別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-2

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2005 年 9 月 26 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称			研究報告の公表状況	Chronic Wasting Disease of Elk: Transmissibility to HumansExamined by Transgenic Mouse Models Qingzhong Kong et al. The Journal of Neuroscience, August 31, 2005; 25(35):7944 - 7949	公表国 米国	
販売名（企業名）						

研究報告の概要

慢性摩耗病(CWD)は、米国およびカナダで広がっている、野生のシカのプリオン病である。米国では野生シカの数が多く、シカ肉を食べることは一般的であり、また CWD が明らかに広がりつつあることから、人と CWD に感染したシカが接触する機会が増えていると考えられる。BSE が人に感染したように CWD も人に感染するのかどうかは不明である。そこで CWD の人への伝染可能性について検討するために、著者らは、ヘラジカまたはヒトのプリオン蛋白(PrP)を発現するように遺伝子操作したトランスジェニックマウスをそれぞれ作成した。すなわち、シカ化トランスジェニックマウスとして Tg12 系統を 25 匹、ヒト化トランスジェニックマウスとして Tg40 および Tg1 系統をそれぞれ 29 匹および 22 匹、作成した。これらのマウスに CWD に感染したヘラジカの脳抽出物を脳内に接種したところ、「ヒト化」トランスジェニックマウスの 2 系統の計 51 匹全てが平均 657 日以上または 756 日以上経過してもプリオン病の症状を発現しなかった。一方、「シカ化」トランスジェニックマウスは、26 匹中 25 匹が、それぞれ接種材料別に、平均 118 日、142 日、125 日後にプリオン病に特徴的な運動失調を発現した。ポジティブコントロールとしてヒト化トランスジェニックマウスに散発性 CJD の患者の脳由来物質を接種した群では 17 匹中 16 匹が発症した。以上の実験結果から、シカの CWD には、BSE が超えた人との種の壁よりも厚い「種の壁」がヒトとシカとの間で存在することが示唆された。すなわち、シカの CWD がヒトに感染するリスクは極めて低いと考えられる。

使用上の注意記載状況・その他参考事項等

BYL-2005-0196
Chronic Wasting Disease (http://www3.gov.ab.ca/srd/fw/diseases/CWD/index.html)
Chronic Wasting Disease Found in Hampshire County Deer (http://www.wydnr.gov/2005news/05news167.shtm)
More instances of chronic wasting disease discovered (http://www.wildlife.utah.gov/news/05-10/cwd_found.php),
TWO NEW MEXICO ELK TEST POSITIVE FOR CHRONIC WASTING DISEASE (http://www.wildlife.state.nm.us/publications/press_releases/documents/10-9cwdelk.htm),
Deer disease remains a concern to IDNR (http://www.belleville.com/mld/belleville/sports/13607269.htm)
Wasting disease confirmed in Kansas (http://www.kansas.com/mld/kansas/13696983.htm)
Current Distribution of CWD among Captive Cervid Herds http://www.aphis.usda.gov/vs/nahps/cwd/cwd-distribution.html

報告企業の意見	今後の対応
米国で報告が増加している CWD が、ヒトに感染するリスクはほとんどないことを実験的に示唆した報告である。さらに、弊社の血漿分画製剤の製造工程におけるプリオン除去能は 4 log を上回ることが確認されている。現時点では CWD がヒトに感染し、さらに血漿分画製剤に CWD の病原プリオンが混入するリスクは極めて低いと考えられる	現時点で弊社において新たな安全対策上の措置を講じる必要はないと考える。

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Neurobiology of Disease

Chronic Wasting Disease of Elk: Transmissibility to Humans Examined by Transgenic Mouse Models

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Chronic wasting disease (CWD), a prion disease affecting free-ranging and captive cervids (deer and elk), is widespread in the United States and parts of Canada. The large cervid population, the popularity of venison consumption, and the apparent spread of the CWD epidemic are likely resulting in increased human exposure to CWD in the United States. Whether CWD is transmissible to humans, as has been shown for bovine spongiform encephalopathy (the prion disease of cattle), is unknown. We generated transgenic mice expressing the elk or human prion protein (PrP) in a PrP-null background. After intracerebral inoculation with elk CWD prion, two lines of "humanized" transgenic mice that are susceptible to human prions failed to develop the hallmarks of prion diseases after >657 and >756 d, respectively, whereas the "cervidized" transgenic mice became infected after 118–142 d. These data indicate that there is a substantial species barrier for transmission of elk CWD to humans.

Key words: chronic wasting disease; CWD; transmissibility to humans; transgenic mice; prion; cervids; deer; elk; species barrier

Introduction

Prion diseases are neurodegenerative diseases that affect humans and animals, including cattle, sheep, cervids (deer and elk), and mink (Sigurdson and Miller, 2003; Jeffrey and Gonzalez, 2004; Kong et al., 2004). They pose a serious threat to public health because they can be transmitted between humans and from animals to humans. Animal to human transmission was dramatically exemplified by the sudden appearance of a new form of prion disease, identified as variant Creutzfeldt–Jakob disease (vCJD), in the United Kingdom, after the emergence of a large outbreak of bovine spongiform encephalopathy (BSE) (Will, 2003). Compelling evidence indicates that vCJD is acquired after the consumption of beef or beef products from BSE-infected cattle (Scott et al., 1999; Will, 2003). To date, five cases of indigenous BSE, but no case of locally acquired vCJD, have been reported in North America.

