Abnormal Activation of Glial Cells in the Brains of Prion Protein-deficient Mice Ectopically Expressing Prion Protein-like Protein, PrPLP/Dpl

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Abstract

Background: Some lines of mice homozygous for a disrupted prion protein gene (*Prnp*), including Ngsk *Prnp*$^{0/0}$ mice, exhibit Purkinje cell degeneration as a consequence of the ectopic overexpression of the downstream gene for prion protein-like protein (PrPLP/Dpl) in the brain, but others, such as Zrch I *Prnp*$^{0/0}$ mice, show neither the neurodegeneration nor the expression of PrPLP/Dpl. In the present study, we found that Ngsk *Prnp*$^{0/0}$, but not Zrch I *Prnp*$^{0/0}$ mice, developed gliosis involving both astrocytes and microglia in the brain.

Materials and Methods: The brains from wild-type (*Prnp*$^{+/+}$), Ngsk *Prnp*$^{0/0}$, Zrch I *Prnp*$^{0/0}$, and reconstituted Ngsk *Prnp*$^{0/0}$ mice carrying a mouse PrP transgene, designated Tg(P) Ngsk *Prnp*$^{0/0}$ mice, were subjected into Northern blotting and *in situ* hybridization using probes of glial fibrillary acidic protein (GFAP) and lysozyme M (LM) specific for astrocytes and microglia, respectively. Immunohistochemistry was also performed on the brain sections using anti-GFAP and anti-F4/80 antibodies.

Results: Northern blotting demonstrated upregulated expression of the genes for GFAP and LM in the brains of Ngsk *Prnp*$^{0/0}$, but not in Zrch I *Prnp*$^{0/0}$ mice. A transgene for normal mouse PrP^C successfully rescued Ngsk *Prnp*$^{0/0}$ mice from the glial activation. *In situ* hybridization and immunohistochemistry revealed activated astrocytes and microglia mainly in the white matter of both the forebrains and cerebella. In contrast, there was no evidence of neuronal injury except for the Purkinje cell degeneration. Moreover, the glial cell activation was notable well before the onset of the Purkinje cell degeneration.

Conclusions: These findings strongly suggest that ectopic PrPLP/Dpl in the absence of PrP^C is actively involved in the glial-cell activation in the brain.

Introduction

The cellular isoform of prion protein (PrP^C), a membrane glycoprotein anchored by a glycosyl-phosphatidylinositol moiety, is highly expressed in the central nervous system (CNS), in particular on neurons. PrP^C is known to play a crucial role in the pathogenesis of prion diseases (1,2), but its physiological function remains to be clarified. We previously reported a line of PrP^C-deficient mice, designated Ngsk *Prnp*$^{0/0}$ mice, that exhibited ataxia due to cerebellar Purkinje cell degeneration and demyelination in the spinal cord and peripheral nervous system at old age (3,4). Subsequently, two other

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independent mouse lines, Rcm0 and Zrch II *Prnp*$^{0/0}$ mice, were confirmed to develop similar neurodegeneration (5,6). In contrast, another independent line, Zrch I *Prnp*$^{0/0}$, revealed the demyelination and has never exhibited Purkinje cell degeneration (4). Behavioral and electrophysiological studies of the Zrch I *Prnp*$^{0/0}$ mice demonstrated abnormal circadian rhythms (7) and impaired long-term potentiation in the hippocampus (8), respectively. In spite of the discrepancy in the ataxic phenotype, all of the neurological abnormalities, including the Purkinje cell degeneration in these PrP^C-deficient mice were subsequently rescued by the introduction of a transgene encoding wild-type PrP^C (4,7,9), indicating the crucial role of PrP^C in survival and various functions of neurons.

Others and we recently identified a novel gene, *Prnd*, that is localized 16 kb downstream of the murine PrP gene (*Prnp*) and encodes a PrP-like protein, PrPLP/Dpl (5,10). This novel membrane glycoprotein is homologous to the C-terminal two-thirds of PrP

with a 23% identity in the amino acid sequence. Interestingly, in the brains of Ngsk, Rcm0, and Zrch II $Prnp^{0/0}$ mice, but not in those of Zrch I $Prnp^{0/0}$ mice, unusual intergenic splicing between $Prnp$ and $Prnd$ occurred as a result of the deletion of a part of the $Prnp$ intron 2 sequence, including its splicing acceptor (5,10). As a consequence, the PrPLP/Dpl was ectopically expressed in neurons, under the control of the $Prnp$ promoter (10). It is thus most likely that both the loss of PrP^C and the ectopic expression of PrPLP/Dpl are necessary for the Purkinje cell degeneration in Ngsk $Prnp^{0/0}$ mice. Rossi *et al.* recently confirmed the causal involvement of the ectopic PrPLP/Dpl in the Purkinje cell degeneration by demonstrating that the Zrch II $Prnp^{0/0}$ mice transgenic for $Prnd$ developed the Purkinje cell degeneration much earlier than Zrch II $Prnp^{0/0}$ mice (6).

