Prevention of Scrapie Pathogenesis by Transgenic Expression of Anti–Prion Protein Antibodies

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Variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy are initiated by extracerebral exposure to prions. Although prion transmission from extracerebral sites to the brain represents a potential target for prophylaxis, attempts at vaccination have been limited by the poor immunogenicity of prion proteins. To circumvent this, we expressed an anti-prion protein (anti-PrP) μ chain in *Prnp*$^{o/o}$ mice. Transgenic mice developed sustained anti-PrP titers, which were not suppressed by introduction of *Prnp*$^{+}$ alleles. Transgene expression prevented pathogenesis of prions introduced by intraperitoneal injection in the spleen and brain. Expression of endogenous PrP (PrP^{C}) in the spleen and brain was unaffected, suggesting that immunity was responsible for protection. This indicates the feasibility of immunological inhibition of prion disease in vivo.

Prion diseases are slow, lethal transmissible neurodegenerative illnesses that affect humans and many animal species. Although human prion diseases are rare, the incidence of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom appears to be increasing exponentially (*1*), probably as the result of exposure to bovine spongiform encephalopathy (BSE) prions (*2*, *3*). Given the large amount of BSE-infected material that may have entered the human food chain, and the many people who may eventually develop vCJD (*4*), it will be important to introduce strategies to prevent the development of symptoms in these individuals. For many conventional infectious agents, vaccination is an effective method of infection control. However, there has been little evidence that vaccines might be effective for protection against prion diseases. The endogenous cellular prion protein (PrP^{C}) is expressed by most tissues of the body and is therefore known to be a poor immunogen, probably because of host tolerance. However, ablation of the *Prnp* gene (*5*), which encodes PrP^{C}, makes it possible to immunize mice with prions (*6*). We reasoned that genes encoding high-affinity anti-PrP antibodies generated in *Prnp*$^{o/o}$ mice might be used to redirect B cell responses of prion-susceptible mice that express PrP^{C}.

Epitope-binding variable regions of the immunoglobulin heavy (V_H) and light chains (V_L) from cDNA of hybridoma 6H4, which expresses an immunoglobulin G1 (IgG1) monoclonal antibody (mAb) recognizing murine PrP^{C}, were amplified by polymerase chain reaction (*7*). The same method was used to amplify the V_H and V_L chains of 15B3, which expresses an IgM antibody recognizing PrP^{Sc} (*7*, *8*). These sequences were then expressed as single-chain variable fragment (scFv), tested for their anti-PrP binding capacity, and were finally expressed as transgenes (*7*). To avoid autoimmunity, we used fertilized eggs from *Prnp*$^{o/o}$ Sv129 × C57BL/6 mice (*5*), which express IgM/D of the a or b allotype. The resulting mice expressed transgenic IgM^{a} μ chain combined with a repertoire of endogenous λ and κ light chains, but no detectable endogenous IgM/D, indicating that the transgene induced allelic exclusion of endogenous heavy chains (Fig. 1A) (*9*). When compared with nontransgenic *Prnp*$^{o/o}$ or *Prnp*$^{+/o}$ mice, the B cell compartments of 6H4μ and 15B3μ transgenic mice were reduced (*7*), whereas T cell compartments (*7*) were normal (Fig. 1B). Transgenic *Prnp*$^{o/o}$-6H4μ mice had consistently high spontaneous anti-PrP titers at the age of 4 weeks, whereas 15B3μ transgenic mice did not display signals above background (Fig. 1C) (*10*). In addition, 15B3μ sera showed no titer when tested in an enzyme-linked immunosorbent assay with a target peptide recognized by mAb 15B3 (*11*). Because 15B3μ mice do not contain the original 15B3 light chain, their combinatorial repertoire may differ in specificity from that of mAb 15B3. Therefore, 15B3μ was operationally regarded as of irrelevant specificity. Sera from *Prnp*$^{o/o}$-6H4μ, but not *Prnp*$^{o/o}$-15B3μ mice, were able to decorate Western blot membranes loaded with brain extracts of wild-type mice and with PrP^{REC} (*12*) (Fig. 1D). In addition, *Prnp*$^{o/o}$-6H4μ, but not *Prnp*$^{o/o}$-15B3μ sera, bound to splenocytes from *tg94* transgenic mice, which overexpress PrP^{C} on lymphocytes (*13*) (Fig. 1E). The above data indicate that the 6H4μ transgene, but not 15B3μ, conferred anti-PrP titers to *Prnp*$^{o/o}$ mice.

