The cellular prion protein PrP\textsuperscript{C} confers susceptibility to transmissible spongiform encephalopathies, yet its normal function is unknown. Although PrP\textsuperscript{C}-deficient mice develop and live normally, expression of amino proximally truncated PrP\textsuperscript{C} (ΔPrP) or of its structural homolog Doppel (Dpl) causes cerebellar degeneration that is prevented by coexpression of full-length PrP\textsuperscript{C}. We now report that mice expressing ΔPrP or Dpl suffer from widespread leukoencephalopathy. Oligodendrocyte-specific expression of full-length PrP\textsuperscript{C} under control of the myelin basic protein (MBP) promoter repressed leukoencephalopathy and vastly extended survival but did not prevent cerebellar granule cell (CGC) degeneration. Conversely, neuron-specific PrP\textsuperscript{C} expression under control of the neuron-specific enolase (NSE) promoter antagonized CGC degeneration but not leukoencephalopathy. PrP\textsuperscript{C} was found in purified myelin and in cultured oligodendrocytes of both wild-type and MBP-PrP transgenic mice but not in NSE-PrP mice. These results identify white-matter damage as an extraneuronal PrP-associate pathology and suggest a previously unrecognized role of PrP\textsuperscript{C} in myelin maintenance.

Key words: cerebellum; spinal cord; prion protein; Doppel; leukoencephalopathy; neurodegeneration

Introduction

The infectious agent causing transmissible spongiform encephalopathies (TSE) was termed prion (Prusiner, 1982). Its only known constituent is PrP\textsuperscript{Sc}, a TSE-associated isoform of the cellular prion protein PrP\textsuperscript{C}. Ablation of the Prnp gene, which encodes PrP\textsuperscript{C}, abrogates prion replication (Büeler et al., 1993) and scrapie pathogenesis (Brandner et al., 1996), but the physiological function of PrP\textsuperscript{C} has remained elusive (Aguzzi and Polymenidou, 2004). Prnp\textsuperscript{o/o} mice show no obvious developmental defects and enjoy a normal life expectancy (Büeler et al., 1992). Subtle changes in circadian rhythms (Tobler et al., 1996), alterations of hippocampal function (Collinge et al., 1994; Colling et al., 1997; Herrms et al., 1999; Mallucci et al., 2002), and behavioral abnormalities have been described. However, some of these phenotypes were not reproduced by others (Lledo et al., 1996), and none were clarified in molecular terms. PrP\textsuperscript{C} binds copper (Brown et al., 1997; Kretzschmar et al., 2000) and may have antiapoptotic properties (Kuwahara et al., 1999; Bouhkar et al., 2001; Chiarini et al., 2002; Zanata et al., 2002) but could also sensitize neurons to apoptotic stimuli (Paitel et al., 2003). A possible dismutase activity of PrP\textsuperscript{C} was not confirmed in vivo (Waggoner et al., 2000; Hutter et al., 2003). Several factors bind PrP\textsuperscript{C}, including the laminin receptor precursor protein (Riegger et al., 1997), heparan sulfate (Caughey et al., 1994), neural cell adhesion molecule (NCAM) (Schmitt-Ulms et al., 2001), and bcl-2 (Kurschner et al., 1995). However, none of these interactions were shown to be functionally significant. Hence, no unified view of PrP\textsuperscript{C} function has emerged from the above observations.

Transgenic expression of amino proximally truncated PrP\textsuperscript{C} mutants causes early-onset ataxia associated with progressive cerebellar granule cell (CGC) degeneration and death at 3–4 months of age, also referred to as Shmerling’s syndrome (Shmerling et al., 1998). Only truncated versions of PrP\textsuperscript{C} encompassing half (amino acids Δ34–121) or all (amino acids Δ34–134) of the highly conserved hydrophobic transmembrane domain 1 (TM1) region (amino acids 113–134) (collectively termed ΔPrP) induce disease. A similar neurodegenerative phenotype affecting cerebellar Purkinje cells was observed in compound heterozygous Prnp\textsuperscript{o/ZHII} mice and in homozygous Prnp\textsuperscript{ZHII/ZHII} mice (Rossi et al., 2001), whose targeted Prnp\textsuperscript{ZHII} allele (termed “ZHII”) because it was the second targeting event of Prnp executed in Zurich) leads to overexpression of the PrP\textsuperscript{C} homolog Doppel (Dpl) (Weissmann and Aguzzi, 1999). This phenotype is only observed in Prnp\textsuperscript{o/o} mice that overexpress ΔPrP or Dpl and is fully reverted by substoichiometric coexpression of full-length PrP\textsuperscript{C}, as either the original endogenous Prnp locus or a transgene under transcriptional control of a “half-genomic” miniversion of Prnp (Fischer et al., 1996). It follows that ΔPrP and Dpl interfere with a physiological function of PrP\textsuperscript{C}. 