In the present study, we demonstrate glial-cell activation in the brains of Ngsk $Prnp^{0/0}$ mice, but not in those of Zrch I $Prnp^{0/0}$ mice. The glial cell activation was not evident in the reconstituted Ngsk $Prnp^{0/0}$ mice harboring a transgene encoding wild-type PrP^C. The results suggest an active role of the ectopic PrPLP/Dpl in the glial-cell activation in Ngsk $Prnp^{0/0}$ mice.

Materials and Methods

Mice

Ngsk $Prnp^{0/0}$ mice were generated as described previously (2). The reconstituted Ngsk $Prnp^{0/0}$ mice, designated Tg(P) Ngsk $Prnp^{0/0}$ mice, were generated by mating Ngsk $Prnp^{0/0}$ mice with Tg(MoPrP-A)/FVB-B4053 mice (4,11) carrying a mouse PrP-A transgene on the Zrch I $Prnp^{0/0}$ mouse background. Zrch I $Prnp^{0/0}$ mice were obtained by mating Tg(MoPrP-A)/FVB-B4053 breeding pairs. Ngsk $Prnp^{0/0}$ and Zrch I $Prnp^{0/0}$ mice were intercrossed to obtain Ngsk/Zrch I $Prnp^{0/0}$ mice.

Northern Blot Analysis

Total RNA was extracted using Trizol reagent (Gibco BRL Life Technologies, Inc., Grand Island, NY, USA), separated on a formaldehyde-denatured agarose gel, and blotted onto a Hybond N membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK) with $10 \times$ SSC. The RNA was fixed onto the membrane by ultraviolet light at 70,000 microjoules per cm^2 for 2 min (UVP, Ltd., Upland, USA). Next, the membrane was subjected to a prehybridization procedure for 4 hr at 45°C in the buffer ($5 \times$ SSPE/0.5% SDS/50% formamide/$5 \times$ Denhardt's solution/10% dextran sulphate/100μg/ml salmon sperm DNA), and then hybridized using an appropriate ^{32}P-labelled DNA probe (BcaBEST Labelling Kit, TaKaRa, Tokyo, Japan) overnight at 45°C in the same buffer. The membrane was washed twice in $2 \times$ SSC/0.1% SDS at room temperature for 10 min, once in $1 \times$ SSC/0.1% SDS, and twice in $0.1 \times$ SSC/0.1% SDS at 65°C for 15 min. Signals were detected by BAS 5000 (Fuji, Tokyo, Japan) or autoradiography on Konica X-ray film.

In Situ Hybridization

Brains were fixed for 16 hr in 4% buffered-paraformaldehyde (pH 7.4) at 4°C, embedded in paraffin and sliced to 5-μm thickness. The sections were deparaffinized, digested with 8 mg/ml pepsin and 10 mg/ml proteinase K for 10 min at 37°C, and soaked for 10 min in 0.25% acetic anhydride/0.1 mM triethanolamine hydrochloride (pH 8.0)/0.9% NaCl. The cRNA probes were labelled with digoxigenin (DIG)-UTP (Roche Diagnostics, Mannheim, Germany) using T7 or T3 polymerase (Gibco BRL Life Technologies, Inc.). The tissue sections were subjected to hybridization with the labelled cRNA probes in buffer (50% formamide/10 mM Tris-HCl [pH 7.5]/1 mM EDTA/0.6M NaCl/0.5 mg/ml yeast tRNA/0.25 mg/ml salmon sperm DNA/1% skim milk/0.25% SDS/$5 \times$ Denhart's solution) at 50°C for 16 hr. The sections were washed several times in $4 \times$ SSC, and immersed in 50% formamide/$2 \times$ SSC at 50°C for 30 min. They were digested by 20 μg/ml RNase A at 37°C for 30 min and washed in $0.2 \times$ SSC at 60°C for 20 min. Signals were detected by an enzyme-linked immunosorbent assay using alkaline phosphatase conjugated anti-DIG Fab fragments (1:500, Roche Diagnostics) and nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP).

Probes

The LM probe used has been described previously (12). The GFAP probe was a 791-bp DNA fragment spanning nucleotide 466 to 1256 of the cDNA (Accession No. K01347), the IP_3R1 probe a 502-bp fragment from nucleotide 2854 to 3355 of the cDNA (Accession No. X15373), the synaptophysin probe a 677-bp fragment from nucleotide 1413 to 2088 of the cDNA (Accession No. X95818), the MBP probe a 590-bp fragment from nucleotide 340 to 930 of the cDNA (Accession No. M15060), the PLP probe a 309-bp fragment from nucleotide 834 to 1142 of the cDNA (Accession No. M37335), and the GAPDH probe a 677-bp fragment from nucleotide 1413 to 2088 of the cDNA (Accession No. M32599).

Immunohistochemistry

Deparaffinized sections were digested with 1 mg/ml trypsin for 15 min at 37°C, and then placed in 3% H_2O_2 in methanol for 30 min at room temperature to abolish endogenous peroxidase activity. After treatment with normal rabbit serum for 30 min, the tissue sections were incubated overnight at 4°C with anti-GFAP (1:50 [Dako, Kyoto, Japan]) and anti-F4/80 antigen (1:20 [Serotec, Ltd., Oxford, United Kingdom]). To detect GFAP immunoreactivity we used the Polymer-Immuno Complex method, in accordance with the manufacturer's recommendations (Dako). For F4/80, the tissue sections were subsequently incubated with biotinylated rabbit anti-rat immunoglobulin (mouse adsorbed, [Dako]) at room temperature for 30 min. The antibody-bound

peroxidase was revealed with 0.04% diamino-benzidine (Sigma Chemical Co., St. Louis, MO).