Because prion replication requires PrP^{C} (*14*), we reintroduced one or two *Prnp* alleles by crossbreeding transgenic mice with wild-type C57BL/6 mice. The number of transgenic IgM^{a+}IgD^{-} B cells (*7*), as well as IgM^{a} surface expression levels in *Prnp*$^{+/o}$-6H4μ mice, were slightly higher than those of *Prnp*$^{o/o}$-6H4μ mice (Fig. 2A). *Prnp*$^{+/o}$-

Table 1. Intraperitoneal prion challenge of transgenic mice. Spleen infectivity titers were reduced by >4 logarithmic units in the presence of the 6H4μ transgene. dpi, days after inoculation; LD_{50}, median lethal dose.

Prnp host genotype of individual mice	Transgene	dpi	Attack rate	Incubation time of *tga20* mice (mean ± SD)	log(LD_{50})/g spleen tissue
o/o	–	35	0/4	>200	<0
o/o	–	50	0/4	>200	<0
o/o	6H4μ	35	0/4	>200	<0
o/o	6H4μ	50	0/4	>200	<0
+/o	6H4μ	35	0/3	>200	<0
+/o	6H4μ	50	0/4*	>200	<0
+/o	–	50	4/4	82 ± 6.3	4.2
+/o	–	50	6/6	75 ± 5	4.9
o/o	15B3μ	35	0/4	>200	<0
o/o	15B3μ	50	0/4	>200	<0

*One mouse died at 91 days due to intercurrent disease.

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6H4μ and *Prnp*$^{+/+}$-6H4μ mice developed substantial spontaneous anti-PrP serum levels, albeit more slowly than *Prnp*$^{o/o}$-6H4μ mice (Fig. 2B). Because unresponsiveness of B cells may correlate with the level of self-antigen (*15*) and B cell receptor affinity/avidity (*16*), the delayed build-up of anti-PrP serum titers in *Prnp*$^{+/o}$-6H4μ mice may have reflected negative selection of B cells expressing combinations of 6H4μ with light chains yielding high-avidity binding to PrP^{C} that was not permissive for B cell survival. If this were the case, it would be expected that (i) the B cell repertoires of *Prnp*$^{o/o}$-6H4μ, *Prnp*$^{+/o}$-6H4μ, and *Prnp*$^{+/+}$-6H4μ mice should differ; (ii) the unexpected expansion of B cells in *Prnp*$^{+/o}$-6H4μ and *Prnp*$^{+/+}$-6H4μ mice may consist of clones expressing lower affinities; and (iii) peripheral 6H4μ-expressing B cells may be susceptible to tolerization in PrP-overexpressing mice.

To test the latter hypothesis, 6H4μ transgenic mice were bred onto *tg94* transgenic mice that overexpress PrP in B and T cells ~1000-fold (*13*). Whereas *Prnp*$^{+/o}$-6H4μ and *Prnp*$^{+/+}$-6H4μ mice were indistinguishable from each other (*7*), *tg94*-6H4μ mice exhibited almost complete loss of B cells in peripheral blood (Fig. 2C) (*7*). In the blood of *tg94*-6H4μ mice, we found a residual population of B220^{dull}IgM^{a}-positive B cells (Fig. 2D) that was CD11b^{-}CD5^{-}CD62L^{+} (*11*) and may represent immature/transitional B cells that recently emigrated from the bone marrow (*17*, *18*). Therefore, PrP can be co-expressed with PrP-specific antibodies in *Prnp*$^{+/o}$-6H4μ or *Prnp*$^{+/+}$-6H4μ transgenic mice without inducing autoimmune disease or hematological disorders. Only when PrP^{C} was expressed at nonphysiologically high levels were 6H4μ-expressing B cells strongly reduced.

Transgenic mice (*Prnp*$^{o/o}$ and *Prnp*$^{+/o}$) were next inoculated intraperitoneally with scrapie prions (RML strain, passage 5) (*19*). Spleens were harvested at 35 and 50 days after inoculation (dpi), and prion titers were determined by bioassay with *tga20* mice (*20*, *19*). Although nontransgenic *Prnp*$^{+/o}$ mice developed measurable prion titers, no infectivity could be detected in *Prnp*$^{+/o}$-6H4μ or in *Prnp*$^{o/o}$ mice at any time (Table 1).