Chronic wasting disease (CWD) is the prion disease that affects free-ranging and captive cervids, including white-tail deer, mule deer, and Rocky Mountain elk (Miller and Williams, 2004). First reported in 1967, CWD was once considered a rare and geographically contained disease; however, recent data support the presence of CWD among free-ranging and captive cervids in at least 13 states in the United States and 2 provinces in Canada, with a prevalence of up to 20% in some endemic areas (Miller and Williams, 2003; Miller et al., 2004; Prusiner et al., 2004). These findings, along with other considerations such as the high cervid population in the United States (estimated at 22 million), the several million deer and elk hunters, and the widespread consumption of elk and deer meat, underscore the likely increasing risk of human exposure to CWD. These considerations, along with the recognition that the outbreak of BSE led to the emergence of vCJD, have heightened concerns about possible direct-contact and food-borne CWD transmission to humans. In fact, many people are known to have consumed venison from confirmed CWD-affected cervids. Twenty-seven patients with CJD who regularly consumed elk and deer meat were reported to the National Prion Disease Pathology Surveillance Center at Case Western Reserve University, but none of these cases appeared to have a novel form of prion disease (Belay et al., 2001, 2003, 2004; P. Gambetti, unpublished observation); however, human disease acquired from CWD might have an unusual phenotype or a phenotype that is difficult to distinguish from that of sporadic CJD (sCJD). This uncertainty is a serious public health concern in the United States.

To assess the transmissibility of CWD to humans, we generated transgenic (Tg) Friend leukemia virus B (FVB) mice express-

Received June 16, 2005; revised July 18, 2005; accepted July 19, 2005.

This work was supported by National Institutes of Health Grant 1P01 AG14359-06 (P.G.), Centers for Disease Control and Prevention (CDC) Grant UR8\CU515004 (P.G.), and funding from the Center for Food Safety and Applied Nutrition of the Food and Drug Administration through the CDC (P.G.). We thank B. Chakraborty, K. Edmonds, D. Kofskey, P. Scalzo, and F. Scalzo for technical support as well as M. E. Potter for help and encouragement.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

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DOI:10.1523/JNEUROSCI.2467-05.2005

 0270-6474/05/257944-06$15.00/0

ing either the human prion protein (PrP) or Rocky Mountain elk PrP in a PrP-null background. Here we show that, after intracerebral inoculation with elk CWD prion, the two lines of "humanized" Tg mice failed to develop the hallmarks of prion diseases after >657 and >756 d, respectively, whereas the "cervidized" Tg mice became infected after 118–142 d. These data indicate that there is a substantial species barrier for the transmission of elk CWD to humans.

Materials and Methods

Construction of transgenes expressing human PrP-129M or elk PrP-132M. The transgene constructs are based on the murine half-genomic PrP clone in plasmid pHGPRP (Fischer et al., 1996). The HuPrP-129M open reading frame (ORF) was amplified from the human genomic DNA PAC (P1-derived artificial chromosome) clone RP5–1068H6 (obtained from the Sanger Center, Cambridge, UK) with primers HRM-F (TATGTGGACTGATGTCGGCCTCTGCAAGAAGCGC) and HRM-R (CCACCTCAATTGAAAGGGCTGCAGGTGGATAC). The PCR product was digested with *Psh*AI and *Mfe*I and used to replace the corresponding 0.97 kb *Psh*AI–*Mfe*I fragment in pHGPRP to create pHGHuPrP-129M. In the resulting pHGHuPrP-129M clones, the signal-peptide sequence is still from mouse, but the rest of the PrP ORF and the first 76 bp after the stop codon are from human PRNP (prion protein) genomic DNA. The inserted 0.97 kb *Psh*AI–*Mfe*I fragment in pHGHuPrP-129M was then sequenced with the primers HRM-R, HRM-F, and HP306R (CATGTTGGTTTTTGGCTTACTC). One error-free clone was chosen for the creation of transgenic mice. To create the transgene construct expressing elk PrP-132M, the mouse PrP ORF in pHGPRP was first replaced with the restriction sites for *Cla*I and *Nru*I by using conventional recombinant DNA techniques to create pHGD3. The ElPrP-132M ORF (eGMSE allele) was selected to make cervidized transgenic mice because it was reported that all CWD-affected elk and some deer carried this allele (Raymond et al., 2000). The ElPrP-132M ORF was amplified from the genomic DNA of an American elk with primers DePrP-F (CAGTCTAGACCGCGGCATGGTGAAAAGCCACATAGG) and DePrP-R (ACCTCTAGACCTATCCTACTATGAGAAAAATGAG), and cloned into pSTBlue 1 (Novagen, Madison, WI). The 0.78 kb ElPrP ORF thus cloned was released by *Sac*II–*Xba*I double digestion, blunted with T4 DNA polymerase, and inserted into the *Nru*I site of pHGD3 to create pHGDePrP-132M. The final pHGElPrP-132M construct was confirmed by sequencing. One error-free clone was chosen for the creation of transgenic mice.

Generation, screening, and characterization of transgenic Tg(HuPrP-129M)Prnp$^{0/0}$ and Tg(ElPrP-132M)Prnp$^{0/0}$ mice. The 12.2 kb HuPrP-129M and elk PrP-132M transgene constructs were microinjected into fertilized FVB/NJ eggs, and planted into the oviducts of pseudopregnant CD-1 mice at the transgenic mouse facility of Albert Einstein College of Medicine (Bronx, NY). Founder pups were screened by tail DNA PCR. All founder mice that carry the transgene were bred with FVB/*Prnp*$^{0/0}$ mice (Fischer et al., 1996) (kindly provided by the Prusiner laboratory, University of California, San Francisco) to obtain Tg mice in PrP-null background. Transgenic PrP expression in the brain and other tissues of the Tg mice were examined by Western blot analysis with monoclonal antibodies (mAbs) 3F4 and 8H4 for humanized and cervidized Tg mice, respectively. All animal experiments in this study were approved by the Institutional Animal Use and Care Committee and the Institutional Biosafety Committee, and the use of human brain tissues was authorized by the Institutional Review Board.

