Transmission of Creutzfeldt–Jakob disease from humans to transgenic mice expressing chimeric human–mouse prion protein

(species barrier/prion propagation/human growth hormone/scrapie/bovine spongiform encephalopathy)

GLENN C. TELLING*, MICHAEL SCOTT*, KAREN K. HSIAO*†, DALLAS FOSTER*, SHU-LIAN YANG‡, MARILYN TORCHIA*, KATIE C. L. SIDLE§, JOHN COLLINGE§, STEPHEN J. DEARMOND‡, AND STANLEY B. PRUSINER*¶

Departments of *Neurology, †Biochemistry and Biophysics, and ‡Pathology, University of California, San Francisco, CA 94143; and §Prion Disease Group, Department of Biochemistry and Molecular Genetics, St. Mary's Hospital Medical School, Imperial College, Norfolk Place, London W2 1PG, United Kingdom

Contributed by Stanley B. Prusiner, June 16, 1994

ABSTRACT Transgenic (Tg) mice were constructed that express a chimeric prion protein (PrP) in which a segment of mouse (Mo) PrP was replaced with the corresponding human (Hu) PrP sequence. The chimeric PrP, designated MHu2MPPrP, differs from MoPrP by 9 amino acids between residues 96 and 167. All of the Tg(MHu2M) mice developed neurologic disease ≈200 days after inoculation with brain homogenates from three patients dying of Creutzfeldt–Jakob disease (CJD). Inoculation of Tg(MHu2M) mice with CJD prions produced MHu2MPPrP^{Sc} (where PrP^{Sc} is the scrapie isoform of PrP); inoculation with Mo prions produced MoPrP^{Sc}. The patterns of MHu2MPPrP^{Sc} and MoPrP^{Sc} accumulation in the brains of Tg(MHu2M) mice were different. About 10% of Tg(HuPrP) mice expressing HuPrP and non-Tg mice developed neurologic disease >500 days after inoculation with CJD prions. The different susceptibilities of Tg(HuPrP) and Tg(MHu2M) mice to Hu prions indicate that additional species-specific factors are involved in prion replication. Diagnosis, prevention, and treatment of Hu prion diseases should be facilitated by Tg(MHu2M) mice.

The presentation of human (Hu) prion diseases as sporadic, genetic, and infectious illnesses initially posed a conundrum that has been explained by the cellular genetic origin of the prion protein (PrP) (1). Four prion diseases of humans have been identified: (i) kuru, (ii) Creutzfeldt–Jakob disease (CJD), (iii) Gerstmann–Sträussler–Scheinker disease (GSS), and (iv) fatal familial insomnia (2–5). Over the last 30 years, 300 cases of CJD, kuru, and GSS have been transmitted to a variety of apes and monkeys (6). The scarcity of these primates, the expense of their long-term care, and the increasing ethical objections to such experiments have limited investigations.

Because of the “species barrier” (7), the initial passage of prions from humans to rodents requires prolonged incubation times with relatively few animals developing illness (8–13). Subsequent passage in the same species occurs with high frequency and shortened incubation times. The molecular basis for the species barrier between Syrian hamster (SHa) and mouse (Mo) was found to reside in the sequence of the PrP gene using transgenic (Tg) mice (14); SHaPrP differs from MoPrP at 16 of 254 amino acid residues (15, 16).

Since mice expressing HuPrP transgenes have not given abbreviated incubation times similar to Tg(SHaPrP) mice when inoculated with homologous prions, we constructed a chimeric Hu/Mo transgene similar to the chimeric SHa/Mo transgene labeled MH2M, which carries 5 amino acid substitutions found in SHaPrP lying between codons 94 and 188

(17). Hu PrP differs from Mo PrP at 28 of 254 positions (18) while chimeric Hu/Mo PrP, designated MHu2M, differs at 9 residues. We report that mice expressing the MHu2M transgene are susceptible to Hu prions and exhibit abbreviated incubation times.

METHODS

Tg mice were produced as described (14, 19). The HuPrP and MHu2MPPrP open reading frame (ORF) expression cassettes are flanked by *Sal* I and *Xho* I sites, which allowed cleavage immediately adjacent to the initiation and termination codons of the PrP ORF, respectively, followed by subcloning into the cosSHa.Tet expression vector (19). The wild-type HuPrP allele encoding valine at codon 129 was used for the production of the Tg(HuPrP) mice, whereas the MHu2MPPrP transgene encodes methionine. Genomic DNA, isolated from tail tissue of weanling animals, was screened for the presence of incorporated MHu2M transgene using probes that hybridize to the 3' untranslated region of the SHaPrP gene contained in the cosSHa.Tet vector (19); in the case of Tg(HuPrP) mice, the HuPrP ORF was used.

