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Genetics

Serial transmission in rodents of neurodegeneration from transgenic mice expressing mutant prion protein

(*de novo* prion synthesis/Gerstmann–Sträussler–Scheinker disease/scrapie/protein conformation/inherited prion diseases)

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ABSTRACT **Two lines of transgenic (Tg) mice expressing high (H) levels of the mutant P101L prion protein (PrP) developed a neurologic illness and central nervous system pathology indistinguishable from experimental murine scrapie; these mice were designated Tg(MoPrP-P101L)H. Brain homogenates from Tg(MoPrP-P101L)H mice were inoculated intracerebrally into CD-1 Swiss mice, Syrian hamsters, and Tg196 mice, Tg mice expressing the MoPrP-P101L transgene at low levels. None of the CD-1 mice developed central nervous system dysfunction, whereas ≈10% of hamsters and ≈40% of the Tg196 mice manifested neurologic signs between 117 and 639 days after inoculation. Serial transmission of neurodegeneration in Tg196 mice and Syrian hamsters was initiated with brain extracts, producing incubation times of ≈400 and ≈75 days, respectively. Although the Tg(MoPrP-P101L)H mice appear to accumulate only low levels of infectious prions in their brains, the serial transmission of disease to inoculated recipients argues that prion formation occurs *de novo* in the brains of these uninoculated animals. These Tg mouse studies, taken together with similar findings in humans dying of inherited prion diseases, provide additional evidence that prions lack a foreign nucleic acid.**

The inherited prion diseases were first recognized in families suffering from Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler–Scheinker disease (GSS) (1–6), but these observations remained perplexing until mutations in the prion protein (PrP) gene were identified and shown to be genetically linked to development of the disease (7–11).

Scrapie infectivity and a sialoglycoprotein designated PrP^{Sc} copurify, and many lines of evidence argue that PrP^{Sc} is the major macromolecular component of the infectious prion particle (12). PrP^{Sc} is encoded by a single-copy chromosomal gene and not by a putative nucleic acid carried within the infectious scrapie prion particle (13–15). In humans dying of prion diseases, PrP^{Sc} is often found in brain (16–20), but in some cases it has been difficult to detect, especially in the inherited prion diseases (21, 22). PrP^{Sc} has been generally distinguished from cellular PrP (PrP^{C}) by its relative protease resistance, its insolubility in nondenaturing detergents, its enhanced antigenicity after denaturation, and its posttranslational biogenesis; spectroscopic studies show that the two proteins differ in their conformations (23). When amyloid plaques are present in these patients, the plaques stain with anti-PrP antibodies (24–26).

Tg(MoPrP-P101L)H mice express high levels of a mouse PrP transgene product containing the P102L mutation causing GSS; aa 102 in human PrP corresponds to aa 101 in mouse PrP (MoPrP). We report here that serial transmission of neurodegeneration to Tg196 mice (which express the MoPrP-P101L transgene at low levels) and Syrian hamsters was initiated with brain extracts from Tg(MoPrP-P101L)H mice. Although the Tg(MoPrP-P101L)H mice appear to accumulate only low levels of infectious prions, the serial transmission of disease to inoculated recipients argues that prion formation occurs *de novo* in these uninoculated animals.

METHODS

Transgenic (Tg) mice expressing MoPrP(P101L) were constructed, assessed for signs of central nervous system (CNS) dysfunction, and inoculated as described (7, 27). Brain tissue was fixed by immersion in buffered 10% formalin, embedded in paraffin, and sectioned (8 μm). Sections mounted on polylysine-coated slides were deparaffinized in xylene followed by two washes in 100% ethanol. Serial sections were stained with periodic acid/Schiff reagent, hematoxylin and eosin, and alkaline Congo red dye (28). The vacuolation scores (27) are visual estimates of the number and size of vacuoles in a region. For immunohistochemistry, endogenous peroxidase activity was blocked with 2 ml of 30% H_2O_2 in 200 ml of methanol. To enhance PrP immunoreactivity, the sections were immersed in 1.3 mM HCl and autoclaved at 121°C for 10 min (29).