We next quantitated the level of deposition of PrP^{Sc} in the spleens of scrapie-infected 6H4μ and 15B3μ transgenic mice (*12*). PrP^{Sc} accumulated in *Prnp*$^{+/o}$ and *Prnp*$^{+/o}$-15B3μ spleens at various time points investigated, but was undetectable in *Prnp*$^{+/o}$-6H4μ spleens (Fig. 3, A and B). The sensitivity of Western blot analyses may be inadequate if PrP^{Sc} is distributed inhomogeneously in tissue, because PrP^{Sc}-free material may dilute the PrP^{Sc}-positive regions. We controlled for this possibility with histoblots of spleen and brain sections (*21*, *22*). Protease-resistant PrP^{Sc} was distributed in-

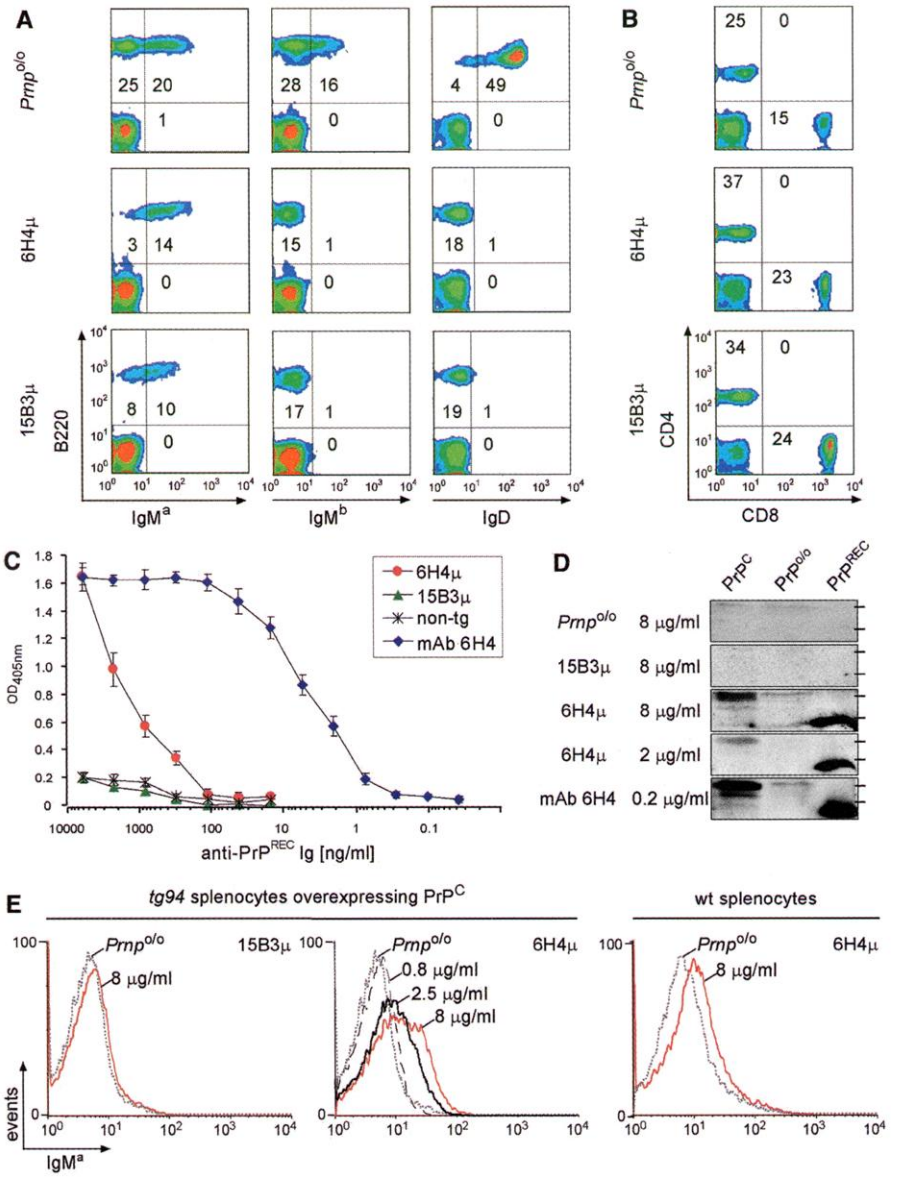

Fig. 1. Expression of anti-PrP μ in transgenic mice. (**A**) FACS analysis of surface immunoglobulins on B220^{+} peripheral blood B cells. Transgenic 6H4μ and 15B3μ mice expressed only immunoglobulins of the IgM^{a}, but not of the IgM/D, class, indicating allelic exclusion of endogenous heavy chains. Instead, nontransgenic littermates expressed IgM^{a}/D and/or IgM^{b}/D. Relative B cell numbers were reduced in both 6H4μ and 15B3μ mice. (**B**) FACS analysis of peripheral blood revealed no alteration in CD4^{+} and CD8^{+} T cell subsets in transgenic mice. (**C**) ELISA showing anti-PrP^{REC} titers in sera of 6H4μ mice (red circles), but not of 15B3μ mice (green triangles), nor of nontransgenic littermates (black crosses). Each data point represents the average of four mice. Immunoreactivity of 6H4μ mice was only detectable with anti-IgM and anti-IgM+A+G, but not with anti-IgG secondary antibodies (*11*). Based on the concentration of anti-PrP^{REC} immunoglobulins (6H4μ mice: IgM^{a}; mAb 6H4 control: IgG1), the anti-PrP titer of transgenic 6H4μ immunoglobulins is ~500-fold lower than that of mAb 6H4 (blue diamonds). Because IgM is about fives times heavier than IgG, the total anti-PrP avidity of IgM^{a} in 6H4μ serum (M^{-1}) is ~100-fold lower than that of mAb 6H4. (**D**) Transgenic IgM^{a} immunoglobulins of 6H4μ mice, in contrast to 15B3μ mice or nontransgenic littermates, recognized