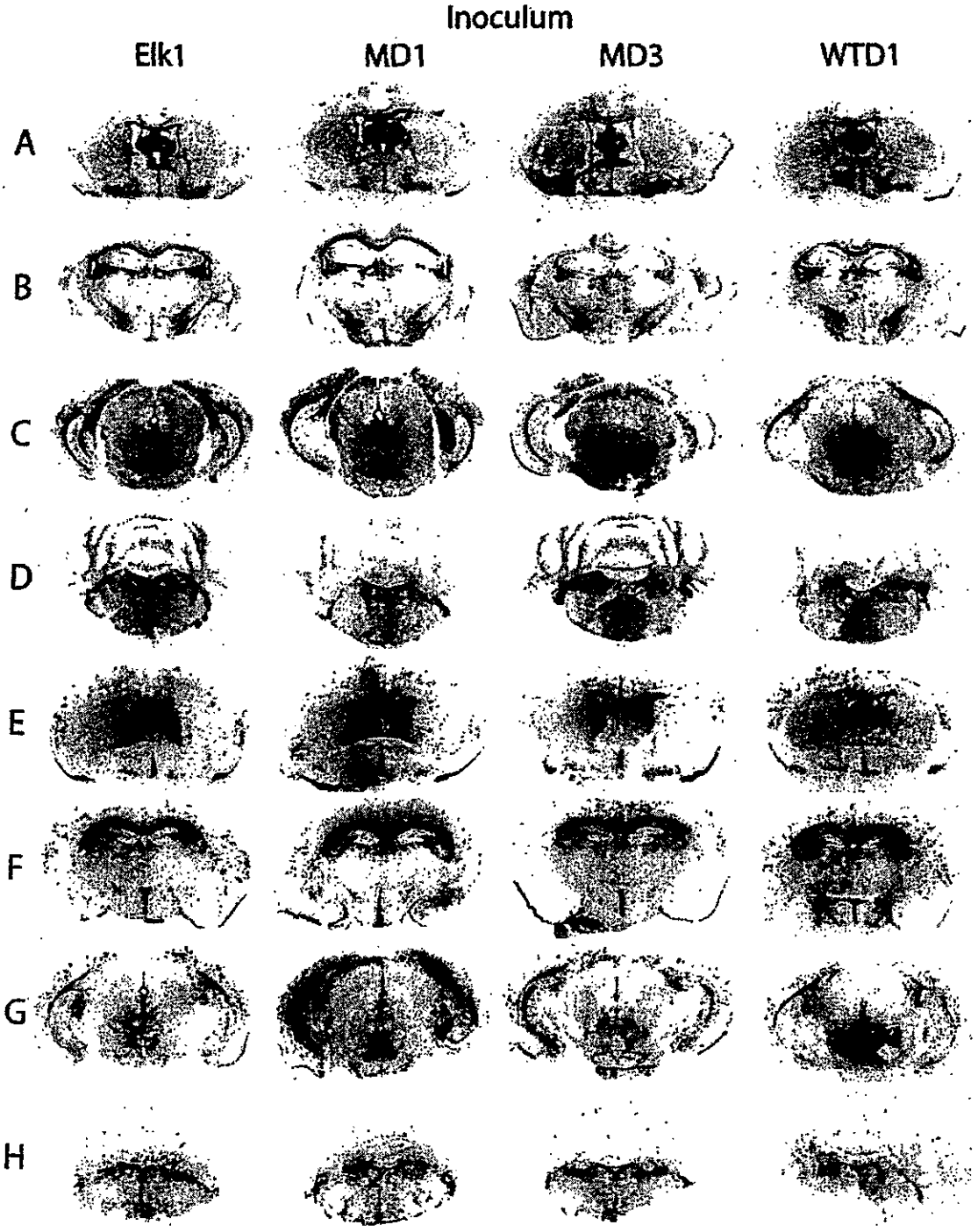

FIG. 7. Histoblot analysis shows the anatomic deposition of protease-resistant PrP^{Sc} in Tg(ElkPrP)12577 (A to D) and Tg(DePrP)10945 (E to H) mice following inoculation with different CWD isolates, as indicated. Coronal sections from four different brain levels are shown: (A and E) head of the caudate nucleus; (B and F) dorsal hippocampus and thalamus; (C and G) midbrain; and (D and H) cerebellum and pons.

that found in cattle brain. When inoculated with BSE prions, Tg(BoPrP)4092 mice and Tg(BoPrP)4125 mice died of prion disease in ~240 days. In contrast, Tg(BoPrP)4092 mice remained healthy after inoculation with four CWD brain samples from elk (>600 days) and two samples each from mule deer and white-tailed deer (>500 days). Neither of the two elk samples inoculated into Tg(BoPrP)4125 mice resulted in prion disease (>600 days). Similarly, inoculation of three elk, two mule deer, and three white-tailed deer CWD samples into Tg(OvPrP,VRQ)338 mice did not transmit prion disease after >500 days. These Tg mice express OvPrP at a level 12-fold higher than that found in sheep

TABLE 4. Comparison of cervid prion inocula in Tg mice expressing human, bovine, ovine, and murine PrP genes