For Western immunoblotting, brains were disrupted in lysis buffer [10 mM Tris·HCl, pH 8.0/0.15 M NaCl/2 mM $MgCl_2$/0.5% (vol/vol) Nonidet P-40/0.5% (wt/vol) sodium deoxycholate] yielding a homogenate with protein at ≈5 mg/ml of protein (BCA assay, Pierce); after 5 min on ice, it was centrifuged at 463 × *g* for 5 min in a Beckman J-6 centrifuge. The supernatant was adjusted to 500 μg of protein per ml in 10 mM Tris·HCl, pH 8.0/100 mM NaCl/1% (wt/vol) *N*-lauroylsarcosine. Proteinase K (1 mg/ml) from a freshly prepared stock was added to 1-ml samples (final concentration, 20 μg/ml) for 0–3 hr at 37°C. Limited digestions were terminated by 2 mM phenylmethylsulfonyl fluoride. Samples were centrifuged at 56,800 × *g* in a Beckman TL-100 ultracentrifuge using a TLA 100.3 rotor for 1 hr at 4°C. The pellets were resuspended in 10 mM Tris·HCl, pH 8.0/100 mM NaCl/1% *N*-lauroylsarcosine and the ultracentrifugation was repeated. After the pellets were resuspended in 20 μl of lysis buffer and an equal volume of 2× SDS/PAGE buffer was added, they were boiled for 5 min. A 40-μl aliquot was loaded into each well of a 12.5% polyacrylamide slab gel, 1.5 mm

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Abbreviations: CJD, Creutzfeldt–Jakob disease; CNS, central nervous system; GSS, Gerstmann–Sträussler–Scheinker disease; PrP, prion protein; PrP^{C}, cellular isoform of PrP; PrP^{Sc}, scrapie isoform of PrP; MoPrP, mouse PrP; Tg, transgenic.

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thick (30). After SDS/PAGE, proteins were electrotransferred for 1.5–2 hr to Immobilon (Millipore). PrP was detected with a rabbit antiserum R073 diluted 1:5000. Bound anti-PrP antibodies were detected by the Amersham ECL method.

RESULTS

Two Tg(MoPrP-P101L)H lines with ≈60 copies of the transgene and ≈8-fold overexpression of mutant MoPrP^C spontaneously developed ataxia, lethargy, bradykinesia, and rigidity (31). These lines were designated Tg174 and Tg87. The age of onset of illness was 195 ± 42 (mean ± SEM) days and 152 ± 33 days in 193 Tg174 and 20 Tg87 mice, respectively. The duration of disease from the onset of illness to death was 12 ± 8 and 14 ± 11 days in Tg174 and Tg87 mice, respectively. Ninety-nine percent of Tg174 mice and 100% of Tg87 mice developed disease by 300 days of age. Tg(wtPrP-A) mice harboring ≈60 copies of the PrP-A transgene and overexpressing wtPrP^C-A ≈8-fold have not developed illness spontaneously at >600 days of age (G. Telling and S.B.P., unpublished work).

Both Tg174 and Tg87 mice developed prominent vacuolar degeneration in the neocortex and hippocampus (Fig. 1). Plaques observed in both Tg(MoPrP-P101L)H lines stained with periodic acid/Schiff reagent (Fig. 2) and were similar to the "kuru plaques" (33, 34). These PrP plaques measured 20–90 μm in diameter and were found in 3 of 8 Tg174 mice and 2 of 16 Tg87 mice in the caudate nucleus, Ammon's horn and the cerebellum. The development of indistinguishable neurologic disorders in two independent Tg lines argues that mutant transgene expression rather than insertional mutagenesis is responsible for the CNS dysfunction.

Tg196 mice harbor ≈9 haploid copies of the transgene, express low levels of the P101L mutant transgene, and do not develop CNS dysfunction spontaneously. None of 36 uninoculated Tg196 mice observed for up to 2 years developed neurologic signs. None of 23 Tg196 mice inoculated with brain homogenates prepared from the brains of non-Tg littermates or CD-1 mice had amyloid plaques or spongiform degeneration when sacrificed at 600 days of age or older (Fig. 1). The P101L mutation was present in the PrP open reading frame amplified by PCR and sequenced from DNA of a Tg196 mouse, indicating that the absence of spontaneous illness in this line is not due to a reversion of the mutation. PrP^C levels in brains of Tg196 mice were ≈2-fold higher than in non-Tg littermates, as determined by dot immunoblotting.