PrP^{C} in immunoblotted wild-type brain homogenates (left column) and full-length PrP^{REC}, but yielded no signal with *Prnp*$^{o/o}$ brain homogenate. mAb 6H4 produced a pattern similar to that of the serum of 6H4μ mice exhibiting the un-, mono- and diglycosylated band of PrP^{C}. Markers on the right indicate molecular size at 35 and 28 kD. (**E**) FACS analysis. (Left) Twenty-five–fold diluted serum of 15B3μ mice (red line, corresponding to 8 μg/ml IgM^{a}) or of nontransgenic *Prnp*$^{o/o}$ littermates (gray dotted line) did not bind to native PrP^{C} of *tg94* splenocytes that overexpress PrP^{C}. (Middle) In contrast, 25-fold–diluted serum of 6H4μ transgenic mice recognized native PrP^{C} of *tg94* splenocytes (red line, 8 μg/ml IgM^{a}) and produced a shift of >1 logarithmic unit of fluorescence intensity. (Right) Twenty-five–fold diluted 6H4μ serum (red line, corresponding to 8 μg/ml IgM^{a}) yielded only a faint signal when wild-type (wt) splenocytes expressing low levels of PrP^{C} were used as targets. Gray dotted lines: 25-fold–diluted serum of nontransgenic *Prnp*$^{o/o}$ mice.

homogenously in prion-inoculated *Prnp*$^{+/o}$ and *Prnp*$^{+/o}$-15B3μ spleens (Fig. 3C), reflecting accumulation within splenic germinal centers (*23*). Again, no signal was detectable in *Prnp*$^{+/o}$ -6H4μ mice (Fig. 3C), confirming that 6H4μ effectively prevented splenic accumulation of protease-resistant PrP.

We next tested whether anti-PrP humoral immunity in 6H4μ mice could inhibit prion transport from peripheral sites to the central nervous system. The brains of scrapie-inoculated *Prnp*$^{o/o}$, *Prnp*$^{+/o}$, *Prnp*$^{+/o}$-15B3μ, and *Prnp*$^{+/o}$-6H4μ mice were analyzed by Western blot. PrP^{Sc} was not detected in any transgenic *Prnp*$^{+/o}$-6H4μ brain, whereas there was a strong signal in the brains of all nontransgenic *Prnp*$^{+/o}$ and *Prnp*$^{+/o}$-15B3μ mice as early as 170 dpi (Fig. 3D). Histoblots confirmed PrP^{Sc} accumulation in the brains of *Prnp*$^{+/o}$-15B3μ mice, whereas there was no signal in *Prnp*$^{+/o}$-6H4μ mice (Fig. 3C).