Human brain inocula were from patients dying of CJD or GSS; the diagnosis was confirmed by histopathology and immunoblotting for PrP^{CJD}. In some cases, the PrP gene was amplified by PCR of DNA isolated from blood and the PrP sequence was determined. No HuPrP mutations were detected in cases of sporadic or iatrogenic CJD. A 10% (wt/vol) homogenate of thawed human brain tissue was prepared in phosphate-buffered saline (PBS) lacking Ca²⁺ and Mg²⁺. The tissue was dissociated by using a sterile disposable homogenizer followed by repeated extrusion first through an 18- and then through a 22-gauge needle. Samples for inoculation into test animals were diluted 10-fold in PBS. Rocky Mountain Laboratory (RML) prions (20) were previously passaged in Swiss mice. Mice were inoculated intracerebrally with 30 μl of extract using a 27-gauge needle inserted into the right parietal lobe. Criteria for diagnosis of central nervous system dysfunction in mice have been described (21).

The relative levels of PrP expression in Tg mouse and human brains were determined by immuno dot blots (17). For Western blots (22, 23), an enhanced chemiluminescent (ECL)

Abbreviations: BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; GSS, Gerstmann–Sträussler–Scheinker disease; PrP, prion protein; PrP^C, cellular isoform of PrP; PrP^{Sc}, scrapie isoform of PrP; Hu, human; HuGH, human growth hormone; Tg, transgenic; Mo, mouse; SHa, Syrian hamster; mAb, monoclonal antibody; RML, Rocky Mountain Laboratory.

†Present address: Department of Neurology, University of Minnesota, Minneapolis, MN 55455.

¶To whom reprint requests should be addressed at: Department of Neurology, HSE-781, University of California, San Francisco, CA 94143-0518.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

detection method (Amersham) was used. The blot was exposed to x-ray film for 5–60 sec. Histoblots were made by pressing 16- μ m-thick unfixed cryostat sections of brain to nitrocellulose paper (24). Histoblots were exposed to 400 μ g of proteinase K per ml for 18 hr at 37°C to eliminate PrP^C (cellular isoform of PrP) followed by 3 M GdnSCN to denature the PrP^{Sc} (scrapie isoform of PrP).

Brains were immersion fixed in 10% buffered formalin and embedded in paraffin; 8- μ m-thick histological sections were stained with the hematoxylin/eosin method or with peroxidase immunohistochemically for glial fibrillary acidic protein (25).

RESULTS

Tg(MHu2MP_{Pr}) Mice Are Susceptible to Hu Prions. We constructed a chimeric Hu/Mo PrP gene analogous to MH2M (17), designated MHu2M, that differs from MoPrP at nine positions, all of which lie between codons 96 and 167. Mice harboring two to four copies of the MHu2M PrP transgene expressed MHu2MP^{Pr} in their brains at a level similar to that of HuPrP^C in human brain. The Tg(MHu2M)FVB-B5378 mice were inoculated with brain homogenates from three unrelated Caucasian patients who had died of CJD. Two of the three patients died of sporadic CJD: one (RG) was a 56-year-old American female; the other (EC) was a 61-year-old Canadian female. In both cases cerebrocortical biopsies showed severe spongiform degeneration. The third (no. 364) was a British youth who had contracted iatrogenic CJD after treatment for hypopituitarism with human growth hormone (HuGH) derived from cadaveric pituitaries (26). Brain homogenates from all three CJD patients contained PrP^{CJD}.

All of the Tg(MHu2M) mice inoculated with homogenates from the three CJD patients developed signs of central nervous system dysfunction $\approx$ 200 days after inoculation (Table 1). Two of eight uninoculated Tg(MHu2M) mice are now >650 days of age and remain well; the youngest of the other six is now >350 days of age. Inoculation of Tg(MHu2M) mice with RML prions passaged in mice produced disease in 178 ± 3 days (mean $\pm$ SE), which is $\approx$ 40 days longer than RML prions in non-Tg mice; in contrast, incubation times for RML prions in Tg(MH2M) mice were the same as those of the non-Tg mice (17).

Tg(HuPrP) Mice Are Resistant to Human Prions. Tg mice designated Tg(HuPrP)B6SJL-110 and Tg(HuPrP)FVB-152 that harbor 2–4 and 30–50 copies of the HuPrP transgene, respectively, were constructed; the level of PrP^C in the brains of the Tg152 mice was 4- to 8-fold higher than HuPrP^C in

human brain. These Tg(HuPrP) mice were inoculated with brain extracts from 18 patients dying of sporadic CJD, iatrogenic CJD, familial CJD, or GSS. The transmission rate of 8.3% (14/169 mice) for Tg(HuPrP) mice was similar to that of 10.3% (6/58 mice) for control, non-Tg mice (Table 2); incubation times ranged from 590 to 840 days. Interpretation of transmissions occurring after extremely long incubation periods is difficult since aged mice develop a variety of illnesses and there is the heightened potential for artifactual results due to low levels of contaminating prions.