Western blot analysis. Mouse tissues were homogenized in 10 vol of lysis buffer (10 mM Tris, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 5 mM EDTA, pH 8.0) with or without 1 mM phenylmethylsulfonyl fluoride (PMSF). The immunoblotting was performed as described previously (Pan et al., 2001), with minor modifications. The homogenate was cleared at 12,000 rpm for 10 min, and the supernatant was then stored at −80°C. To detect Proteinase K (PK)-resistant PrP fragments (PrP^{Sc}), brain extracts without PMSF were incubated with 100 μg/ml PK for 1 h at 37°C, and PMSF was added to a final concentration of 3 mM to terminate the digestion. The extracts were mixed with PAGE loading buffer (160 mM Tris, 4% SDS, 4% 2-ME, 20% glycerol, 0.04% bromophenol blue, pH 6.8), loaded onto 12% Tris-glycine SDS-PAGE or 10–20% Tris-tricine SDS-PAGE gels, transferred to polyvinylidene difluoride membrane, and probed with 8H4 or 3F4 in conjunction with horseradish peroxidase-conjugated goat anti-mouse IgG Fc antibody.

Inoculation of transgenic mice. Brain tissues from humans with sCJD or from elk with CWD were homogenized and inoculated into the brains of Tg(HuPrP-129M)*Prnp*$^{0/0}$ or Tg(ElPrP-132M)*Prnp*$^{0/0}$ mice. Brain tissues were homogenized in cold PBS, and the homogenate was centrifuged at 1000 × *g* for 10 min at 4°C. The supernatant was diluted to 10-fold of the brain tissue volume in cold PBS to obtain 10% brain homogenate, frozen at −80°C for storage, and diluted to 1% with PBS just before inoculation. After anesthetization with isoflurane, 30 μl of the 1% brain homogenate was injected into each mouse brain with a 26 gauge needle inserted to a depth of ~2 mm at the left parietal region of the cranium.

Monitoring of symptoms and examination of PrP^{Sc}. After intracerebral inoculations, the animals were visually examined daily for symptoms such as coarse coat, waddling gait, tail plasticity, and bradykinesia. Within 2–3 d after the appearance of these symptoms or at death, the brain was removed; one-half was frozen for biochemical studies, and the other half was stored in formalin for histology and immunohistochemistry analysis as described previously (Taraboulos et al., 1992). Total PrP as well as PrP^{Sc} (PK-resistant PrP) were examined by Western blotting in Tris-glycine and/or Tris-Tricine SDS-PAGE gels, as described above. Sodium phosphotungstate precipitation of PrP^{Sc} was performed as described previously (Safar et al., 1998).

Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling assay. Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) staining of paraffin-embedded brain sections was performed as described previously (Shi et al., 1990) with the *In Situ* Cell Death Detection Kit-peroxidase (POD) (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. Paraffin-embedded sections were dried at 60°C. After the paraffin was removed with xylene, tissues were rehydrated in serial solutions of ethanol (100, 95, and 75%), washed in PBS, and subjected to microwave treatment for 20 min in 60 mM HCl. The slides were then air dried at room temperature, treated with 5% Triton X-100 at room temperature, washed in PBS several times, and incubated with TUNEL reagent for 2 h at 37°C in a humid chamber. The slides were then washed in PBS, incubated with converter-POD for 30 min at 37°C, developed with diaminobenzidine substrate according to the manufacturer's protocol, counterstained with hematoxylin QS (Vector Laboratories, Burlingame, CA), and mounted. The TUNEL-positive cells were stained brown.

Results

We used two lines of humanized Tg mice, Tg40 and Tg1, and one line of cervidized Tg mice, Tg12, that express the transgene PrP in brain approximately onefold, twofold, and twofold, respectively, the level of brain PrP in wild-type FVB mice. Both humanized and cervidized Tg mice were inoculated intracerebrally with brain homogenates from two CWD-affected elk. The humanized Tg1 and Tg40 mice were also similarly inoculated with brain homogenates from human subjects with a type of sporadic CJD identified as sCJDMM1 (Parchi et al., 1996).

Thirteen of 14 cervidized Tg12 mice inoculated intracerebrally with CWD elk 1 brain homogenates developed ataxia after an average of 118 ± 6 d postinoculation (dpi) (range, 83–142 dpi) (Table 1, Fig. 1). All seven cervidized Tg12 mice inoculated intracerebrally with CWD elk 2 brain homogenates developed ataxia after an average of 142 ± 7 dpi (range, 124–178 dpi) (Table 1, Fig. 1). Histologically, all ataxic mice contained severe and widespread spongiform degeneration throughout the cerebral cortex and basal ganglia, as well as neuronal loss in the hippocampus and granule cell layer of the cerebellum (Fig. 2*a*,*b*). Neuronal apoptosis was detected by TUNEL staining in the hippocampus (Fig. 2*f*), other cortical regions, and the cerebellum (data not shown). Amyloid plaques were not present; however, immuno-

histochemical staining for PrP revealed spotted, round PrP deposits, the so-called plaque-like PrP deposits, similar to those found in CWD-affected elk, in the cerebral cortex (Fig. 2*d*) and the molecular layer of the cerebellum (Fig. 2*e*). Symptomatic mice also accumulated large amounts of PK-resistant PrP with gel mobility and glycoform ratios matching those of the original CWD inoculum (Fig. 3); therefore, the cervidized Tg12 mice replicated the main features of the elk CWD PrP^{Sc}. Furthermore, secondary transmission of the elk 1 CWD prion passaged once in Tg12 mice required an incubation period of 125 ± 4 d (range, 115–138 d) (Table 1), indicating that there is no species barrier for elk CWD transmission to the Tg12 mice.

Table 1. Prion transmission in transgenic mice

Prion inoculum	Mice	PrP (level)	Incubation time (SEM)	Transmission rate[a]
CWD (elk 1)	Tg12	Elk PrP-132M (2×)	118 ± 6 d	13/14
CWD (elk 2)	Tg12	Elk PrP-132M (2×)	142 ± 7 d	7/7
CWD (Tg12)	Tg12	Elk PrP-132M (2×)	125 ± 4 d	5/5
CWD (elk 1/2)	Tg40	HuPrP-129M (1×)	>756 d	0/29
CWD (elk 1/2)	Tg1	HuPrP-129M (2×)	>657 d	0/22
sCJDMM1	Tg40	HuPrP-129M (1×)	263 ± 13 d	9/10
sCJDMM1	Tg1	HuPrP-129M (2×)	226 ± 5 d	7/7

[a]Number of affected/total inoculated animals.

