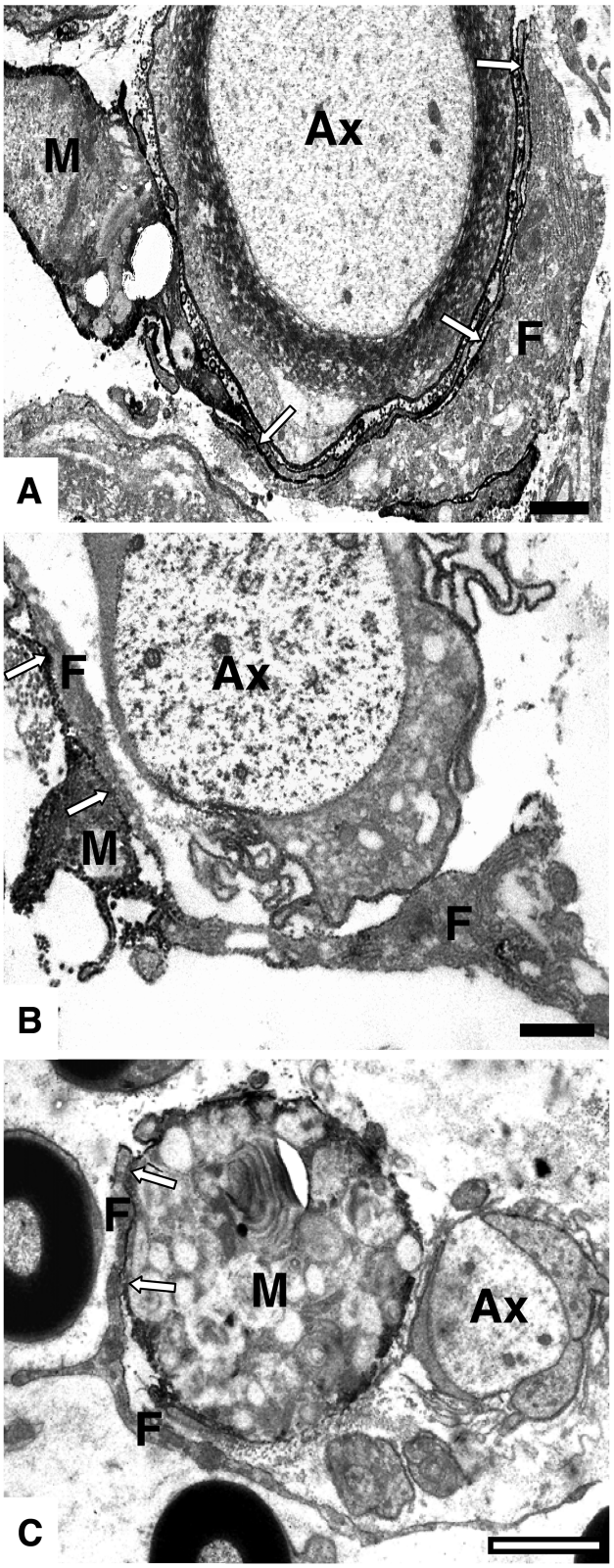


Fig. 5. Cell contacts between macrophages and endoneurial fibroblasts. Fluorescence microscopy of F4/80+ endoneurial macrophages (red) and CD34+ endoneurial fibroblasts (green) in a peripheral nerve of a 6-mo-old P0+/– mouse. Note contacts between macrophages and fibroblasts. Cells positive for both markers are not detectable. Bar = 10 μm.



mouse mutant deficient in the *GJB1* gene that is a model for the X-linked dominant form of CMT (CMT1X) (Anzini et al., 1997; Scherer et al., 1998).

The features indicative of demyelination, such as thinly remyelinated axons and supernumerary Schwann cells, are very similar to those in P0+/– mice (Fig. 1). However, there are a couple of features that are unique to the GJB1-deficiency, such as abnormally enlarged periaxonal collars and axonal damage and resprouting (Fig. 1) (Anzini et al., 1997; Scherer et al., 1998).