Transgenic line	PrP expression (*n*-fold)	Inoculum	Incubation time ± SEM (days)	No. of animals ill/no. of animals inoculated[a]
Tg(HuPrP)440	2[b]	Elk4	>540	0/11 A
		Elk5	>540	0/13 B
		Elk6	>532	0/8
		Elk7	>538	0/7
		MD4	>512	0/9
		MD5	>512	0/6
		WTD4	>512	0/5
		WTD5	>539	0/8
		sCJD, M/M129	154 ± 2.6	18/18
Tg(BoPrP)4092	10[c]	Elk4	>622	0/6
		Elk5	>600	0/11 C
		Elk6	>610	0/3 D
		Elk7	>610	0/6
		MD4	>512	0/8
		MD5	>532	0/8 E
		WTD4	>532	0/7
		WTD5	>532	0/7
		BSE PG31/90	244 ± 4.8	9/9
Tg(BoPrP)4125	8 to 16[d]	Elk4	>610	0/10 F
		Elk5	>620	0/8
		BSE PG31/90	340 ± 5.3	41/41
Tg(OvPrP,VRQ)338	12[e]	Elk4	>516	0/5 G
		Elk5	>509	0/8 H
		Elk6	>509	0/11
		MD4	>516	0/9
		MD5	>516	0/11
		WTD4	>505	0/8
		WTD5	>505	0/11 I
		WTD6	>526	0/12
		Scrapie 027	178 ± 9.3	16/16
Tg(MoPrP)4053	8[f]	Elk4	542 ± 32.1	6/8
		Elk5	>590	0/10
		Elk6	>512	0/7 J
		Elk7	>512	0/8
		MD4	>539	0/5
		MD5	>512	0/5
		WTD4	>512	0/3 K
		WTD5	>532	0/10
		RML	59 ± 1.5	10/10

[a] Animals with intercurrent illness at later stages of the experiments died on the indicated days postinoculation (unless otherwise noted, one animal died on the indicated day): A, 428 days; B, 481 days; C, 589 days; D, 588 days (two animals); E, 399 days; F, 384 days; G, 446 and 494 days; H, 232 days (two animals); I, 291 days; J, 518 days; K, 224 days. These animals were determined to be free of prion disease after neuropathologic analysis for spongiform degeneration and immunohistochemistry for PrP and GFAP.

[b] Compared to PrP^C in human brain (47).

[c] Compared to PrP^C in wild-type FVB mouse brain.

[d] Compared to PrP^C in bovine brain (43).

[e] Compared to PrP^C in sheep brain, listed as 6× for the hemizygous line Tg(OvPrP,$VRQ^{+/0}$)338 (26).

[f] Compared to PrP^C in wild-type FVB mouse brain (46).

brain. When inoculated with sheep scrapie prions, the Tg(OvPrP,VRQ)338 mice developed neurologic dysfunction in <200 days. Occasional Tg mice expressing HuPrP, BoPrP, or OvPrP that had been inoculated with CWD prions died at an older age (Table 4). The brains of those mice were examined histologically for spongiform changes and immunohistochemically for PrP and GFAP staining. None of the Tg mice showed evidence of prion disease.

In contrast to Tg mice expressing human, bovine, or ovine PrP, Tg(MoPrP)4053 mice were susceptible to one CWD prion isolate, albeit after prolonged incubation periods (Table 4). Tg(MoPrP)4053 mice express wild-type MoPrP-A at eightfold-higher levels than WT FVB mice. Six of eight mice became ill after inoculation with the Elk4 CWD sample beginning at 460 days, with a mean incubation period of ~540 days. Inoculation with three other CWD samples from elk, two from mule deer, and two from white-tailed deer did not produce disease after >500 days. Neuropathologic examination of diseased Tg(MoPrP)4053 mouse brain showed spongiform degeneration, PrP^{Sc} deposition, and PrP amyloid plaques (Fig. 6I).

DISCUSSION

The overexpression of cervid PrPs in mice did not have any deleterious effect on the Tg lines described here. Uninoculated mice from one such Tg line was observed for ~650 days (Table 1). The absence of spontaneous disease in these Tg mice allowed us to use them to bioassay prions in the brains of elk and deer that died of CWD.

Brainstem samples from elk, mule deer, and white-tailed deer with CWD were inoculated into five Tg lines expressing ElkPrP and two lines expressing DePrP. Bioassay of the Elk1 inoculum in the seven Tg cervid PrP lines showed that the length of the incubation time is inversely proportional to the level of cervid PrP expression in the brain (Fig. 5; Table 2). When the level of cervid PrP^C expression was similar to that of $MoPrP^C$ in WT mice, it was designated 1×. In Tg(DePrP) mice expressing DePrP at 1×, the incubation time was ~300 days, whether the CWD inoculum was from mule deer (MD1) or elk (Elk1). Doubling the level of cervid PrP^C to 2× resulted in a reduction of the incubation time to ~200 days for the Elk1 and MD1 inocula while tripling the expression of cervid PrP^C reduced the incubation time to ~100 days for the Elk1 inoculum. A similar relationship was described earlier for Tg mice expressing $SHaPrP^C$ (35); however, both MoPrP and SHaPrP were coexpressed in those Tg lines. In the studies reported here (Fig. 5), the MoPrP gene was disrupted (10) so that the only PrP being expressed was cervid PrP^C. In a recent study, Tg mice expressing DePrP at 5× and 3× the level of PrP expressed in WT FVB mice developed neurologic deficits at ~235 days after intracerebral inoculation with CWD prions from elk and at 225 to 264 days with CWD prions from mule deer (9). In another study, two lines of Tg mice expressing ElkPrP at 2× developed CNS disease 118 or 142 days after inoculation with CWD prions from elk (23).