Brain homogenates from the two CWD-affected elk used for the cervidized mice were also inoculated intracerebrally into 29 Tg40 humanized mice and 22 Tg1 humanized mice. None of the 29 Tg40 mice or the 22 Tg1 mice showed signs of prion diseases after >756 and >657 dpi, respectively. Three Tg40 mice appeared to be mildly ataxic before being killed at 420–509 dpi. A total of 18 Tg40 mice and 12 Tg1 mice died naturally of old age or were killed because of other illnesses; however, none of the Tg40 and Tg1 mice examined, including the three ataxic Tg40 mice, had PK-resistant PrP^{Sc} as detected by immunoblotting of PrP^{Sc}-enriched preparations after precipitation with sodium phosphotungstate (Fig. 4). Histopathological and PrP immunohistochemical examinations also were negative (data not shown). Furthermore, the PrP immunoprecipitates obtained from the three ataxic mice with the mAb OCD4, which immunoreacts with both PK-resistant and PK-sensitive abnormal PrP present in human and animal prion diseases but not with normal PrP^{C} (Zou et al., 2004), were not different from the corresponding immunoprecipitates obtained from other elk CWD-inoculated or noninoculated Tg40 mice. Therefore, all CWD-inoculated humanized mice appeared to be free of detectable prion, and the cause of the mild ataxia in these three Tg40 mice did not appear to be related to prion disease.

As positive controls, 10 Tg40 and 7 Tg1 humanized mice were also inoculated intracerebrally with brain homogenate from a human subject with sCJDMM1. Nine of the 10 Tg40 mice and all 7 Tg1 mice became symptomatic, with an average incubation time of 263 ± 13 d (range, 213–315 d) for the Tg40 mice and 226 ± 5 d (range, 213–244 d) for the Tg1 mice. The affected mice revealed fine spongiform degeneration in the cerebral cortex (Fig. 5*a*) but not in the cerebellum (Fig. 5*b*), and the vacuoles were different in size and distribution from those of the cervidized mice inoculated with elk CWD prion. Apoptosis of neuronal cells was present but was less prominent than in the CWD-affected Tg12 cervidized mice (data not shown). After immunohistochemical staining, fine PrP deposits mimicking the "synaptic" pattern of sCJDMM1 were found in the cerebral cortex (Fig. 5*c*) and cerebellum (Fig. 5*d*) of the CJD-affected humanized mice, whereas the plaque-like deposits observed in the elk CWD-inoculated cervidized mice were not present. Abundant PK-resistant PrP^{Sc} with gel mobility matching that of the sCJDMM1 inoculum was shown in the brain of the infected mice (Fig. 6*a*). The glycoform ratio also was similar to that of sCJDMM1, with underrepresentation of the diglycosylated PK-resistant PrP^{Sc} fragment (Fig. 6*b*), but it was quite different from that of CWD.

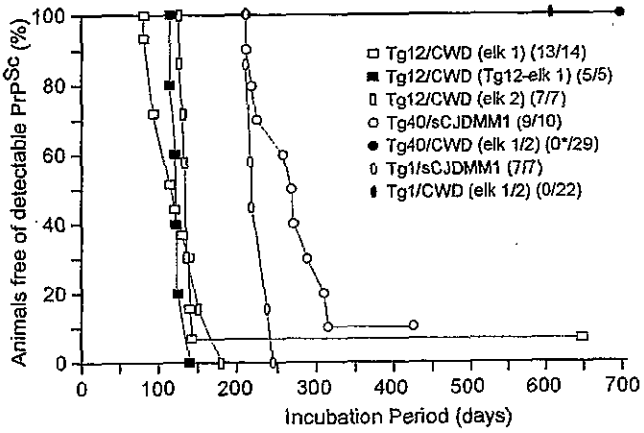

Figure 1. Survival curves of humanized and cervidized transgenic mice. Six- to eight-week-old cervidized Tg12 and humanized Tg1 and Tg40 mice were inoculated intracerebrally with 30 μl of 1% brain homogenate from two CWD-affected elk or a subject with sCJDMM1. The Tg12 mice were also inoculated with brain homogenates from elk 1 CWD-affected Tg12 mice to evaluate the species barrier of elk CWD transmission to the Tg12 mice. The average incubation times were as follows: 118 ± 6 d for elk 1 CWD-inoculated Tg12 mice (open squares); 125 ± 4 d for secondary transmission of elk 1 CWD in Tg12 mice (filled squares); 142 ± 7 d for elk 2 CWD-inoculated Tg12 mice (open rectangles); 263 ± 13 d for the sCJDMM1-inoculated Tg40 mice (open circles); and 226 ± 5 d for the sCJDMM1-inoculated Tg1 mice (open ovals). None of the 29 CWD-inoculated Tg40 mice (filled circle) or the 22 CWD-inoculated Tg1 mice (filled oval) had detectable PK-resistant PrP^{Sc} or substantial histopathology after >756 and >657 dpi, respectively. The asterisk indicates that three Tg40 mice became ataxic between 420 and 509 dpi but were free of prion infection. Parentheses indicate prion-positive mice per total inoculated mice.