To confirm the clinical diagnosis of prion disease, five ill Tg(HuPrP) mice and one non-Tg mouse were sacrificed and brain extracts were examined for the presence of PrP^{Sc} by Western blotting with the anti-PrP monoclonal antibody (mAb) 3F4 and RO73 antiserum (30, 31). The 3F4 mAb reacts specifically with HuPrP, allowing discrimination from MoPrP. HuPrP^{Sc} was detected in the brains of two Tg(HuPrP) mice that developed clinical signs 589 days after inoculation with iatrogenic CJD case no. 170 (Fig. 1A). MoPrP^{Sc} was detected in the brain of a non-Tg mouse that developed clinical signs 756 days after inoculation with sporadic CJD case no. 87011. The absence of either RO73- or 3F4-reactive PrP^{Sc} in the brains of three of the six ill mice may reflect the difficulty of accurately diagnosing prion disease in elderly animals (Fig. 1A and B).

At $\approx$ 400 days after the experiments described in Table 2 had been initiated and none of the Tg(HuPrP) mice inoculated with CJD prions were ill, a second study was undertaken to

Table 2. Inoculation of Tg(HuPrP), Tg(MH2M), and non-Tg mice with CJD prions from humans

Host*	Inoculum	(n/n _o)†	Incubation time,‡ days
Tg 152	Sporadic CJD (no. 87011)	1/10	706
Non-Tg	Sporadic CJD (no. 87011)	3/5	697 $\pm$ 51
Tg 152	Sporadic CJD (no. 88037)	3/10	680 $\pm$ 28
Non-Tg	Sporadic CJD (no. 88037)	0/5	>800
Tg 152	Sporadic CJD (RG)	0/10	>500
Non-Tg	Sporadic CJD (RG)	0/10	>500
Tg 152	Sporadic (RG) rods	0/8	>470
Non-Tg	Sporadic (RG) rods	0/8	>470
Tg 152	Codon 102 GSS (no. 87027)	4/10	724 $\pm$ 16
Non-Tg	Codon 102 GSS (no. 87027)	1/5	679
Tg 152	Codon 102 GSS (no. 87031)	0/10	>800
Non-Tg	Codon 102 GSS (no. 87031)	1/5	742
Tg 152	Codon 178 CJD	0/8	>570
Non-Tg	Codon 178 CJD	0/8	>570
Tg 152	Sporadic CJD (no. 87036)	0/8	>650
Non-Tg	Sporadic CJD (no. 87036)	1/5	838
Tg 110	Iatrogenic CJD (no. 703)§	0/10	>800
Non-Tg	Iatrogenic CJD (no. 703)§	0/5	>800
Tg 110	Iatrogenic CJD (no. 170)§	2/10	589 $\pm$ 0
Non-Tg	Iatrogenic CJD (no. 170)§	0/5	>800
Tg 110	Iatrogenic CJD (no. 364)§	0/10	>800
Non-Tg	Iatrogenic CJD (no. 364)§	0/5	>800
Tg 110	Codon 200 CJD	1/8	791
Tg 110	Codon 217 GSS¶	1/8	874
Tg 110	Codon 102 GSS (A)	0/10	>850
Tg 110	Codon 102 GSS (B)	1/8	694
Tg 110	Codon 117 GSS**	0/8	>650
Tg 92	Iatrogenic CJD (no. 364)	0/9	>350
Tg 92	Iatrogenic CJD (no. 170)	0/10	>450

*Tg 152 and Tg 110 mice harbor the wild-type HuPrP (valine at codon 129) transgene. Tg 92 mice express the chimeric MH2MP_{Pr} transgene.

†Number of sick animals divided by the total number of animals inoculated.

‡Mean $\pm$ SE.

§Ref. 26. ¶Ref. 27. ||Ref. 28. **Ref. 29.

Table 1. Incubation times for Tg(MHu2M) mice inoculated with CJD prions from humans or RML prions from mice

Inoculum	Source	No.†	Incubation time,* days	
			Illness	Death‡
RG	Sporadic CJD	8/8	238 $\pm$ 3.2	240 $\pm$ 5.4 (3)
EC	Sporadic CJD	7/7	218 $\pm$ 4.6	ND
364	Iatrogenic CJD	9/9	232 $\pm$ 2.9	235 $\pm$ 3.9 (5)
RML	Scrapie mouse	19/19	178 $\pm$ 3.3	203 $\pm$ 2.0 (14)

*Mean $\pm$ SE.

