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## Transmission Barriers for Bovine, Ovine, and Human Prions in Transgenic Mice

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**Transgenic (Tg) mice expressing full-length bovine prion protein (BoPrP) serially propagate bovine spongiform encephalopathy (BSE) prions without posing a transmission barrier. These mice also posed no transmission barrier for Suffolk sheep scrapie prions, suggesting that cattle may be highly susceptible to some sheep scrapie strains. Tg(BoPrP) mice were also found to be susceptible to prions from humans with variant Creutzfeldt-Jakob disease (vCJD); on second passage in Tg(BoPrP) mice, the incubation times shortened by 30 to 40 days. In contrast, Tg(BoPrP) mice were not susceptible to sporadic, familial, or iatrogenic CJD prions. While the conformational stabilities of bovine-derived and Tg(BoPrP)-passaged BSE prions were similar, the stability of sheep scrapie prions was higher than that found for the BSE prions but lower if the scrapie prions were passaged in Tg(BoPrP) mice. Our findings suggest that BSE prions did not arise from a sheep scrapie strain like the one described here; rather, BSE prions may have arisen spontaneously in a cow or by passage of a scrapie strain that maintains its stability upon passage in cattle. It may be possible to distinguish BSE prions from scrapie strains in sheep by combining conformational stability studies with studies using novel Tg mice expressing a chimeric mouse-BoPrP gene. Single-amino-acid substitutions in chimeric PrP transgenes produced profound changes in incubation times that allowed us to distinguish prions causing BSE from those causing scrapie.**

Prion diseases are fatal degenerative disorders of the central nervous system (CNS). The pathogenesis of prion diseases involves conversion of the host-encoded prion protein (PrP), denoted PrP<sup>C</sup>, into an insoluble isoform (PrP<sup>Sc</sup>) that accumulates in the CNS. PrP<sup>Sc</sup> is the only known component of the infectious pathogen (43). PrP<sup>C</sup> undergoes a profound conformational change as PrP<sup>Sc</sup> is formed; this structural transition is characterized by an increase in  $\beta$ -sheet content and the disappearance of several surface epitopes (12, 33, 37, 41, 46, 49, 51, 67). Moreover, this conformational change is the fundamental event in prion replication.

To distinguish PrP<sup>Sc</sup> from PrP<sup>C</sup>, limited digestion with proteases is commonly employed, during which PrP<sup>C</sup> is readily digested and PrP<sup>Sc</sup> is N-terminally truncated to form PrP 27-30 (4, 45, 46). Concerned that proteolytic digestion was destroying a significant portion of PrP<sup>Sc</sup>, one of us (S.B.P.) developed the conformation-dependent immunoassay (CDI) for measuring PrP<sup>Sc</sup>. The CDI uses antibodies directed to epitopes that are exposed in PrP<sup>C</sup> but buried in native PrP<sup>Sc</sup> (52). The buried epitope in PrP<sup>Sc</sup> becomes exposed upon denaturation, and the difference in immunoreactivities between the denatured sample and the native sample is equal to PrP<sup>Sc</sup>. From studies with the CDI, we learned that a minority of PrP<sup>Sc</sup> molecules are protease resistant and that we had underestimated the level of

PrP<sup>Sc</sup> by as much as 90% in some instances. These results indicated that PrP<sup>Sc</sup> adopts both protease-resistant (r) and -sensitive (s) conformations.

Bovine spongiform encephalopathy (BSE) (26) of cattle and chronic wasting disease of deer and elk (68) were recognized as new prion diseases in the 1980s. It has been estimated that ~750,000 cattle infected with BSE prions were slaughtered for human consumption in Great Britain during a period spanning from 1980 to 1996 (1, 17). It now seems clear that during this period, the disease passed to humans through the consumption of contaminated beef products, leading to the emergence of a new, variant form of Creutzfeldt-Jakob disease (vCJD). While epidemiological studies (65, 66) and prion strain typing experiments (7, 15, 24) suggested that vCJD is caused by the transmission of BSE prions to humans, compelling evidence arguing for an etiologic link between BSE and vCJD came from the demonstration that human vCJD prions precisely recapitulate the properties of bovine BSE prions following transmission to transgenic (Tg) mice expressing bovine prion protein (BoPrP) (58).

Although it is clear that vCJD in humans is caused by BSE prions, it is unknown what proportion of those exposed to BSE prions will eventually develop vCJD. Moreover, it is not known whether consumption of chronic wasting disease-tainted venison poses a risk to humans. Although prion diseases are not generally communicable, experimental transmission of prion diseases between hosts of the same mammalian species is typically highly efficient, with a uniform and consistent incubation period. In contrast, prion transmission between animals of different species often fails to cause disease even after prolonged incubation periods. This comparative resistance to in-

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fection, as seen in the first passage to a new species, has been termed the species barrier (38).

Studies with Tg mice (47, 48, 54, 55, 57, 62), mice with deficient endogenous PrP gene (*Prnp*<sup>0/0</sup>) expression (9, 44), and homologous recombinants (3, 34) established the role of the PrP sequence in modulating susceptibility to foreign prions. However, the work presented here and elsewhere (29) demonstrate that the strain of prion may eliminate the transmission barrier imposed by sequence differences and vice versa. In some cases, a chimeric or mutated gene may be able to confer greater susceptibility to prions, provided that the new PrP sequence is compatible with the prion strain in the inoculum.

Tg(BoPrP) mice are highly susceptible to BSE prions, and the infected animals faithfully recapitulate many of the features of BSE in cattle (57). These same mice are also highly susceptible to both vCJD and natural sheep scrapie prions (58). We developed a new line of Tg mice homozygous for the BoPrP transgene, and this facilitated the breeding of large numbers of animals required for an endpoint titration study (53). The titers obtained, which were equal to or greater than 10<sup>7</sup> infectious units per gram of tissue, indicated that these Tg mice are ~10-fold more susceptible than cattle and 10<sup>4</sup>-fold more sensitive than wild-type mice to BSE prions (53). We presume that the heightened sensitivity of these Tg mice to BSE prions compared to cattle is a consequence of the overexpression of BoPrP (11, 47, 63). Overexpression of PrP in mouse neuroblastoma cells also appears to increase the susceptibility of these cells to infection with mouse prion strains (36).

In the study reported here, we demonstrate that some isolates of sheep scrapie prions transmit very efficiently to Tg(BoPrP) mice, with no evidence of a prion transmission barrier. In contrast, vCJD prions transmit less efficiently, with an ~30-day change in incubation period from first to second passage. Tg(BoPrP) mice were resistant to all other human prions tested. We next created a series of chimeric mouse-BoPrP transgenes and showed that they confer various degrees of susceptibility to BSE, vCJD, and scrapie prions. One line of Tg mice appear highly susceptible to BSE prions but not to scrapie prions and may be suitable for selectively identifying BSE strains in sheep.

#### MATERIALS AND METHODS

**Chimeric transgenes and production of transgenic mice.** Tg(BoPrP)<sup>4125</sup> and Tg(BoPrP)<sup>+/+</sup>4092 mice have been described previously (53, 57, 58). The Bo3M open reading frame (ORF) was created by replacing the N-terminal coding sequence of the MBo2M chimera (58) with the corresponding sequence from the native BoPrP ORF cassette (58). This exchange of sequences exploited the presence of a single PstI restriction enzyme cleavage site that cleaves within the homologous BoPrP-derived sequences. Additional chimeric PrP transgenes were created by replacing regions of the Bo3MPrP ORF with synthetic double-stranded DNA oligonucleotides to create a series of chimeras (Fig. 1). Complete sequences of the chimeric ORF constructs in GenBank-compatible format were determined (data not shown). Plasmid manipulations were prototyped, and sequences were archived by using Vector NTI software (Informax, Inc., Frederick, Md.).