Concerning the presence of immune cells in peripheral nerves, we found, as in P0+/– mutants, increased numbers of CD8+ T lymphocytes and macrophages in the endoneurium. In addition, macrophages were found within endoneurial tubes and contacted degenerating myelin reminiscent of a macrophage-mediated demyelinating neuropathy (Fig. 3) (Kobsar et al., 2002). Furthermore, macrophages were often in contact with fibroblasts (Fig. 6). Cross-breeding of GJB1-deficient mice with RAG-1–/– mice led to reduced numbers of endoneurial macrophages and to a substantial mitigation of features indicative of myelin degeneration and axonopathic changes (Fig. 2) (Kobsar et al., 2003). However, hallmarks for GJB1-deficiency, such as enlarged periaxonal Schwann cell collars, were not reduced (Kobsar et al., 2003). Thus, the immune system appears to be a possibly widespread modulator of the severity of pathological changes in myelin mutants.

Transgenic Mice Mildly Overexpressing the Myelin Protein PMP22

CMT1A, the most frequent demyelinating form of inherited neuropathies is predominantly caused by a 1.5 Mb genomic duplication within chromosome 17 encompassing the gene encoding PMP22

Fig. 6. Electron microscopic view of cell contacts between macrophages and endoneurial fibroblasts in different myelin mutants. Immunoelectron microscopic identification of F4/80+ macrophages (M) contacting endoneurial fibroblasts (F) in adult P0+/– mice **(A)**, GJB1–/– mice **(B)** and transgenic mice mildly overexpressing PMP22 **(C)**. The fibroblasts are void of F4/80-positivity and lack a basement membrane. Arrows mark electron-dense F4/80-immunoreactivity; Ax, axons. Bar = 1 μm **(A, B)**; 2 μm **(C)**.

(Lupski et al., 1991; Raeymaekers et al., 1991; Lupski and Chance, 2005). This extra gene copy leads to an overexpression of PMP22 resulting in peripheral nervous system demyelination as demonstrated by slowed nerve conduction velocities and segmental demyelination on nerve biopsies (Lupski and Chance, 2005).

As an approach to investigate the mechanism of this demyelinating CMT1A neuropathy, several transgenic rats and mice expressing altered levels of PMP22 were generated (Adlkofer et al., 1995; Huxley et al., 1996; Magyar et al., 1996; Sereda et al., 1996; Huxley et al., 1998; Niemann et al., 1999; Perea et al., 2001; Robertson et al., 2002; Robertson and Huxley, 2005). Some of these transgenic animals express multiple copies of PMP22 and are, thus, characterized by dysmyelination (Robertson and Huxley, 2005). As a model for CMT1A, a transgenic rat mutant with three additional mouse *PMP22* genes (Sereda et al., 1996) and a mouse mutant with four additional human *pmp22* genes (C61) (Huxley et al., 1998; Robertson and Huxley, 2005) displayed a mild PMP22 overexpression and showed histopathological features similar to those seen in CMT1A patients, such as de- and remyelinating profiles as well as hypermyelination and onion bulb formation. Additionally, the overall progression of the disorder was slow.

Our electron microscopic and electrophysiological investigations confirmed that this mouse strain develops pathological features similar to those found in CMT1A patients. Moreover, we detected an upregulation of CD8+ and F4/80-positive lymphocytes and macrophages, respectively, in peripheral nerves (Kobsar et al., 2005). The observation that macrophages enter endoneurial tubes of the mutants and obviously phagocytose morphologically normal myelin strongly suggests that the myelin degeneration is at least partially mediated by these phagocytic cells (Fig. 3). Additionally, and similar to the other myelin mutants, cell contacts between macrophages and endoneurial fibroblasts were seen (Fig. 6). By gene-array technology and real-time PCR of peripheral nerve homogenates from PMP22 mutants MCP-1 could be identified as a putative factor to attract or activate macrophages that attack myelin sheaths in this model of CMT1A (Kobsar et al., 2005). This is a striking similarity to P0+/– mice regarding the early molecular signals that might attract and activate macrophages. Further studies are needed to prove the functional role of the immune cells in C61 mice mimicking the most common inherited demyelinating neuropathies in humans.

Mouse Models for Inherited Dysmyelination

Apart from late-onset, demyelinating forms of inherited neuropathies, we investigated the influence of immune cells on early-onset dysmyelination in mice homozygously deficient of the myelin component P0. In contrast to demyelinating mutants in which myelin is almost normally formed for a limited time period, myelination in P0–/– mutants is abnormal from the outset.