Transmission of Neurodegeneration. Sixty-two of 138 Tg196 mice inoculated with brain homogenates from Tg(MoPrP-P101L)H mice developed neurologic signs between 226 and 712 days (mean ± SEM = 442.5 ± 10.5 days) consisting of kyphosis, foot clasp, tremor, and ataxia (Table 1). These Tg196 mice progressed to death over a period ranging from 1 to 217 days (mean ± SEM = 62 ± 4.6 days). None of 26 Tg196 mice inoculated with brain homogenates from the three presymptomatic Tg(MoPrP-P101L)H mice developed neurologic signs. One of 88 Tg196 mice inoculated with brain homogenates from 9 healthy CD-1 or non-Tg(MoPrP-P101L)H littermates developed signs of neurologic dysfunction. This mouse was 552 days old when it developed symptoms 476 days after inoculation. None of the other 9 mice injected with the same control inoculum developed recognizable signs of neurologic dysfunction. Unfortunately, tissues from this single symptomatic Tg196 mouse were not taken for pathological evaluation. Transmission of disease to Tg196 mice from 11 of the 14 brain homogenates prepared from symptomatic Tg(MoPrP-P101L)H mice versus healthy CD-1 or non-Tg(MoPrP-P101L)H littermates is statistically significant ($\chi^2 = 44.021$, $P = 3 \times 10^{-11}$).