**Prion isolates and transmission studies.** BSE isolates from samples GJ248/85, SE1809/11, 97/1612, 97/1997, and PG31/90 were obtained from John Wilesmith at the Central Veterinary Laboratory, Weybridge, United Kingdom. Brain samples from vCJD cases RU96/02, RU96/07, RU96/70, RU96/80, and RU96/110 were obtained from Robert Will and James Ironside at the United Kingdom National CJD Surveillance Unit. Sheep scrapie isolates Oreo, T97-1104, no. 15,

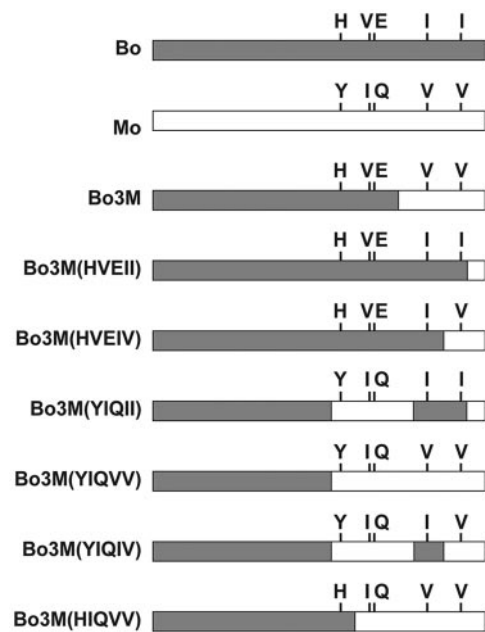


FIG. 1. Chimeric PrP transgenes. Constructs were created as described in Materials and Methods. The name of each construct, as referenced in the text, is shown at the left of each diagram illustrating the corresponding PrP ORFs. Regions corresponding to BoPrP are shown in gray, and regions derived from MoPrP are shown in white. The five residues that vary in the chimeric ORFs are diagrammed in their respective single-letter amino acid codes.

no. 139, and no. 340 have been described elsewhere (58, 64), and all are derived from Suffolk sheep with the ARQ genotype. Tg(BoPrP)-passaged BSE, vCJD, and scrapie isolates were produced in this laboratory and have been described in previous publications (53, 57, 58). Transmissions of the BSE, vCJD, and scrapie isolates to Tg mice were performed as previously described (57). Brain samples used for transmission studies are listed in the figure legends. Brain homogenates were prepared by repeated extrusion through syringe needles of successively smaller sizes, from 22 to 18 gauge. Mice were inoculated with 30  $\mu$ l of a 1% homogenate (wt/vol) in sterile phosphate-buffered saline without Ca<sup>2+</sup> or Mg<sup>2+</sup> by using a 27-gauge, disposable hypodermic needle inserted into the right parietal lobe. Following inoculation, the status of the mice was monitored daily while the neurologic status was assessed three times per week. Clinical diagnosis was performed as previously described (11, 55).

**Neuropathology.** Brains were removed rapidly from animals and either perfusion fixed in 10% buffered formalin or snap-frozen. The brains were embedded in paraffin, and histological sections were stained with hematoxylin and eosin for evaluation of vacuolar (spongiform) degeneration. Vacuolation scores, which are semiquantitative estimates of a brain region occupied by vacuoles (10), were determined by a single rater (S.J.D.). Immunohistochemical localization of PrP<sup>Sc</sup> on formalin-fixed, paraffin-embedded tissue sections was determined by the formic acid-hydrated autoclaving method (35). Sections of formalin-fixed, paraffin-embedded tissue on glass slides were immersed in undiluted formic acid for 2 min followed by autoclaving in 1.3 mM HCl in distilled H<sub>2</sub>O for 10 min at 121°C. Sections were then incubated overnight at 4°C with the rabbit BoPrP-specific 9095 antiserum (57). After rinsing, the sections were incubated for 30 min at room temperature with biotinylated anti-rabbit antiserum (Vector Laboratories, Burlingame, Calif.) diluted 1:100 in 5% nonfat milk. Following rinses, color was developed with diaminobenzidine.

**Preparation of brain homogenates and conformational stability assays.** Aliquots of 1% (wt/vol) brain homogenates were added to an equal volume of stock solutions containing increasing concentrations of GdnHCl, chosen to produce the range of final concentrations of GdnHCl indicated in the figure legends. GdnHCl stock solutions were prepared from an 8 M solution (Pierce, Rockford, Ill.) diluted in water. Following 1 h of incubation at room temperature, all samples were diluted with Tris-buffered saline containing 2% Sarkosyl to a final concentration of 0.4 M GdnHCl. Proteinase K was added in a ratio of 1:500,

proteinase K-protein (wt/wt), and digested for 1 h at 37°C. The reaction was stopped with 2 mM phenylmethylsulfonyl fluoride. Protease-resistant, insoluble proteins were collected by centrifugation at 50,000 × g for 1 h at 4°C. Pellets were resuspended in lysis buffer (10 mM Tris-Cl [pH 8.0], 0.15 M NaCl, 0.5% NP-40 [wt/vol], 0.5% sodium deoxycholate [wt/vol]), then an equal volume of 2× sodium dodecyl sulfate sample buffer (30) was added, and the mixture was boiled for 3 min prior to electrophoresis. Sodium dodecyl sulfate gel electrophoresis and Western blotting were performed as previously described (57, 58). PrP was detected by using the humanized clone P recombinant antibody (53) and developed by using the enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, N.J.) as previously described (58).

## RESULTS

**Susceptibility of Tg(BoPrP) mice to BSE prions.** In earlier studies, we presented evidence that transgene-directed expression of BoPrP in *Pmp*<sup>0/0</sup> mice conferred susceptibility to BSE prions (57, 58). Originally, we reported the development of two Tg lines, Tg(BoPrP)4125 and Tg(BoPrP)4092 (57, 58). Of the two, the Tg(BoPrP)4125 line, consistently yielded shorter incubation periods with BSE inocula, a result we attribute to the ~2-fold higher level of BoPrP expression, as expected from previous studies on the relationship between the level of PrP expression and the length of the incubation period (10, 47).

Tg(BoPrP)4125 mice were inoculated with multiple isolates containing BSE prions, derived from both infected cattle and Tg(BoPrP) mice (Fig. 2). In these mice, the average incubation period of BSE prions derived from infected cows (240 days) is not significantly different from that obtained with prions derived from BSE-infected Tg(BoPrP) mouse brain (Fig. 2A). Furthermore, when the number of surviving animals was plotted against the incubation period, the curves obtained were superimposable (Fig. 2A). The neuropathologic changes caused by bovine-derived and Tg(BoPrP) mouse-passaged BSE inocula were also indistinguishable (58). By all available criteria, BSE prions were unchanged by serial passage in Tg(BoPrP) mice. This result contrasts with corresponding studies with non-Tg mice (7, 31, 32), in which a change in incubation period from >300 to ~150 days was typically observed from the first to second passage. Recent evidence suggests that both the physical properties and pathogenicity of a prion strain may change dramatically when a change in incubation period is observed from first to second passage (40).

Breeding large numbers of Tg(BoPrP<sup>+0</sup>)4125 and Tg(BoPrP<sup>+0</sup>)4092 mice is hampered by the need to screen all progeny for the presence of the transgene because the mice are hemizygous for the BoPrP transgene. In an effort to establish a mouse model better suited for large-scale bioassays of BSE prions, we attempted to breed both of these lines to homozygosity for the BoPrP transgene array. We were not successful with the Tg(BoPrP<sup>+0</sup>)4125 line, presumably because of a deleterious insertion site, but were able to establish a line from Tg(BoPrP<sup>+0</sup>)4092 that is homozygous for the transgene (53). We have already reported that these new Tg(BoPrP<sup>+/+</sup>)4092 mice are up to 10× more sensitive to BSE prions than cattle when used for endpoint titration (53). When Tg(BoPrP<sup>+/+</sup>)4092 mice were challenged with BSE prions from infected cow brain and from infected Tg(BoPrP) mouse brain, we observed incubation periods of 244 and 247 days, respectively (Fig. 2B), similar to incubation periods for Tg(BoPrP<sup>+0</sup>)4125 mice (Fig. 2A).

## Susceptibility of Tg(BoPrP) mice to human CJD prions.

Expression of the BoPrP transgene conferred susceptibility to vCJD prions (58): incubation periods and characteristic neuropathologic features of Tg(BoPrP) mice infected with vCJD prions closely resembled those found in Tg(BoPrP) mice inoculated with BSE prions. Having performed additional studies resulting in a much larger sample size, it is now evident that the average incubation period (270 days) of Tg(BoPrP)4125 mice infected with vCJD prions (Fig. 2C) is ~30 days longer than the 240-day average obtained with the same mice infected with BSE prions from cow brain (Fig. 2A). Although the incubation period for BSE prions did not change upon serial transmission (Fig. 2A), a shortening of incubation period was observed when brains taken from vCJD-infected Tg(BoPrP) mice were retransmitted to Tg(BoPrP<sup>+0</sup>)4125 mice (Fig. 2C). In Tg(BoPrP<sup>+0</sup>)4125 mice, we found that the incubation period of Tg(BoPrP)-passaged vCJD prions (224 days) was slightly shorter than that obtained with either prions from a BSE-infected cow (240 days) or from BSE-infected Tg(BoPrP) mice (236 days). However, this shortening is probably not significant and may be a reflection of a much smaller sample size in this data set (Fig. 2A and C). When Tg(BoPrP<sup>+/+</sup>)4092 mice were challenged with prions from vCJD-infected human brain and vCJD-infected Tg(BoPrP) mouse brain, we observed incubation periods of 274 and 240 days, respectively (Fig. 2D), consistent with the results obtained with Tg(BoPrP<sup>+0</sup>)4125 mice (Fig. 2C). Whether these changes result from a transmission barrier or a difference in the relative titer of prions in the inoculum is currently unknown.