Results

Upregulation of Glial Cell-specific Gene Expression in the Brains of Aged Ngsk Prnp$^{0/0}$ Mice

We first examined expression levels of the glial fibril-lary acidic protein (GFAP) and lysozyme M (LM) mR-NAs specific to astrocytes and microglia, respectively, in the forebrains and cerebella of 102-week-old Ngsk *Prnp*$^{0/0}$ mice. Total RNA isolated from the pooled fore-brains and cerebella of Ngsk *Prnp*$^{0/0}$ and age-matched wild-type (*Prnp*$^{+/+}$) mice was subjected to Northern blotting. As shown in Fig. 1a, the GFAP and LM mRNA levels were elevated in both tissue types in the Ngsk *Prnp*$^{0/0}$ mice. Densitometric analysis estimated that, compared with those of the *Prnp*$^{+/+}$ mice, GFAP mRNA was increased 2.5- and 2.7-times, and LM mRNA 2.7- and 3.0-times, in the forebrains and cerebella, respectively. In contrast, the expression of inositol 1,4,5-trisphosphate receptor type 1 (IP$_3$R1) mRNA, a specific marker for Purkinje cells, was de-creased in the cerebella of Ngsk *Prnp*$^{0/0}$ mice (Fig. 1a).

In situ hybridization and immunohistochemistry on the brain sections of Ngsk *Prnp*$^{0/0}$ mice at 92 weeks of age revealed numerous astrocytes expressing GFAP mRNA and its product mainly in the major white mat-ter areas including the corpus callosum, fimbria hip-pocampus, and internal and external capsule, and the cerebellum (Fig. 2). While a number of astrocytes was also seen in the brain tissues of the age-matched
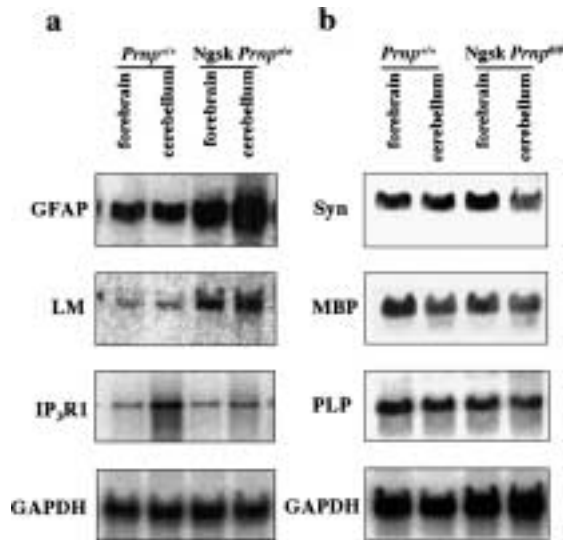

Fig. 1. Glia-specific genes in the forebrain and cerebellum are upregulated in aged Ngsk *Prnp*$^{0/0}$ mice. 10 μg of total RNA extracted from the pooled brain tissues of 102-week-old Ngsk *Prnp*$^{0/0}$ and *Prnp*$^{+/+}$ mice were subjected to Northern blotting using appropriate probes. The GFAP, LM, and IP$_3$R1 probes were used in (**a**) and synaptophysin (Syn), MBP, and PLP probes in (**b**). The integrity and quantity of RNA were verified with a glyceraldehydes-3-phosphate dehydrogenase (GAPDH) probe on the same membrane.
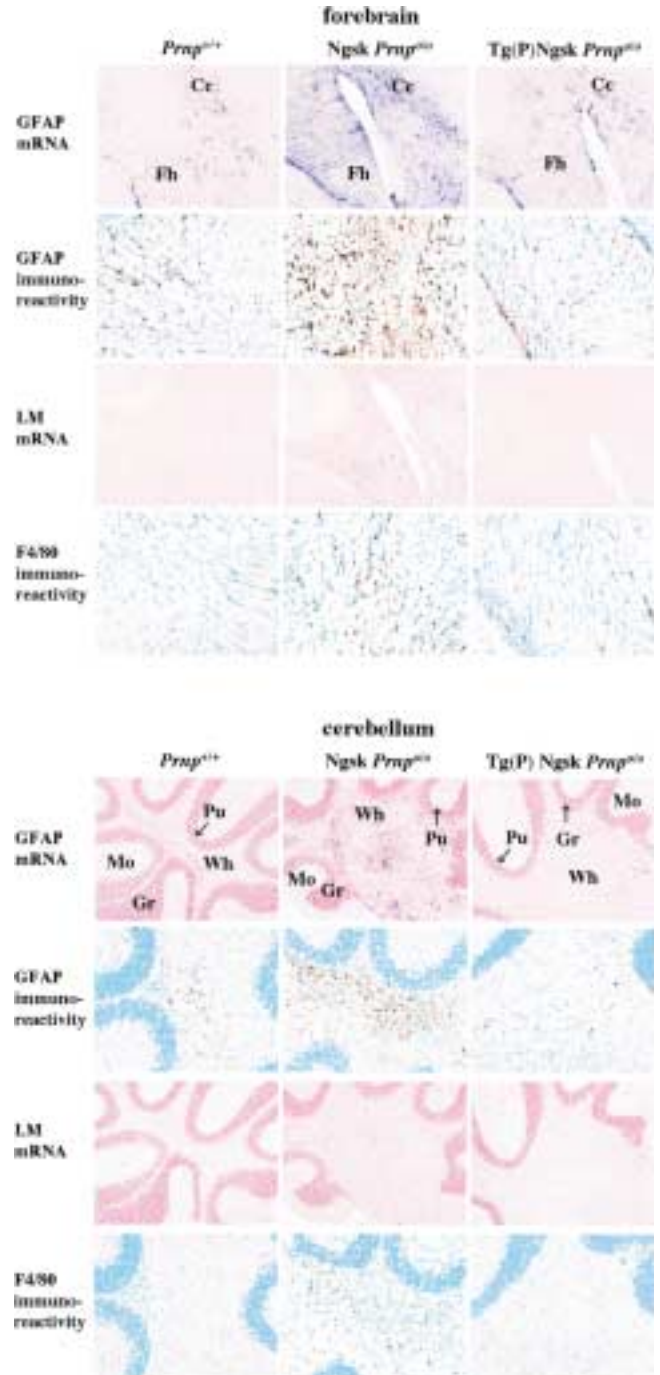