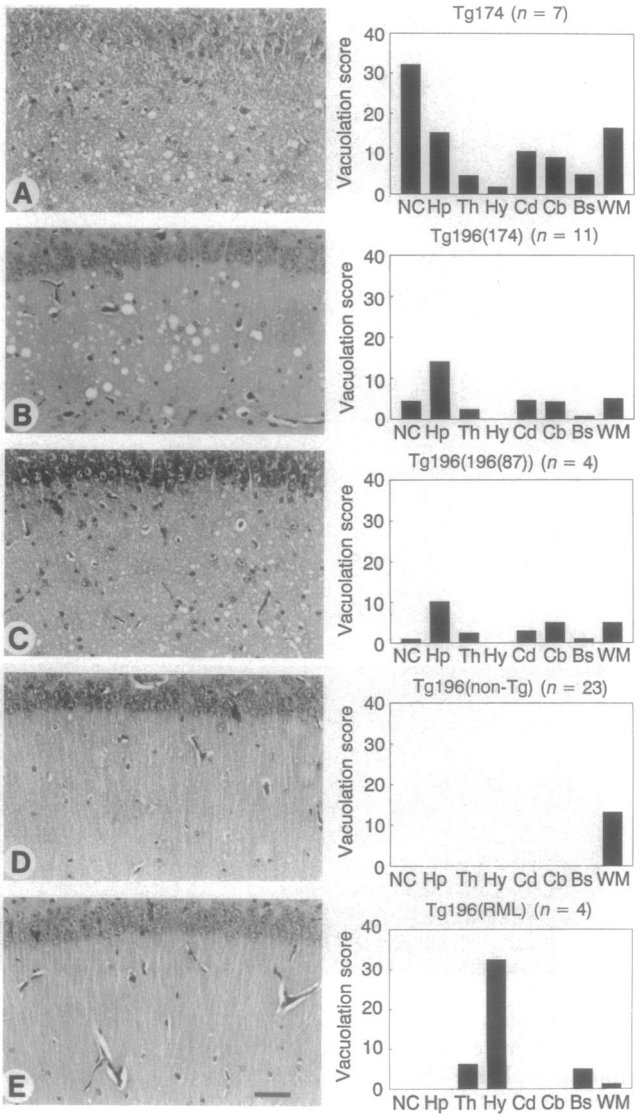

FIG. 1. Neuropathological changes in Tg mice with genetic and infectious prion diseases. (*Left*) Photomicrographs of representative sections through the same region of the hippocampus (CA1 region) stained with the hematoxylin and eosin. (Bar = 50 μm). (*Right*) Bar graphs depicting the extent of vacuolation in eight regions of the brain. (*A*) Tg174 mice expressing high levels of MoPrP-P101L that developed clinical signs of neurologic dysfunction spontaneously (n = 7). (*B*) Tg196 mice which developed clinical signs following inoculation with brain homogenates from Tg174 mice (n = 11). (*C*) Tg196 inoculated with brain homogenates from ill Tg196 mice that were previously inoculated with brain homogenates from ill Tg87 mice (n = 4). (*D*) Tg196 mice inoculated with brain homogenates from normal, non-Tg mice; no signs of CNS dysfunction or neuropathologic change occurred except for age-related white matter vacuolation (n = 23). (*E*) Tg196 mice inoculated with RML scrapie prions passaged in CD-1 mice (n = 4). No spongiform degeneration was found in the hippocampus. NC, neocortex; Hp, hippocampus; Th, thalamus; Hy, hypothalamus; Cd, caudate nucleus; Cb, cerebellar cortex; Bs, brainstem; WM, white matter.

All 350 Swiss CD-1 mice inoculated with homogenates from either clinically ill Tg(MoPrP-P101L)H mice or healthy control mice remained free of neurologic disease for >500 days. All 60 Swiss CD-1 mice inoculated with RML scrapie prions developed signs of scrapie within 155 days (Fig. 3).

Nine of 148 Syrian hamsters inoculated with brain homogenates from 3 of the 16 symptomatic Tg(MoPrP-P101L)H mice developed signs indistinguishable from experimental scrapie 117–411 days after inoculation (mean ± SEM = 253 ± 30 days) (Table 1). No Syrian hamsters inoculated with brain