†Number of animals developing signs of neurological dysfunction divided by the total number of animals inoculated. In the case of inoculum RG, three animals were found dead after 224, 238, and 243 days before a clinical diagnosis could be made. In the case of inoculum EC, two animals were found dead after 225 and 226 days before a clinical diagnosis could be made. In each case, these animals died at the same time that clinical diagnosis was made in other inoculated animals.

‡The number of mice dying of scrapie is shown in parentheses. Mice sacrificed for pathological examination were excluded from these calculations. ND, not determined.

FIG. 1. Analysis of brain homogenates from Tg(HuPrP) and Tg(MHu2M) mice exhibiting signs of neurologic dysfunction. Samples of 10% brain homogenates from ill mice were either undigested (odd-numbered lanes) or digested with proteinase K at a final concentration of 20 μ g/ml for 1 hr at 37°C (even-numbered lanes). Samples were resolved by SDS/PAGE and analyzed by Western blot. (A) Blot of Tg(HuPrP) mice and a non-Tg littermate developed with anti-PrP 3F4 mAb. Lanes 1 and 2, non-Tg152 inoculated with sporadic CJD (no. 87011), diagnosed ill at 740 days; lanes 3 and 4, Tg110 inoculated with iatrogenic CJD (no. 170), diagnosed at 589 days; lanes 5 and 6, second Tg110 animal inoculated with iatrogenic CJD (no. 170), also diagnosed at 589 days; lanes 7 and 8, Tg152 inoculated with sporadic CJD (no. 88037), diagnosed at 645 days; lanes 9 and 10, Tg152 inoculated with GSS (no. 87027), diagnosed at 679 days; lanes 11 and 12, Tg152 inoculated with GSS (no. 87027), diagnosed at 747 days. (B) Same samples as in A stained with polyclonal rabbit anti-PrP RO73 antiserum. (C) Blot of ill Tg(MHu2M) mice inoculated with three CJD inocula developed with anti-PrP 3F4 mAb. Lanes 1 and 2, brain homogenate from a CD-1 mouse inoculated with RML prions; lanes 3 and 4, Tg(MHu2M) mice inoculated with iatrogenic CJD (no. 364); lanes 5 and 6, Tg(MHu2M) mice inoculated with sporadic CJD (EC); lanes 7 and 8, Tg(MHu2M) mice inoculated with sporadic CJD (RG); lanes 9 and 10, brain homogenate from sporadic CJD case (EC); lanes 11 and 12, brain homogenate from iatrogenic CJD case (no. 364). (D) Same samples as in C but stained with anti-PrP RO73 antiserum. Positions of protein molecular mass standards corresponding to molecular masses (from top to bottom) of 31, 21, and 14 kDa are shown.

examine additional inocula and the effect of transgene homozygosity. In this second study, which has been in progress for about a year, three of six Tg(HuPrP)152 mice developed signs of central nervous system dysfunction at $\approx$ 280 days after inoculation with the iatrogenic CJD case no. 170. It

seems likely that the transmission of neurodegeneration after $\approx$ 280 days results from the higher copy number of the HuPrP transgene in Tg152 compared to Tg110 mice that developed neurodegeneration at 589 days after inoculation with case no. 170 (Table 2). In addition, the Tg(HuPrP) mice in Table 2 were all hemizygous for the transgenes; the three ill Tg(HuPrP)152 mice in this ongoing study may be homozygous since both parents carried the transgenes.

In contrast to Tg(MHu2M) mice, Hu prions from patient RG have not transmitted to either Tg(HuPrP) or non-Tg mice after >500 days (Table 2). Attempts to transmit preparations enriched for Hu prions prepared from the brain of patient RG have likewise been negative for >470 days. In addition, inoculum from the iatrogenic CJD case (no. 364) has not produced illness in either Tg(HuPrP) or non-Tg mice after >800 days or in Tg(MHu2M) mice after >350 days (Table 2). The lack of disease in Tg(MHu2M)92 mice demonstrates the specificity of the chimeric transgene in the transmission of Hu prions to mice (17).

PrP^{Sc} and Neuropathology in Tg(MHu2M) Mice. Western blots of brain homogenates from Tg(MHu2M) mice infected with Hu prions showed MHu2M PrP^{Sc} as demonstrated by reactivity with the 3F4 mAb (Fig. 1C), which does not react with MoPrP (32). The polyclonal rabbit anti-PrP RO73 antiserum diluted 1:5000 was poorly reactive with MHu2MPrP^{Sc} as well as HuPrP^C and HuPrP^{Sc} from diseased human brains (Fig. 1B and D). Brain homogenates from Tg(MHu2M) mice infected with RML prions contained PrP^{Sc}, which was detectable only with RO73 and not 3F4 anti-PrP antibodies (Fig. 1D), indicating that Tg(MHu2M) mice are capable of producing MoPrP^{Sc} but not MHu2MPrP^{Sc} in response to RML prions previously passaged in mice.

