Published in final edited form as:

Science. 2006 July 7; 313(5783): 94–97.

Prion-Induced Amyloid Heart Disease with High Blood Infectivity in Transgenic Mice

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Abstract

We investigated extraneural manifestations in scrapie-infected transgenic mice expressing prion protein lacking the glycoposphatidylinositol membrane anchor. In the brain, blood, and heart, both abnormal protease-resistant prion protein (PrPres) and prion infectivity were readily detected by immunoblot and by inoculation into nontransgenic recipients. The titer of infectious scrapie in blood plasma exceeded 10^7 50% infectious doses per milliliter. The hearts of these transgenic mice contained PrPres-positive amyloid deposits that led to myocardial stiffness and cardiac disease.

In humans and animals, transmissible spongiform encephalopathies (TSEs), or prion diseases, cause neurodegeneration and death following ingestion or experimental inoculation of infected material. Prion diseases are characterized by the conversion of the normal protease-sensitive host prion protein (PrPsen) to a disease-associated protease-resistant form (PrPres). Although prion disease damages the central nervous system (CNS), infectivity and PrPres can be detected within peripheral tissues, including lymphoid organs in humans, sheep, and deer (1, 2), as well as skeletal muscle (3), kidney, and pancreas (4) of some transgenic rodent models. Despite the toxic effect on the CNS, few if any histopathological changes have been observed at peripheral sites.

Transmission of TSE disease to humans has resulted from cannibalism, contaminated surgical instrumentation, and tainted growth hormone (5–7). A human disease termed variant Creutzfeldt-Jakob disease (vCJD) has occurred more recently, apparently through the ingestion of bovine spongiform encephalopathy (BSE)-infected cattle products (8). Recent evidence suggests that transmission of vCJD between humans may occur through blood transfusion (9, 10), and this conclusion is supported by experimental transmission of BSE between sheep via blood transfusion (11). TSE infectivity has been demonstrated in blood by intracerebral-

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Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5783/94/DC1

Materials and Methods

Tables S1 and S2

References

inoculation in mouse, mink, hamster, and goat models (7, 12–20). However, infectivity in such cases is low, $\leq 10^2$ 50% infectious doses (ID_{50}) per ml of blood compared to 10^6 to 10^{10} ID_{50} /g in the brain.

Normal prion protein, PrP^{sc}, is expressed primarily as a membrane-bound, glycosylphosphatidylinositol (GPI)-anchored protein. The role of cellular PrP membrane anchoring in prion disease has been studied in transgenic mice expressing GPI-negative anchorless PrP, which is secreted from cells (21). Intracerebral inoculation of these GPI-negative anchorless PrP transgenic (tg) mice with murine scrapie results in scrapie replication and deposition of PrP^{sc} within the brain. Although wild-type (WT) mice infected with scrapie usually develop a nonamyloid form of PrP^{sc}, in these tg mice the PrP^{sc} is primarily in the form of amyloid plaques (21). At the same time, these mice do not manifest the clinical and pathologic alterations normally associated with prion disease, thus demonstrating a separation between PrP^{sc} amyloid accumulation and clinical CNS disease (21). In the brain of these infected tg mice, PrP^{sc} was located primarily within and around endothelial cells (21) (Fig. 1A), leading to the hypothesis that anchorless PrP^{sc} may be secreted in the blood. Here we examined this possibility.

To determine whether PrP^{sc} and/or scrapie infectivity was present in blood, four infected tg mice were bled between 450 and 512 days postinfection (dpi) with the RML strain of scrapie. Inoculation of a 1:500 dilution of blood from all four mice induced scrapie in WT (C57BL/6) recipients in ~145 days. In addition, blood of two mice analyzed by serial dilution titration gave titers of $\geq 1.6 \times 10^7$ and $\geq 1.6 \times 10^5$ ID_{50} /ml blood (Table 1).

Blood was also tested by immunoblotting for PrP^{sc}. At 500 dpi, blood from infected tg mice had detectable PrP^{sc} in both plasma and cellular fractions (Fig. 1B). PrP^{sc} in the plasma was four times as abundant as in the cellular fraction (B and F). With 250 to 500 μ l of plasma, five out of six additional infected tg mice analyzed between 160 and 500 dpi displayed PrP^{sc} (Fig. 1C). PrP^{sc} was not detected at 160 dpi but was found in all tg mice at 281 dpi and thereafter.