The finding that Tg(BoPrP) mice appear relatively susceptible to human vCJD prions prompted us to test whether they were equally susceptible to other known human prion isolates. Neither Tg(BoPrP<sup>+0</sup>)4125 nor Tg(BoPrP<sup>+/+</sup>)4092 mice displayed any significant sensitivity to multiple isolates of sporadic CJD (21, 62), familial CJD bearing the E200K mutation (13, 22, 27, 59), or iatrogenic CJD (8) (Table 1). These data clearly demonstrate that susceptibility of Tg(BoPrP) mice to human prions is highly strain dependent. A slight difference in incubation periods was observed when Tg(BoPrP)4125 mice were inoculated with vCJD prions (Tables 1 and 2). The difference was slightly greater than expected from the standard errors, but we do not know the cause for this observation. Whether the inocula differ slightly in titer because different portions of the same brain were used to prepare homogenates for inoculation, and this is reflected in different incubation times, remains to be established.

**Sensitivity of Tg(BoPrP) mice to challenge with sheep scrapie prions.** In an earlier report, we found that the BoPrP transgene conferred susceptibility to 5 sheep scrapie isolates tested, which were all of the ARQ genotype (58). Compiling all of the available data for transmission of sheep scrapie prions in Tg(BoPrP<sup>+0</sup>)4125 mice (Fig. 2E), we found that the average incubation period was 225 days, shorter than the duration for BSE prions (Fig. 2A and C), and 100% of inoculated animals developed disease. Although ~80% of the infected Tg(BoPrP<sup>+0</sup>)4125 mice exhibited symptoms of disease at ~200 days, a plot of the data displayed a notable shoulder at 250 to 300 days. Because most of the animals within this group were originally challenged with a single scrapie sample (data not shown), it is possible that these longer incubation periods

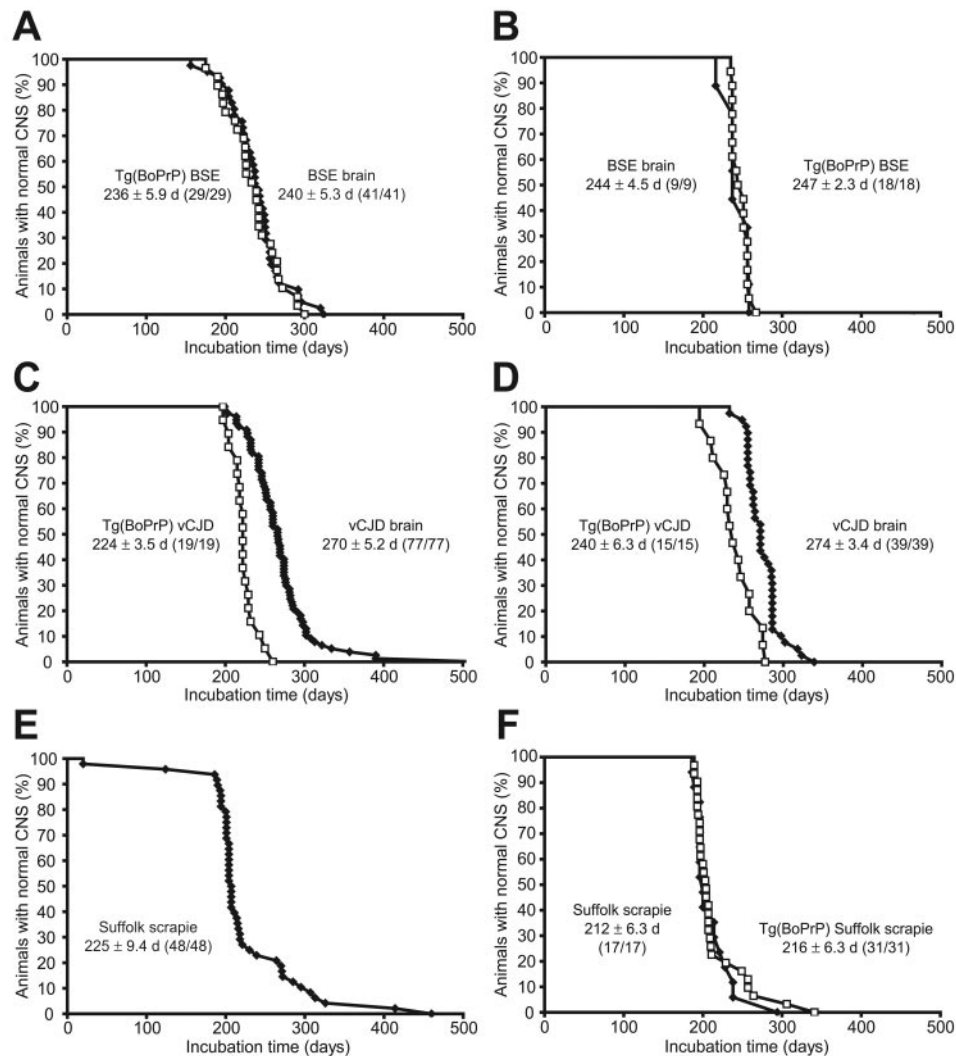


FIG. 2. Transmissions of native and Tg(BoPrP)-passaged BSE, vCJD, and scrapie prions to Tg(BoPrP)4125 (A, C, E) and Tg(BoPrP<sup>+/+</sup>)4092 (B, D, F) mice. After inoculation with 1% homogenates of brain tissue, Tg mice were placed under observation for appearance of clinical signs of neurologic disease. The data represent a plot of the number of animals surviving without presenting signs of clinical disease versus time in days. The derivation of BSE, vCJD, and scrapie inocula used were described previously (57, 58). (A) Survival curves after inoculation with BSE prions. BSE brain (filled diamonds) indicates that brain tissue was derived from five independent cases of BSE: GJ248/85, SE1809/11, 97/1612, 97/1997, and PG31/90. Tg(BoPrP) BSE (open squares) indicates that brain tissue was derived from four separate Tg(BoPrP) mice infected with BSE prions. (B) Survival curves after inoculation with BSE case PG31/90 (filled diamonds). Tg(BoPrP) BSE (open squares) indicates that brain tissue was derived from two separate Tg(BoPrP) mice infected with BSE prions. (C and D) Survival curves after inoculation with vCJD prions. vCJD brain (filled diamonds) indicates that brain tissue was derived from five independent cases of vCJD: RU96/02, RU96/07, RU96/70, RU96/80, and RU96/110. Tg(BoPrP) vCJD (open squares) indicates that brain tissue was derived from three separate Tg(BoPrP) mice infected with vCJD prions. (E) Survival curve after inoculation with scrapie prions. Suffolk scrapie (filled diamonds) indicates that brain tissue was derived from five independent cases of scrapie: Oreo, T97-1104, no. 15, no. 139, and no. 340. (F) Survival curves after inoculation with scrapie prions. Suffolk scrapie (filled diamonds) indicates that brain tissue was derived from two independent cases of scrapie: no. 139 and no. 340. Tg(BoPrP) Suffolk scrapie (open squares) indicates that brain tissue was derived from four separate Tg(BoPrP) mice infected with sheep scrapie prions. For each panel, the mean incubation periods  $\pm$  standard errors of the means are indicated; the values in parentheses signify the number of ill mice over the number of inoculated mice.

are indicative of a different scrapie strain. We do not have serial passage data available for the Tg(BoPrP<sup>+/+</sup>)4125 line.

We challenged Tg(BoPrP<sup>+/+</sup>)4092 mice with multiple inocula, including both sheep scrapie prions and Tg(BoPrP) mouse-passaged scrapie prions (Fig. 2F). Tg(BoPrP<sup>+/+</sup>)4092 mice exhibited an abbreviated incubation time (212 days) following inoculation with scrapie prions isolated from Suffolk sheep (Fig. 2F). It is notable that the incubation period of

sheep scrapie is 20 to 30 days shorter than is routinely observed with BSE inocula in both Tg(BoPrP) lines (Fig. 2A, B, D, and E). Significantly, the incubation periods obtained with Tg(BoPrP<sup>+/+</sup>)4092 mice inoculated with Tg(BoPrP)-passaged scrapie prions were essentially identical (216 days) to those observed after inoculation with native scrapie prions (Fig. 2F). We conclude that Tg(BoPrP) mice present no detectable transmission barrier to at least some strains of sheep scrapie prions;

TABLE 1. Comparison of human prion inocula in Tg(BoPrP) mice

Inoculum <sup>b</sup>	Recipient	Mean incubation period ± SEM (days)	n/n <sub>0</sub> <sup>a</sup>
vCJD	Tg(BoPrP <sup>+/+</sup> )4092	274 ± 3.4	39/39
	Tg(BoPrP <sup>+/0</sup> )4125	270 ± 5.2	77/77
sCJD	Tg(BoPrP <sup>+/+</sup> )4092	>427	0/29
	Tg(BoPrP <sup>+/0</sup> )4125	>500	2/27*
fCJD	Tg(BoPrP <sup>+/+</sup> )4092	>447	1/18**
	Tg(BoPrP <sup>+/0</sup> )4125	>483	3/16***
iCJD	Tg(BoPrP <sup>+/+</sup> )4092	>434	0/10

<sup>a</sup> n, number of ill animals; n<sub>0</sub>, number of inoculated animals. \*, two animals diagnosed with prion disease at 472 and 487 days; \*\*, one animal diagnosed with prion disease at 391 days, \*\*\*, three animals diagnosed with prion disease at 190, 354, and 404 days.