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Here we show that, in addition to CGC degeneration, mice expressing ΔPrP or Dpl in their brain suffer from widespread cerebellar leukoencephalopathy. Oligodendrocyte-specific expression of PrPΔC repressed this pathology and restored long-term survival but did not rescue CGC death. Conversely, neuron-specific expression of PrPΔC delayed disease onset and antagonized, to some extent, CGC degeneration but did not prevent white-matter degeneration. Therefore, white-matter disease and CGC degeneration are independent of each other. Surprisingly, white-matter disease appears to represent a major survival-limiting pathology induced by a toxic PrP mutant.

Materials and Methods

Generation of myelin basic protein-PrP and neuron-specific enolase-PrP transgenic mice. Mice hemizygmously transgenic for the F35(PrPΔ32–134)Δ/Δ allele (Shmerling et al., 1998) and hemizygous for the ablated endogenous Prnp locus (PrnpΔ/Δ) (Büeler et al., 1992) are referred to as ΔPrP mice. Myelin basic protein (MBP)-PrPΔ/Δ:mPrnpΔΔ mice (Prinz et al., 2004) were crossed to ΔPrP or to compound PrnpΔΔ:ZHI/ZHII-Lox1 mice (Rossi et al., 2001). The wild-type and neo-targeted Prnp loci were detected by PCR analysis of tail DNA as described previously (Büeler et al., 1992), the F35(PrPΔ32–134)Δ transgenic was detected as described previously (Shmerling et al., 1998), and the ZHI-Lox1 locus was also detected as described previously (Rossi et al., 2001). To detect the MBP-PrP transgene, the following primers were used: 5′-gcgg acag gggt att gtt c-3′ and 5′-tccta ccag tcta cca ccag ggtg-3′ (annealing at 58°C). For the generation of neuron-specific enolase (NSE)-PrPΔ/Δ:mPrnpΔΔ mice, NSE-PrPΔ/Δ:mPrnpΔΔ mice were crossed with F35(PrPΔ32–134)Δ/Δ:mPrnpΔΔ animals. Primers used were as follows: Prnpnt2, 5′-ATA CTG GCC GAC GAT CCC TGT TTC CTA C; P10rev, 5′-GCT GGG CTT GTT CCA GTT CCA CTA CTG ATT GTA GGT AC. These primers amplify selectively the wild-type Prnp allele but not the NSE-PrP transgene and were used to identify animals negative for wild-type Prnp. Standard PCR for Prnp (Büeler et al., 1992) detected both the NSE-PrP and the wild-type Prnp allele and was used to confirm the inheritance of the NSE-PrP in animals lacking wild-type Prnp. Onset of disease was declared when animals exhibited unambiguous tremor or unsteady gait that was blindly recognizable from healthy littermates in the same cage by two different observers. Animals were considered to be at a terminal disease stage when they developed hindlimb paralysis and were unable to reposition for 10 s after having been turned back-down.

Histopathology. Organs were fixed in 4% paraformaldehyde (PFA) in PBS, pH 7.5, paraffin embedded, and cut into 2 μm sections. Brain sections were stained with hematoxylin-eosin, Luxol-Fast Blue (myelin and neurons), and commercial antibodies to GFAP (Dako, Carpinteria, CA), microtubule-associated protein-2 (MAP-2) (Chemicon, Temecula, CA), CNPase (Roche, Basel, Switzerland), or phosphorylated neurofilament protein (Sigma, St. Louis, MO). For major histocompatibility complex class II (MHC-II) and CD11b stains, tissues were snap frozen in OCT, cut on a vibratome, and stained with antibodies to CD11b (Pharmingen, San Diego, CA) or MHC-II (Serotec, Oxford, UK). Antibody stainings were performed as described previously (Shmerling et al., 1998), and the ZHII-lox1 locus was also detected as described previously onto glass or plastic culture chambers coated, respectively, with 100 or 10 μg/mL poly-L-lysine. Oligodendrocyte precursors were expanded with PDGF- and FGF-supplemented SATO medium [400 ng/mL T3, 400 ng/mL T4, 2 mmol/L glutamine, 50 U/mL penicillin, 50 g/mL streptomycin, and 5 mL of N2 supplement (Invitrogen, Paisley, UK)] for 2 d and subsequently differentiated with 1% horse serum-supplemented SATO medium. After 3 d of differentiation, cells were washed with PBS and homogenized in PBS containing 0.5% NP-40, 0.5% sodium deoxycholate and protease inhibitors (Roche). Homogenate was then incubated on ice for 30 min and centrifuged at 10,000 rpm for 15 min at 4°C. Equal amounts of lysate were then processed for immunoblotting with a mouse monoclonal antibody to PrP (ICSM-18) (White et al., 2003) and with a mouse monoclonal anti-actin antibody (Chemicon).