In these tg mice, similar brain manifestations are induced by all three scrapie strains tested (RML, 22L, and ME7) (21). However, between 300 and 500 dpi with strain 22L, PrP^{sc} was not detected in blood cells or plasma. Thus, the RML strain appeared to be exceptionally effective in inducing PrP^{sc} in the blood of these tg mice.

Next we examined whether PrP^{sc} could deposit within extraneural tissues. By immunoblot, PrP^{sc} was found in the spleen and heart, but not in the thymus, of infected tg mice at 400 dpi (Fig. 1D). In infected WT mice with clinical disease at the time near death (160 dpi), PrP^{sc} was found in the spleen; however, no PrP^{sc} was found in the heart or thymus. PrP^{sc} was also not detected in the spleen, heart, thymus, or brain of mock-infected tg mice at any times tested (200 to 425 dpi). Thus, accumulation of PrP^{sc} in the heart occurred in infected tg mice only.

To determine the timing of PrP^{sc} accumulation in the heart, infected tg mice were killed at 100 to 650 dpi, and tissues were examined by immunoblot (Fig. 1E). Infected tg mice had detectable PrP^{sc} by 300 dpi, with levels steadily increasing over the 650 days of observation (Fig. 1, E and G). At 600 dpi, the amount of PrP^{sc} in the heart tissue was about one-half of that in brain homogenates of RML-infected WT mice (Fig. 1G). Mock-infected tg mice up to 575 dpi ($n = 10$) and scrapie-infected WT mice up to 160 dpi ($n = 10$) showed no detectable PrP^{sc} in their hearts by immunoblot or by immunohistochemistry. To determine whether scrapie strains differed in PrP^{sc} deposition in the heart, we also injected murine scrapie strains 22L and ME7 into groups of tg mice. Both these strains induced significant amounts of PrP^{sc} in the heart, which was detected by 350 dpi.

We analyzed infectivity titers of heart tissue by using two tg mice infected with RML scrapie. Whereas mock-infected tg mice and scrapie-infected WT mice had no detectable infectivity in heart, titers in infected tg mice at 480 and 507 dpi exceeded 3.0×10^6 ID₅₀ per heart (Table 1). Previously, titers from 2×10^6 to $>4 \times 10^8$ ID₅₀ per brain have been observed in infected tg mice (21).

We next performed immunohistopathologic studies to characterize the PrPres distribution within cardiac tissue. Immunohistochemical staining revealed the presence of PrPres in heart by 300 dpi. Most PrPres staining was between myocardial cells, often around or in the walls of capillaries (Fig. 2, B to D). Staining of consecutive sections also revealed thioflavin S (Fig. 2, C and D) and Congo red between myocardial cells, indicating amyloid deposition in the corresponding areas. By 500 dpi, PrPres and thioflavin staining was seen throughout the heart (Fig. 2, E and F) and occasionally was observed on or within myocardial cells (Fig. 2G). By light microscopy, myocardial tissue was predominantly normal; however, by Trichrome staining, a few scattered foci of fibrosis were observed, although these did not exceed 1 to 5% of the total heart tissue.

Mice infected with strain 22L also developed similar PrPres amyloid deposition in cardiac tissue, primarily in areas of microvasculature between the cardiac myocytes and around larger blood vessels (Fig. 2H).

A hallmark of cardiac amyloidosis is a restrictive cardiomyopathy characterized by reduced distensibility or increased stiffness of the heart during diastole. This reduced distensibility is best characterized by the diastolic pressure-volume relation obtained by catheterization of the intact left ventricle. To study possible disordered cardiac function in scrapie-infected tg mice, we compared infected versus uninfected mice by using a series of physiological tests. We found no significant differences in ventricular chamber sizes during end-diastole (left ventricular diastolic diameter) or end-systole (left ventricular systolic diameter), wall thickness (interventricular septum thickness and posterior wall thickness), or fractional shortening, according to echocardiographic analysis (tables S1 and S2). Pressure-volume analysis was performed with catheterization of the left ventricle to determine diastolic and systolic function as described (22). Load-independent systolic function was significantly decreased in the infected PrPres tg mice, as demonstrated by a decrease in the slope of the end-systolic pressure-volume relation (E_{max}) (Fig. 3A). In addition, there was a significant increase in end-diastolic pressure at baseline without an increase in end-diastolic volume in infected tg mice (Fig. 3A). However, the time constant of relaxation, tau, an index for cardiac active relaxation, showed no significant differences between the two groups.