The distributions of the Mo and MHu2M PrP were examined by histoblotting of coronal sections through the hippocampus and thalamus of Tg(MHu2M) mice inoculated with RML or CJD prions. When inoculated with RML prions, a diffuse pattern of MoPrP^{Sc} deposition in the hippocampus, thalamus, hypothalamus, and all layers of the neocortex was found (Fig. 2A); in contrast, inoculation of Tg(MHu2M) mice with CJD prions produced intense immunostaining for MHu2MPrP^{Sc} in the ventral posterior lateral and medial nuclei of the thalamus as well as the deep layers of the cerebral cortex (Fig. 2B). While the hippocampus was completely devoid of MHu2MPrP^{Sc}, this region showed the most intense MHu2MPrP^C signal (data not shown). The same pattern of MHu2MPrP^{Sc} deposition was consistently observed in histoblots of Tg(MHu2M) mice inoculated with all three CJD prion isolates prepared from human brain.

Inoculation of Tg(MHu2M) mice with RML prions produced a mild to moderate degree of vacuolation throughout the cerebral hemispheres, cerebellum, and brainstem, which was accompanied by a mild to moderate degree of reactive astrocytic gliosis. Inoculation with CJD prions produced little or no detectable PrP^{Sc} in the hippocampus (Fig. 2B) with a corresponding absence of spongiform degeneration. In the head of the caudate as well as the thalamus, particularly in the ventral posterior lateral and medial nuclei where the most intense PrP^{Sc} was found, there was moderate to severe spongiform degeneration with microglial cell (macrophage) infiltration and intense reactive astrocytic gliosis (Fig. 3). In the cerebellum, PrP^{Sc} accumulation was confined to the cerebellar cortex; vacuolation was confined to the cerebellar cortex and was not present in the cerebellar white matter in contrast to mice inoculated with RML prions.

DISCUSSION

To assess the universality of the MHu2M transgene for laboratory transmission of Hu prions requires further investigation. For example, it may be necessary to match the PrP

FIG. 2. Regional distributions of PrP^{Sc} in Tg(MHu2M) mice exhibiting neurologic dysfunction after inoculation with RML or CJD prions as detected by histoblotting. Shown are coronal sections at the level of the hippocampus and thalamus in Tg(MHu2M) mice inoculated with RML prions and immunostained with the RO73 antiserum (A) or with human CJD prions (RG) and stained with 3F4 mAb (B). Am, amygdala; Hp, hippocampus; Hy, hypothalamus; Nc, neocortex; Th, thalamus. ($\times \approx 4.5$.)

genotype of the Tg(MHu2M) mouse with that of the CJD patient from whom the inoculum was derived. The 200-day incubation times in Tg(MHu2M) mice inoculated with Hu prions can probably be reduced substantially. In Tg(SHaPrP) mice, the level of SHaPrP transgene expression was found to be inversely proportional to the length of the scrapie incubation time after inoculation with SHa prions (33). Thus, producing Tg(MHu2M) mice with higher levels of transgene expression should markedly reduce the incubation times; in addition, ablation of the MoPrP gene may also reduce the incubation time (34, 35). By systematically altering the extent and position of the substitutions in chimeric Hu/Mo PrP constructs, it may be possible to enhance further the susceptibility of Tg mice to Hu prions.

From Tg(SHaPrP) mouse studies, prion propagation is thought to involve the formation of a complex between PrP^{Sc} and the homotypic substrate PrP^C (33). Attempts to mix PrP^{Sc} with PrP^C have failed to produce or inefficiently produce nascent PrP^{Sc} (36, 58), raising the possibility that proteins such as chaperones might be involved in catalyzing the conformational changes that feature in the formation of PrP^{Sc} (37). One explanation for the difference in susceptibility of Tg(MHu2M) and Tg(HuPrP) mice to Hu prions may be that

FIG. 3. Spongiform degeneration and reactive astrocytic gliosis in Tg(MHu2M) mice exhibiting signs of neurologic dysfunction after inoculation with CJD Hu prions. (A) Spongiform degeneration in the ventral posterior medial thalamic nucleus (VP); hematoxylin/eosin stain. (B) Intense reactive astrocytic gliosis in the VP; peroxidase immunohistochemistry for glial fibrillary acidic protein. (Bar = 100 μ m.)

mouse chaperones catalyzing the refolding of PrP^C into PrP^{Sc} can readily interact with the MHu2MPrP^C/HuPrP^{CJD} complex but not with HuPrP^C/HuPrP^{CJD}. Another possibility is that sequences at the N or C terminus of HuPrP inhibit the formation of PrP^{Sc} in murine cells. To test this possibility, HuPrP sequences can be substituted for the Mo sequences at each terminus of MHu2MPrP. Comparison of the PrP sequences in many mammals around the glycosyl phosphatidylinositol anchor addition site (codons 227–235) reveals an interesting difference of 4 amino acids between rodents and primates (38).