<sup>b</sup> sCJD, sporadic CJD; fCJD, familial CJD; iCJD, iatrogenic CJD.

moreover, Tg(BoPrP) mice are slightly more susceptible to scrapie prions than to BSE prions.

**Molecular properties of Tg(BoPrP)-passaged scrapie prions and BSE prions.** To distinguish among different prion strains, we developed a method to characterize prion strains biochemically by assessing the relative conformational stability of PrP<sup>Sc</sup> (39, 40). To compare the relative conformational stabilities of Tg(BoPrP)-passaged BSE and scrapie prions, we employed a variation of this technique. Samples of brain homogenates obtained from BSE- or scrapie-infected Tg(BoPrP) mice were exposed to increasing concentrations of GdnHCl, digested with protease to hydrolyze denatured PrP, and subjected to electrophoresis and Western blotting to detect protease-resistant fragments (Fig. 3A). When PrP band intensities were assessed

by densitometric analysis, we were able to determine an approximate [GdnHCl]<sub>1/2</sub> value, the concentration at which half of PrP<sup>Sc</sup> in the sample is denatured (39, 40) for each of the Tg(BoPrP) mouse-passaged isolates (Fig. 3B). Tg(BoPrP) mouse-passaged BSE prions displayed a higher relative conformational stability (mean [GdnHCl]<sub>1/2</sub> value of ~2.8 M) than Tg(BoPrP) mouse-passaged scrapie prions ([GdnHCl]<sub>1/2</sub> value of ~2.3 M) (Fig. 3A and B).

These initial data allowed us to develop a simple procedure for biochemical strain typing based on the resistance of PrP<sup>Sc</sup> to denaturation after exposure to specific concentrations of GdnHCl. For discriminating BSE and scrapie prions, we opted to compare the relative resistance to denaturation with 0, 2.8, and 3.0 M GdnHCl (Fig. 3C to F). As expected from our transmission studies, the relative susceptibilities of BSE prions derived from bovine brain (Fig. 3C) and Tg(BoPrP) mouse brain (Fig. 3E) appeared similar, with a nearly identical proportion of PrP<sup>Sc</sup> surviving denaturation with 3.0 M GdnHCl. With 2.8 M GdnHCl, PrP<sup>Sc</sup> derived from bovine brain appeared to be slightly more resistant to denaturation than PrP<sup>Sc</sup> derived from mouse brain (Fig. 3C and E). Consistent with the data shown in Fig. 3A, we found that scrapie prions passaged in Tg(BoPrP) mice were much more susceptible to GdnHCl denaturation than BSE prions from either cattle or infected Tg(BoPrP) mice. Only a small PrP<sup>Sc</sup> fraction survived exposure to 2.8 M GdnHCl, and almost all PrP<sup>Sc</sup> was denatured after treatment with 3.0 M GdnHCl. However, we were very surprised to find that scrapie prions from ovine brain appeared highly resistant to denaturation with 3.0 M GdnHCl (Fig. 3D).

TABLE 2. Comparison of bovine, human, and ovine inocula in Tg mice expressing native and chimeric BoPrP genes

Transgenic line	PrP expression (fold)	Inoculum	Mean incubation period ± SEM (days)	n/n <sub>0</sub> <sup>a</sup>
BoPrP 4125	8–16	BSE	240 ± 5.3	41/41
		vCJD	270 ± 5.2	77/77
		Scrapie	225 ± 9.4	48/48
Bo3M(HVEII)7177	16–32	BSE	254 ± 7.5	10/10
		vCJD	304 ± 13.4	7/7
		Scrapie	317 ± 10.4	9/9
Bo3M(HVEII)7187	8–16	BSE	235 ± 5.0	9/9
		vCJD	273 ± 25.6	8/8
		Scrapie	325 ± 19.0	7/7
Bo3M(HVEIV)14926	8	BSE	>580	0/9
		vCJD	>580	0/9
		Scrapie	>648	1/7*
Bo3M(YIQII)23939	8	BSE	248 ± 7.8	6/6
		vCJD	277 ± 7.4	9/9
		Scrapie	449 ± 8.0	7/7
Bo3M(YIQII)23953	8	BSE	232 ± 3.6	17/17
		vCJD	255 ± 6.9	10/10
		Scrapie	347 ± 8.2	19/19
Bo3M(YIQVV)12138	8	BSE	260 ± 13.5	10/10
		vCJD	226 ± 8.0	8/8
		Scrapie	>502	1/9**
Bo3M(YIQIV)10667	8–16	BSE	351 ± 34.1	5/5
		vCJD	319 ± 16.2	6/6
		Scrapie	514 ± 71.5	6/6
Bo3M(HIQVV)29285	8	BSE	432 ± 12.0	6/6
		vCJD	437 ± 9.8	9/9
		Scrapie	>655	1/6***

<sup>a</sup> n, number of ill animals; n<sub>0</sub>, number of inoculated animals. \*, one animal diagnosed with prion disease at 600 days; \*\*, one animal diagnosed with prion disease at 483 days; \*\*\*, one animal diagnosed with prion disease at 603 days.

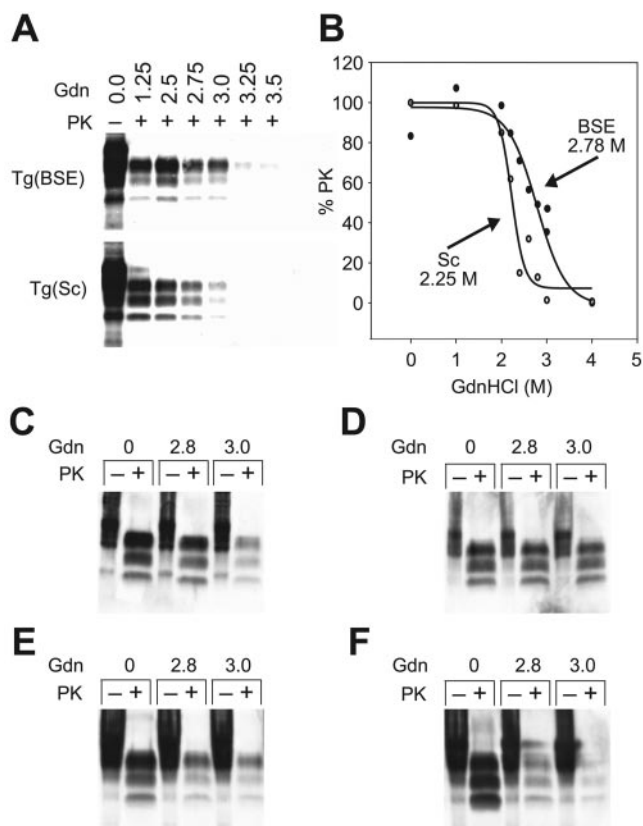


FIG. 3. Conformational stabilities of native and Tg(BoPrP)-passaged BSE and scrapie prions. Sample processing, electrophoresis, and blotting were performed as described in Materials and Methods. Samples were denatured with different concentrations of GdnHCl and then either treated with proteinase K (+) or left untreated (-), as indicated. (A) Tg(BSE) indicates brain tissue derived from two Tg(BoPrP) mice infected with BSE prions, diluted five times with brain tissue from uninfected Tg(BoPrP) mice. Tg(Sc) indicates brain tissue derived from two Tg(BoPrP) mice infected with scrapie prions. The Tg(BSE) brain tissue was diluted with uninfected Tg(BoPrP) brain homogenate because scrapie-infected Tg(BoPrP) mice contain ~5-times-lower amounts of PrP<sup>Sc</sup> than BSE-infected Tg(BoPrP) mice (M. Scott and H.-O. Nguyen, unpublished data). (B) Densitometric analysis of the Western blots from panel A showing the percentage of protease-resistant protein plotted as a function of GdnHCl concentration. (C) Brain homogenate of a cow (case PG31/90) diagnosed with BSE. (D) Brain homogenate of a sheep (case no. 27) diagnosed with scrapie. (E) Brain homogenate derived from two Tg(BoPrP) mice infected with BSE prions and diluted five times with brain tissue from uninfected Tg(BoPrP) mice. (F) Brain homogenate derived from two Tg(BoPrP) mice infected with scrapie prions.

Although the sheep scrapie samples used for conformational stability studies and the inocula for Tg(BoPrP) mice were all from U.S. Suffolk sheep, insufficient amounts of sheep brain prevented us from performing conformational stability studies on matched pairs. Thus, this condition must temper our conclusions.