Volume loading (0.5-ml normal saline bolus injection) was used to further assess diastolic distensibility. End-diastolic pressure after volume loading increased in both groups, but remained significantly higher in infected tg mice (Fig. 3B). After volume loading, the preload was altered by transient occlusion of the inferior vena cava, allowing determination of the end-diastolic pressure-volume relation at the increased volumes. This demonstrated a significant increase ($P \leq 0.05$) in the slope of the end-diastolic pressure-volume relation in the infected tg mice, indicating an increase in stiffness of the left ventricle during diastole (23) (Fig. 3B). The increased stiffness and decreased systolic function are characteristic of findings in the early phase of human cardiac amyloidosis (24). Thus, the tg mice described in this study appear to have a functional cardiac amyloidosis induced by scrapie infection.

The tg mice used in the present study expressed GPI-negative anchorless PrP, which was secreted from cells (21). After scrapie infection, these tg mice produced a soluble form of PrPres found in plasma and blood cells, and the blood contained high levels of scrapie infectivity. The synthesis of secretable anchorless PrP in the infected tg mice is most likely

responsible for the accumulation of infectivity and PrPres in the blood. After RML scrapie infection, tg mice produced much greater amounts of blood PrPres than did infected WT mice (25), in which 10 million-fold cyclic amplification was required for detection.

Concern about TSE transmission through blood transfusion has grown, and hence the need for reliable tests to screen the human blood supply has become increasingly important. The tg mouse model described in this work reliably produces detectable PrPres and prion infectivity within the blood, so it should be useful for determining the sensitivity of new diagnostic kits and for testing the effectiveness of methods for removal of infectivity.

Evaluation of hearts from infected tg mice indicated the presence of PrPres, amyloid, and scrapie infectivity. Although PrPres was occasionally noted within or on myocardial cells late in infection, most PrPres was found along capillaries in the interstitial spaces. The deposition of amyloid fibrils probably causes the abnormalities in systolic and diastolic ventricular function that limit motion of the ventricular myocytes in this model. However, there was minimal microscopic evidence for myocardial cell damage. These observations are consistent with the modest severity of the cardiac disease in the infected tg mice and suggest that considerable time is required for the phenotype to develop. This is typical of the presentation of amyloid heart disease in humans, in whom diagnosis of the disease before 30 years of age is rare (23).

Recently, the deposition of PrPres within a diseased human heart of a 43-year-old CJD patient was reported (26). Low amounts of PrPres have also been detected by enzyme-linked immunosorbent assay in heart tissue of monkeys inoculated intracerebrally with vCJD (27). Together, these studies suggest that humans and nonhuman primates might occasionally deposit cardiac PrPres, similar to the results obtained with our tg mice. Future human and primate studies should consider more extensive testing for this possibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References and Notes

1. Fraser H, Dickinson AG. *Nature* 1970;226:462. [PubMed: 4985820]
2. Prusiner SB. *Science* 1982;216:136. [PubMed: 6801762]
3. Bosque PJ, et al. *Proc Natl Acad Sci USA* 2002;99:3812. [PubMed: 11904434]
4. Heikenwalder M, et al. *Science* 2005;307:1107. [PubMed: 15661974]
5. Alperovitch A, et al. *Lancet* 1999;353:1673. [PubMed: 10335789]
6. Gajdusek DC, Gibbs CJ Jr. *Res Publ Assoc Res Nerv Ment Dis* 1968;44:254. [PubMed: 4978852]
7. Gibbs CJ Jr, et al. *Science* 1968;161:388. [PubMed: 5661299]
8. Scott MR, et al. *Proc Natl Acad Sci USA* 1999;96:15137. [PubMed: 10611351]
9. Llewelyn CA, et al. *Lancet* 2004;363:417. [PubMed: 14962520]
10. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. *Lancet* 2004;364:527. [PubMed: 15302196]
11. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. *Lancet* 2000;356:999. [PubMed: 11041403]
12. Casaccia P, Ladogana A, Xi YG, Pocchiari M. *Arch Virol* 1989;108:145. [PubMed: 2512893]
13. Clarke MC, Haig DA. *Vet Rec* 1967;80:504. [PubMed: 4962208]
14. Collis SC, Kimberlin RH, Millson GC. *J Comp Pathol* 1979;89:389. [PubMed: 528692]
15. Tateishi J, Sato Y, Koga M, Doi H, Ohta M. *Acta Neuropathol (Berlin)* 1980;51:127.
16. Brown P, et al. *Transfusion* 1999;39:1169. [PubMed: 10604242]
17. Brown P, et al. *Transfusion* 1998;38:810. [PubMed: 9738619]
18. Cervenakova L, et al. *Transfusion* 2003;43:1687. [PubMed: 14641865]