A major constraint in these mutants is abnormal myelin compaction and myelin degeneration in peripheral nerves (Fig. 1) (Giese et al., 1992; Anzini et al., 1997). Another striking feature is the substantial loss of myelinated axons, particularly at the distal ends of long nerves. First evidence came from electrophysiological investigations revealing not only features indicative of abnormal myelination, but also of axonal impairment owing to the strongly reduced amplitudes of compound muscle action potentials (Martini et al., 1995; Zielasek et al., 1996). Consequent morphometric analyses then revealed loss of axons in the toes of the hind feet (Frei et al., 1999) and in plantar nerves of P0-deficient mice (Samsam et al., 2003). Strikingly, the most prominent rate of axon loss was found within the first 3 mo.

Because axonal loss is clinically the most relevant pathological alteration in de- and dysmyelinating disorders, we investigated this issue regarding an impact of immune cells (Berghoff et al., 2005). In peripheral nerves of P0–/– mice, CD8+ T-lymphocytes were detected and the cells increased with age. Macrophages showed different dynamics than observed in demyelinating mutants in that they did not constantly increase with age, but peaked at 3 mo of age, followed by a substantial decline. Another difference to demyelinating mutants was the origin of the macrophages. Whereas in demyelinating mutants, both hematogeneous and resident macrophages contributed to the elevated cell number, in P0–/– mice, the macrophages were almost completely of hematogenous origin (Berghoff

et al., 2005). Furthermore, and in contrast to our previous observations in demyelinating P0+/–, GJB1–/– and PMP22-overexpressing mice (Carenini et al., 2001; Kobsar et al., 2002, 2005), macrophages were exclusively located in the endoneurium and did not penetrate the Schwann cell basal laminae to invade the endoneurial tubes (Fig. 3).

In order to evaluate the functional role of immune cells, we cross-bred P0–/– mutants with RAG-1-deficient mice. As expected, cross-breeding completely abolished the lymphocytes from peripheral nerves of the mutants. At 3 mo, the number of endoneurial macrophages did not differ from macrophage numbers of immunocompetent myelin mutants, but a later decline of macrophages was not observed.

Morphologically, the absence of lymphocytes and the altered regulation of macrophage numbers in the P0–/–/RAG-1–/– mutants did not change the dysmyelinating histopathological phenotype, as revealed by morphometric studies at the electron microscopic level (Berghoff et al., 2005). However, RAG-1 deficiency enhanced the typical axonal loss by 25% when compared with axonal loss in immunocompetent P0–/– mice. These facts differ from our observations in demyelinating P0+/– and GJB1–/– mice, in which the genetic ablation of T and B lymphocytes led to a significant amelioration of the pathological phenotype (Schmid et al., 2000; Kobsar et al., 2003; Martini and Toyka, 2004). Thus, a neuroprotective net effect of T lymphocytes on axon survival in inherited, early onset dysmyelination has to be postulated.

Discussion

Possible Role of Immune Cells in Demyelinating Mutants

Our studies have shown that in at least two models of inherited demyelinating neuropathies (P0+/–, GJB1–/– mice) immune cells play comparable roles during pathogenesis. This was proven by cross-breeding P0+/– and GJB1–/– mutants, respectively, with mice deficient in RAG-1 resulting in an ameliorated pathological phenotype. Although this formal proof is still lacking for PMP22-overexpressing mice, we assume that in this mutant the immune system plays a similar role. This view is based on the observation that the type and number of immune cells is comparable to the situation found in P0+/– and GJB1–/– mutants. For instance, it is the CD8+ T-lymphocyte population that is found elevated in the peripheral nerves of the PMP22 mice and the number of these cells is enhanced to a similar extent as in the other mutants. Additionally, the number of macrophages resembles that seen in the P0+/– and GJB1–/– mutants. Furthermore, the penetration of the Schwann cell basal laminae by macrophages and their association with demyelinating profiles or naked axons is also comparable to the corresponding features seen in the P0+/– and GJB1–/– mutants (Kobsar et al., 2005).