Flotation assays. Flotation of detergent-insoluble complexes was performed as described previously (Naslavsky et al., 1997). Appropriate brain homogenates were extracted for 1 h on ice in cold lysis buffer (150 mM NaCl, 25 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 1% Triton X-100; total protein, 30 μg in 100 μl). Extracts were mixed with two volumes (200 μl) of 60% Optiprep (Axis Shield, Oslo, Norway) to reach a final concentration of 40%. All lysates were loaded at the bottom of Beckman Instruments (Fullerton, CA) ultracentrifuge tubes. A 5–30% Optiprep step gradient in TNE (in mm: 150 NaCl, 25 Tris-HCl, pH 7.5, and 5 mM EDTA) was then overlaid onto the lysate (1300 μl of 30% Optiprep and 300 μl of 5% Optiprep). Tubes were centrifuged for 12 h at 4°C in a TLS55 Beckman Instruments rotor at 100,000 × g. Fractions (200 μl) were collected from the top of the tube and processed for immunoblotting and visualization with an anti-PrP antibody [ICSM-18 (White et al., 2003) and anti-PrD (Tomomori-Sato et al., 2001)] and an antibody against β-actin (Sigma). Both detect the PrP core and display identical patterns and an anti-flotillin 1 antibody (BD Transduction Laboratories, Lexington, KY).

Western blot analysis of myelin proteins. Brains and spinal cords were removed and homogenized in lysis buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mm sodium orthovanadate (Fluka, Basel, Switzerland), and protease inhibitors (Roche) in PBS. Homogenates were incubated on ice for 30 min and centrifuged at 10,000 rpm for 10 min at 4°C. Equal amounts of lysate (10 μg of protein for spinal cord and 20 μg of protein for brain homogenate) were separated by electrophoresis on 12% SDS-PAGE gels and transferred to nitrocellulose membranes. The membranes were probed with antibodies to MBP (Chemicon), myelin-associated glycoprotein (MAG) (Chemicon), proteolipid protein (PLP) (Chemicon), 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) (Abcam, Cambridge, UK), and actin (Chemicon). The amount of MBP, MAG, and actin was quantified using a VersaDoc model 4000 Imaging System (Bio-Rad, Zurich, Switzerland).

Northern blots. Brains were collected and immediately snap frozen in liquid nitrogen before storage at −80°C until required. Total RNA was isolated using Trizol (Invitrogen, Basel, Switzerland) with a T18 Basic Disperser (Ika Works, Staufen, Germany). Northern blot hybridization experiments were performed according to standard procedures (Sambrook and Russell, 2001) using 15 μg of total RNA. Random primed ([32P]deoxyCTP) probes were prepared from 20 ng of gel-purified DNA.
Results

Myelinopathy and axonal degeneration in ΔPrP<sup>–</sup> mice

Hemizygous hg-PrP<sub>Δ(32–134)tg</sub> transgenic mice, which express amino proximally truncated PrP<sup>ΔC</sup> under transcriptional control of a half-genomic Prnp minigene (Fischer et al., 1996) (henceforth termed ΔPrP<sup>Δ</sup> mice) were bred to Prnp<sup>−/−</sup> mice. The resulting mice (termed ΔPrP<sup>Δ</sup>) were studied in the context of both endogenous Prnp alleles, developed CGC degeneration as described previously (Shmerling et al., 1998) (Fig. 1a,b). In addition, histological analysis of terminally sick ΔPrP<sup>Δ</sup> brains revealed additional coarse vacuolar degeneration of the intrapontine part of cranial nerves (Fig. 1d,e). Also, substantial areas of white matter in the cerebellum, the brainstem, as well as the anterior and lateral spinal cord columns exhibited large vacuoles, astrogliosis, and activated microglia/macrophages (Fig. 1m–r).