**Chimeric PrP transgenes.** We were surprised to find that Tg(BoPrP) mice are highly susceptible to sheep scrapie prions in the brains of asymptomatic U.S. Suffolk sheep and that these mice posed no transmission barrier to sheep prions. Because of heightened concerns that BSE prions may have been passed to the sheep population via contaminated feed, we sought to

devise a transgenic mouse that could be used to discriminate BSE prions in sheep from scrapie prions in sheep, even if the BSE strain was present in a mixture of one or more scrapie prion strains. A chimeric PrP transgene, termed MH2M, comprised of sequences derived from Syrian hamster PrP (SHaPrP) fused to N- and C-terminal sequences from mouse (Mo) PrP (56), was shown to exhibit a transmission barrier when challenged with two different SHa prion strains (40). Tg (MH2M) mice showed no transmission barrier when challenged with the SHa drowsy strain, but transmission of the SHa Sc237 strain was prolonged on the first passage compared to subsequent transmission (40). These results suggested that it may be possible to construct an analogous chimeric mouse-BoPrP transgene with the ability to confer a high degree of susceptibility to BSE prions while retaining relative resistance to infection with most strains of sheep scrapie. However, we had previously created Tg mice expressing a chimeric construct analogous to MH2M, designated MBo2M and differing only in that it contained BoPrP instead of SHaPrP sequences (57). Tg (MBo2M) mice were resistant to infection with BSE prions, leading us to conclude that a C-terminal epitope controls susceptibility to BSE (57). We therefore elected to create a series of new mouse-BoPrP chimeric transgenes, with the goal of furthering our understanding of the relationship between PrP sequence and susceptibility.

In creating a series of transgenes, we used variations of the Bo3M construct (Fig. 1), which is comprised of the N-terminal region of BoPrP fused to the C terminus of MoPrP. The C-terminal MoPrP subregion is identical to that in MBo2M. We next identified five residues in Bo3M PrP that lie within the three-helical C-terminal folded domain of PrP<sup>C</sup> and that differ between BoPrP and MoPrP (Fig. 1). The residues are at positions 166, 195, 197, 214, and 226 and correspond to residues 154, 183, 185, 202, and 214, respectively, in MoPrP. We then substituted each of these residues in Bo3M with its corresponding residue in either BoPrP or MoPrP and named the resulting transgenes by the amino acid residues present at these five locations (Fig. 1). Tg mouse lines expressing each of the chimeric PrPs were established in a *Pmp*<sup>0/0</sup> background, expanded, and then inoculated with BSE, scrapie, or vCJD prions (Table 2). Wherever possible, we selected lines that showed similar levels of expression of chimeric PrP<sup>C</sup>. When tested with BoPrP-specific antisera, most were found to express 8 to 16 times the amount of BoPrP<sup>C</sup> found in brains of control cattle. Whereas most lines fell into this category, the Tg (Bo3M,HVEII)7177 line had a higher level of expression (16 to 32 times).

We were surprised to find that relatively small changes in the sequence could cause dramatic differences in relative susceptibility to the three different inocula used. The Bo3M(HVEII) chimera represents BoPrP up to and including residue 226 fused to the C terminus of MoPrP. It therefore comprises the N terminus, helices A and B from BoPrP, and a hybrid helix C comprising BoPrP sequence but with Arg substituted for Lys at residue 231, as found in MoPrP. All remaining C-terminal sequences including the GPI anchor signal sequence are derived from MoPrP. The Tg(Bo3M,HVEII)7187 line showed incubation periods similar to those of Tg(BoPrP)4125 mice when inoculated with BSE prions (235 days) and vCJD prions (273 days), reproducing the characteristic 35- to 40-day differ-

ence in incubation period between these two inocula observed in Tg(BoPrP) mice (Table 2). However, we found that Tg(Bo3M,HVEII) mice exhibited a longer incubation period (317 days) than Tg(BoPrP)4125 mice (~225 days) after inoculation with sheep scrapie prions (Table 2). Another line, Tg(Bo3M,HVEII)7177, harboring this construct but with a slightly higher level of chimeric PrP expression, gave similar results but showed longer incubation periods with BSE and vCJD prions and a slightly shorter incubation period with scrapie prions (Table 2). It is interesting that despite the high level of expression, this line showed slightly longer incubation periods for 2 of the 3 inocula than the Bo3M(HVEII)7187 line, which had the same transgene but expressed approximately half the amount of PrP<sup>C</sup> (Table 2). These data show that transmissions between hosts encoding PrP<sup>C</sup> of different sequences do not necessarily exhibit an inverse relationship between PrP<sup>C</sup> expression and incubation period with all inocula.

We obtained dramatically different results with the Bo3M(HVEIV) chimera, a construct that is identical to Bo3M(HVEII), except Ile was changed to Val at residue 226, as found in MoPrP. Of the three residues within helix C of Bo3M(HVEII) that differ between BoPrP and MoPrP, two are identical to MoPrP. The third residue, position 214 at the beginning of helix C, is Ile, as found in BoPrP; the corresponding residue in MoPrP is Val at position 202. Tg(Bo3M,HVEIV)14926 mice were resistant to all three inocula, with no animals showing clinical signs at >550 days postinfection. Susceptibility to prion infection was partially restored by replacing an internal region of BoPrP corresponding to the region between helices A and B with corresponding sequences from MoPrP, resulting in the Bo3M(YIQIV) construct. The Tg(Bo3M,YIQIV)10677 line was susceptible to all three inocula but exhibited prolonged incubation periods (Table 2). An additional substitution of Val for Ile at residue 214 (in helix C) produced the Bo3M(YIQVV) chimera (Fig. 1), which harbors the N terminus of BoPrP, including helix A, fused to the two C-terminal helices of MoPrP. The Tg(Bo3M,YIQVV)12138 line produced yet another susceptibility profile, showing shorter incubation periods with vCJD prions than BSE prions but displaying complete resistance to scrapie prions.

**Bo3M(YIQII) chimeric transgenes.** The foregoing results demonstrate how minute differences in the sequence of PrP can dramatically alter susceptibility to foreign prions. We became particularly interested in results we obtained with the Bo3M(YIQII) chimera (Table 2). The Tg(Bo3M,YIQII)23953 line exhibited a short (232 days) incubation period after inoculation with BSE prions comparable to our Tg(BoPrP) and Tg(Bo3M,HVEII) mice (Table 2). The Tg(Bo3M,YIQII)23953 line also gave a slightly shorter incubation period (~255 days) after inoculation with vCJD prions. However, it appeared relatively resistant to scrapie prions, with an incubation period of ~350 days. Such a susceptibility profile may be useful in distinguishing scrapie prions from BSE prions in affected sheep.

To characterize these Tg mice further, we performed a serial transmission study (Fig. 4). We found that BSE prions from infected Tg(Bo3M,YIQII) mice gave even shorter incubation periods (170 days) than BSE prions derived from cattle (232 days), whereas only a slight shift in incubation period (from 347 to 313 days) was observed between scrapie prions from sheep and from Tg(Bo3M,YIQII) mice, respectively. We in-

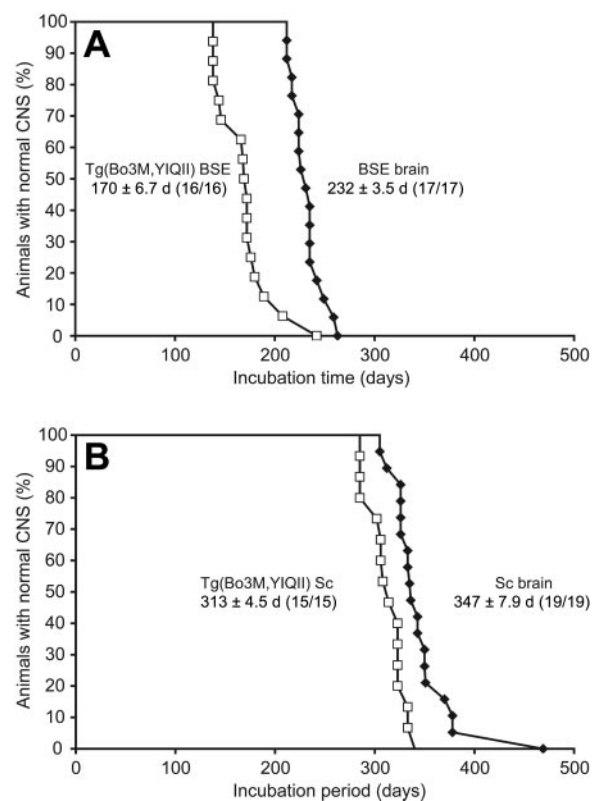


FIG. 4. Survival curves from serial transmissions of BSE prions (A) and scrapie prions (B) to Tg(Bo3M,YIQII)23953 mice. Tg mice were inoculated with 1% brain homogenates. (A) BSE brain (filled diamonds) indicates inocula derived from BSE case PG31/90. Tg(Bo3M,YIQII) BSE (open squares) indicates inocula derived from two Tg(Bo3M,YIQII) mice infected with BSE prions. (B) Sc brain (filled diamonds) indicates inocula derived from two cases of scrapie: no. 139 and no. 27. Case no. 27 was described previously (64). Tg(Bo3M,YIQII) Sc (open squares) indicates inocula derived from two Tg(Bo3M,YIQII) mice infected with scrapie prions. Mean incubation periods  $\pm$  standard errors of the means are indicated for each inoculum; the values in parentheses signify the number of ill mice over the number of inoculated mice.