Assuming that the contribution of immune cells to inherited demyelination is a wide-spread phenomenon, it is of particular interest which signals might activate the immune system in the demyelinating mutants. It is possible that total (GJB1) or partial disruption (P0) or mild overexpression (PMP22) of peripheral myelin genes leads to cellular stress within the Schwann cell when myelination starts and/or when myelin has to be maintained. This could possibly result in the expression of the chemokine MCP-1, as found in P0+/– (Fischer, Kleinschnitz, Berghoff, Weishaupt, Troppmair and Martini unpublished) and PMP22 mutants (Fig. 7) (Kobsar et al., 2005). MCP-1 has been shown to be expressed by Schwann cells under various pathological conditions (Toews et al., 1998; Taskinen and Roytta, 2000; Tofaris et al., 2002). Once attracted by MCP-1, the macrophages could attract T cells, which develop either into effector cells or into CD8+ regulatory cells that, in turn, may operate as positive regulators of locally activated resident macrophages (Hartung et al., 1998; Gold et al., 2005). This hypothesis is supported by the observation that in RAG-1-deficient P0+/– or GJB1–/– double mutants not only T lymphocytes are lacking but also macrophages did not show an upregulation as seen in myelin mutants with an intact immune system (Schmid et al., 2000; Kobsar et al., 2003). Furthermore, in P0+/–, GJB1–/– and in the PMP22 mutants, macrophages by far outnumber CD8+ T cells. Therefore, we presently favor the idea that the regulation of phagocytic macrophages could be the major task of endoneurial CD8+ lymphocytes in myelin mutants.

Up to now, endoneurial fibroblasts have been poorly recognized. We could demonstrate in the P0+/– mutants that endoneurial fibroblasts are of hematogeneous origin, express the fibroblast

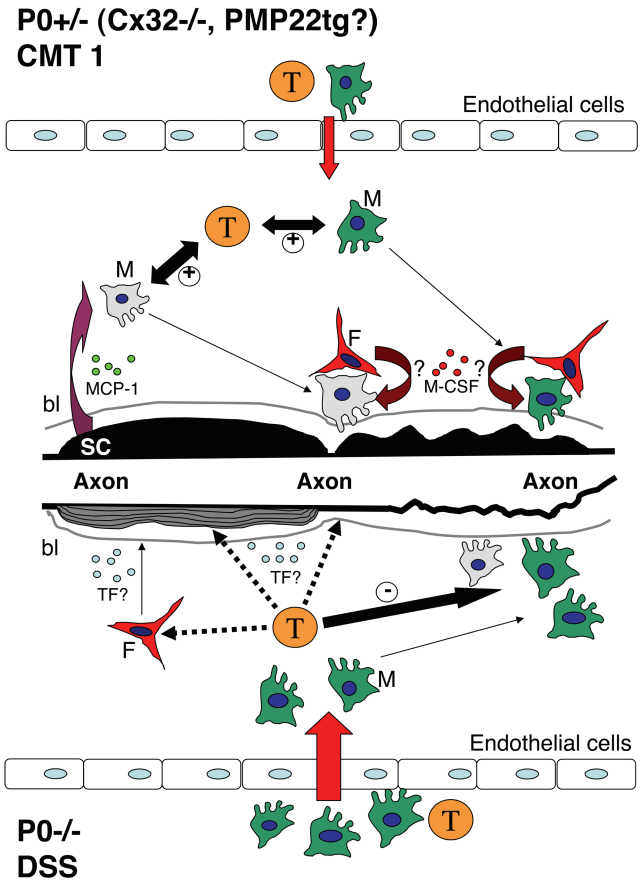


Fig. 7. Role and origin of immune cells in models for demyelinating (P0+/–, GJB1–/–; CMT-1; upper part of the scheme) and dysmyelinating forms (P0–/–; Dejerine-Sottas syndrome, DSS; lower part of the scheme) of inherited neuropathies. P0+/– mice show a demyelinating phenotype that is mainly mediated by resident macrophages (M, gray color). The macrophages penetrate the basal lamina (bl) of Schwann cells (SC) and are activated by the CD8+ T-lymphocytes (T) that are observed in demyelinating nerves. Assumingly, macrophage activation is also dependent on various chemokines/cytokines like Schwann cell-derived MCP-1. The important role of M-CSF has been proven for P0+/– mice; the cytokine may be derived from endoneurial fibroblasts (F). These cells are often in close association with macrophages. The activating role of CD8+ T lymphocytes has also been demonstrated in GJB1–/– mice. The role of these cells in PMP22 overexpressing mice is still hypothetical, as well as the role of macrophage-activating mechanisms in GJB1–/– mice and in PMP22 overexpressing mice. P0–/– mice show a dysmyelinating phenotype with pronounced axonopathy. Increased macrophage number is primarily owing to immigration of haematogenous macrophages (M, green color). They do not penetrate Schwann cell basal laminae, may foster axonopathic changes by secreting proinflammatory cytokines and may be negatively regulated by T-lymphocytes. T-lymphocytes could also secrete trophic facors (TF) or trigger their expression in Schwann cells and, thus, act neuroprotective. Another putative source for TF are endoneurial fibroblasts.