The CDI studies of the CWD inocula indicated that the Elk1 and MD1 inocula contained similar levels of PrP^{Sc} (Fig. 1A), which is consistent with the indistinguishable incubation times for these inocula in Tg(ElkPrP)12577, Tg(DePrP)10945, and Tg(DePrP)10969 mice (Table 2). Interestingly, the levels of PrP^{Sc} varied over a >100-fold range for the first nine cervid brain specimens examined (Fig. 1A). Assessing the level of PrP^{Sc} in brain samples in advance of our transmission studies proved to have been quite useful in retrospect (Table 2).

Serial passage of CWD prions in the Tg(ElkPrP) mice resulted in modest reductions in the incubation times, i.e., up to ~70 days (Fig. 3A to D; Table 3). This shortening was seen with prions from elk, mule deer, and white-tailed deer. These

results contrast with those for the serial passaging of BSE prions in Tg(BoPrP) mice and the serial passaging of CWD prions in Tg12 mice, for which no changes in the incubation times were observed (23, 42, 44). There are several possible explanations for the shortening of the incubation times upon serial passage in Tg(ElkPrP)12577 mice. First, the level of prions in the brains of cervids may be lower than in Tg(Elk PrP)12577 mice. If that were the case, then the first Tg mouse-to-Tg mouse passages would be expected to exhibit shorter incubation times than those found with passages from the cervids to Tg mice. A corollary to this scenario is that the incubation times upon subsequent passage in a given Tg line should remain constant. Second, the cervid brain inocula may be composed of a mixture of strains, and one strain may emerge as the predominant strain over the length of the incubation time. In this case, the predominant strain in the Tg mouse brain exhibits a shorter incubation time during the next passage, because it exists at a higher titer in the mouse brain than in the cervid brain sample. Third, within a mixture of prion strains, some slow strains may be inhibitory for faster ones as previously reported (13, 22). If this were the case and transmission to Tg mice resulted in the elimination of the slower strain, then on subsequent passage in Tg mice, the incubation time would shorten. Fourth, a posttranslational modification in cervid PrP, such as the N-linked oligosaccharides or the glycosylphosphatidylinositol anchor, might slow replication of cervid prions in the Tg mice. If this were the case, then on subsequent passage, CWD prions formed in a mouse would exhibit shorter incubation times.

Except for the first possibility, for which endpoint titrations can be used to establish the titers of CWD prions in cervid and Tg mouse brains, distinguishing among the possibilities listed above may be difficult. Interpreting such a titration study will be facilitated if endpoint titrations in cervids give results similar to those obtained with the Tg mice. It is notable that endpoint titrations performed with cattle resulted in a titer of 10^6 50% infective dose units/g of brain tissue from the obexes of BSE-infected cattle, whereas endpoint titrations performed with Tg(BoPrP) mice resulted in a titer of $10^{6.9}$ 50% infective dose units/g of brain tissue (39, 42, 50).

Both the glycoform abundance patterns and the distribution of neuropathologic lesions in CWD-inoculated Tg(ElkPrP) and Tg(DePrP) mice argue for a single prion strain. The molecular masses of the di-, mono-, and unglycosylated PrP^{Sc} fragments from all CWD isolates were similar before (Fig. 2A) and after passaging in Tg(ElkPrP)12577 (Fig. 2B and D) and Tg(DePrP)10945 (Fig. 2C) mice. Moreover, the relative abundance of these glycoforms did not change upon passage in the Tg mice (see Fig. S1 in the supplemental material).

Neuropathologic examination of ill Tg(ElkPrP) and Tg(DePrP) mice demonstrated similar CNS lesions in all of the mice inoculated with CWD prions from elk, mule deer, and white-tailed deer (Fig. 6). The deposition patterns and neuroanatomic distribution of both PrP^{Sc} deposition (Fig. 7) and florid PrP amyloid plaques (Fig. 6) were similar for all inocula but differed somewhat in intensity. Upon serial passage in Tg(Elk PrP) mice, the CNS lesions remained unchanged (data not shown). Overall, our neuropathologic findings for CWD-infected Tg mice expressing DePrP or ElkPrP did not differ substantially from those reported by others (9, 23).

In Tg(MoPrP)4053 mice inoculated with CWD prions, both the morphology of the lesions and distribution of PrP amyloid plaques (Fig. 6I) were different from those found in Tg(Elk PrP) and Tg(DePrP) mice. In contrast to RML prions that produce finely granular deposits of PrP^{Sc} in the absence of amyloid plaques, the CWD prion strain was amyloidogenic in the brains of Tg(MoPrP)4053 mice.