It has been observed that ablation of B-lymphocytes prevents neuropathogenesis of prion disease after intraperitoneal inoculation (*24*, *25*). This is probably due to impaired lymphotoxin-dependent maturation of follicular dendritic cells (FDCs) (*26*), which are a major extracerebral prion reservoir (*23*). Neuropathogenesis is also impaired by ablation of complement factors and receptors that mediate opsonization and capture by FDCs (*27*). We therefore tested whether antiprion protection of 6H4μ may result from altered B cell physiology, development, or histoarchitecture and cellular composition of germinal centers. Such alterations may impair antiviral responses. However, 6H4μ and 15B3μ (*Prnp*$^{o/o}$ or *Prnp*$^{+/o}$) mounted IgM and IgG responses to vesicular stomatitis virus or lymphocytic choriomeningitis virus with similar kinetics (*7*). Therefore, expression of transgenic μ chains did not alter the immune system (*7*) in a way that would suppress B cell responses, although

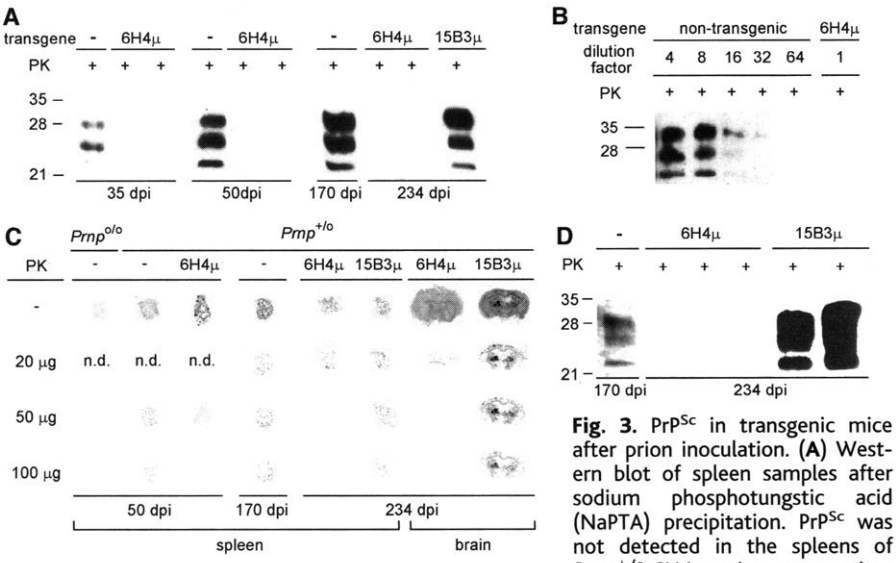

Fig. 3. PrP^{Sc} in transgenic mice after prion inoculation. **(A)** Western blot of spleen samples after sodium phosphotungstic acid (NaPTA) precipitation. PrP^{Sc} was not detected in the spleens of *Prnp*$^{+/o}$-6H4μ mice at any time point investigated (35 to 234 dpi), whereas nontransgenic littermates and *Prnp*$^{+/o}$-15B3μ showed substantial PrP^{Sc} deposition. All samples were digested with proteinase K (PK). All mice were *Prnp*$^{+/o}$. **(B)** Determination of the sensitivity of Western blots after NaPTA precipitation. In the spleens of *Prnp*$^{+/o}$ mice 170 dpi, the PrP^{Sc}-specific signal was detected in 32-fold–diluted samples. Therefore, PrP^{Sc} accumulation in the spleens of *Prnp*$^{+/o}$-6H4μ mice was ≤3% that of nontransgenic *Prnp*$^{+/o}$ mice. **(C)** Histoblot of spleens (lanes 1 to 6) and brains (lanes 7 and 8) 50 to 234 dpi. Discrete PrP^{Sc} deposits were detected in the spleens of *Prnp*$^{+/o}$-15B3μ and nontransgenic *Prnp*$^{+/o}$ mice, whereas there was no PrP^{Sc} accumulation in *Prnp*$^{+/o}$-6H4μ mice. The brains of *Prnp*$^{+/o}$-6H4μ displayed no PrP^{Sc} deposits, whereas *Prnp*$^{+/o}$-15B3μ controls exhibited strong PrP^{Sc} accumulation at 234 dpi. **(D)** PrP^{Sc} deposition in the brains of nontransgenic littermates and *Prnp*$^{+/o}$-15B3μ was detected as early as 170 dpi and, to a larger extent, at 234 dpi. In contrast, no signal was detected in the brains of 6H4μ mice, even when 1 mg of protein was used and the blots were overexposed. (+) indicates proteinase K (PK) digestion.