19. Holada K, et al. J Virol 2002;76:4649. [PubMed: 11932431]
20. Kuroda Y, Gibbs CJ Jr, Amyx HL, Gajdusek DC. Infect Immun 1983;41:154. [PubMed: 6407995]
21. Chesebro B, et al. Science 2005;308:1435. [PubMed: 15933194]
22. Bale TL, et al. Proc Natl Acad Sci USA 2004;101:3697. [PubMed: 14990799]
23. Gaasch WH. JAMA 1994;271:1276. [PubMed: 8151903]
24. Zile MR, Brutsaert DL. Circulation 2002;105:1387. [PubMed: 11901053]
25. Castilla J, Saa P, Soto C. Nat Med 2005;11:982. [PubMed: 16127436]
26. Ashwath ML, Dearmond SJ, Culclasure T. Arch Intern Med 2005;165:338. [PubMed: 15710800]
27. Herzog C, et al. J Virol 2005;79:14339. [PubMed: 16254368]
28. We acknowledge the technical assistance of A. Andaya, J. Garber, and F. Last. This research was supported by NIH grants AGO4342 (M.B.A.O. and M.J.T.) and 5R01HL66424-04 (K.U.K.) and by NIH training grant NS041219-05 (M.J.T.).

Fig 1.

Detection of PrPres in blood and extraneural tissues of tg mice infected with RML scrapie. (A) PrPres lining blood vessels within the corpus callosum of a tg mouse at 500 dpi with scrapie strain RML (PrPres staining in red). (B to E) PrPres was detected on immunoblots with monoclonal antibody D13. Similar results were observed with R30 antibody. (B) Immunoblot showing PrPres expression in blood cellular (C) and plasma (P) fractions following scrapie infection. For comparison, a sample (pos) equivalent to 10 mg of brain tissue isolated from the brain of a clinically sick RML scrapie-infected C57BL/6 (wt) mouse is shown. (C) Immunoblot showing 250 to 400 μl of plasma from six RML-infected tg mice at times ranging from 160 to 500 dpi. (D) Immunoblot analysis of PrPres in 20 mg of spleen, thymus, and heart from a tg mouse infected for 420 days. Similar results were observed with 10 additional mice. (E) Immunoblot analysis of heart homogenates from individual tg mice at various times after scrapie inoculation. Equivalent results were observed in at least three mice per time point. (F) Quantification of PrPres in blood cellular (C) and plasma (P) fractions relative to the amount of PrPres found in the brains of clinically sick WT mice after scrapie infection. Four times as much PrPres was identified in the plasma fraction as in the cellular fraction (* $P \leq 0.05$). (G)

Quantification of PrPres in the hearts of scrapie-infected tg mice relative to the amount of PrPres in the brains of clinically sick scrapie-infected WT mice. (◆) Each diamond represents data from a single mouse.

Fig 2.

Detection of PrPres deposits in cardiac samples from scrapie-infected tg mice using PrP monoclonal antibody D13. **(A)** Absence of PrPres in heart tissue in a scrapie-infected clinically ill WT mouse at 160 dpi. Similar results were obtained from an additional 10 scrapie-infected WT mice. **(B)** PrPres (red staining) in heart tissue at 400 days after RML scrapie infection of tg mice. PrPres deposition was observed primarily between myocytes and often around capillaries. **(C and D)** Adjacent sections from two mice at 450 dpi with RML scrapie were evaluated for PrPres (red) and amyloid (Thio-flavin S; green). PrPres most often localized with amyloid. A similar association between PrPres and amyloid was seen in more than 20 individual hearts from RML scrapie-infected tg mice. **(E)** Detection of widespread deposition of PrPres

(pink) and **(F)** amyloid (Thioflavin S; green) from adjacent sections of heart from an infected tg mouse at 500 dpi. **(G)** PrPres could be found in or around myocytes at 500 dpi or later. **(H)** Detection of PrPres between myocardial myocytes and around blood-vessel endothelia in the heart of a mouse infected with 22L scrapie 715 days earlier.