Discussion

We have shown that CWD of elk can be transmitted to Tg12 cervidized mice with a relatively short average incubation time of 118 ± 6 d for elk 1 and 142 ± 7 d for elk 2. The PK-resistant PrP^{Sc} of the elk CWD-inoculated Tg12 mice has the same gel mobility and glycoform ratio as the PK-treated PrP^{Sc} of the CWD-affected elk. The pattern of PrP immunostaining is also similar in the two conditions. In addition, the secondary transmission of elk 1 CWD passaged in Tg 12 mice to naive Tg12 mice required an incubation time of 125 ± 4 d, which is not substantially different from that of the primary transmissions. Furthermore, it is associated with a histopathology and a PrP immunostaining pattern that is similar to that of the primary infection. These data argue that there is no species barrier in the transmission of elk CWD to the Tg12 mice and that CWD-inoculated Tg12 mice reproduced major strain characteristics of the PrP^{Sc} associated with elk CWD. Recently, Browning et al. (2004) inoculated both hemizygous and homozygous cervidized Tg(CerPrP) mice that express mule deer PrP (S2 allele) with brain homogenates from CWD-affected mule deer and elk. The inoculated mice developed a disease with histopathological and PrP immunohistochemical

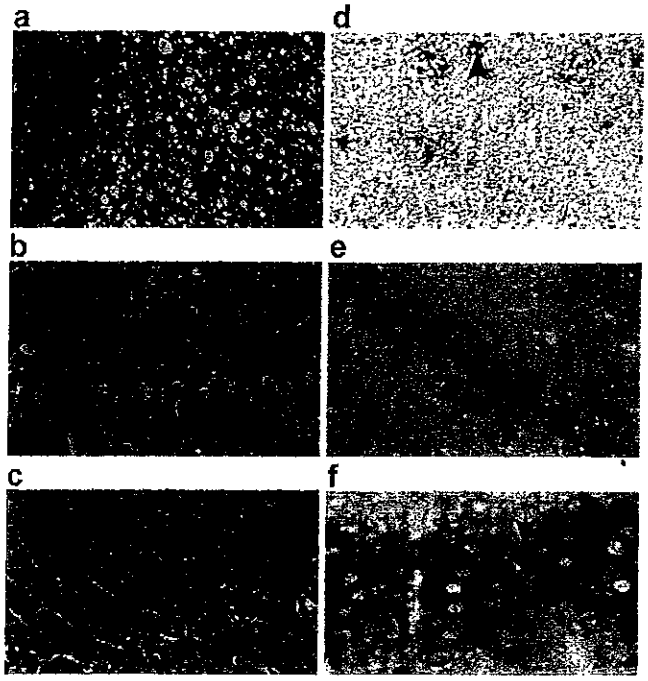

Figure 2. Histopathology, PrP immunohistochemistry, and apoptosis examination in CWD-affected Tg12-cervidized mice. Prominent spongiform degeneration was present in the cerebral cortex (*a*) along with marked neuronal loss in the granule cell layer of the cerebellum (*b*) when compared with age-matched control Tg12 mice (*c*) (hematoxylin and eosin staining; 20× magnification). PrP deposits formed a fine granular pattern interspersed with plaque-like deposits (arrow) in the cerebral cortex (*d*) and in the granule cell layer of the cerebellum (*e*) (mAb 8H4; 20× magnification). Neuronal apoptosis (arrow) was present in the granule cells of the hippocampus (*f*) and in other brain regions (TUNEL; 40× magnification).

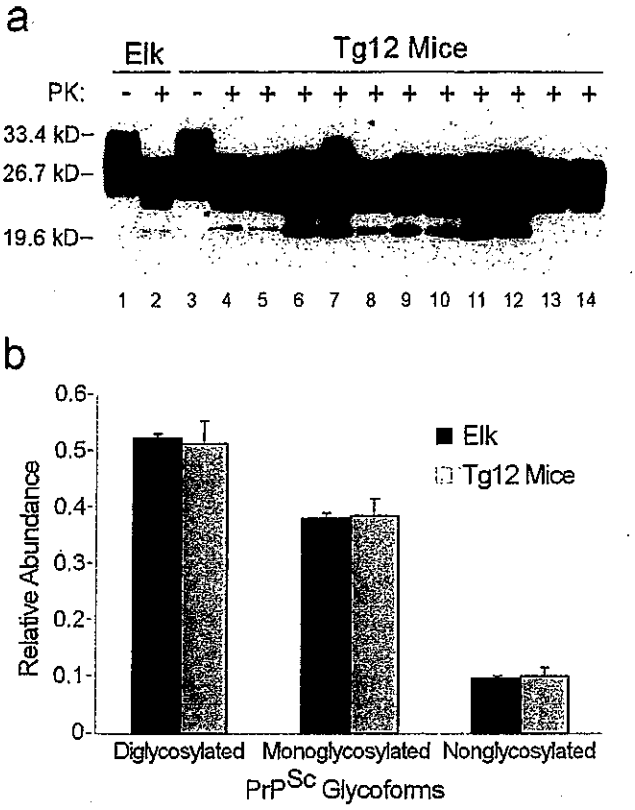

Figure 3. Characterization of PK-resistant PrP^{Sc} from the donor CWD-affected elk and CWD-affected Tg12 mice. *a*, Immunoblot of PrP. Lanes 1 and 2, PK-untreated (lane 1) and PK-treated (lane 2) PrP from one of the donor elks with CWD. Lanes 3–14, PK-untreated (lane 3) and PK-treated (lanes 4–14) PrP from 11 CWD-affected Tg12 mice. *b*, Glycoform ratio analysis of PK-resistant PrP^{Sc}. The blots were probed with mAb 8H4. Error bars are based on quantitative analyses of digital chemiluminescence images of triplicate Western blots of brain homogenates from an elk with CWD (the inoculum) and duplicate Western blots of 11 Tg12 mice infected with elk CWD. kD, Kilodalton.