We also bred heterozygous and homozygous hg-PrPΔ<sub>(32–121)tg</sub> transgenic mice, which express a Prnp version with a smaller deletion than the hg-PrPΔ<sub>(32–134)</sub> transgene, to the Prnp<sup>−/−</sup> genetic background. We found that both the hg-PrPΔ<sub>(32–121)</sub> and hg-PrPΔ<sub>(32–134)</sub> transgenes induced analogous white-matter lesions in Prnp<sup>−/−</sup> mice (data not shown). Therefore, in all experiments described below, we used ΔPrP<sup>Δ</sup> mice as a general paradigm for the toxicity of amino proximally truncated PrP<sup>Δ</sup>.

Myelinated fibers in spinal cord and cerebellar white matter displayed severe axonal loss associated with large vacuoles and degeneration of myelin sheaths into condensed spheroids (Fig. 2a–f). Semithin sections and electron microscopy did not evidence any abnormality in myelin thickness, spacing, and periodicity in ΔPrP<sup>Δ</sup> and ΔPrP<sup>−</sup> mice (Fig. 2i), but many individual sheaths displayed vacuoles, especially around large axons. Axonal pathology was evident in the latter areas. Some axons were swollen and featured accumulation of mitochondria and dense bodies (Fig. 2j,k). Other axons suffered from compromised adhesion to their myelin sheaths, evident as periaxonal splitting over broad portions of the axolemma and detachment from the myelin wrap (Fig. 2l,m). Many axons had completely degenerated, leaving behind empty myelin rings or collapsed and condensed residual myelin ghosts (Fig. 2n,o).

Axon–myelin pathology may result from developmental myelinization failure or, alternatively, from degeneration of myelinated axons after completion of normal development. This question was addressed by studying semithin spinal cord and cerebellar white-matter sections from postnatal day 25 mice,
may also be affected by cord neurons (Fig. 3), implying that neurons other than CGCs visible in degenerating CGCs, as well as in brainstem and spinal sections of perikaryal phosphorylated neurofilament proteins were described previously (Shmerling et al., 1998), abnormal accumulation of oligodendrocyte origin. White-matter pathology in account for white-matter pathology.

Although florid apoptosis was only found in CGCs, as described previously (Shmerling et al., 1998), abnormal accumulations of perikaryal phosphorylated neurofilament proteins were visible in degenerating CGCs, as well as in brainstem and spinal cord neurons (Fig. 3), implying that neurons other than CGCs may also be affected by ΔPrP toxicity. Additionally, this suggests that axonal damage and pathologic cytoplasmic accumulation of phosphorylated neurofilament are a primary consequence of ΔPrP expression rather than secondary to neuronal cell death. We considered whether axon–myelin degeneration might result from oligodendrogial damage. However, no apoptosis was found in the white matter of diseased animals (Fig. 3), suggesting that it is a late-onset pathology. Scale bar, 20 μm. Electron microscopic analysis showed regular spacing and periodicity of PrP α myelin (i), whereas ΔPrP α white matter presented with axonal swellings and spheroids with accumulation of membrane organelles, mitochondria, and undefined dense bodies (j, k), as well as defects in axon–gial adhesion (l, m) with axon–myelin detachment and optically empty myelin cavities (asterisk). n, o, More advanced stages of degeneration included axonal degeneration with empty myelin rings (arrow) and collapse of myelin in condensed multilamellar spheroid structures (arrowheads). Scale bar, 5 μm.

Figure 2. Axon and myelin degeneration in ΔPrP α and PrP α/ZHII mice. a–f, Toluidine blue-stained semithin sections of spinal cord (a–c) and cerebellar white matter (d–f) of clinically healthy ΔPrP α (a, d) and terminally sick ΔPrP α (b, e) and PrP α/ZHII (c, f) mice. Axonal loss, vacuolation, and axon–myelin degeneration were visible in ΔPrP α and PrP α/ZHII mice but absent from ΔPrP t mice. No axon–myelin degeneration was seen in 25-d-old ΔPrP α mice (g, h), suggesting that it is a late-onset pathology. Scale bar, 20 μm. 