Fig. 2. Gliosis of astrocytes and microglia in the brains of aged Ngsk *Prnp*$^{0/0}$ mice. Localizations of mRNAs for GFAP and LM, and immunoreactivities to GFAP and F4/80 in the forebrains and cerebella of 92-week-old *Prnp*$^{+/+}$, Ngsk *Prnp*$^{0/0}$ and Tg(P) Ngsk *Prnp*$^{0/0}$ mice are shown. Both hy-bridized and immunoreactive signals were localized mainly in the cerebral and cerebellar white matter. The number of positive cells and the intensity of their signals were markedly increased in the Ngsk *Prnp*$^{0/0}$ mice compared with *Prnp*$^{+/+}$ and Tg(P) Ngsk *Prnp*$^{0/0}$ mice. Cc; corpus callosum, Fh; fimbria hippocampus, Gr; granular layer, Mo; molecular layer, Pu; Purkinje cell layer, Wh; cerebellar white matter. Original magnification: ×13.2 for *in situ* hybridization and ×25 for immunohistochemistry.

Prnp^{+/+} mice, Ngsk *Prnp*[0/0] mice revealed much larger numbers of hypertrophic astrocytes stainable for GFAP. LM mRNA and F4/80 protein expressed by microglia were also concentrated in the white matter, and the number of positive cells was markedly increased in the Ngsk *Prnp*[0/0] mice.

No Evidence for Neuronal Injury Other Than Purkinje Cell Degeneration in the Brains of Aged Ngsk Prnp[0/0] Mice

Glial cell activation usually occurs as a reaction to neuronal injury. However, extensive histological studies have failed to identify any neuronal damage other than Purkinje cell degeneration in the brains of Ngsk *Prnp*[0/0] mice. Luxol fast blue staining of myelin sheaths in the forebrains of mice aged 92 weeks was normal (data not shown). To further examine whether there is any submicroscopic injury to the neuronal architecture in the brains of Ngsk *Prnp*[0/0] mice, we evaluated the levels of mRNA for a neuronal protein, synaptophysin, and two major components of myelin, myelin basic protein (MBP) and proteolipid protein (PLP) by Northern blotting using total RNA

extracted from the pooled forebrains and cerebella of 102-week-old Ngsk *Prnp*[0/0] mice. Similarly to the IP₃Rl mRNA, the expression of synaptophysin was normal in the forebrains but reduced in the cerebella of 102-week-old Ngsk *Prnp*[0/0] mice (Fig. 1b). On the other hand, there was no difference in the levels of MBP and PLP mRNAs between Ngsk *Prnp*[0/0] and age-matched *Prnp*^{+/+} mice. These observations were also confirmed by *in situ* hybridization (data not shown).

Glial-cell Activation Occurs Well Before the Onset of Purkinje Cell Degeneration in the Brains of Ngsk Prnp[0/0] Mice