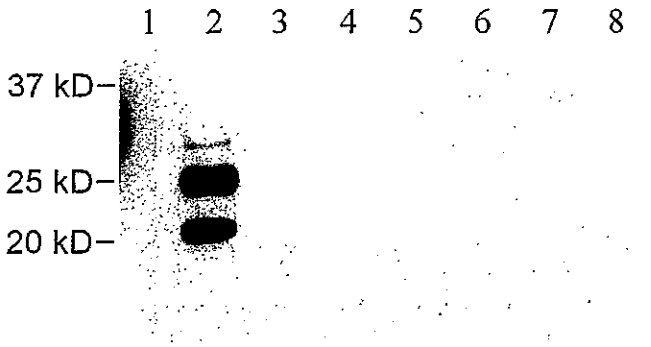

Figure 4. Absence of PK-resistant PrP^{Sc} in elk CWD-inoculated humanized Tg mice. Brain homogenates were subjected to sodium phosphotungstate treatment to precipitate PrP^{Sc}, digested with 20 μg/ml PK for 30 min, and analyzed by Western blotting with the mAb 3F4. Lane 1, An uninoculated Tg40 mouse; lane 2, an sCJDMM1-infected Tg40 mouse; lanes 3–5, three ataxic CWD-inoculated Tg40 mice; lanes 6–8, three CWD-inoculated Tg40 mice killed because of other aging-related illnesses. Lanes 1, 3–8, Forty microliters of 10% brain homogenates were loaded; lane 2, a total of 2 μl was loaded. kD, Kilodalton.

characteristics that appear overall to mimic the histopathology and PrP immunohistochemistry observed in our elk CWD-inoculated Tg12 mice. The gel mobility of the PK-resistant fragments of PrP^{Sc} recovered from the Tg(CerPrP) mice also is apparently similar to that observed in our Tg12 mice; however, Browning et al. (2004) reported a difference in the glycoform ratio between the PK-resistant PrP^{Sc} fragments recovered from the Tg(CerPrP) mice and that of the original elk and deer inocula. At variance with this finding, the PrP^{Sc} glycoform ratio of our affected Tg12 mice reproduced precisely that of the elk CWD inoculum (Fig. 3*b*). The incubation time of the CWD-inoculated Tg(CerPrP) mice varied between 220 and 270 d for the hemizygous mice and was 160 ± 3 d for the homozygous mice despite the 3- to 5-fold and 6- to 10-fold PrP expression of the hemizygous and homozygous mice, respectively. The difference in incubation time between the Tg(CerPrP) mice and the Tg12 mice could be caused by the polymorphism of codon 226 [226Q for the S2 allele used in Tg(CerPrP) mice and 226E for the eGMSE allele used in Tg12 mice], the PrP genotype difference in the cervid CWD samples, or the difference in transgene vectors. The short incubation time combined with the only slightly elevated PrP expression make the Tg12 mice suitable for bioassay and other studies on CWD. The short incubation time for elk CWD prion in Tg12 mice also compares favorably with the incubation time of 238 d reported for BSE transmission to the Tg mice expressing bovine PrP (Scott et al., 1999).

In contrast to the efficient CWD transmission in the cervidized Tg12 mice, the same CWD inocula failed to infect the humanized Tg40 and Tg1 mice after >756 and >657 dpi, respectively, the approximate lifespan of these mice, which were ~2 months old at the time of inoculation; however, sCJD was transmitted to these humanized mice with average incubation times of 263 and 226 d, respectively. At variance with CWD, BSE has been transmitted to a similar humanized Tg mouse model that, like our humanized Tg mice, expressed human PrP-129M at a level

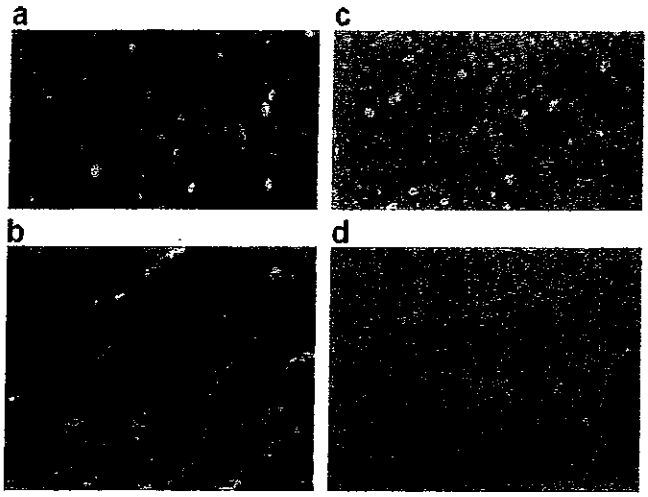

Figure 5. Histopathology and PrP^{Sc} deposition in the brains of sCJDMM1-infected Tg40 mice. In the brains of sCJDMM1-infected Tg40 mice, hematoxylin and eosin staining revealed moderate spongiform degeneration with fine vacuoles in the cerebral cortex (*a*) accompanied by even milder spongiosis in other brain areas (20× magnification), but the granule cell layer of the cerebellum (*b*) appears mostly intact (40× magnification). Immunohistochemistry for PrP^{Sc} with 3F4 revealed fine PrP^{Sc} deposits in the cerebral cortex (*c*), with more intense PrP^{Sc} deposits in the cerebellum (*d*) (20× magnification).