Unexpectedly, white-matter disease was not restricted to cerebellar degeneration, possibly by similar mechanisms as ΔPrP (Moore et al., 1999; Genoud et al., 2004). We therefore analyzed the white matter of 18-month-old Prnp α/ZHII mice with advanced clinical disease. We found white-matter vacuolation similar to that of ΔPrP α mice (Fig. 1f, l). Unexpectedly, white-matter disease was not restricted to cerebellum, brainstem, and spinal cord, but extended into the forebrain white matter, the pyramidal projections, and the corpus callosum (Fig. 1j–l).

Oligodendroglial PrP C represses leukoencephalopathy but not granule cell degeneration

Axons and their myelin sheaths are functionally dependent on each other: degeneration in one compartment may result in secondary damage to the other compartment. Alternatively, damage may result from parallel primary toxicity to both compartments. If myelin-producing oligodendrocytes were a primary target of mutant PrP C and Dpl, selective oligodendrocytic expression of PrP C should repress white-matter pathology. We therefore crossed ΔPrP α mice to tg640 mice, which express full-length PrP C under transcriptional control of a myelin basic protein promoter fragment (Fig. 4a) (Prinz et al., 2004). Hemizygous expression of the MBP-PrP transgene significantly delayed the onset of tremor and ataxia (mean time of onset, 74 ± 3 vs 43 ± 2 d; p < 0.0001) (Table 1, Fig. 4a,c) and reduced the typical wasting of ΔPrP α mice (body weight at 8 weeks of age, 22.2 ± 0.39 vs 14.8 ± 0.29 g; p < 0.0001). ΔPrP α mice reached terminal disease at 3–4 months of age, with severe wasting and hindlimb paralysis. In contrast, ΔPrP α/MBP-PrP tg/– littermates were ataxic, but weight loss was not as severe as in

White-matter pathology in Prnp α/α mice overexpressing Dpl

The prion protein homolog Dpl bears structural resemblance to ΔPrP (Luhrs et al., 2003) and is encoded by the Prnd gene, which is situated 16 kb 3′ of Prnp. In homozygous Prnp ZHII/ZHII and in compound heterozygous Prnp ZHII/loxp mice, loxp-mediated deletion of the entire third Prnp exon induces atypical intergenic splicing patterns that place Dpl under transcriptional control of the Prnp promoter, thereby leading to Dpl overexpression in brain (Weissmann and Aguzzi, 1999; Rossi et al., 2001). Overexpression of Prnd leads to cerebellar degeneration, possibly by similar mechanisms as ΔPrP (Moore et al., 1999; Genoud et al., 2004). We therefore analyzed the white matter of 18-month-old Prnp α/ZHII mice with advanced clinical disease. We found white-matter vacuolation similar to that of ΔPrP α mice (Fig. 1f, l). Unexpectedly, white-matter disease was not restricted to cerebellum, brainstem, and spinal cord, but extended into the forebrain white matter, the pyramidal projections, and the corpus callosum (Fig. 1j–l).
ΔPrP mice. The weight of 1-year-old ΔPrP<sup>−/−</sup>PrP<sup>°</sup>MMP<sup>−/−</sup> was still 17–19 g. Remarkably, these mice achieved long-term survival (>400 d), with stable or minimally progressing disease, whereas the mean survival time of ΔPrP<sup>−/−</sup>PrP<sup>°</sup> littermates was 99 ± 5.3 d (Fig. 4a,c). Histopathological analysis showed repression, but not complete rescue, of axon–myelin degeneration and white-matter vacuolization (Fig. 4g–j). Instead, no rescue of CGC was observed (Fig. 4e,f), confirming that white-matter disease is unrelated to CGC degeneration. These results strengthen the conjecture that myelin is a direct target of ΔPrP toxicity.

Compound heterozygous Pmp<sup>ZHII/ZHII</sup> mice develop neurological symptoms at 13–21 months and survive 18–24 months (Rossi et al., 2001). Also in these mice, intercross with tg640 mice delayed onset of disease, prolonged survival (Table 1, Fig. 4b,d), and repressed axon–myelin degeneration (Fig. 4m–p), adding support to the hypothesis that Dpl and ΔPrP share the same pathological basis. However, expression of the MBP-PrP transgene did not rescue Purkinje cell degeneration (Fig. 4k,l).