The patterns of PrP^{Sc} in Tg(MHu2M) mice were remarkably similar for the three inocula from humans dying of CJD (Fig. 2). Whether these three inocula all represent the same "strain" of prions or whether differences among them are obscured by passage in the Tg(MHu2M) mice remains to be established (39). Studies in rodents have shown that prion strains produce different patterns of PrP^{Sc} accumulation (40, 41) that can be dramatically changed by the expression of different PrP sequences (17, 42). Transmission studies of Hu prions from patients dying of CJD or kuru to nonhuman primates suggested that each case might be due to a different strain (9).

The sensitivity of Tg(MHu2M) mice to Hu prions suggests that a similar approach to the construction of Tg mice susceptible to bovine spongiform encephalopathy (BSE) and sheep scrapie prions may prove fruitful. The BSE epidemic has led to considerable concern about the safety for humans consuming beef and dairy products. Although epidemiologic studies over the past two decades argue that humans do not contract CJD from scrapie-infected sheep products (43–45), it is unknown whether any of the seven amino acid substitutions that distinguish bovine from sheep PrP render bovine prions permissive in humans (46). Whether Tg(MHu2M) mice are susceptible to bovine or sheep prions is unknown.

Since >45 young adults previously treated with HuGH derived from human pituitaries have developed CJD (47–51), testing of pharmaceuticals prepared from human and animal cells for prion contamination using Tg(MHu2M) mice would seem prudent. That the HuGH prepared from pituitaries was contaminated with prions is supported by the transmission of prion disease to a monkey 66 months after inoculation with a suspect lot of HuGH (52). The full extent of iatrogenic CJD in thousands of people treated with HuGH worldwide will not be revealed for decades due to long incubation times that may exceed 30 years in humans. Iatrogenic CJD also developed in four infertile women treated with human pituitary-derived gonadotrophin hormone (53, 54) as well as more than a dozen patients receiving corneal, eardrum, or dura mater grafts (49, 55–57).

The remarkable susceptibility of Tg(MHu2M) mice to Hu prions raises the possibility that chimeric Hu/Mo transgenes encoding proteins other than PrP might prove useful in investigations of the more common central nervous system degenerative disorders such as Alzheimer and Parkinson diseases.

We thank Drs. J. Anderson, H. Baker, T. Bird, G. Davidson, L. Duchon, R. Gabizon, G. Glenner, R. Moxley, and J. Tateishi for providing human specimens and R. Kascsak for 3F4 mAb. This study was supported by grants from the National Institutes of Health, British Agriculture and Food Research Council, the Wellcome Trust, and the American Health Assistance Foundation as well as by a gift from the Sherman Fairchild Foundation.

1. Prusiner, S. B. (1989) *Annu. Rev. Microbiol.* **43**, 345–374.
2. Gajdusek, D. C., Gibbs, C. J., Jr., & Alpers, M. (1966) *Nature (London)* **209**, 794–796.
3. Gibbs, C. J., Jr., Gajdusek, D. C., Asher, D. M., Alpers, M. P., Beck, E., Daniel, P. M., & Matthews, W. B. (1968) *Science* **161**, 388–389.