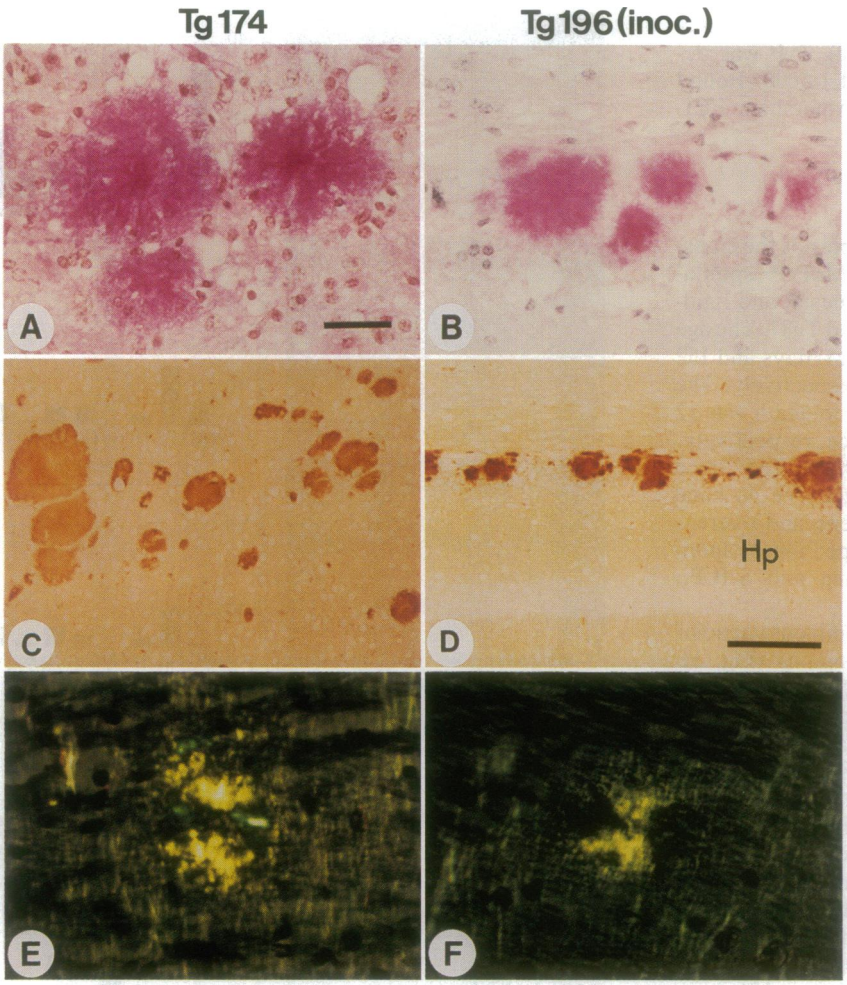

FIG. 2. PrP amyloid plaques in the brains of uninoculated Tg174 (*A*, *C*, and *E*) and inoculated Tg196 (*B*, *D*, and *F*) mice. Similarities shared by the plaques were strong histochemical staining by the periodic acid/Schiff method (*A* and *B*), immunoreactivity with PrP-specific antibodies (*C* and *D*), and green-gold birefringence when stained with Congo red and viewed with polarized light (*E* and *F*). The main differences were that Tg174 plaques were primarily in the caudate nucleus and were as large as 90 μm, whereas those in inoculated Tg196 mice were primarily in the subcallosal region (*D*) over the hippocampus (Hp) and smaller. Peroxidase immunohistochemistry with anti-PrP antibodies (R073) stained amyloid plaques in the caudate nucleus (*C*) and subcallosal plaques (*D*). Tg196 mice in *B*, *D*, and *F* were inoculated with homogenates prepared from the brains of clinically ill Tg174 mice. (Bar in *A* = 50 μm for *A*, *B*, *E*, and *F*; bar in *D* = 100 μm for *C* and *D*.)

homogenates from three presymptomatic Tg(MoPrP-P101L)H mice (n = 24) or from healthy CD-1 mice or non-Tg(MoPrP-P101L)H littermates (n = 120) developed signs of CNS dysfunction. The transmission to hamsters from symptomatic Tg(MoPrP-P101L)H mice compared with healthy CD-1 mice or non-Tg(MoPrP-P101L)H littermates is statistically significant (χ^2 = 7.551, P = 0.006). Results in Syrian hamsters were scored 400–550 days after inoculation, at which time experiments were terminated due to the large percentage of atypical deaths that occur in aged laboratory hamsters. Results in CD-1 and Tg196 mice were scored 500–700 days following inoculation.

Serial Transmissions. Brain homogenates prepared from 3 affected Tg196 mice initially inoculated with Tg(MoPrP-P101L)H brain extracts were inoculated into 30 Tg196 mice. After more than a year, 83% of inoculated Tg196 mice showed signs of CNS dysfunction (Fig. 3) as well as spongiform degeneration (Fig. 1). Tg196 mice inoculated with RML prions developed scrapie with a mean incubation time of 245 ± 3 days (mean ± SEM; n = 27), considerably longer than the 155-day incubation time in wt(*Prn-p*^{a}) mice (Fig. 3). PrP^{Sc} was detected in Tg196 mice which developed scrapie after RML inoculation but at lower levels than detected in inoculated CD-1 mice (Fig. 4). Brain homogenates from Tg196 mice inoculated with RML prions were passaged into CD-1 mice, which had incubation times indistinguishable from CD-1 mice inoculated with RML prions (Fig. 3).

Neurologic disease from Syrian hamsters inoculated with brain homogenates H034 and K28 from Tg174 mice, ill at 170 and 160 days, respectively, was serially transmitted to Syrian hamsters after 75 ± 1 days (mean ± SEM, n = 24). When the H034 isolate was passaged a second time into hamsters, signs of scrapie developed after 68 ± 2 days (n = 13). Propagation of infectivity was demonstrated by endpoint titrations: ham-

Table 1. Transmission of neurodegeneration to animals by inoculation of brain extracts from spontaneously ill Tg(MoPrP-P101L)H mice