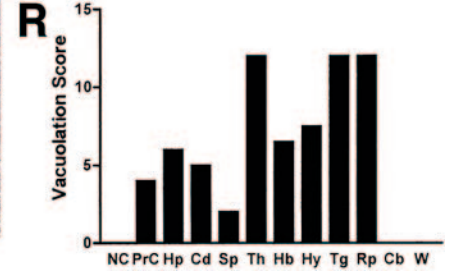
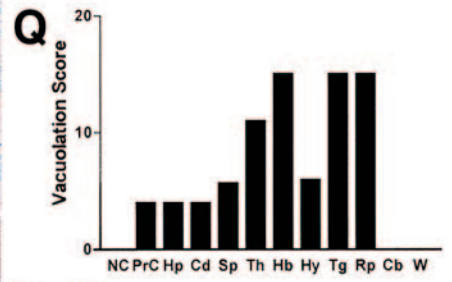
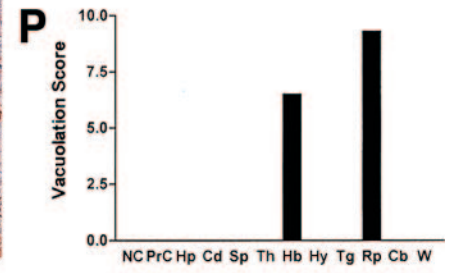
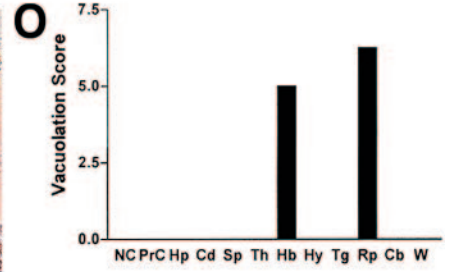
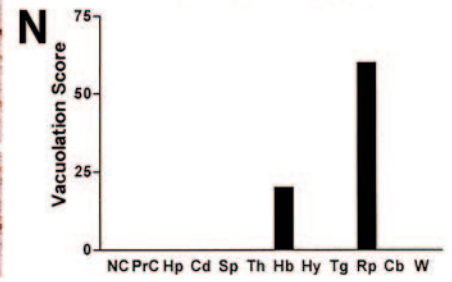
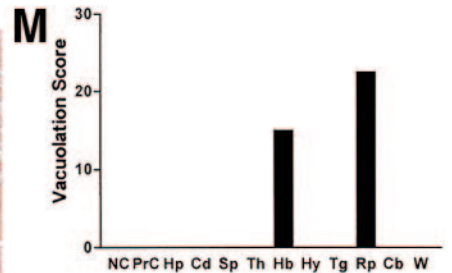
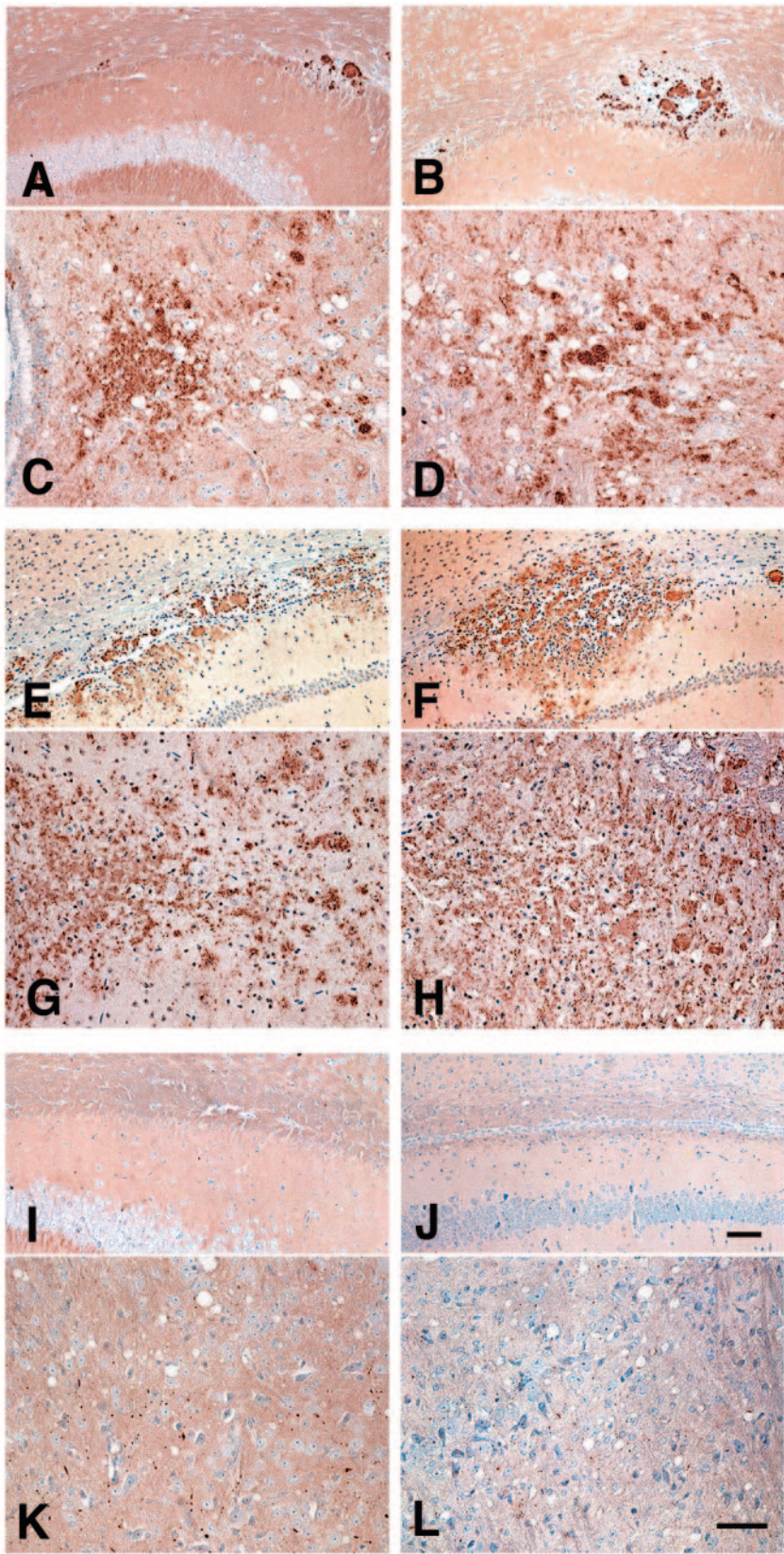
terpret this to mean that there is a smaller strain barrier for transmission of scrapie prions to these mice compared to BSE prions.

#### Neuropathology in Tg(BoPrP) and Tg(Bo3M,YIQII) mice.

Based on the foregoing data, we performed a detailed comparison of the neurohistopathologic changes caused by BSE, vCJD, and scrapie prions during serial passaging in Tg(BoPrP<sup>+/+</sup>)4092 (Fig. 5) and Tg(Bo3M,YIQII)23953 mice (Fig. 6). Four relevant findings were made: (i) the neuropathologic changes caused by bovine-derived BSE and human-derived vCJD prions were similar in both Tg mouse lines and were significantly different than those caused by sheep-derived scrapie prions; (ii) these similarities and differences were maintained during the second passage; (iii) large aggregates of PrP amyloid plaques were induced by BSE and vCJD prions but not by scrapie prions; (iv) PrP amyloid plaques were exceptionally large in Tg(Bo3M,YIQII)23953 mice infected with BSE and vCJD prions.