Neuronal PrP<sup>C</sup> expression antagonizes CGC degeneration but does not prevent leukoencephalopathy

Neuronal processes may conceivably represent the primary target of mutant PrP toxicity, whereas myelinopathy may be secondary to axonal degeneration. If so, oligodendrocytes may provide PrP<sup>C</sup> to the axon–myelin interface, which would abrogate any cell-autonomous axonal ΔPrP toxicity in trans. Intercellular transfer of glycosylphosphatidylinositol (GPI)-anchored protein (a phenomenon called “cell painting”) was described for CD4 (Anderson et al., 1996) and for PrP<sup>C</sup> in vitro (Liu et al., 2002). Alternatively, PrP<sup>C</sup> may act as a ligand and trigger signaling through receptors expressed by neighboring cells.

These scenarios were tested by crossing ΔPrP<sup>°</sup> mice with mice transgenic for ΔPrP<sup>−/−</sup>PrP<sup>°</sup>.
Table 1. Genetic, clinical, and histopathological features of ΔPrP- and Dpl-induced diseases

<table>
<thead>
<tr>
<th>Name</th>
<th>Genotype</th>
<th>Age at disease onset (days)</th>
<th>Survival (days)</th>
<th>Neuronal degeneration</th>
<th>White-matter disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPrP−</td>
<td>Hg-PrP(Δ32–134); Prnp−/+</td>
<td>43 ± 2 (n = 11)</td>
<td>&gt;600</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>ΔPrP0</td>
<td>Hg-PrP(Δ32–134); Prnp−/−</td>
<td>99 ± 5.3</td>
<td>&gt;600</td>
<td>Full CGC degeneration at 3 months</td>
<td>None</td>
</tr>
<tr>
<td>MBP-PrP</td>
<td>Prnp+/−; MBP-PrP+/−</td>
<td>No disease (n = 11)</td>
<td>600</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>NSE-PrP</td>
<td>Prnp+/−; NSE-PrP+/−</td>
<td>No disease (n = 12)</td>
<td>&gt;600</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>ΔPrP0NSE-PrP−</td>
<td>Hg-PrP(Δ32–134); Prnp+/−; MBP-PrP+/−</td>
<td>74 ± 2 (n = 12); p &lt; 0.0001</td>
<td>&gt;400; p &lt; 0.0001</td>
<td>Full CGC degeneration at 3 months</td>
<td>Mild and stable</td>
</tr>
<tr>
<td>ΔPrP0NSE-PrP0</td>
<td>Prnp(Δ32–134); Prnp+/−; NSE-PrP+/−</td>
<td>418 ± 30 (n = 10); p &lt; 0.0001</td>
<td>&gt;600; p &lt; 0.0001</td>
<td>Partial CGC degeneration at 8–20 months</td>
<td>Severe at 8–20 months</td>
</tr>
<tr>
<td>Pmp0/ZHII</td>
<td>Heterozygous Pmp0/ZHII; overexpresses Dpl</td>
<td>700</td>
<td>None</td>
<td>Purkinje cell degeneration</td>
<td>None</td>
</tr>
<tr>
<td>Pmp0/ZHII MBP-PrP</td>
<td>ZHI/ZHII PrP alleles; deficient in PrPC</td>
<td>None (n = 7)</td>
<td>700</td>
<td>Purkinje cell degeneration</td>
<td>None</td>
</tr>
<tr>
<td>Prnp0/ZHII MBP-PrP/−</td>
<td>ZHI/ZHII; Prnp0/ZHII; MBP-PrP+/−</td>
<td>660 ± 17.4 (n = 15); p &lt; 0.005</td>
<td>&gt;700; p &lt; 0.0001</td>
<td>Purkinje cell degeneration</td>
<td>Mild and stable</td>
</tr>
</tbody>
</table>

Significance p values were derived by comparing mean survival or disease-onset times of transgenic and control mice (unpaired Student’s t-test).