	No. diseased/no. inoculated		
	CD-1 mice	Syrian hamsters	Tg196 mice
Inocula from symptomatic Tg(MoPrP-P101L)H mice			
Groups	0/16	3/16	11/14
Animals	0/160	9/148	62/138
		(P = 0.006)*	($P = 3 \times 10^{-11}$)*
[Incubation time, days]		[117–411]	[226–712]
Inocula from presymptomatic Tg(MoPrP-P101L)H mice			
Groups	0/3	0/3	0/3
Animals	0/30	0/24	0/26
			(P = 0.00007)†
Inocula from non-Tg mice			
Groups	0/15	0/15	1/9
Animals	0/150	0/120	1‡/88
[Incubation time, days]	[>500]	[>500]	[>500]

Mice were inoculated intracerebrally with 30 μl of 10% (wt/vol) brain homogenates via a 27-gauge disposable hypodermic needle inserted into the right parietal lobe; hamsters received 50 μl intracerebrally. Criteria for diagnosis of neurologic disease in mice and hamsters were ataxia, as manifested by a tremulous gait and difficulty with the righting reflex; immobility; and rigidity.

*Compared with inocula from non-Tg mice.

†Compared with inocula from symptomatic Tg(MoPrP-P101L)H mice.

‡Animal diagnosed with a scrapie-like neurological disease 476 days after inoculation.

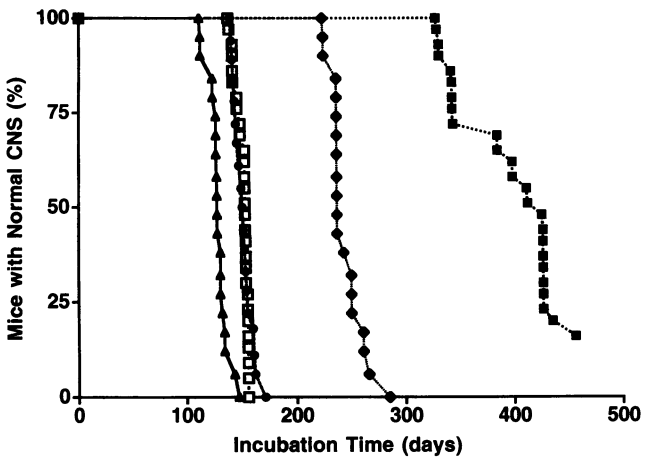

FIG. 3. Incubation times for Tg196 mice inoculated with RML mouse scrapie prions or GSS prions generated *de novo* in Tg(MoPrP-P101L)H mice. RML mouse prions were inoculated intracerebrally into non-Tg mice (▲) (n = 19), CD-1 Swiss mice (●) (n = 30) and Tg196 mice (◆) (n = 27), and transmissions from three Tg196 mice were inoculated with RML prions into CD-1 mice (□) (n = 30). Prions generated *de novo* in Tg(MoPrP-P101L)H mice were transmitted to Tg196 mice and serially passaged in Tg196 mice (■) (n = 25 of 30 mice inoculated).

sters inoculated with 1–10 ID_{50} units produced $\approx 10^6$ ID_{50} units. The H034 isolate produced an illness in Tg(SHaPrP)7 mice after 52 ± 2 days (n = 12) and upon serial passage after 53 ± 2 days (n = 16). Syrian hamsters inoculated with the K28 isolate developed scrapie after 75 ± 2 days (n = 8).

Neuropathology of Tg196 Mice Inoculated with Prions Generated *de Novo*. Spongiform changes were found in all 18 Tg196 mice inoculated with brain homogenates from symptomatic Tg(MoPrP-P101L)H mice; most intense was the hippocampus. No spongiform change except for the white matter was found in the 23 Tg196 mice inoculated with control brain homogenates (Fig. 1); similar lesions have been seen in the brains of aged, uninoculated mice. PrP amyloid plaques were observed in 7 of 18 of the Tg196 mice in the experimental group and in 0 of 23 of the Tg196 mice in the control group (Fig. 2). These plaques were confined largely to the subcallosal region overlying the hippocampus, whereas the PrP plaques in the brains of Tg(MoPrP-P101L)H mice were found primarily in the caudate nucleus.