In contrast to Tg mice expressing cervid PrP, Tg mice expressing human, bovine, or ovine PrP did not succumb to prion disease after inoculation with CWD prions after >500 days (Table 4). Our results agree with those of others who reported that Tg mice expressing human PrP were resistant to CWD prions but susceptible to sCJD prions (23). Despite our confirmation of an earlier study demonstrating that Tg mice expressing HuPrP(M129) do not develop prion disease when inoculated with CWD prions, any conclusions from such negative data need to be tempered (7), especially in light of a recent study with squirrel monkeys. Intracerebral inoculation of CWD prions into squirrel monkeys (*Saimiri sciureus*) demonstrated transmission to a nonhuman primate, arguing that humans might be susceptible to CWD prions (27).

While our studies of Tg(BoPrP) mice inoculated with CWD prions also gave negative results, a recent study reported that five of 13 cattle inoculated with CWD prions produced PrP^{Sc}, based on limited proteolysis and immunohistochemical staining of brain sections (16). These studies were terminated 6 years after intracerebral inoculation, before any of the cattle developed neurologic dysfunction. In other studies, sheep scrapie prions were inoculated into elk (17). After more than three years, PrP^{Sc} was found in the brains of three elk that developed neurologic deficits before dying. Our negative results with CWD prions inoculated into Tg(HuPrP), Tg(Bo PrP), and Tg(OvPrP) mice might reflect low levels of prion replication that are insufficient to produce disease during the 500-day observation period. Several investigators have described situations in which prions from one species replicate too slowly in another species to cause signs of neurologic dysfunction but do produce disease with serial passage (19, 36). In the studies reported here, we did not choose to passage serially the brains of asymptomatic, CWD-inoculated Tg(Hu PrP), Tg(BoPrP), and Tg(OvPrP) mice (Table 4). An alternative explanation for our negative results may reside in the strain(s) of CWD prions that we used for inoculation. While our CWD prions were unable to initiate the conversion of $HuPrP^C$, $BoPrP^C$, or $OvPrP^C$ into PrP^{Sc}, some other CWD strains might be able to do so. Precedent for the latter has been seen with human prions: human vCJD prions replicate well in Tg(BoPrP) mice but multiply slowly in Tg(HuPrP) mice and in Tg(MHu2M) mice expressing a chimeric human-mouse PrP (2, 24, 42, 44).

Whether hunters, cervid farmers, and aficionados of venison are at increased risk for prion disease remains to be established. Recently, CWD prions were also reported in the skeletal muscles of infected deer, indicating a possible hazard for the oral transmission of CWD prions (1). Tg(ElkPrP) and Tg(DePrP) mice provide a sensitive and specific bioassay for determining the levels of CWD prion infectivity in cervid tissues and for studying the biology of these particular prions. These Tg mice make it possible to determine the levels of CWD prion titers in brain, muscle, liver, pancreas, kidney, and

other tissues as well as in the blood, urine, feces, and saliva of both elk and deer. Elucidating the mode of CWD prion spread among elk, deer, and moose will be important for understanding why CWD prions are so contagious for domesticated and free-ranging cervids. Such information may prove useful in learning how to restrict the epizootic spread of CWD prions to humans and livestock.

ACKNOWLEDGMENTS

G.T. was supported by a fellowship from the German Research Foundation. This work was supported by grants from the National Institutes of Health (AG02132) as well as the Rockefeller Brothers Fund and Goldman Philanthropic Partnerships.

We thank Pierre Lessard and the staff at the Hunters Point animal facility for support with the transgenic animal experiments, Giuseppe Legname for antibodies, Hang Nguyen for editorial assistance, Jian Yang and Peter Nelken for assistance in neuropathology, Svetlana Didorenko and Camille Deering for assistance with the CDI, the late Allen L. Jenny (National Veterinary Services Laboratory, Ames, Iowa) and the late Elizabeth S. Williams (Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, Wyo.) for providing CWD-infected brain samples, and Hubert Laude (Unité de Virologie Immunologie Moléculaires, Institut National de la Recherche Agronomique, Jouy-en-Josas Cedex, France) for his gift of Tg(OvPrP,VRQ)338 mice.

J.S., K.G., S.J.D., and S.B.P. have financial interest in InPro Biotechnology.