4. Masters, C. L., Gajdusek, D. C. & Gibbs, C. J., Jr. (1981) *Brain* **104**, 559–588.
5. Medori, R., Tritschler, H.-J., LeBlanc, A., Villare, F., Manetto, V., Chen, H. Y., Xue, R., Leal, S., Montagna, P., Cortelli, P., Tinuper, P., Avoni, P., Mochi, M., Baruzzi, A., Hauw, J. J., Ott, J., Lugaresi, E., Autilio-Gambetti, L. & Gambetti, P. (1992) *N. Engl. J. Med.* **326**, 444–449.
6. Brown, P., Gibbs, C. J., Jr., Rodgers-Johnson, P., Asher, D. M., Sulima, M. P., Bacote, A., Goldfarb, L. G. & Gajdusek, D. C. (1994) *Ann. Neurol.* **35**, 513–529.
7. Pattison, I. H. (1965) in *Slow, Latent and Temperate Virus Infections: NINDB Monograph 2*, eds. Gajdusek, D. C., Gibbs, C. J., Jr., & Alpers, M. P. (U.S. Government Printing, Washington, DC), pp. 249–257.
8. Manuelidis, E., Gorgacz, E. J. & Manuelidis, L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3422–3436.
9. Gibbs, C. J., Jr., Gajdusek, D. C. & Amyx, H. (1979) in *Slow Transmissible Diseases of the Nervous System*, eds. Prusiner, S. B. & Hadlow, W. J. (Academic, New York), Vol. 2, pp. 87–110.
10. Prusiner, S. B. (1987) in *Prions—Novel Infectious Pathogens Causing Scrapie and Creutzfeldt–Jakob Disease*, eds. Prusiner, S. B. & McKinley, M. P. (Academic, Orlando, FL), pp. 83–112.
11. Kitamoto, T., Tateishi, J., Sawa, H. & Doh-Ura, K. (1989) *Lab. Invest.* **60**, 507–512.
12. Tateishi, J., Doh-ura, K., Kitamoto, T., Tranchant, C., Steinmetz, G., Warter, J. M. & Boellaard, J. W. (1992) in *Prion Diseases of Humans and Animals*, eds. Prusiner, S. B., Collinge, J., Powell, J. & Anderton, B. (Horwood, London), pp. 129–134.
13. Muramoto, T., Kitamoto, T., Tateishi, J. & Goto, I. (1992) *Brain Res.* **599**, 309–316.
14. Scott, M., Foster, D., Miranda, C., Serban, D., Coufal, F., Wälchli, M., Torchia, M., Groth, D., Carlson, G., DeArmond, S. J., Westaway, D. & Prusiner, S. B. (1989) *Cell* **59**, 847–857.
15. Basler, K., Oesch, B., Scott, M., Westaway, D., Wälchli, M., Groth, D. F., McKinley, M. P., Prusiner, S. B. & Weissmann, C. (1986) *Cell* **46**, 417–428.
16. Loch, C., Chesebro, B., Race, R. & Keith, J. M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6372–6376.
17. Scott, M., Groth, D., Foster, D., Torchia, M., Yang, S.-L., DeArmond, S. J. & Prusiner, S. B. (1993) *Cell* **73**, 979–988.
18. Kretzschmar, H. A., Stowring, L. E., Westaway, D., Stubblebine, W. H., Prusiner, S. B. & DeArmond, S. J. (1986) *DNA* **5**, 315–324.
19. Scott, M. R., Köhler, R., Foster, D. & Prusiner, S. B. (1992) *Protein Sci.* **1**, 986–997.
20. Chandler, R. L. (1961) *Lancet* **1**, 1378–1379.
21. Carlson, G. A., Kingsbury, D. T., Goodman, P. A., Coleman, S., Marshall, S. T., DeArmond, S. J., Westaway, D. & Prusiner, S. B. (1986) *Cell* **46**, 503–511.
22. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
23. Barry, R. A. & Prusiner, S. B. (1986) *J. Infect. Dis.* **154**, 518–521.
24. Taraboulos, A., Jendroska, K., Serban, D., Yang, S.-L., DeArmond, S. J. & Prusiner, S. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7620–7624.
25. DeArmond, S. J., Mobley, W. C., DeMott, D. L., Barry, R. A., Beckstead, J. H. & Prusiner, S. B. (1987) *Neurology* **37**, 1271–1280.
26. Collinge, J., Palmer, M. S. & Dryden, A. J. (1991) *Lancet* **337**, 1441–1442.
27. Hsiao, K., Dlouhy, S., Farlow, M. R., Cass, C., Da Costa, M., Conneally, M., Hodes, M. E., Ghetti, B. & Prusiner, S. B. (1992) *Nat. Genet.* **1**, 68–71.
28. Hsiao, K., Baker, H. F., Crow, T. J., Poulter, M., Owen, F., Terwilliger, J. D., Westaway, D., Ott, J. & Prusiner, S. B. (1989) *Nature (London)* **338**, 342–345.
29. Hsiao, K. K., Cass, C., Schellenberg, G. D., Bird, T., Devine-Gage, E., Wisniewski, H. & Prusiner, S. B. (1991) *Neurology* **41**, 681–684.
30. Kascsak, R. J., Rubenstein, R., Merz, P. A., Tonna-DeMasi, M., Fersko, R., Carp, R. I., Wisniewski, H. M. & Diringer, H. (1987) *J. Virol.* **61**, 3688–3693.