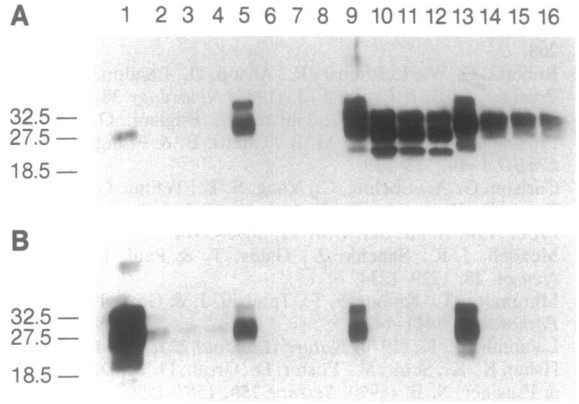

FIG. 4. Western blots of fractions prepared from the brains of Tg174 mice developing CNS dysfunction spontaneously, as well as Tg196 mice inoculated with Tg174 brain homogenates and RML prions. Immunoblots show brain fractions digested with proteinase K (20 μg/ml) at 37°C for 0 hr (lanes 1, 5, 9, and 13), 1 hr (lanes 2, 6, 10, and 14), 2 hr (lanes 3, 7, 11, and 15), or 3 hr (lanes 4, 8, 12, and 16). (*A*) CD-1 mouse (lanes 1–4), non-Tg174 littermate (lanes 5–8), CD-1 mouse inoculated with RML prions and exhibiting clinical signs of scrapie (lanes 9–12), and Tg196 mouse inoculated with RML prions and exhibiting clinical signs of scrapie (lanes 13–16). (*B*) Tg174 mouse spontaneously developing clinical signs of CNS dysfunction (lanes 1–4), Tg196 mouse inoculated with a brain homogenate prepared from an ill Tg174 mouse and exhibiting clinical signs of CNS dysfunction (lanes 5–8), Tg196 mouse inoculated with a brain homogenate prepared from an ill Tg196 mouse previously inoculated with brain homogenate from an ill Tg174 mouse and exhibiting clinical signs of CNS dysfunction (lanes 9–12), and uninoculated Tg196 mouse (lanes 13–16). Bio-Rad prestained markers were used: carbonic anhydrase, M_r 32,500; soybean trypsin inhibitor, M_r 27,500; lysozyme, M_r 18,500.

Serial passage of prions in Tg196 mice produced intense spongiform degeneration within the hippocampus. The distribution of spongiform change in the 4 mice examined was indistinguishable from that found in first-passage Tg196 mice (Fig. 1). No plaques were found in the brains of these 4 Tg196 mice. Inoculation of Tg196 mice with RML prions passaged in CD-1 mice did not produce vacuolation in hippocampus or neocortex, but intense spongiform change was found in hypothalamus, basal forebrain, and rostral cingulate gyrus (limbic lobe) (Fig. 1). The lesions contrast sharply with the mild to moderate vacuolation found in multiple regions of the gray matter of non-Tg mice inoculated with RML prions and of Tg196 mice inoculated with extracts from ill Tg(MoPrP-P101L)H mice (Fig. 1).

Immunoblotting for PrP^{Sc}. PrP^{Sc} was not detected on Western immunoblots of brain homogenates from Tg(MoPrP-P101L)H mice with clinical signs of CNS dysfunction indistinguishable from experimental scrapie (Fig. 4). The PrP remaining in samples, which had been digested with proteinase K, had a molecular weight similar to that of PrP^C and, thus, probably represented PrP^C which was undigested under conditions of limited proteolysis in the setting of transgene overexpression. This residual PrP was present in both healthy and ill Tg(MoPrP-P101L)H mice.