REFERENCES

1. **Angers, R. C., S. R. Browning, T. S. Seward, C. J. Sigurdson, M. W. Miller, E. A. Hoover, and G. C. Telling.** 2006. Prions in skeletal muscles of deer with chronic wasting disease. Science 311:1117.
2. **Asante, E. A., J. M. Linehan, M. Desbruslais, S. Joiner, I. Gowland, A. L. Wood, J. Welch, A. F. Hill, S. E. Lloyd, J. D. Wadsworth, and J. Collinge.** 2002. BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. EMBO J. 21:6358–6366.
3. **Bahmanyar, S., E. S. Williams, F. B. Johnson, S. Young, and D. C. Gajdusek.** 1985. Amyloid plaques in spongiform encephalopathy of mule deer. J. Comp. Pathol. 95:1–5.
4. **Bartz, J. C., R. F. Marsh, D. L. McKenzie, and J. M. Aiken.** 1998. The host range of chronic wasting disease is altered on passage in ferrets. Virology 251:297–301.
5. **Belay, E. D., R. A. Maddox, E. S. Williams, M. W. Miller, P. Gambetti, and L. B. Schonberger.** 2004. Chronic wasting disease and potential transmission to humans. Emerg. Infect. Dis. 10:977–984.
6. **Bellworthy, S. J., G. Dexter, M. Stack, M. Chaplin, S. A. Hawkins, M. M. Simmons, M. Jeffrey, S. Martin, L. Gonzalez, and P. Hill.** 2005. Natural transmission of BSE between sheep within an experimental flock. Vet. Rec. 157:206.
7. **Bosque, P. J.** 2002. Bovine spongiform encephalopathy, chronic wasting disease, scrapie, and the threat to humans from prion disease epizootics. Curr. Neurol. Neurosci. Rep. 2:488–495.
8. **Brayton, K. A., K. I. O'Rourke, A. K. Lyda, M. W. Miller, and D. P. Knowles.** 2004. A processed pseudogene contributes to apparent mule deer prion gene heterogeneity. Gene 326:167–173.
9. **Browning, S. R., G. L. Mason, T. Seward, M. Green, G. A. Eliason, C. Mathiason, M. W. Miller, E. S. Williams, E. Hoover, and G. C. Telling.** 2004. Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. J. Virol. 78:13345–13350.
10. **Büeler, H., M. Fisher, Y. Lang, H. Bluethmann, H.-P. Lipp, S. J. DeArmond, S. B. Prusiner, M. Aguet, and C. Weissmann.** 1992. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 356:577–582.
11. **Carlson, G. A., P. A. Goodman, M. Lovett, B. A. Taylor, S. T. Marshall, M. Peterson-Torchia, D. Westaway, and S. B. Prusiner.** 1988. Genetics and polymorphism of the mouse prion gene complex: control of scrapie incubation time. Mol. Cell. Biol. 8:5528–5540.
12. **Chandler, R. L.** 1961. Encephalopathy in mice produced by inoculation with scrapie brain material. Lancet 1:1378–1379.
13. **Dickinson, A. G., H. Fraser, V. M. H. Meikle, and G. W. Outram.** 1972. Competition between different scrapie agents in mice. Nat. New Biol. 237: 244–245.
14. **Eloit, M., K. Adjou, M. Coulpier, J. J. Fontaine, R. Hamel, T. Lilin, S. Messiaen, O. Andreoletti, T. Baron, A. Bencsik, A. G. Biacabe, V. Beringue, H. Laude, A. Le Dur, J. L. Vilotte, E. Comoy, J. P. Deslys, J. Grassi, S. Simon, F. Lantier, and P. Sarradin.** 2005. BSE agent signatures in a goat. Vet. Rec. 156:523–534.
15. **Guiroy, D. C., E. S. Williams, R. Yanagihara, and D. C. Gajdusek.** 1991. Immunolocalization of scrapie amyloid (PrP27-30) in chronic wasting disease of Rocky Mountain elk and hybrids of captive mule deer and white-tailed deer. Neurosci. Lett. 126:195–198.
16. **Hamir, A. N., R. A. Kunkle, R. C. Cutlip, J. M. Miller, K. I. O'Rourke, E. S. Williams, M. W. Miller, M. J. Stack, M. J. Chaplin, and J. A. Richt.** 2005. Experimental transmission of chronic wasting disease agent from mule deer to cattle by the intracerebral route. J. Vet. Diagn. Investig. 17:276–281.
17. **Hamir, A. N., J. M. Miller, R. C. Cutlip, R. A. Kunkle, A. L. Jenny, M. J. Stack, M. J. Chaplin, and J. A. Richt.** 2004. Transmission of sheep scrapie to elk (*Cervus elaphus nelsoni*) by intracerebral inoculation: final outcome of the experiment. J. Vet. Diagn. Investig. 16:316–321.
18. **Heaton, M. P., K. A. Leymaster, B. A. Freking, D. A. Hawk, T. P. Smith, J. W. Keele, W. M. Snelling, J. M. Fox, C. G. Chitko-McKown, and W. W. Laegreid.** 2003. Prion gene sequence variation within diverse groups of U.S. sheep, beef cattle, and deer. Mamm. Genome 14:765–777.