We monitored kinetics of the GFAP and LM mRNA levels in the pooled forebrains and cerebella of Ngsk *Prnp*[0/0] and wild-type mice at various ages between 3 and 102 weeks. In the Ngsk *Prnp*[0/0] mice, however, increased levels of GFAP mRNA appeared as early as 8 and 3 weeks in the forebrains and cerebella, respectively, and the transcript accumulated more rapidly in the tissues (Fig. 3a, b). Increased levels of LM mRNA were also seen from 22 and 8 weeks in
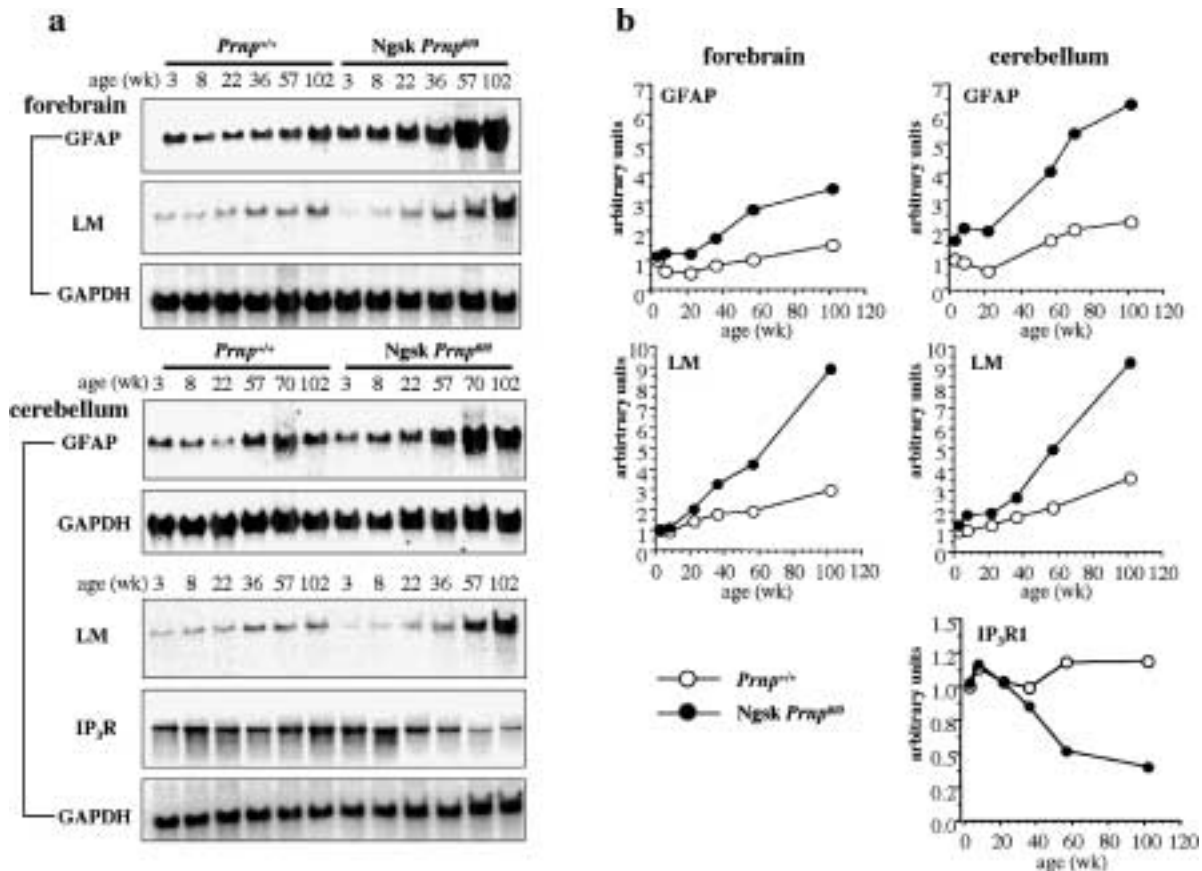

Fig. 3.   Kinetic analysis of the glial cell-specific gene expression in the forebrains and cerebella of Ngsk *Prnp*[0/0] mice. (a) The levels of transcripts for GFAP, LM, and IP₃R1 were compared between Ngsk *Prnp*[0/0] and *Prnp*^{+/+} brain tissues at different ages (wk). 10 μg of total RNA extracted from the pooled tissues were subjected to Northern blotting. The integrity and quantity of the RNA were verified with GAPDH probe on the same membrane. **(b)** Kinetics of the mRNAs for GFAP, LM, and IP₃R1 were compared between Ngsk *Prnp*[0/0] (closed circles) and *Prnp*^{+/+} (open circles) mice. The intensities of Northern blot signals shown in (a) were quantified using BAS 5000, standardized with GAPDH mRNA level, and expressed as arbitrary units in comparison with the value of *Prnp*^{+/+} mice at 3 weeks of age.

the forebrains and cerebella, respectively, and levels continued to rise until 102 weeks. In contrast, the decline of IP$_3$R1 mRNA in the cerebella of Ngsk *Prnp*[0/0] mice became evident at 36 weeks, suggesting that glial-cell activation occurs before the onset of the Purkinje cell degeneration.

A Transgene Encoding Wild-type PrP^C Normalizes GFAP and LM mRNA Levels in the Brains of Ngsk Prnp$^{0/0}$ Mice