marker platelet-derived growth factor-receptor-α and the hematogenous stem cell marker CD34, but never the macrophage marker F4/80 (Mäurer et al., 2003). Their association with macrophages is most interesting. Although the relevance of this association is not yet clear, the fibroblasts might execute an important function concerning macrophage regulation. An example for a regulatory interaction between fibroblast-like cells and macrophages is found within bone tissue in which osteoblasts, representing a population of sophisticated fibroblast-like cells, contact osteoclasts, which represent a cell population of highly specialized macrophages. This interaction is crucial for the differentiation of monocytic cells into osteoclasts via the interaction of the receptor of activation nuclear factor-κB with its ligand receptor of activation nuclear factor-L. Additionally, the osteoblasts secrete M-CSF that activates the osteoclasts (Teitelbaum, 2000). Interestingly, we could show that P0+/– mutants with a defect in the M-CSF gene failed to upregulate endoneurial macrophages and showed a milder demyelinating phenotype within motor nerves and ventral roots (Carenini et al., 2001). Therefore, it is conceivable that the interaction of macrophages and endoneurial fibroblasts might be critical for the pathogenetic mechanisms leading from the genetic defect to myelin loss.

Possible Role of Immune Cells in Dysmyelinating Mutants

Regarding our findings in demyelinating mutants, it was unexpected that the immune system appears to play a different role in the dysmyelinating P0–/– mice. In this context, it is important to stress that the severity and progression of the disorder is much more pronounced in P0–/– mice than in demyelinating mutants. In P0+/– and GJB1–/– mice, myelination is normal in the first 3–4 mo of age and is accompanied by a gradual increase of

Table 1
Impact of RAG-1 Deficiency in Demyelinating (P0+/– or GJB1–/–) and Dysmyelinating (P0–/–) Mouse Mutants

	P0+/– or GJB1–/–		P0–/–	
	RAG-1 +/?	RAG-1 –/–	RAG-1 +/?	RAG-1 –/–
Macrophages	++	+	++	+++
T-cells (CD8+)	+	0	+	0
De-/Dysmyelination	++	+	+++	+++
Axonopathy	++[a]	(+)[a]	++	+++

In the demyelinating models, RAG-1 deficiency leads to a reduction of macrophage number and to an amelioration of demyelination and axonopathy. In contrast, RAG-1 deficiency in the dysmyelinating model impedes downregulation of macrophage numbers at 6 mo and fosters axonal loss. Because RAG-1+/+ and RAG-1+/– mice cannot be discriminated by FACS analysis showing similar T-lymphocyte numbers, all mice containing T-lymphocytes are designated as RAG-1+/?

[a]Among the two demyelinating models, a pronounced axonopathy is observed only in peripheral nerves of GJB1–/– mice.

macrophages beginning at postnatal month 2 (Schmid et al., 2000; Kobsar et al., 2002; Kobsar et al., 2003). This early upregulation of macrophages is followed by an increase of CD8+ T-lymphocyte numbers first seen at postnatal month 6. Similar dynamics in macrophage and lymphocyte increase have been shown in PMP22-overexpressing mice (Kobsar et al., 2005). In the dysmyelinating P0–/– mutants, however, the severe and early-onset phenotype is accompanied by an early upregulation of both macrophages and CD8+ T lymphocytes. In addition, the increase of endoneurial macrophages in the dysmyelinating model is caused by a distinct influx of macrophages from the blood pool, whereas increase in macrophage numbers in the demyelinating P0+/– mouse is mainly the result of a local proliferation of resident macrophages (Fig. 7) (Mäurer et al., 2003).