19. **Hill, A. F., and J. Collinge.** 2003. Subclinical prion infection in humans and animals. Br. Med. Bull. 66:161–170.
20. **Johnson, C., J. Johnson, M. Clayton, D. McKenzie, and J. Aiken.** 2003. Prion protein gene heterogeneity in free-ranging white-tailed deer within the chronic wasting disease affected region of Wisconsin. J. Wildl. Dis. 39:576–581.
21. **Kim, T. Y., H. J. Shon, Y. S. Joo, U. K. Mun, K. S. Kang, and Y. S. Lee.** 2005. Additional cases of chronic wasting disease in imported deer in Korea. J. Vet. Med. Sci. 67:753–759.
22. **Kimberlin, R. H., and C. A. Walker.** 1985. Competition between strains of scrapie depends on the blocking agent being infectious. Intervirology 23: 74–81.
23. **Kong, Q., S. Huang, W. Zou, D. Vanegas, M. Wang, D. Wu, J. Yuan, M. Zheng, H. Bai, H. Deng, K. Chen, A. L. Jenny, K. O'Rourke, E. D. Belay, L. B. Schonberger, R. B. Petersen, M. S. Sy, S. G. Chen, and P. Gambetti.** 2005. Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models. J. Neurosci. 25:7944–7999.
24. **Korth, C., K. Kaneko, D. Groth, N. Heye, G. Telling, J. Mastrianni, P. Parchi, P. Gambetti, R. Will, J. Ironside, C. Heinrich, P. Tremblay, S. J. DeArmond, and S. B. Prusiner.** 2003. Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. Proc. Natl. Acad. Sci. USA 100:4784–4789.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. Nature 227:680–685.
26. **Laude, H., D. Vilette, A. Le Dur, F. Archer, S. Soulier, N. Besnard, R. Essalmani, and J. L. Vilotte.** 2002. New *in vivo* and *ex vivo* models for the experimental study of sheep scrapie: development and perspectives. C. R. Biol. 325:49–57.
27. **Marsh, R. F., A. E. Kincaid, R. A. Bessen, and J. C. Bartz.** 2005. Interspecies transmission of chronic wasting disease prions to squirrel monkeys (*Saimiri sciureus*). J. Virol. 79:13794–13796.
28. **Miller, M. W., and E. S. Williams.** 2003. Prion disease: horizontal prion transmission in mule deer. Nature 425:35–36.
29. **Muramoto, T., S. J. DeArmond, M. Scott, G. C. Telling, F. E. Cohen, and S. B. Prusiner.** 1997. Heritable disorder resembling neuronal storage disease in mice expressing prion protein with deletion of an α-helix. Nat. Med. 3:750–755.
30. **Muramoto, T., T. Kitamoto, J. Tateishi, and I. Goto.** 1992. The sequential development of abnormal prion protein accumulation in mice with Creutzfeldt-Jakob disease. Am. J. Pathol. 140:1411–1420.
31. **O'Rourke, K. I., T. E. Besser, M. W. Miller, T. F. Cline, T. R. Spraker, A. L. Jenny, M. A. Wild, G. L. Zebarth, and E. S. Williams.** 1999. PrP genotypes of captive and free-ranging Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease. J. Gen. Virol. 80:2765–2769.
32. **O'Rourke, K. I., T. R. Spraker, L. K. Hamburg, T. E. Besser, K. A. Brayton, and D. P. Knowles.** 2004. Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer. J. Gen. Virol. 85:1339–1346.
33. **Peretz, D., R. A. Williamson, K. Kaneko, J. Vergara, E. Leclerc, G. Schmitt-Ulms, I. R. Mehlhorn, G. Legname, M. R. Wormald, P. M. Rudd, R. A. Dwek, D. R. Burton, and S. B. Prusiner.** 2001. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. Nature 412:739–743.
34. **Prusiner, S. B.** 1998. Prions. Proc. Natl. Acad. Sci. USA 95:13363–13383.
35. **Prusiner, S. B., M. Scott, D. Foster, K.-M. Pan, D. Groth, C. Mirenda, M. Torchia, S.-L. Yang, D. Serban, G. A. Carlson, P. C. Hoppe, D. Westaway, and S. J. DeArmond.** 1990. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell 63:673–686.
36. **Race, R., K. Meade-White, A. Raines, G. J. Raymond, B. Caughey, and B. Chesebro.** 2002. Subclinical scrapie infection in a resistant species: persistence, replication, and adaptation of infectivity during four passages. J Infect. Dis. 186:S166–S170.
37. **Safar, J., H. Wille, V. Itri, D. Groth, H. Serban, M. Torchia, F. E. Cohen, and**