In order to elucidate whether the activation of GFAP and LM genes is associated with the loss of PrP^C, we examined the reconstituted mice, Tg(P) Ngsk *Prnp*[0/0], which carried several copies of a transgene encoding wild-type mouse PrP^C on the genetic background of Ngsk *Prnp*[0/0]. *In situ* hybridization exhibited a high level of PrP mRNA widely distributed in the neurons and glial cells thoughout the CNS of Tg(P) Ngsk *Prnp*[0/0] mice (data not shown). Neurons, especially in the hippocampus and cerebellar Purkinje cell layer, were heavily hybridized. Histologic examination confirmed that Purkinje cells were preserved in all areas of the cerebellum of the Tg(P) Ngsk *Prnp*[0/0] mice (data not shown). Total RNAs from the brains of three each of 80-week-old *Prnp*^{+/+}, Ngsk *Prnp*[0/0], and Tg(P) Ngsk *Prnp*[0/0] mice were subjected to Northern blotting with the GFAP and LM probes. The elevated expression of GFAP and LM mRNAs found in the Ngsk *Prnp*[0/0] mice declined in the Tg(P) Ngsk *Prnp*[0/0] mice to a level similar to that of the *Prnp*^{+/+} mice (Fig. 4a, b). Additionally, the number of astrocytes and microglia in the Tg(P) Ngsk *Prnp*[0/0] mice was decreased to the level seen in the *Prnp*^{+/+} mice, as shown by *in situ* hybridization and immunohistochemistry (Fig. 2).

The Level of Glial-cell Activation Correlates with that of Ectopic Expression of PrPLP/Dpl

To further elucidate the mechanisms for the glial-cell activation, we examined the expression levels of GFAP and LM mRNAs in the brains of another independent mouse line, Zrch I *Prnp*[0/0], which has neither exhibited ataxia nor Purkinje cell degeneration, and heterozygous Ngsk/Zrch I *Prnp*[0/0] mice. The heterozygous mice developed the Purkinje cell degeneration much later than the Ngsk *Prnp*[0/0] mice (data not shown). Although the Ngsk *Prnp*[0/0] mice expressed abundant PrPLP/Dpl mRNA in the brains, the Zrch I *Prnp*[0/0] mice expressed no detectable PrPLP/Dpl mRNA and the Ngsk/Zrch I *Prnp*[0/0] mice revealed half the level of PrPLP/Dpl mRNA compared with the Ngsk *Prnp*[0/0] mice (Fig. 5a). The expression levels of GFAP and LM mRNAs correlated well with that of PrPLP/DpL mRNA. The Zrch I *Prnp*[0/0] mice aged 70 weeks showed no upregulation of the GFAP and LM mRNAs, exhibiting levels similar to those of age-matched *Prnp*^{+/+} mice (Fig. 5a, b). The Ngsk/Zrch I *Prnp*[0/0] mice exhibited levels of GFAP and LM mRNAs between those of the Ngsk *Prnp*[0/0] and *Prnp*^{+/+} mice (Fig. 5a, b).
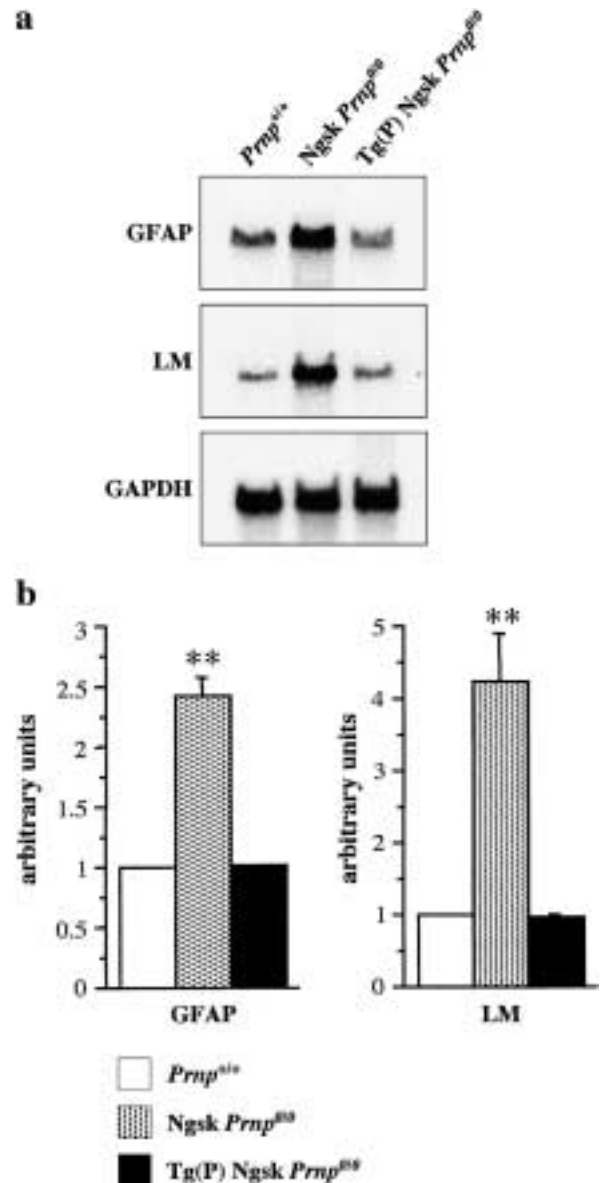

Fig. 4.   A wild-type mouse PrP transgene normalizes the level of glial cell-specific gene expression in the brains of Ngsk *Prnp*[0/0] mice. (a) 10 μg of total RNA extracted from the total brain tissues of 80-week-old *Prnp*^{+/+}, Ngsk *Prnp*[0/0], and Tg(P) Ngsk *Prnp*[0/0] mice were subjected to Northern blotting using the GFAP and LM probes. The integrity and quantity of RNA were verified with GAPDH probe on the same membrane. (b) The intensities of Northern blot signals shown in (a) were quantified using BAS 5000, the mRNA levels standardized with GAPDH mRNA, and expressed as arbitrary units in comparison with the values of *Prnp*^{+/+} mice. Three brains from each line were analyzed by ANOVA. **represents p < 0.01.