Scrapie, BSE, and vCJD prions could be distinguished by the





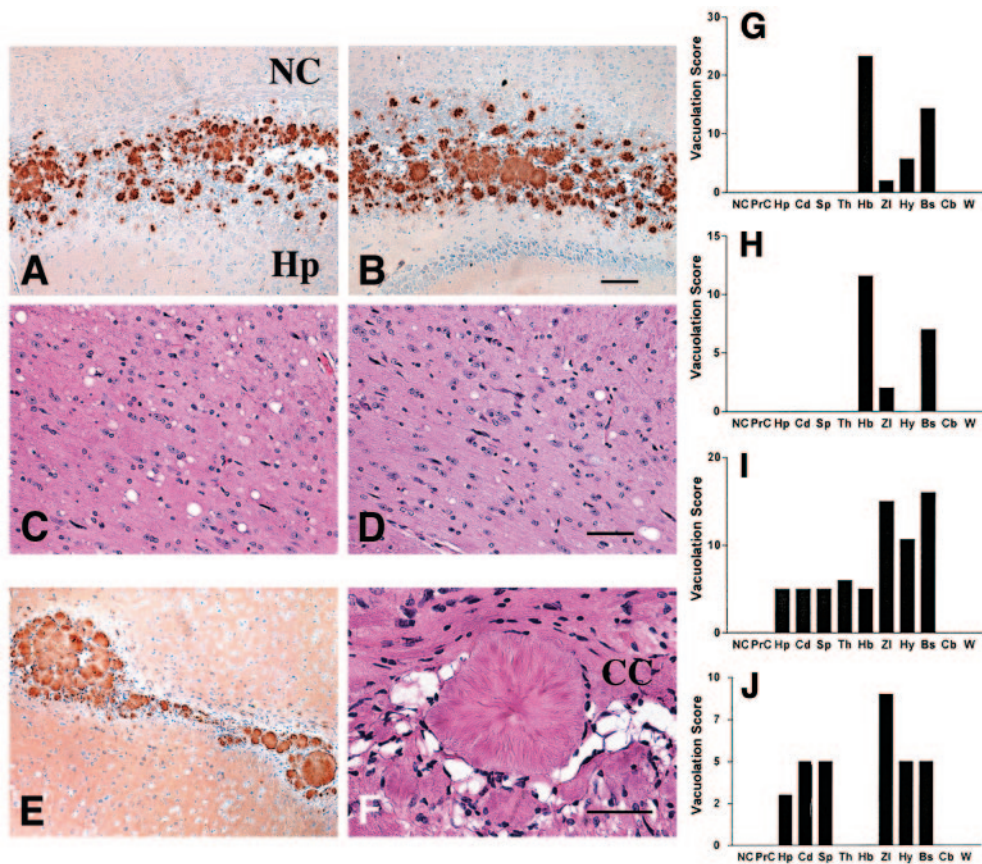


FIG. 6. Neuropathology and vacuolation in Tg(Bo3M,YIQII)23953 mice after passage of BSE, vCJD, and scrapie prions. (A to F) Neuropathology as seen by immunohistochemistry for PrP<sup>Sc</sup> in Tg(Bo3M,YIQII)23953 mice after inoculation with BSE (A, B), scrapie (C, D), and vCJD prions (E, F) on first (left column) and second (right column) passage. (A, B, E) Hippocampal sections show massive aggregates of amyloid plaques in the subcallosal region after inoculation with BSE (A, B) and vCJD (E) prions. The BoPrP-specific 9095 antibody with a hematoxylin counterstain was used (58). The bar in panel B represents 100  $\mu$ m and also applies to panels A and E. Hp, hippocampus; NC, neocortex. (C, D) Sections of the zona incerta stained with hematoxylin and eosin shows mild-to-moderate vacuolation in multiple brain regions after inoculation with scrapie prions. No amyloid plaques were found. The bar in panel D represents 70  $\mu$ m and also applies to panel C. (F) Higher magnification of the large amyloid plaque at the far right edge of panel E. This plaque is  $\sim$ 100  $\mu$ m in diameter, has a radially arranged fibrous substructure like a kuru plaque, and is partially surrounded by vacuoles. Bar, 50  $\mu$ m. CC, corpus callosum. (G to J) Histograms comparing the intensity and neuroanatomic distribution of vacuolation in the neuropil after inoculation with BSE (G, H) and scrapie (I, J) prions on first passage (G, I) and second passage (H, J). (G, H) Primary and secondary transmissions of BSE prions reveal virtually identical distributions of vacuolation in the brain. Little or no vacuolation was seen in the zona incerta and hypothalamus. (I, J) With scrapie prions, vacuolation was more widely spread. Upon serial transmission, vacuolation was absent in the thalamus proper and the habenula. Abbreviations are defined in the legend to Fig. 5.

FIG. 5. Neuropathology and vacuolation in Tg(BoPrP)4092 mice after inoculation with BSE, vCJD, and scrapie prions. (A to L) Neuropathology as seen by immunohistochemistry for PrP<sup>Sc</sup> in Tg(BoPrP)4092 mice inoculated with BSE (A to D), vCJD (E to H), and scrapie (I to L) prions on first passage (left column) and on second passage (right column). (A, B, E, F, I, and J) Sections of the subcallosal region of the hippocampus show small aggregates of PrP amyloid plaques from BSE prions (A, B), large, discontinuous aggregates of PrP amyloid plaques from vCJD prions (E, F), and no amyloid plaques from scrapie prions (I, J). The bar in panel J represents 100  $\mu$ m and applies to panels A, B, E, F, and I. (C, D, G, H, K, and L) Coronal sections of the raphe of the rostral pons show distribution of PrP<sup>Sc</sup> deposits and vacuolation. Note that all sections of the raphe are shown lying horizontally, with the dorsal raphe to the left and the more ventral raphe to the right. Numerous PrP<sup>Sc</sup> deposits surround vacuolation of the neuropil from BSE (C, D) and vCJD (G, H) prions. Only minute, punctate PrP<sup>Sc</sup> deposits are scattered among the small number of vacuoles from scrapie prions (K, L). The bar in panel L represents 50  $\mu$ m and applies to panels C, D, G, H, and K. (M to R) Histograms comparing the intensity and neuroanatomic distribution of vacuolation in the neuropil after inoculation with BSE (M, N), vCJD (O, P), and scrapie (Q, R) prions on first passage (M, O, Q) and second passage (N, P, R). Vacuolation is confined largely to the habenula and the raphe of the rostral pons in both the first and second passage of BSE (M, N) and vCJD (O, P) prions. Note the degree of vacuolation in the raphe is twice as intense during the second passage. Inoculation with scrapie prions (Q, R) resulted in widespread, mild-to-moderate vacuolation of multiple brain regions without amyloid plaque formation. NC, neocortex; PrC, pyriform cortex; Hp, hippocampus; Cd, head of caudate nucleus; Sp, septal nuclei; Th, thalamus proper; Hb, habenula; Hy, hypothalamus; Tg, overall tegmentum of the brainstem including the raphe; Rp, raphe of the brainstem; Cb, cerebellum; W, white matter.

distribution of vacuolation in the gray matter neuropil. Scrapie was characterized by mild to moderate degrees of vacuolation in multiple cerebral hemisphere and brainstem regions, whereas vacuolation in BSE and vCJD prions was confined largely to the raphe nuclei of the brainstem and to the habenula (Fig. 5 M to R and 6B). In Tg(BoPrP<sup>+/+</sup>)4092 mice, vacuolation of the brainstem caused by BSE and vCJD prions was largely confined to the raphe nuclei in the midline of the tegmentum (Fig. 5C and D). In contrast, scrapie-associated vacuolation was found throughout the brainstem tegmentum. In Tg(Bo3M, YIQII)23953 mice, BSE prions caused vacuolation throughout the brainstem tegmentum, similar to scrapie prions. Because we have reviewed only one vCJD primary transmission (Fig. 6E and F), we remain tentative about the pattern of vacuolation in Tg(Bo3M, YIQII)23953 mice.

Cattle-derived BSE and human-derived vCJD prions induced deposition of large amounts of PrP amyloid beneath the corpus callosum and ependyma of the rostral half of the cerebral hemispheres in both Tg(BoPrP<sup>+/+</sup>)4092 and Tg(Bo3M, YIQII)23953 mice (Fig. 5A and E; Fig. 6A and E). Mature amyloid was not found in other brain regions. Upon second passage, similar-sized aggregates of PrP amyloid developed in the same rostral cerebral hemisphere locations (Fig. 5B and F; Fig. 6B). The individual plaques forming these aggregates bound Congo red dye, which showed green-gold birefringence in polarized light, verifying that they are amyloid (data not shown). The plaques varied in size from 10 to 100  $\mu$ m in diameter (Fig. 6F). The amyloid aggregates were associated with vacuoles and therefore resemble the florid plaques that are characteristic of vCJD in humans (66) (Fig. 6F). The subcallosal amyloid aggregates in Tg(BoPrP<sup>+/+</sup>)4092 and Tg(Bo3M, YIQII)23953 mice differed in size. Aggregates were relatively small in the former group, whereas they were massive in the latter group, in which they extended continuously as much as 2 mm in a single coronal section. In contrast to BSE and vCJD prions, no amyloid plaques were formed by scrapie prions upon the first or second passage (Fig. 5I and J).

In addition to mature amyloid plaques, primitive plaque-like deposits of PrP<sup>Sc</sup> arranged singly or in aggregates were also formed by BSE and vCJD prions in both the primary and secondary passages in both Tg mouse lines (Fig. 5C, D, G, and H). These deposits were associated with vacuoles in the gray matter neuropil. In contrast, scrapie prions were associated with small, punctate deposits of PrP<sup>Sc</sup> in association with neuropil vacuolation (Fig. 5K and L).