expressing murine PrPC+ under transcriptional control of the NSE promoter (Fig. 5a). NSE-PrP were crossed to mice lacking Prnp, thus yielding NSE-PrP−/−; Prnp+/− mice that are hemizygous for the NSE-PrP transgene and homozygous for the ZHI Prnp null allele. The latter mice (henceforth termed NSE-PrP−/−) express PrPC in neurons including CGC at higher levels than wild-type mice and do not develop any spontaneous pathological phenotype (O. T. Giger, B. Navarro, and A. Aguzzi, unpublished data). Instead, ΔPrP0−/−NSE-PrP mice developed tremor and ataxia only at 9–18 months and survived >500 d. This confirms that neuronal PrPC expression prolongs survival of ΔPrP0 mice (Table 1, Fig. 5b). However, histological analysis of clinically sick animals revealed leukoencephalopathy of similar severity in ΔPrP0 and ΔPrP0NSE-PrP mice but not in age-matched ΔPrP0 animals (Fig. 5c–h). Leukoencephalopathy was found also in pyramidal tracts, corpus callosum, and optic tracts (data not shown), suggesting that neuronal protection, by prolonging survival, allowed for more extensive development of myelin pathology. These findings indicate that neuron-restricted expression, even at supraphysiological levels, does not suffice to prevent ΔPrP-induced leukoencephalopathy and adds strength to the contention that ΔPrP0 leukoencephalopathy is a primary myelin pathology. ΔPrP0NSE-PrP mice suffered also from CGC degeneration, although to a lesser extent than ΔPrP0 mice. This sug-
bands in MBP-PrP mice might reflect degeneration of unglycosylated PrP C, because glycosylation is known to influence the stability of proteins.

**ΔPrP and PrP C in MBP-PrP mice are localized in lipid rafts**

PrP C localizes to lipid rafts (Naslavsky et al., 1997; Meier et al., 2003), in which it may exert a physiological function by interacting with signaling molecules. Dpl is also found in rafts (Massimino et al., 2004). If toxicity of ΔPrP and Dpl results from interference with some PrP C-mediated signaling pathway and toxicity is counteracted by PrP C, these three proteins may compete for the same partners on rafts. Moreover, ΔPrP and Dpl may disrupt normal axon–glia interaction and adhesion, whose integrity depends on GPI-anchored and transmembrane proteins (Falk et al., 2002; Marcus et al., 2002; Bartels, 2003; Vinson et al., 2003). To investigate the topology of ΔPrP, we performed flotation assays on Optiprep gradients with cold Triton X-100-treated ΔPrP C brain extracts. ΔPrP was detected in low-density, detergent-insoluble fractions and displayed buoyancy similar to that of PrP and flotillin (Fig. 6f–j). These characteristics strongly suggest that ΔPrP is indeed localized in lipid rafts and colocalized with wild-type PrP C. Therefore, cytotoxicity is unlikely to be attributable to inappropriate membrane targeting of ΔPrP. In addition, PrP C from MBP-PrP mice associated with rafts similarly to PrP C from wild-type mice (Fig. 6i). Unglycosylated PrP C was the dominant moiety, similar to PrP C isolated from myelin.

Neither ΔPrP nor Dpl affect myelin protein expression

Leukoencephalopathy in ΔPrP C and Prnp H9004 mice had features suggestive of compromised axon–glial adhesion similar to those described in mice lacking MAG and myelin galactolipids (Marcus et al., 2002), as well as in mice deficient for PLP (Griffiths et al., 1998). In addition, we found axonal swellings similar to those of Cnp1−/− mice (Lappe-Siefke et al., 2003). Moreover, in mice deficient for these diverse myelin proteins, oligodendrocytes produce appropriately thick myelin sheaths. These findings raised the question as to whether ΔPrP and Dpl might affect the synthesis or the stability of the above myelin components. Therefore, we analyzed the myelin content in whole brain and spinal cord homogenates of Prnp H9004, Prnp H9004/H9004, ΔPrP C, and Prnp H9004/H9004 mice (age, 2 months). MAG, MBP, PLP, and CNP transcription and protein content were assayed with Northern and Western blots (Fig. 7a,b). The amount of MAG and MBP protein was normalized against actin (Fig. 7c). These analyses revealed that both the transcription and the steady-state protein concentration of all investigated myelin proteins were unchanged in ΔPrP C and Prnp H9004 mice.

**Discussion**

Overexpression of amino proximally truncated PrP C variants collectively termed ΔPrP (Shmerling et al., 1998), or of the structural PrP C homolog Dpl (Weissmann and Aguzzi, 1999; Behrens
et al., 2002), triggers neuronal degeneration. We now report that both ΔPrP and Dpl elicit additional leukoencephalopathy. These pathologies were not entirely expected, because PrP-related diseases are thought to affect primarily neurons (Chiesa et al., 1998, 2000; Hegde et al., 1998, 1999; Shmerling et al., 1998; Rossi et al., 2001). Conversely, myelin sheath damage and neuroaxonal dystrophy (Liberski et al., 2002), as well as cytopathic changes in oligodendrocytes (El Hachimi et al., 1998), have been noted in human and animal TSEs.