Discussion

In the present study, we demonstrated glial-cell activation, or gliosis, with the upregulated expression of both astrocyte- and microglia-specific genes encoding GFAP and LM, respectively, in the brains of Ngsk *Prnp*[0/0] mice. A transgene for wild-type PrP^C rescued
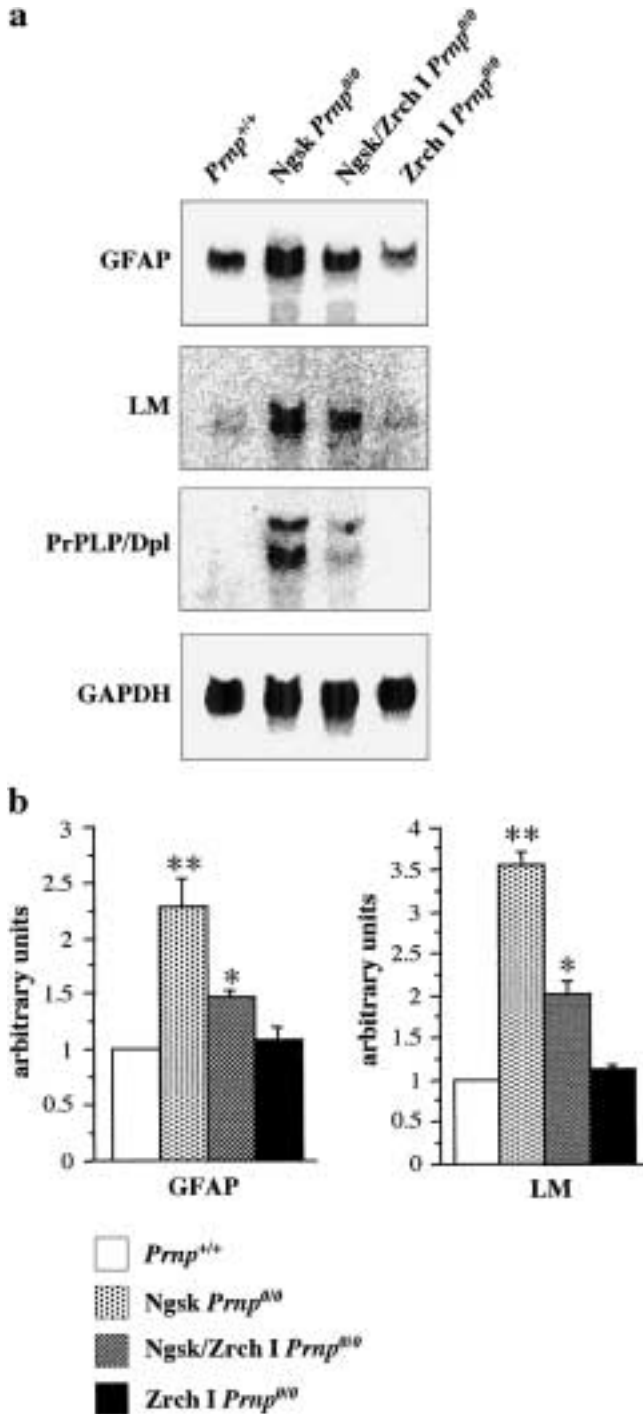
a

b

Fig. 5. Glial cell-specific genes are not upregulated in the brains of Zrch I *Prnp^{0/0}* mice lacking Purkinje cell degeneration. (a) The levels of transcripts for GFAP and LM were compared by Northern blotting among total brain tissues from *Prnp^{+/+}* (lane 1), Ngsk *Prnp^{0/0}* (lane 2), Ngsk/Zrch I *Prnp^{0/0}* (lane 3), and Zrch I *Prnp^{0/0}* (lane 4) at 70 weeks of age. 10 μg of total RNA were blotted on each lane, and the integrity and quantity were verified with GAPDH probe on the same membrane. **(b)** The intensities of Northern blot signals were quantified and plotted as described. The levels of transcripts for GFAP and LM were compared among *Prnp^{+/+}*, Ngsk *Prnp^{0/0}*, Ngsk/Zrch I *Prnp^{0/0}*, and Zrch *Prnp^{0/0}* mice at 70 weeks of age by using arbitrary units and analyzed by ANOVA (n = 3). **represents $p < 0.01$; *represents $p < 0.05$.

the mice from the abnormal glial-cell activation. The finding that Zrch I *Prnp^{0/0}* mice did not reveal such a phenotype, however, indicates that the loss of PrP^C is indispensable, but not the only factor involved, in the glial cell activation. The heterozygous Ngsk/Zrch I *Prnp^{0/0}* mice exhibited levels of GFAP and LM expression in the brains between those of Ngsk *Prnp^{0/0}* and Zrch I *Prnp^{0/0}* mice, strongly suggesting the involvement of an additional factor(s) in the Ngsk *Prnp^{0/0}* mice. We previously demonstrated that the disrupted Ngsk *Prnp* allele but not Zrch I *Prnp^{0/0}* allele expressed PrPLP/Dpl ectopically in brains, through intergenic mRNA splicing between *Prnp* and *Prnd* (10). The ectopically expressed PrPLP/Dpl is likely to play an active role in the glial-cell activation in Ngsk *Prnp^{0/0}* mice.