## DISCUSSION

Studies of prion transmission barriers are important in elucidating the fundamental mechanisms of prion replication and critical with respect to the safety of the human food supply. In extreme cases, the host may be incapable of supporting propagation of prions from another species; rabbits appear to be resistant to all prion inocula derived from other species (2, 20) and may present such a bona fide species barrier. More typically, however, a relative difference in susceptibility is manifest by a shortening of the incubation period observed when the first passage is compared to the second and subsequent passages. In recent years, several groups (23, 25, 60) have recognized the need to adopt the more generic term transmission

barrier to refer to this difference in susceptibility as defined by a difference in incubation period following transmission to a different species or strain of transgenic animal.

As we have shown, in some cases, a transmission barrier may be evident with certain strains but not others, so we introduce the term strain transmission barrier, or simply strain barrier, to denote this. We propose that the majority of the so-called species barriers observed upon interspecies prion transmissions are actually strain barriers. This notion of strain barriers has significant implications: one cannot assume that because one or more strains derived from a given species, such as sheep, do not appear to be pathogenic in another species, such as humans, that this association will extend to all strains.

**Properties of BSE, vCJD, and scrapie prions in Tg(BoPrP) mice.** The vCJD strain appears unique among human prion strains, since all others tested were not transmissible to Tg(BoPrP) mice (Table 1). These data are consistent with the proposition that prion strains represent different conformers of PrP<sup>Sc</sup> (5, 40, 61). As vCJD appears to be BSE that has passed from cattle to humans (7, 15, 24, 58, 65, 66), we infer that the vCJD prions exist in a PrP<sup>Sc</sup> conformation that is compatible with the sequence of both BoPrP and human (Hu) PrP and that other human prion strains are conformers that are incompatible with the sequence of BoPrP, presumably due to steric hindrances. In contrast to vCJD prions, scrapie prions of Suffolk sheep transmitted to Tg(BoPrP) mice with no evidence of a transmission barrier and consistently produced shorter incubation periods than BSE prions in these mice (Fig. 2D and E). Notably, scrapie prions have also been experimentally transmitted to cattle (16, 50). Together, these data suggest that cattle possess some potential to propagate scrapie prions efficiently, although other factors may prevent pathogenesis by natural modes of transmission.

When the conformational stabilities of BSE and scrapie prions passaged in Tg(BoPrP) mice were compared, BSE prions were much more resistant to GdnHCl denaturation (Fig. 3), even though the original scrapie prions from sheep showed greater relative conformational stability than BSE prions from cattle (Fig. 3). Unfortunately, we were not able to acquire any samples of sheep-passaged BSE prions for comparison, but our studies suggest that measurement of conformational stability may prove useful as a diagnostic procedure in distinguishing BSE prions from scrapie prions in sheep (Fig. 3).

**Chimeric PrP transgenes.** Our data on the differential susceptibilities of Tg(BoPrP) mice to BSE, scrapie, and various human prion strains led us to consider whether it would be possible to create chimeric mouse-BoPrP transgenes specifically tailored to propagate particular strains. One goal was to create a chimeric transgene that, unlike native BoPrP, is able to confer a high degree of susceptibility to BSE prions while retaining relative resistance to infection with most strains of sheep scrapie. Accordingly, we created the series of chimeric PrPs (Fig. 1) and expressed them in Tg mice. We noted a wide variance in the relative ability of these mice to propagate BSE, vCJD, and scrapie prions (Table 2). On reviewing these results, we were unable to detect any clear relationship between particular segments of PrP and their ability to propagate BSE, vCJD, or scrapie prions. The Bo3M(HVEII) chimera is essentially a perfect replica of BoPrP, with the exception of the extreme C terminus following residue 226 (Fig. 1). Although

Tg(Bo3M,HVEII) mice displayed almost identical susceptibility to BSE and vCJD prions compared to Tg(BoPrP) mice (Table 1), they showed a much longer incubation period with scrapie prions (Table 2). The C-terminal region of Bo3M (HVEII) contains a substitution of Lys for Arg at residue 231 and the motif DRG at the extreme C terminus, preceding the GPI cleavage site. It is not known whether one or another of these sequence differences is responsible for the observed difference in susceptibility to scrapie prions.

In Bo3M(HVEIV), a single additional change was made compared to Bo3M(HVEII): a substitution of Val for Ile at residue 226. Unexpectedly, the Tg(Bo3M,HVEIV) mice were completely resistant to BSE, vCJD, and scrapie prions. This is perplexing because this single change was designed to correct a mismatch in Bo3M(HVEII) with the binding motif of protein X, proposed to be required for the propagation of prions in murine cells (28) and Tg mice (29, 42, 62). All other residues in this motif are conserved between BoPrP and MoPrP. Contrary to expectation, all of the chimeric PrPs featuring Val at residue 226 were relatively resistant to all prion inocula, except for the Bo3M(YIQVV) construct (Table 2), which features an intact C-terminal MoPrP subdomain and two unchanged disulfide-linked helices. Tg mice expressing Bo3M(YIQVV) show complete resistance to scrapie prions, moderately short incubation periods with BSE prions (260 days), and short incubation times with vCJD prions (226 days), which is the shortest duration for vCJD prions in any Tg mouse line tested to date. No efficient Tg model for vCJD has yet been developed with human or chimeric human PrP transgenes (14, 29). It will be interesting to test whether a construct analogous to Bo3M (YIQVV) but containing Hu rather than Bo sequences confers enhanced susceptibility to vCJD or other strains of Hu prions.

**Distinguishing BSE from scrapie prions in sheep.** Three ORFs that we have tested in Tg mice feature Ile at both residues 214 and 226: Bo, Bo3M(HVEII), and Bo3M(YIQII). Mice expressing any one of the three constructs showed short incubation periods (~230 days) when inoculated with BSE prions (Table 2). These data seem to indicate that preserving the sequence of helix B and at least part of helix C is desirable for the most efficient transmission of BSE prions. Mice expressing the Bo3M(YIQII) construct showed a substantial difference in susceptibility to BSE and scrapie prions, with incubation periods of 232 and 347 days, respectively, in the Tg (Bo3M,YIQII)23953 line, and 248 and 449 days, respectively, in Tg(Bo3M,YIQII)23939 mice (Table 2). Based on these differences, we selected the Tg(Bo3M,YIQII)23953 line for serial transmission studies and found that the incubation time for Tg (Bo3M,YIQII)-passaged BSE prions shortened from 232 to 170 days, whereas Tg(Bo3M,YIQII)-passaged scrapie prions showed a smaller decrease in the incubation period, from 347 to 313 days (Fig. 4). In light of concerns that BSE prions may have transmitted to sheep, Tg(Bo3M,YIQII) as well as the Tg (Bo3M,YIQVV) mice noted above may be of utility in screening sheep samples for the presence of BSE prions.

**PrP amyloid deposition and florid plaques.** BSE and vCJD prions caused similar neuropathologic phenotypes in Tg(BoPrP<sup>+/+</sup>)4092 and Tg(Bo3M,YIQII)23953 mice that were substantially different than those caused by scrapie prions (Fig. 5 and 6). These results agree with those we reported for transmission of BSE, vCJD, and scrapie prions in earlier studies with Tg

(BoPrP) mice (58); unlike most established scrapie prion strains (6, 18, 19), BSE and vCJD prions are highly amyloidogenic (58). With respect to amyloid plaque formation, our present understanding is that an amyloidogenic conformation of an infecting PrP<sup>Sc</sup> is imposed on the host's PrP<sup>C</sup>. Perhaps this amyloidogenic conformation favors natural truncation of PrP<sup>Sc</sup> or presents surfaces that favor polymerization of PrP<sup>Sc</sup> into amyloid filaments. Interestingly, the amyloid plaques formed in Tg(BoPrP<sup>+/+</sup>)4092 and Tg(Bo3M,YIQII)23953 mice resemble the florid plaques that are pathognomonic for vCJD in humans (66).

Earlier studies indicated that both the prion strain and the host genotype determine whether or not amyloid plaques form (6, 18, 19). Therefore, it is reasonable to hypothesize that the amino acid sequence of Bo3M(YIQII) expressed in Tg(Bo3M,YIQII)23953 mice enhances amyloid plaque formation, thereby causing the massive deposits of amyloid found from BSE and vCJD prions (Fig. 6A, B, E, and F).

Transgenes optimized for propagation of specific prion strains may provide faster and more efficient bioassays with animals, cultured cells, or cell-free systems. Unfortunately, it is not yet possible to predict whether a particular PrP sequence will confer susceptibility to any prion strain originating in a species with a different PrP sequence, although our work suggests that slight variations in or near all three helices of PrP can profoundly affect susceptibility in a strain-dependent manner. Our studies raise concerns that differences in the sequence of PrP may not be relied upon for protection from infection from prions of other species. Instead, susceptibility to prions appears to depend on both the sequence of PrP and the strain of prion, a conclusion that has serious implications for assessing the potential of prions to jump from one species, such as sheep or cattle, to another species, such as humans.

#### ACKNOWLEDGMENTS

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