Myelinopathy, rather than neuronal damage, may be a major determinant of lethality in Shmerling’s syndrome, because oligodendrocyte-restricted expression of PrP^C selectively suppresses leukoencephalopathy and restores long-term survival. Instead, neuron-restricted expression of PrP^C, even at supraphysiological levels, does not prevent white-matter degeneration. These data indicate that leukoencephalopathy is not just a consequence of neurodegeneration but suggest a primary role of oligodendrocytes in white-matter disease. Oligodendrocyte-restricted PrP^C may repress in trans the axonal pathology elicited by mutant PrP. A cellular signal might be transduced through PrP^C from oligodendrocytes to axons, analogously to other GPI-anchored proteins (such as F3 and NCAM 120) that play a role in axon–myelin interactions (Falk et al., 2002). Because PrP^C binds in vitro to the transmembrane and GPI-linked forms of NCAM (Schmitt-Ulms et al., 2001), it might contribute to the very same pathways. Alternatively, PrP^C may instruct oligodendrocytes to release factors necessary for axonal survival (Wilkins et al., 2003). Direct toxicity of ΔPrP and Dpl to oligodendrocytes seems to be unlikely, because we did not observe oligodendrocytic cell death or ultrastructural abnormalities in oligodendrocytes. Both oligodendrocyte- and neuron-restricted expression delayed the clinical course of Shmerling’s disease, yet only endogenous PrP^C expression, which occurs on both neurons and glia (Moser et al., 1995), brought about complete rescue. Therefore, both neuronal and oligodendrocyte PrP^C expression are needed for maintenance of the axon–myelin interface in ΔPrP mice.

Surprisingly, neuron-specific PrP^C expression, even at supraphysiological levels, did not completely rescue CGC degeneration. Perhaps ongoing degeneration of white-matter fibers innervating the granule cell layer participates indirectly in granule cell degeneration, e.g., by deafferentation and neuronal “dying back.” The delayed onset of myelinopathy in ΔPrP^C/NSE-PrP mice might be related to the presence of PrP^C in NSE-PrP myelin which, in turn, might be attributable to GPI cell painting or to high-affinity interaction of axonal PrP^C with myelin components.

Leukoencephalopathy in ΔPrP^C and PrP^CZHII/o mice had features suggestive of compromised axon–glial adhesion, including axonal swellings similar to those described in mice lacking MAG and myelin galactolipids (Marcus et al., 2002), PLP (Griffiths et al., 1998), and Cnp1 (Lappe-Siefke et al., 2003). However, expression of MAG, MBP, PLP, and CNP was not affected in ΔPrP^C and PrP^CZHII/o mice, suggesting that disturbance of myelin and axonal physiology is directly mediated by ΔPrP and Dpl rather than by dysmetabolism of myelin proteins.

The overlapping patterns of (1) Dpl- and ΔPrP-induced leukoencephalopathy, (2) their modulation by coexpression of full-length PrP^C (Moore et al., 1999; Li et al., 2000; Rossi et al., 2001), (3) the structural homology between Dpl and ΔPrP (Luhrs et al., 2003), and (4) the abrogation of neurodegeneration by removal of Dpl from PrP^CZHII/o mice (Genoud et al., 2004) all suggest that ΔPrP and Dpl trigger similar pathogenetic mechanisms. What might be the common structural basis of such pathologies? Of all amino proximal deletions that were tested (Shmerling et al., 1998), only those that disrupt the highly conserved hydrophobic TM1 region (amino acids 113–134) provoked pathologies that were prevented by wild-type PrP^C.

Two possible molecular mechanism of toxicity have been put forward. Shmerling et al. (1998) proposed that the amino proximal region of PrP represents an effector domain, whose deletion...
In addition to its role in prion disease pathogenesis, repression of $\Delta PrP/Dpl$-associated neurodegenerative pathologies is likely to represent the most thoroughly characterized function of PrP<sup>C</sup>. As shown by the present study, the neurodegenerative syndromes induced by $\Delta PrP$ provide an accessible experimental system for studying function of PrP<sup>C</sup> <em>in vivo</em> by reverse genetics.

**References**


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