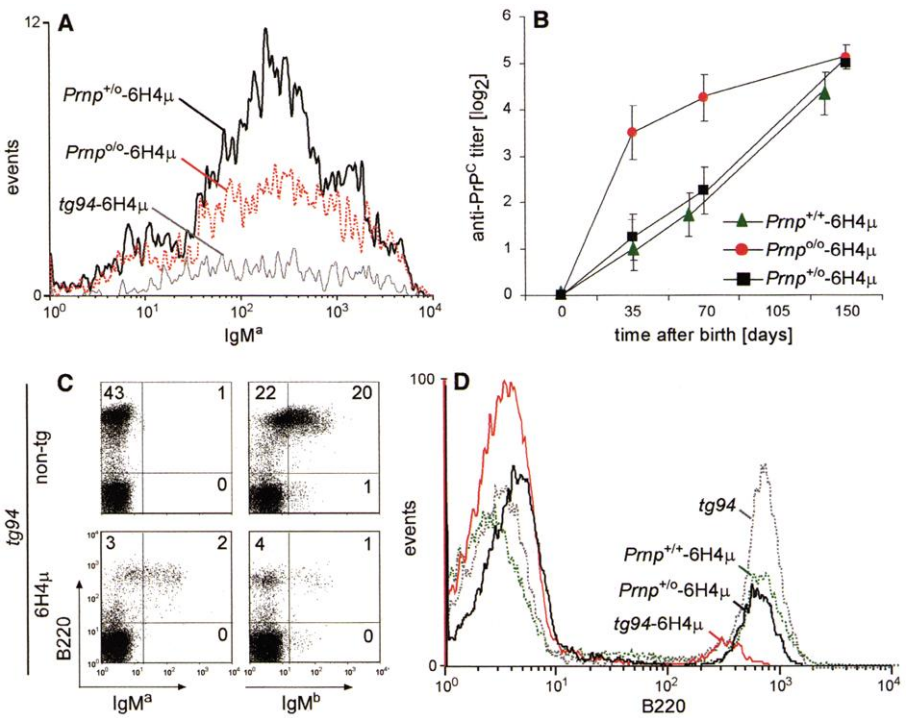

Fig. 2. Coexpression of PrP^C and anti-PrP μ chain: **(A)** FACS analysis of peripheral blood gated on B220$^+$ B cells. Transgenic IgM^a surface expression in *Prnp*$^{+/o}$-6H4μ (black line) was slightly higher than in *Prnp*$^{o/o}$-6H4μ mice (dotted red line), whereas *tg94*-6H4μ mice (gray line) displayed drastically reduced IgM^a expression. **(B)** Anti-PrP^{REC} titers determined by ELISA. *Prnp*$^{o/o}$-6H4μ mice (red circles), *Prnp*$^{+/o}$-6H4μ mice (black squares), and *Prnp*$^{+/+}$-6H4μ mice (green triangles) showed similar titers at the age of 4 to 5 months. However, *Prnp*$^{o/o}$-6H4μ mice already had substantial titers at the age of 1 month, whereas *Prnp*$^{+/o}$-6H4μ mice and *Prnp*$^{+/+}$-6H4μ mice displayed delayed kinetics in reaching considerable anti-PrP serum levels. Each data point represents the average of four mice. **(C)** FACS analysis of peripheral blood. *Tg94*-6H4μ mice displayed only a small residual population of B220$^+$ B cells expressing IgM^a (lower left) but not IgM^b (lower right), suggesting partial clonal deletion of self-reactive B cells. *Tg94* mice without the 6H4μ transgene displayed normal numbers of IgM^b-positive B cells (upper right). Numbers indicate the percentage of cells. **(D)** The residual population of B cells in *tg94*-6H4μ mice (red line) appeared to be B220^{dull}, whereas *Prnp*$^{+/o}$-6H4μ mice (black line) or *Prnp*$^{+/+}$-6H4μ mice (green dotted line) with physiological PrP^C levels or *tg94* controls (gray dotted line) displayed B cells with a normal B220 expression pattern.

a slight reduction of B cell counts per volume unit was seen in peripheral blood and spleen (*7*). *Prnp*$^{+/o}$-6H4μ and *Prnp*$^{+/o}$-15B3μ mice displayed germinal centers of slightly smaller size than nontransgenic *Prnp*$^{+/o}$ mice, but had normal FDC clusters that were found to colocalize with PrP^{C} (Fig. 4A), implying that mature B cells were present (*28*). Splenic B and T cell subsets, dendritic cells, and macrophages were unaltered in all transgenic mice (*7*). All of the above data indicate that 6H4μ prevents, or drastically delays, scrapie pathogenesis.