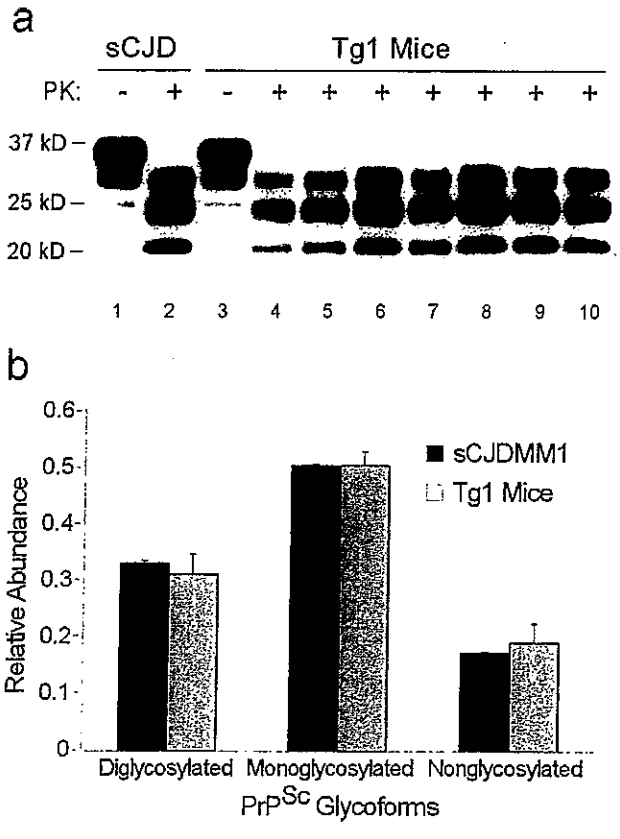

Figure 6. Immunoblots and glycoform ratios of PK-resistant PrP^{Sc} from the sCJDMM1 donor and sCJDMM1-inoculated Tg1 mice. *a*, Immunoblot of PrP. PK-untreated (lane 1) PrP and PK-treated (lane 2) PrP were obtained from the sCJDMM1 donor; PK-untreated (lane 3) PrP and PK-treated (lanes 4–10) PrP were obtained from the seven sCJDMM1-inoculated Tg1 mice. *b*, Glycoform ratio analysis of PK-resistant PrP^{Sc}. PK-untreated and PK-treated brain homogenates were processed as in Figure 2, but blots were probed with 3F4. Error bars are based on quantitative analyses of digital chemiluminescence images of triplicate Western blots of brain homogenates from a subject with sCJDMM1 (the inoculum) and duplicate Western blots of the seven Tg1 mice infected with sCJDMM1. kD, Kilodalton.

two times that of pooled normal human brain (Asante et al., 2002). The total attack rate was 14 of 49; of the 14 infected mice, 6 had clinical signs, and the other 8 had subclinical infection only, but all had PrP^{Sc} in the brain. The incubation times were 414 d on average (range, 338–492 d) but as short as ~340 d in several mice (Asante et al., 2002). Combined, these findings point to the presence of a robust species barrier for elk CWD transmission to humans, which is much more effective than that for BSE transmission to humans. Because the most likely route of CWD transmission to humans is through oral consumption of CWD-contaminated meat and the intracerebral route is much more effective than the oral route (Prusiner et al., 1985), the failure to detect elk CWD transmission in the humanized Tg mice after intracerebral inoculations suggests an even lower risk of elk CWD transmission to humans. This conclusion is consistent with the lack of evidence of CWD transmission to CJD patients investigated for a possible causal link of their illness with CWD and with the low efficiency of an *in vitro* conversion of human PrP^{C} by cervid PrP^{Sc} (Raymond et al., 2000). Secondary transmission experiments in naive humanized Tg mice are underway to determine whether the primary CWD-inoculated humanized Tg mice are asymptomatic carriers of prion infectivity, although PrP^{Sc} is undetectable in any of these primary mice on Western blot even after sodium phosphotungstate precipitation or immunoprecipitation with the mAb OCD4 that selectively recognizes abnormal PrP associated with prion diseases (Safar et al., 1998; Zou et al., 2004). Because the CWD prions from deer and elk appear to be indistinguishable (Williams and Young, 1992; Spraker et al., 2002) and there have been no reports of different CWD prion strains, it is likely that CWD prions from mule deer and white-tail deer are, as reported here for CWD prion from elk, of low or no transmissibility in humans.

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別紙様式第 2-1

別紙 3-7

医薬品
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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 1 月 30 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称			研究報告の公表状況	Prions in Skeletal Muscles of Deer with Chronic Wasting Disease Rachel C. Angers, Shawn R. Browning, Tanya S. Seward, Christina J. Sigurdson, Michael W. Miller, Edward A. Hoover, Glenn C. Telling www.sciencexpress.org / 26 January 2006 / Page 1 / 10.1126/science.1122864	公表国 米国	
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研究報告の概要

実験的に慢性摩耗病(CWD)に感染させたマウスの骨格筋に感染力があることが確認され、シカの肉を食べることによりプリオンに曝露される可能性が高まった。北アメリカでは CWD の発生が増加しており、BSE がヒトに感染し vCJD として発症したように CWD もヒトとシカとの間で感染するのではないかという懸念が高まっている。本実験では、骨格筋中に感染性のあるプリオンが含まれるかどうかを、シカプリオン蛋白を発現するトランスジェニックマウスを使用して検討した。CWD に感染したシカと正常シカの骨格筋として半膜様筋および半腱様筋の抽出物をトランスジェニックマウスの脳内に接種した。また中枢神経(CNS)の抽出物を接種する群を設けた。その結果、CWD 感染シカの骨格筋抽出物を接種されたマウスは全て、360～490 日後に進行性の神経症状を発現した。CNS 抽出物投与群では、神経症状の発症は 230～280 日後に認められた。これらのマウス全ての脳において PrP^{Sc} が検出された。以上の結果は、CNS 組織と同様に、骨格筋にも感染性のあるプリオンが含まれることを示している。また、発症までの期間が、CNS 抽出物投与群よりも筋抽出与群の方が長かったことから、プリオンの感染価は CNS よりも骨格筋の方が低いことが示された。現在プリオンの感染価を正確に評価するための実験を実施中である。筋肉中のプリオン摂取後の CWD の感染リスクは経口経路では伝播されるプリオンが比較的少ないが、本実験の結果はヒトが食用する可能性のある筋肉がプリオンを伝播する原因となりうる可能性を示唆している。

報告企業の意見	今後の対応
本実験では、シカプリオンを発現させたトランスジェニックマウスが用いられている。一方、ヒト化トランスジェニックを用いた Quingzhong Kong らの実験では、CWD が種の壁を超えて感染する可能性は極めて低いことが示されている(別紙 3-2 参照)。 引き続き、関連情報の収集に努める。	現時点で弊社において新たな安全対策上の措置を講じる必要はないと考える。