31. Serban, D., Taraboulos, A., DeArmond, S. J. & Prusiner, S. B. (1990) *Neurology* **40**, 110–117.
32. Rogers, M., Serban, D., Gyuris, T., Scott, M., Torchia, T. & Prusiner, S. B. (1991) *J. Immunol.* **147**, 3568–3574.
33. Prusiner, S. B., Scott, M., Foster, D., Pan, K.-M., Groth, D., Miranda, C., Torchia, M., Yang, S.-L., Serban, D., Carlson, G. A., Hoppe, P. C., Westaway, D. & DeArmond, S. J. (1990) *Cell* **63**, 673–686.
34. Büeler, H., Aguzzi, A., Sailer, A., Greiner, R.-A., Autenried, P., Aguet, M. & Weissmann, C. (1993) *Cell* **73**, 1339–1347.
35. Prusiner, S. B., Groth, D., Serban, A., Koehler, R., Foster, D., Torchia, M., Burton, D., Yang, S.-L. & DeArmond, S. J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 10608–10612.
36. Raeber, A. J., Borchelt, D. R., Scott, M. & Prusiner, S. B. (1992) *J. Virol.* **66**, 6155–6163.
37. Pan, K.-M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z., Fletterick, R. J., Cohen, F. E. & Prusiner, S. B. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 10962–10966.
38. Stahl, N., Baldwin, M. A., Hecker, R., Pan, K.-M., Burlingame, A. L. & Prusiner, S. B. (1992) *Biochemistry* **31**, 5043–5053.
39. Deslys, J.-P., Lasmézas, C. & Dormont, D. (1994) *Lancet* **343**, 848–849.
40. Hecker, R., Taraboulos, A., Scott, M., Pan, K.-M., Torchia, M., Jendroska, K., DeArmond, S. J. & Prusiner, S. B. (1992) *Genes Dev.* **6**, 1213–1228.
41. DeArmond, S. J., Yang, S.-L., Lee, A., Bowler, R., Taraboulos, A., Groth, D. & Prusiner, S. B. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6449–6453.
42. Carlson, G. A., Ebeling, C., Yang, S.-L., Telling, G., Torchia, M., Groth, D., Westaway, D., DeArmond, S. J. & Prusiner, S. B. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 5690–5694.
43. Harries-Jones, R., Knight, R., Will, R. G., Cousens, S., Smith, P. G. & Matthews, W. B. (1988) *J. Neurol. Neurosurg. Psychiatry* **51**, 1113–1119.
44. Cousens, S. N., Harries-Jones, R., Knight, R., Will, R. G., Smith, P. G. & Matthews, W. B. (1990) *J. Neurol. Neurosurg. Psychiatry* **53**, 459–465.
45. Brown, P., Cathala, F., Raubertas, R. F., Gajdusek, D. C. & Castaigne, P. (1987) *Neurology* **37**, 895–904.
46. Prusiner, S. B., Fuzi, M., Scott, M., Serban, D., Serban, H., Taraboulos, A., Gabriel, J.-M., Wells, G., Wilesmith, J., Bradley, R., DeArmond, S. J. & Kristensson, K. (1993) *J. Infect. Dis.* **167**, 602–613.
47. Koch, T. K., Berg, B. O., DeArmond, S. J. & Gravina, R. F. (1985) *N. Engl. J. Med.* **313**, 731–733.
48. Deslys, J.-P., Marcé, D. & Dormont, D. (1994) *J. Gen. Virol.* **75**, 23–27.
49. Brown, P., Cervenáková, L., Goldfarb, L. G., McCombie, W. R., Rubenstein, R., Will, R. G., Pocchiarri, M., Martinez-Lage, J. F., Scalici, C., Masullo, C., Graupera, G., Ligan, J. & Gajdusek, D. C. (1994) *Neurology* **44**, 291–293.
50. Fradkin, J. E., Schonberger, L. B., Mills, J. L., Gunn, W. J., Piper, J. M., Wysowski, D. K., Thomson, R., Durako, S. & Brown, P. (1991) *J. Am. Med. Assoc.* **265**, 880–884.
51. Buchanan, C. R., Preece, M. A. & Milner, R. D. G. (1991) *Br. Med. J.* **302**, 824–828.
52. Gibbs, C. J., Jr., Asher, D. M., Brown, P. W., Fradkin, J. E. & Gajdusek, D. C. (1993) *N. Engl. J. Med.* **328**, 358–359.
53. Healy, D. L. & Evans, J. (1993) *Br. J. Med.* **307**, 517–518.
54. Cochius, J. I., Hyman, N. & Esiri, M. M. (1992) *J. Neurol. Neurosurg. Psychiatry* **55**, 1094–1095.
55. Nisbet, T. J., MacDonaldson, I. & Bishara, S. N. (1989) *J. Am. Med. Assoc.* **261**, 1118.
56. Thadani, V., Penar, P. L., Partington, J., Kalb, R., Janssen, R., Schonberger, L. B., Rabkin, C. S. & Prichard, J. W. (1988) *J. Neurosurg.* **69**, 766–769.
57. Willison, H. J., Gale, A. N. & McLaughlin, J. E. (1991) *J. Neurol. Neurosurg. Psychiatry* **54**, 940.
58. Kocisko, D. A., Come, J. H., Priola, S. A., Chesebro, B., Raymond, G. J., Lansbury, P. T., Jr., & Caughey, B. (1994) *Nature (London)* **370**, 471–474.