Genetic ablation of *Prnp* abrogates susceptibility of mice to scrapie (*14*) and prevents subclinical prion replication in the brain and spleen (*29*). Therefore, we investigated whether 6H4μ repressed PrP^{C} expression in *Prnp*$^{+/o}$ mice. However, Western blot analysis showed that the content of PrP^{C} in the spleens and brains of *Prnp*$^{+/o}$-6H4μ mice before (Fig. 4B) (*7*) and after prion inoculation (*11*) was similar to that of nontransgenic *Prnp*$^{+/o}$ mice (*12*). We conclude that prion resistance of *Prnp*$^{+/o}$-6H4μ mice is not mediated by down-regulation of PrP^{C} expression. Masking of PrP^{C} at critical sites of prion replication may be involved in protection, as suggested by the finding that in *tg94*-6H4μ mice, T cells overexpressing PrP^{C} were highly decorated with transgenic IgM^{a} antibodies (Fig. 4C). This may hinder the interaction of prions with PrP^{C}. Other mechanisms, such as capturing and immune-mediated degradation of the incoming PrP^{Sc} inoculum, steric competition with template-directed refolding, or interference with a seeded PrP^{Sc} nucleation reaction, may also be involved.

Although in vitro preincubation with anti-PrP antisera was reported to reduce the prion titer of infectious microsomes from hamster brain homogenates (*30*) and an anti-PrP antibody was found to inhibit formation of PrP^{Sc} in a cell-free system (*31*), prevention of neuroinvasive scrapie in vivo by specific anti-PrP antibodies has not been previously reported. Because PrP^{C} is broadly expressed, it is possible that induction of anti-PrP immune responses may induce an autoimmune disease and defeat prospects for prion vaccination. However, we observed no overt symptoms of autoimmune disease as a result of anti-prion immunization unless PrP^{C} was expressed at extremely high levels. At the same time, there appears to be no appreciable clonal deletion of autoreactive immune cells, suggesting that B cells are not tolerant to PrP^{C}, similar to other examples of B cells specific for transgenic antigens (*32*). If B cells of wild-type and transgenic 6H4μ mice are not tolerant to PrP^{C}, lack of immunity to prions may be due to T helper cell tolerance—a condition that may be overcome by presenting PrP^{C} in an appropriate, adjuvant context.

From an applied viewpoint, transgenesis is too elaborate as a defensive strategy against prions. However, the findings described here encourage reassessment of the value of active and passive immunization, and perhaps of reprogramming B cell repertoires by μ chain transfer, in prophylaxis or therapy of prion diseases.