Fig 3.

Hemodynamic assessment of RML scrapie-infected tg mice. Systolic and diastolic function was determined in tg mice with (red, $n = 4$) or without (black, $n = 6$) infection (400 to 500 days old or dpi) by pressure-volume analysis. Measurements are shown for a sequence of beats associated with the decrease in preload induced by transient occlusion of the inferior vena cava [left panel of (A) and (B)]. (A) Baseline hemodynamics. Emax, a measure of load-independent systolic function, is defined as the slope of the linear fit line of the end-systolic pressure-volume relation (black and red lines in the left panel). Average baseline indexes for Emax, end-diastolic volume (EDV), and end-diastolic pressure (EDP) are shown. (B) Measurement of passive cardiac relaxation (stiffness) after volume load. Cardiac stiffness was further assessed by infusing 0.5 ml of normal saline into the mouse as a volume load. After volume loading, the preload was altered by transient occlusion of the inferior vena cava. The slope of the linear fit of the end-diastolic pressure-volume relation (EDPVR) (black and red lines in the left panel) is proportional to the diastolic stiffness of the left ventricle. Means $\pm$ SE for the slope of the EDPVR, EDV, and EDP are shown. * $P \leq 0.05$ as compared to uninfected tg mice.

Table 1

Titration of scrapie infectivity from heart and blood of RML scrapie-infected GPI-negative anchorless PrP transgenic (tg) and wild-type (WT) mice.

Mice [*]	Tissue	Dilution [†]	Incubation period (days) [‡]	Infected mice/ total [‡]	Titer [§]
Uninfected tg mouse	Blood	5×10^2	>450	0/6	ND
	Heart	5×10^2	>450	0/6	ND
Infected WT mouse	Blood	5×10^2	>450	0/6	ND
	Heart	5×10^2	>450	0/6	ND
Infected tg mouse #1	Blood	5×10^2	135 ± 10	6/6	$\geq 1.6 \times 10^7$ ID ₅₀ /ml
	Blood	5×10^3	200 ± 15	3/3	$\geq 1.6 \times 10^7$ ID ₅₀ /ml
	Blood	5×10^4	250 ± 12	3/3	$\geq 1.6 \times 10^7$ ID ₅₀ /ml
	Blood	5×10^5	270 ± 18	3/3	$\geq 1.6 \times 10^7$ ID ₅₀ /ml
	Heart	5×10^2	250 ± 12	3/3	$\geq 3.0 \times 10^6$ ID ₅₀ /heart
	Heart	5×10^5	347 ± 6	3/3	$\geq 3.0 \times 10^6$ ID ₅₀ /heart
	Infected tg mouse #2	Blood	5×10^2	6/6	$\geq 1.6 \times 10^5$ ID ₅₀ /ml
		Blood	5×10^3	3/3	$\geq 1.6 \times 10^5$ ID ₅₀ /ml
	Heart	5×10^2	250 ± 14	3/3	$\geq 3.0 \times 10^6$ ID ₅₀ /heart
	Heart	5×10^5	351 ± 8	3/3	$\geq 3.0 \times 10^6$ ID ₅₀ /heart

^{*} WT C57BL/6 mice or GPI-negative anchorless PrP tg mice were inoculated intracerebrally with 1×10^6 ID₅₀ of RML scrapie. Results illustrate data from a WT mouse killed at the time of severe clinical disease (160 dpi) and from two tg mice killed at 480 dpi (#1) and 507 dpi (#2). One uninoculated control tg mouse was killed at 450 days of age.

[†] Mice were exsanguinated under isoflurane anesthesia, and the heart was removed. Heart homogenates and blood were diluted in sterile phosphate-buffered saline, and 30- μ l volumes were inoculated intracerebrally into three to six recipient C57BL/6 mice at the dilutions shown.

[‡] Recipient C57BL/6 mice were monitored for signs of clinical scrapie including altered gait, ataxia, kyphosis, disorientation, and lethargy. The incubation period was recorded as the time when clinical symptoms were observed and mice were moribund. Scrapie was confirmed by Western blot analysis of PrPres in clinically ill mice.

[§] ID₅₀/heart was calculated by dividing the 50% end-point dilution by the volume inoculated and then multiplying by the weight of the heart (180 mg). Blood titers were calculated as ID₅₀/ml. End-point values were not reached, and the titers shown represent the minimum values possible. ND, not detected.