No protease-resistant PrP was detected by Western immunoblotting in brain tissue from 1 or 2 Tg196 mice that had developed neurologic disease following inoculation with brain homogenates from 11 Tg(MoPrP-P101L)H mice, regardless of the presence or absence of PrP immunopositive plaques in the brain. To increase the sensitivity of PrP^{Sc} detection by Western blotting, partial purification of PrP^{Sc} was accomplished by detergent extraction and limited proteolysis. As with homogenates, no protease-resistant PrP was found in the brains of Tg196 mice inoculated with brain extracts from Tg(MoPrP-P101L)H mice compared with control Tg196 mice (Fig. 4). Even serial passage of these mouse GSS prions through Tg196 mice did not produce protease-resistant PrP detectable by this method, yet the mice developed clinical signs of neurologic dysfunction and spongiform degeneration (Fig. 1). In contrast to Tg196 mice, PrP^{Sc} was detected in ill Syrian hamsters inoculated with brain extracts prepared from ill Tg(MoPrP-P101L)H mice.

To address the possibility that undetectable levels of PrP^{Sc} might reflect highly localized deposits, histoblots (35) of ill Tg196 mice inoculated with brain homogenates from ill Tg(MoPrP-P101L)H mice were compared to those with homogenates from non-Tg mice; no differences between the blots were found (data not shown).

DISCUSSION

Our results indicate that the neurologic disease which spontaneously develops in Tg(MoPrP-P101L)H mice is transmissible. Long incubation times and incomplete transmission within experimental sets are consistent with low titers of infectivity, as is the absence of detectable PrP^{Sc} on Western blots in symptomatic Tg(MoPrP-P101L)H mice (Fig. 4). However, PrP amyloid plaques in the brains of some Tg(MoPrP-P101L)H mice (Fig. 2) are evidence for PrP^{Sc} synthesis.

While Tg196 mice do not spontaneously develop neurologic disease, they were positive hosts in nearly 80% of the transmission experiments (Table 1). The Syrian hamsters were positive hosts in ≈20% of experiments, while none of the CD-1 mice were positive. That Syrian hamster PrP^{C} is more readily converted to PrP^{Sc} by mutant MoPrP-P101L^{Sc} than is wild-type MoPrP^{C} was unexpected but is presumably due to the different conformations of these proteins.

It is reasonable to ask whether our results might be due to contaminating exogenous prions. Infectious prions produced *de novo* in Tg(MoPrP-P101L)H mice exhibit properties different from those of prion isolates used for studies of experimental scrapie. (*i*) The neuropathology in neither the primary transmission nor serial passage in Tg196 mice resembled that produced by RML scrapie prions (Fig. 1). (*ii*) The patterns of protease-resistant PrP in the brains of Tg196 mice inoculated with RML scrapie prions were distinct from mice receiving an inoculum prepared from Tg(MoPrP-P101L)H mice (Fig. 4). (*iii*) Serial passage of prions from Tg(MoPrP-P101L)H mice in Tg196 mice produced no protease-resistant PrP as judged by Western immunoblots, in contrast to Tg196 mice inoculated with RML scrapie prions (Fig. 4). Yet the brains from ill Tg196 mice were clearly able to transmit disease to inoculated recipient Tg196 mice (Fig. 3). (*iv*) None of the Swiss CD-1 inoculated with brain homogenates from either Tg(MoPrP-P101L)H mice or non-Tg controls developed neurodegeneration, but they readily developed scrapie after inoculation with RML prions (Table 1). If our results were due to inadvertent laboratory contamination, then some of the 350 CD-1 mice should have developed scrapie. Because CD-1 mice have shorter incubation periods than Tg196 mice after inoculation with RML prions, we would expect them to be more sensitive indicators of contaminating scrapie prions (Fig. 3). (*v*) The apparent low titers of prions in the brains of Tg(MoPrP-P101L)H mice are not without precedent; the inherited human prion diseases are more difficult to transmit than the sporadic or iatrogenic forms of prion disease (20).

The Tg mouse studies reported here, and transmission of neurodegeneration to experimental animals by brain extracts of patients dying of inherited prion diseases (6), argue that prion diseases are both genetic and infectious. These results, and numerous unsuccessful attempts to detect a scrapie-specific nucleic acid (36–40), militate against a foreign nucleic acid as an essential component of the "infectious" prion, despite proposals to the contrary (32, 41–43). Indeed, it seems likely that prions are composed only of PrP^{Sc} molecules.

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