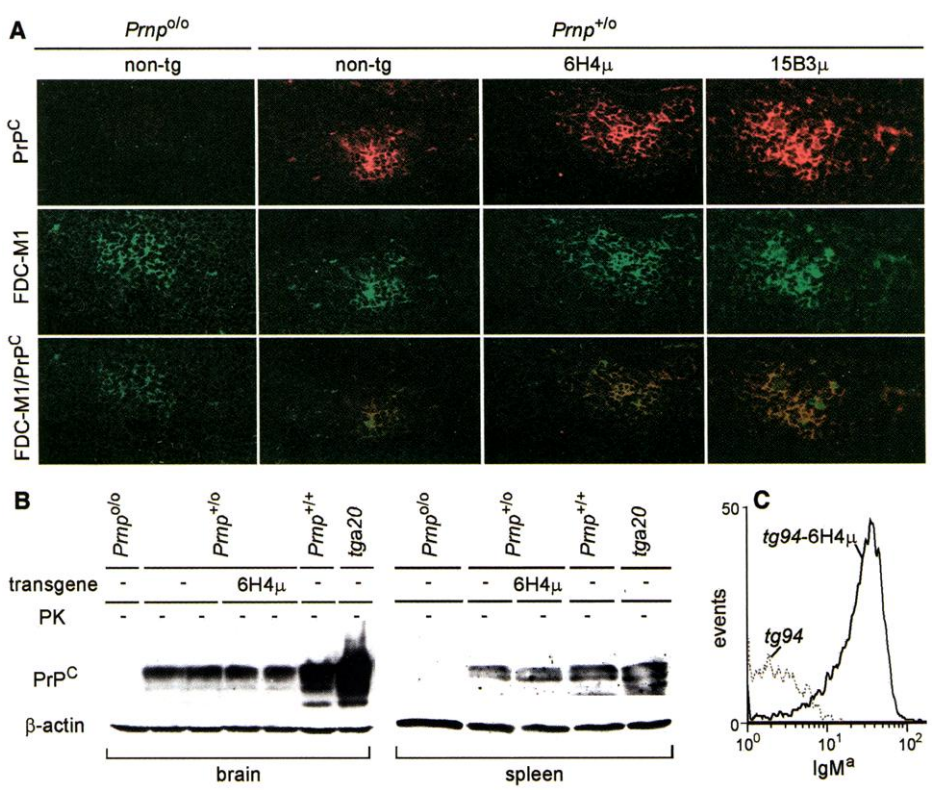

Fig. 4. Histological and biochemical characteristics associated with scrapie protection. (**A**) Histological examination of age-matched transgenic and nontransgenic littermates (genotype as indicated above) revealed normal networks of follicular dendritic cells (FDCs; detected with mAb FDC-M1, middle row) forming networklike structures within germinal centers and colocalized with PrP^{C} expression (detected with polyclonal XN antibody, upper row). (Lower row) Overlay of upper and middle rows. (**B**) Western blot. PrP^{C} expression in the brain (left) and spleen (right) was similar in *Prnp*$^{+/o}$-6H4μ and nontransgenic *Prnp*$^{+/o}$ mice. This was confirmed by quantitative chemiluminescent analysis (*11*). *Prnp*$^{+/+}$ and *tga20* mice served as positive controls, *Prnp*$^{o/o}$ mice as negative controls. Equal amounts of protein were loaded, as assessed by β-actin quantification (lower bands). (**C**) FACS analysis of peripheral blood of *tg94*-6H4μ mice revealed transgenic IgM^{a} immunoglobulins adhering to Thy1.2^{+} T cells, suggesting masking of PrP^{C} (solid black line). Because of the low percentage of B cells in the peripheral blood of *tg94*-6H4μ mice, there was a relative increase in the number of T cells compared with that in *tg94* control mice (gray dotted line) within the sample population (10,000 lymphocytes).

References and Notes

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9. Fluorescence-activated cell sorting (FACS) analysis: Several drops of retroorbital blood were collected in phosphate-buffered saline (PBS), 2% fetal calf serum, 10 mM EDTA (pH 8), 15.3 mM NaN3. The following anti-mouse antibodies were used (all from BD Pharmingen, San Diego, CA): phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)–labeled anti-B220; PE- or FITC-labeled anti-IgMa; PE- or FITC-labeled anti-IgMb; FITC-labeled anti-IgD; PE-labeled anti-CD8; and FITC-labeled anti-CD4. Lysis of red blood cells was performed with FACS lysing solution (Becton Dickinson). Living cells were gated with a combination of forward scatter and side scatter. Data were acquired on a FACS Calibur (Becton Dickinson) with CellQuest software (Becton Dickinson); analyses were performed with Windows Multiple Document Interface (WinMDI, Version 2.8; http://facs.scripps.edu).
10. Enzyme-linked immunosorbent assay (ELISA): For anti-PrP^{REC} ELISA, 384-well plates were coated with 125 ng of recombinant mouse PrP$_{23-231}$ (*33*) in 30 μl of PBS overnight at 4°C. After three washing steps with ELISA buffer (PBS, 0.1% Tween), plates were blocked with 5% bovine serum albumin (BSA) at room temperature. After washing, plates were incubated for 2 hours at room temperature with 25 μl of two- or threefold serially diluted mouse serum in ELISA buffer containing 1% BSA in triplicate, and probed with horseradish peroxidase–conjugated rabbit anti-mouse IgG+A+M (H+L, 1:1000 dilution), rabbit anti-mouse IgM (μ chain–specific, 1:5000 dilution), or rabbit anti-mouse IgG (γ chain–specific, 1:5000 dilution; all from Zymed Laboratories, San Fran-

cisco, CA). Plates were developed with 2.2′-azino-di-ethyl-benzothiazolinsulfonat, and H_2O_2 and optical density (OD) was measured at 405 nm. Titer was defined as the dilution of the OD turning point. Omission of sera, omission of secondary antibody, uncoated wells, and dilutions of purified mAb 6H4 (Prionics, Zurich, Switzerland) (*8*) served as controls.
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19. Prion inoculation of mice and infectivity bioassay: Infectious brain homogenate from mice infected with Rocky Mountain Laboratory scrapie prions (RML, passage 5) was prepared as described (*14*). Mice were inoculated intraperitoneally with 26-gauge needles and 200-μl syringes with dilutions of the brain homogenate (in PBS containing 5% BSA, 100-μl inoculation volume). All inoculated 6H4μ mice had substantial anti-PrP^{REC} titers. Infectivity bioassays were done as described (*20*, *35*).
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