S. B. Prusiner. 1998. Eight prion strains have PrP^{Sc} molecules with different conformations. Nat. Med. 4:1157–1165.
38. Safar, J. G., M. D. Geschwind, C. Deering, S. Didorenko, M. Sattavat, H. Sanchez, A. Serban, M. Vey, H. Baron, K. Giles, B. L. Miller, S. J. DeArmond, and S. B. Prusiner. 2005. Diagnosis of human prion disease. Proc. Natl. Acad. Sci. USA 102:3501–3506.
39. Safar, J. G., M. Scott, J. Monaghan, C. Deering, S. Didorenko, J. Vergara, H. Ball, G. Legname, E. Leclerc, L. Solforosi, H. Serban, D. Groth, D. R. Burton, S. B. Prusiner, and R. A. Williamson. 2002. Measuring prions causing bovine spongiform encephalopathy or chronic wasting disease by immunoassays and transgenic mice. Nat. Biotechnol. 20:1147–1150.
40. Scott, M., D. Groth, D. Foster, M. Torchia, S.-L. Yang, S. J. DeArmond, and S. B. Prusiner. 1993. Propagation of prions with artificial properties in transgenic mice expressing chimeric PrP genes. Cell 73:979–988.
41. Scott, M. R., R. Köhler, D. Foster, and S. B. Prusiner. 1992. Chimeric prion protein expression in cultured cells and transgenic mice. Prot. Sci. 1:986–997.
42. Scott, M. R., D. Peretz, H.-O. B. Nguyen, S. J. DeArmond, and S. B. Prusiner. 2005. Transmission barriers for bovine, ovine, and human prions in transgenic mice. J. Virol. 79:5259–5271.
43. Scott, M. R., J. Safar, G. Telling, O. Nguyen, D. Groth, M. Torchia, R. Koehler, P. Tremblay, D. Walther, F. E. Cohen, S. J. DeArmond, and S. B. Prusiner. 1997. Identification of a prion protein epitope modulating transmission of bovine spongiform encephalopathy prions to transgenic mice. Proc. Natl. Acad. Sci. USA 94:14279–14284.
44. Scott, M. R., R. Will, J. Ironside, H.-O. B. Nguyen, P. Tremblay, S. J. DeArmond, and S. B. Prusiner. 1999. Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc. Natl. Acad. Sci. USA 96:15137–15142.
45. Taraboulos, A., K. Jendroska, D. Serban, S.-L. Yang, S. J. DeArmond, and S. B. Prusiner. 1992. Regional mapping of prion proteins in brains. Proc. Natl. Acad. Sci. USA 89:7620–7624.
46. Telling, G. C., T. Haga, M. Torchia, P. Tremblay, S. J. DeArmond, and S. B. Prusiner. 1996. Interactions between wild-type and mutant prion proteins modulate neurodegeneration in transgenic mice. Genes Dev. 10:1736–1750.
47. Telling, G. C., M. Scott, J. Mastrianni, R. Gabizon, M. Torchia, F. E. Cohen, S. J. DeArmond, and S. B. Prusiner. 1995. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell 83:79–90.
48. Tremblay, P., H. L. Ball, K. Kaneko, D. Groth, R. S. Hegde, F. E. Cohen, S. J. DeArmond, S. B. Prusiner, and J. G. Safar. 2004. Mutant PrP^{Sc} conformers induced by a synthetic peptide and several prion strains. J. Virol. 78:2088–2099.
49. van Duijn, C. M., N. Delasnerie-Laupretre, C. Masullo, I. Zerr, R. de Silva, D. P. Wientjens, J. P. Brandel, T. Weber, V. Bonavita, M. Zeidler, A. Alperovitch, S. Poser, E. Granieri, A. Hofman, R. G. Will et al. 1998. Case-control study of risk factors of Creutzfeldt-Jakob disease in Europe during 1993–95. Lancet 351:1081–1085.
50. Wells, G. A. H. 2002. Report on TSE infectivity distribution in ruminant tissues (state of knowledge, December 2001). European Commission, Brussels, Belgium.
51. Wells, G. A. H., A. C. Scott, C. T. Johnson, R. F. Gunning, R. D. Hancock, M. Jeffrey, M. Dawson, and R. Bradley. 1987. A novel progressive spongiform encephalopathy in cattle. Vet. Rec. 121:419–420.
52. Will, R. G., J. W. Ironside, M. Zeidler, S. N. Cousens, K. Estibeiro, A. Alperovitch, S. Poser, M. Pocchiari, A. Hofman, and P. G. Smith. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347:921–925.
53. Williams, E. S. 2005. Chronic wasting disease. Vet. Pathol. 42:530–549.
54. Williams, E. S., and S. Young. 1980. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. J. Wildl. Dis. 16:89–98.
55. Williams, E. S., and S. Young. 1993. Neuropathology of chronic wasting disease of mule deer (Odocoileus hemionus) and Elk (Cervus elaphus nelsoni). Vet. Pathol. 30:36–45.
56. Xie, Z., K. I. O'Rourke, Z. Dong, A. L. Jenny, J. A. Langenberg, E. D. Belay, L. B. Schonberger, R. B. Petersen, W. Zou, Q. Kong, P. Gambetti, and S. G. Chen. 2006. Chronic wasting disease of elk and deer and Creutzfeldt-Jakob disease: comparative analysis of the scrapie prion protein. J. Biol. Chem. 281:4199–4206.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2006. 12. 26	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Wroe SJ, Pal S, Siddique D, Hyare H, Macfarlane R, Joiner S, Linehan JM, Brandner S, Wadsworth JD, Hewitt P, Collinge J. Lancet. 2006 Dec 9;368(9552):2061-7.	公表国 英国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○輸血に伴う変異型クロイツフェルト・ヤコブ病の臨床像および生前診断:症例報告