Ectopic expression of PrPLP/Dpl is known to be closely associated with the Purkinje cell degeneration in the ataxic lines of *Prnp^{0/0}* mice (5,6,10). It is conceivable that the gliosis in the brains of Ngsk *Prnp^{0/0}* mice occurs as a consequence of neuronal damage. Accumulating evidence has suggested that PrP^C binds copper through its histidine-rich octapeptide repeat region (13–15). Herms *et al.* hypothesized that PrP^C may recapture copper released into the synaptic cleft and take it up into the presynaptic cytosol (16). The reduced activity of cytosolic copper-dependent superoxide dismutase in the brains of *Prnp^{0/0}* mice (17) is consistent with a functional link between copper metabolism and PrP^C in neuronal cells, and is likely to be involved in the increased susceptibility of cultured *Prnp^{0/0}* neurons to oxidative stress. Moreover, Wong *et al.* recently reported that the brains of *Prnp^{0/0}* mice ectopically expressing PrPLP/Dpl revealed much higher levels of protein oxidation and lipid peroxidation than those of *Prnp^{0/0}* mice lacking PrPLP/Dpl (18). Weissmann and colleagues have proposed an active role of PrPLP/Dpl in the neurodegeneration in the *Prnp^{0/0}* mice (19). They hypothesize the existence of a ligand which interacts with PrP^C to elicit a signal essential for the survival of neurons, and another PrP-like molecule capable of binding to the putative ligand but with lower affinity. In Zrch I *Prnp^{0/0}* mice, the association between the PrP-like molecule and the ligand would elicit the survival signal, but in Ngsk *Prnp^{0/0}* mice, the overexpression of PrPLP/Dpl would prevent the interaction, resulting in the Purkinje cell degeneration. It is possible that not only Purkinje cells but also other neurons in Ngsk *Prnp^{0/0}* mice may be affected by the ectopic PrPLP/Dpl to various degrees, leading to the marked gliosis in the brains.

Another intriguing possibility is a direct effect of the ectopic PrPLP/Dpl on the glial cells in the absence of PrP^C. Activated astrocytes and microglia were distributed throughout the forebrains and cerebella of aged Ngsk *Prnp^{0/0}* mice, mainly in the white matter. However, there was no histological evidence for neurodegeneration or demyelination other than

the Purkinje cell degeneration. In contrast to the reduced levels of IP$_3$R1 and synaptophysin mRNAs in the cerebellum, representing the Purkinje cell degeneration, the synaptophysin mRNA level in the forebrain appeared to be normal. Transcripts for two major myelin components, MBP and PLP, were expressed at normal levels in both the forebrain and cerebellum. The expression profiles of these genes argues against the presence of submicroscopic neuronal injury other than the Purkinje cell degeneration. Moreover, a kinetic analysis demonstrated glial-cell activation well before the onset of Purkinje cell degeneration in the Ngsk *Prnp^{0/0}* mice. It is thus conceivable that the glial-cell activation is primarily induced by the ectopic PrPLP/Dpl in the absence of PrP^C rather than as a consequence of neuronal injury.

Astrocytes were reported to physiologically express PrP mRNA *in vivo* (20). We detected abundant PrPLP/Dpl mRNA in the primary culture of astrocytes derived from the Ngsk *Prnp^{0/0}* mice (data not shown). The ectopic PrPLP/Dpl on the surface of glial cells in the absence of PrP^C may be the cause of the abnormal cell activation. An alternative possibility is that PrP^C on the surface of neurons may be involved in the communication with glial cells, and the loss of PrP^C together with ectopic PrPLP/Dpl on the neuronal surface may result in the glial-cell activation. Targeted expression of PrP^C in either glial cells or neurons of Ngsk *Prnp^{0/0}* mice would be useful to elucidate mechanisms of the glial-cell activation.

It is noteworthy that not only astrocytes but also microglia were activated in the brains of Ngsk *Prnp^{0/0}* mice. Activated microglia are thought to be involved in the etiology of various neurodegenerative conditions by their production of neurotoxic substances (21–23). For instance, we recently demonstrated that microglial production of lysosomal hydrolases, a perforin-like protein, and reactive oxygen species was closely correlated with the onset and progression of pathological changes in an experimental prion disease (12). The decreased expression of IP$_3$R1 mRNA was evident well after the activation of LM gene, suggesting that the activated microglia might play a role in the Purkinje cell degeneration in Ngsk *Prnp^{0/0}* mice. The elucidation of the mechanisms of the glial-cell activation in Ngsk *Prnp^{0/0}* mice might provide insights into the understanding of not only the physiological functions of PrP^C and PrPLP/Dpl but also the pathogenesis of neurodegenerative disorders, including prion diseases.

Acknowledgments

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