Another striking difference between demyelinating and dysmyelinating mutants is the location of macrophages within the endoneurial compartment of the peripheral nerves. In all demyelinating mutants investigated, macrophages are frequently laden with myelin debris and occasionally penetrate the basal laminae of the Schwann cells. In P0–/– mutants, macrophages are usually devoid of myelin vacuoles and are very rarely found penetrating the Schwann cell basal lamina. Moreover, the regulation of macrophage numbers is different (Table 1). In the demyelinating mutants, the number of macrophages gradually increases, whereas in the dysmyelinating P0–/– mutant, the number of macrophages peaks at postnatal month 3 followed by a significant decline. In P0–/–/RAG-1–/– mutants, the number of macrophages remains at an unexpected high level and does not decline. This is again in contrast to the demyelinating mutants in which RAG-1 deficiency was always accompanied by a significantly reduced macrophage number in peripheral nerves (Schmid et al., 2000; Kobsar et al., 2003). Thus, recruitment, activation, regulation, and phagocytic behavior of macrophages seem to follow quite different cellular and molecular pathways in demyelinating vs dysmyelinating mutants (Fig. 7; Table 1).

Our observation that RAG-1 deficiency in P0–/– mutants prevents the reduction of endoneurial macrophages resulting in a higher degree of axonal degeneration suggests that macrophages are cytotoxic for dysmyelinated axons. It is conceivable that the complete absence of lymphocytes, including T-suppressor cells, could cause a lack of regulation of cytotoxic macrophages (Hartung et al., 1998). The endoneurial macrophages, which are consequently present in high numbers in the double mutants, could then damage axons by secreting cytotoxic agents and mediators, such as proinflammatory cytokines, free radicals, prostaglandins (Hartung et al., 1993; Gold et al., 2005), nitric oxide (Zielasek et al., 1995), and matrix metalloproteinases (Kieseier et al., 1998; Hartung and Kieseier, 2000; Hartung et al., 2005). It is of note in this context that in P0–/– mice, all

endoneurial macrophages have been identified as cells expressing tumor necrosis factor-α and interleukin-1β (Berghoff et al., 2005).

Regarding our present observations, it is also conceivable that lymphocytes have a neuroprotective role in P0–/– mice. This concept has been proposed for primary immune-mediated inflammatory diseases of the central nervous system (Kerschensteiner et al., 1999, 2003; Hohlfeld et al., 2000) and for central nervous system injury (Hauben et al., 2000; Moalem et al., 2000; Schwartz, 2001). However, it is questionable whether the few CD8+ lymphocytes in the peripheral nerves of the P0–/– mutants could mediate the survival of axons by a direct mechanism, such as secretion of neurotrophic factors (Kerschensteiner et al., 1999, 2003; Hohlfeld et al., 2000). Therefore, it is conceivable that the lymphocytes trigger the expression of such factors in Schwann cells and/or endoneurial fibroblasts as it has been shown for neurotrophic factors after lesion injuries in the PNS (Bandtlow et al., 1987; Heumann et al., 1987a,1987b; Lindholm et al., 1988; Barde, 1989; Stoll and Müller, 1999). Future experiments are needed to clarify this issue in the P0–/– mutant.

Concluding Remarks

Our combined findings suggesting a dual role of immune cells in models for inherited neuropathies may be of clinical relevance. On the one hand, the evidence of a detrimental role of immune cells in mouse mutants mimicking demyelinating forms of human hereditary neuropathies raises hope for an effective immunotherapeutic intervention in these disorders (Mäurer et al., 2002; Martini and Toyka, 2004). On the other hand, according to the observations in dysmyelinating mutants, it is possible that the inhibition of the immune system has a net effect resulting in axonal damage, so that in such cases an unspecific immunotherapeutic intervention could be detrimental. In accordance with these concerns is the notion that the balance between destructive and protective effects of neuroinflammation may vary in different disorders or even within different stages of a distinct disease (Kerschensteiner et al., 2003; Kim and de Vellis, 2005). It is, therefore, important to investigate the parameters that switch the immune system toward a destructive or to a beneficial role during the pathogenesis of inherited peripheral neuropathies. Moreover, understanding the role of the immune system also in other neurodegenerative disorders, such as Alzheimer's (Blasko et al., 2004; Stuchbury and Munch, 2005; Zlokovic, 2005) and Parkinson's diseases (Blum et al., 2004; Hald and Lotharius, 2005; Nagatsu and Sawada, 2005), or in amytrophic lateral sclerosis (Angelov et al., 2003; Blum et al., 2004; Sargsyan et al., 2005), and in leukodystrophies (Berger et al., 2001; Ito et al., 2001) might be an important prerequisite to augment the efficiency of established treatment strategies and to develop novel therapeutic approaches in the future.

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