【背景】変異型クロイツフェルト・ヤコブ病(vCJD)が輸血によって感染する可能性があるという懸念が浮上している。vCJDを発症した供血者の血液を輸血されたことが判明している集団から、プリオン感染症2例が死後に特定され、2004年に報告された。このリスク群の別の1名の患者は、神経学的徴候を発現し、National Prion Clinicへ照会された。

【方法】この1名の患者は検査のため入院となり、National Blood ServiceおよびHealth Protection Agencyから輸血歴の詳細が得られた。当該患者はvCJDと診断された後、MRC PRION-1 trialに登録された。患者が死亡した際、剖検時に脳と扁桃腺の組織を得、免疫ブロッティング法および免疫組織化学検査により異常プリオンの存在を調べた。

【知見】vCJDであることがほぼ確定という臨床診断が下された。扁桃腺の生検は実施しなかった。当該患者は、キナクリンを用いた実験的治療を受けたが、容体は悪化し、典型的なvCJDの臨床経過を経て死亡した。剖検により診断が確認され、扁桃腺のプリオン感染が示された。

【解釈】死亡前に特定された当該輸血関連vCJD症例は、後にvCJDを発症した供血者から輸血を受けて5年以上生存した受血者集団23名からの3例目の患者である。この輸血を受けた残りの受血者のリスクは高いと考えられるため、専門医のフォローアップと検査を受けるべきである。扁桃腺の生検は、ウシ海綿状脳症プリオンの1次感染患者と同様、医原的曝露を被った他の高リスク患者においても、早期の症状発現前診断を可能にする。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
英国で3例目の輸血関連変異型クロイツフェルト・ヤコブ病の症例報告である。	日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ケ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report

Stephen J Wroe, Suvankar Pal, Durrenajaf Siddique, Harpreet Hyare, Rebecca Macfarlane, Susan Joiner, Jacqueline M Linehan, Sebastian Brandner, Jonathan D F Wadsworth, Patricia Hewitt, John Collinge

Summary

Background Concerns have been raised that variant Creutzfeldt-Jakob disease (vCJD) might be transmissible by blood transfusion. Two cases of prion infection in a group of known recipients of transfusion from donors who subsequently developed vCJD were identified post-mortem and reported in 2004. Another patient from this at-risk group developed neurological signs and was referred to the National Prion Clinic.

Methods The patient was admitted for investigation and details of blood transfusion history were obtained from the National Blood Service and Health Protection Agency; after diagnosis of vCJD, the patient was enrolled into the MRC PRION-1 trial. When the patient died, brain and tonsil tissue were obtained at autopsy and assessed for the presence of disease-related PrP by immunoblotting and immunohistochemistry.

Findings A clinical diagnosis of probable vCJD was made; tonsil biopsy was not done. The patient received experimental therapy with quinacrine, but deteriorated and died after a clinical course typical of vCJD. Autopsy confirmed the diagnosis and showed prion infection of the tonsils.

Interpretation This case of transfusion-associated vCJD infection, identified ante-mortem, is the third instance from a group of 23 known recipients who survived at least 5 years after receiving a transfusion from donors who subsequently developed vCJD. The risk to the remaining recipients of such tranfusions is probably high, and these patients should be offered specialist follow-up and investigation. Tonsil biopsy will allow early and pre-symptomatic diagnosis in other iatrogenically exposed individuals at high risk, as in those with primary infection with bovine spongiform encephalopathy prions.

Lancet 2006; 368: 2061–67

See Comment page 2037

National Prion Clinic, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK (S J Wroe FRCP, S Pal MRCP, D Siddique MRCP, H Hyare FRCR, R Macfarlane MRCS, J Collinge FRS); MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, UK (S J Wroe, S Pal, D Siddique, H Hyare, R Macfarlane, J Collinge, S Joiner MSc, J M Linehan BSc, S Brandner MRCPath, J D F Wadsworth PhD); and National Blood Service, London, UK (P Hewitt FRCPath)

Correspondence to:
Prof John Collinge
j.collinge@prion.ucl.ac.uk

Introduction

Since the arrival of variant Creutzfeldt-Jakob disease (vCJD), and the experimental confirmation that it is caused by the same prion strain as that causing epidemic bovine spongiform encephalopathy (BSE), there have been concerns that it might be transmissible by iatrogenic routes including use of blood and blood products.[1] Such concerns were heightened by the recognition that the pathogenesis of vCJD differed substantially from that of sporadic or classical CJD by showing prominent and uniform prion accumulation in lymphoreticular tissues[2,3] akin to ovine scrapie, where prionaemia has since been demonstrated experimentally.[4] All clinically affected patients with vCJD identified to date have been homozygous for methionine at polymorphic codon 129 of the prion protein (PrP) gene, *PRNP*, where either methionine (M) or valine (V) may be encoded.

Several risk reduction measures have been introduced in the UK and some other countries to limit secondary transmission of vCJD prions by blood and surgical instruments. In 1997, a study was set up between the National CJD Surveillance Unit and UK Blood Services so that recipients of blood components from donors who subsequently developed vCJD (ie, vCJD-implicated components) could be identified and evidence sought for possible transmission of vCJD by blood transfusion.[5]

In 2004, two transfusion-associated cases of vCJD prion infection were reported in individuals who had been identified in this way. The first was in a 62-year-old patient who had received a single vCJD-implicated non-leucodepleted unit of red cells and who became symptomatic 6·5 years post-transfusion and died after a progressive neurological illness of estimated 13 months' duration. The pulvinar sign was not seen on brain MRI and tonsil biopsy was not done. The cause of death was recorded as dementia.[5] After autopsy, neuropathology was thought to be suggestive of CJD, and vCJD was subsequently confirmed by neuropathological examination; the clinical presentation of vCJD in the case was judged typical of vCJD.[5]

The second case was in an elderly patient who had also received a single vCJD-implicated unit of non-leucodepleted red cells and who died from an unrelated cause, without evidence of a neurological disorder, 5 years after receiving the transfusion. Western blot analysis for the disease-related prion protein isoform (PrP^{Sc}) and PrP immunohistochemistry of lymphoreticular tissues was consistent with vCJD prion infection.[6] Interestingly, this patient was heterozygous (MV) at *PRNP* codon 129, a genotype that is associated with relative resistance to CJD and other prion diseases.[7] The cohort of identified recipients of blood transfusion from donors who

For UK Blood Services see http://www.cjd.ed.ac.uk/TMER/TMER.htm