使用上の注意記載状況・その他参考事項等

BYL-2005-0209
Chronic Wasting Disease (http://www3.gov.ab.ca/srd/fw/diseases/CWD/index.html)
Chronic Wasting Disease Found in Hampshire County Deer (http://www.wvdnr.gov/2005news/05news167.shtm)
More instances of chronic wasting disease discovered (http://www.wildlife.utah.gov/news/05-10/cwd_found.php),
TWO NEW MEXICO ELK TEST POSITIVE FOR CHRONIC WASTING DISEASE (http://www.wildlife.state.nm.us/publications/press_releases/documents/10-9cwdelk.htm)
,
Deer disease remains a concern to IDNR (http://www.belleville.com/mld/belleville/sports/13607269.htm)
Wasting disease confirmed in Kansas (http://www.kansas.com/mld/kansas/13696983.htm)
Current Distribution of CWD among Captive Cervid Herds http://www.aphis.usda.gov/vs/nahps/cwd/cwd-distribution.html

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Prions in Skeletal Muscles of Deer with Chronic Wasting Disease

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Prions are transmissible proteinaceous agents of mammals that cause fatal neurodegenerative diseases of the central nervous system (CNS). The presence of infectivity in skeletal muscle of experimentally infected mice raised the possibility that dietary exposure to prions might occur through meat consumption (*1*). Chronic wasting disease (CWD), an enigmatic and contagious prion disease of North American cervids, is of particular concern. The emergence of CWD in an increasingly wide geographic area and the interspecies transmission of bovine spongiform encephalopathy (BSE) to humans as variant Creutzfeldt Jakob disease (vCJD) have raised concerns about zoonotic transmission of CWD.

To test whether skeletal muscle of diseased cervids contained prion infectivity, Tg(CerPrP)1536 mice (*2*) expressing cervid prion protein (CerPrP), were inoculated intracerebrally with extracts prepared from the semitendinosus/semimembranosus muscle group of CWD-affected mule deer or from CWD-negative deer. The availability of CNS materials also afforded direct comparisons of prion infectivity in skeletal muscle and brain. All skeletal muscle extracts from CWD-affected deer induced progressive neurological dysfunction in Tg(CerPrP)1536 mice with mean incubation times ranging between 360 and ~490 d, whereas the incubation times of prions from the CNS ranged from ~230 to 280 d (Table 1). For each inoculation group, the diagnosis of prion disease was confirmed by the presence of PrP^{Sc} in the brains of multiple infected Tg(CerPrP)1536 mice (see supporting online material for examples). In contrast, skeletal muscle and brain material from CWD-negative deer failed to induce disease in Tg(CerPrP)1536 mice (Table 1) and PrP^{Sc} was not detected in the brains of sacrificed asymptomatic mice as late as 523 d after inoculation (supporting online material).

Our results show that skeletal muscle as well as CNS tissue of deer with CWD contains infectious prions. Similar analyses of skeletal muscle BSE-affected cattle did not reveal high levels of prion infectivity (*3*). It will be important to assess the cellular location of PrP^{Sc} in muscle. Notably, while PrP^{Sc} has been detected in muscles of scrapie-affected sheep (*4*), previous studies failed to detect PrP^{Sc} by immunohistochemical analysis of skeletal muscle from deer with natural or experimental CWD (*5*, *6*). Since the time of disease onset is inversely proportional to prion dose (*7*), the longer incubation times of prions from skeletal muscle extracts compared to matched brain samples indicated that prion titers were lower in muscle than in CNS where infectivity titers are known to reach high levels. Although possible effects of CWD strains or strain mixtures on these incubation times cannot be excluded, the variable 360 to ~490 d incubation times suggested a range of prion titers in skeletal muscles of CWD-affected deer. Muscle prion titers at the high end of the range produced the fastest incubation times that were ~30% longer than the incubation times of prions from the CNS of the same animal. Since all mice in each inoculation group developed disease, prion titers in muscle samples producing the longest incubation times were higher than the end point of the bioassay, defined as the infectious dose at which half the inoculated mice develop disease. Studies are in progress to accurately assess prion titers. While the risk of exposure to CWD infectivity following consumption of prions in muscle is mitigated by relatively inefficient prion transmission via the oral route (*8*), these

results show that semitendinosus/semimembranosus muscle, which is likely to be consumed by humans, is a significant source of prion infectivity. Humans consuming or handling meat from CWD-infected deer are therefore at risk to prion exposure.

References and Notes

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9. This work was supported by grants from the U.S. Public Health Service 2RO1 NS040334-04 from the National Institute of Neurological Disorders and Stroke and N01-AI-25491 from the National Institute of Allergy and Infectious Diseases.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1122864/DC1
Materials and Methods
Fig. S1

21 November 2005; accepted 13 January 2006
Published online 26 January 2006; 10.1126/science.1122864
Include this information when citing this paper.

Table 1. Incubation times following inoculation of Tg(CerPrP)1536 mice with prions from skeletal muscle and brain samples of CWD-affected deer.

Inocula	Incubation time, mean d ± SEM (n/n0)*	
	Skeletal muscle	Brain
	CWD-affected deer	
H92	360 ± 2 d (6/6)	283 ± 7 d (6/6)
33968	367 ± 9 d (8/8)	278 ± 11 d (6/6)
5941	427 ± 18 d (7/7)	
D10	483 ± 8 d (8/8)	231 ± 17 d (7/7)
D08	492 ± 4 d (7/7)	
Averages	426 d	264 d
	Non-diseased deer	
FPS 6.98	>523 d (0/6)	
FPS 9.98	>454 d (0/7)	>454 d (0/6)
None	>490 d (0/6)	
PBS	>589 d (0/5)	

*The number of mice developing prion disease divided by the original number of inoculated mice is shown in parentheses. Mice dying of intercurrent illnesses were excluded.