occurred 75 km south of the port of Skikda (*10*). The hypothesis of recent importation of the plague bacillus in Kehailia is therefore tempting but is tempered by the fact that 1) the grain is primarily imported from Europe, which is not affected by plague, and from North America where natural foci exist but have very limited areas of overlap with those regions where cereal grains are grown, 2) no higher mortality rate in the murine population of the port was noted, 3) no human cases occurred in this sector of the city, and 4) a 3IS–restriction fragment length polymorphism (*11*) analysis grouped these strains in a cluster clearly distinct from the strains isolated from Africa and America (V. Chenal-Francisque et al., unpub. data).

The geographic concentration of the cases in 2 foci, both contiguous in the mountainous area of Tessala, suggested the existence of a natural focus in this area. Moreover, *Meriones* are present in Tessala, and these rodents are a well-known potential reservoir of *Y. pestis* (*12*). The outbreak occurred at harvest time, and it is possible that the abrupt reduction in the source of food pushed the wild rodents to approach houses in which grain was stored.

The current challenge in terms of public health is to determine if this animal reservoir has disappeared or if it is well established in the ecosystem. The capture of 3 seropositive small mammals (2 *Mus musculus* and 1 *Aleterix algerius*) in July 2004 (J.L. Soares et al., unpub. data) and the identification of several *Y. pestis* infected fleas in the same area (*13*) favor the second option.

Beyond the local problem, the proximity of a possible natural reservoir of plague to Oran, a large international commercial port, raises the possibility of the risk for an urban outbreak. At the time of the investigation, the sanitation in the city and port were poor and rodents proliferated. These urban rodents could come in contact with infected rodents from rural areas in the uncontrolled dumps at the periphery or through a dry riverbed that penetrates as far as the city center. Because of Oran's population density and the commercial activities of its seaport, a plague outbreak would have international implications.

This outbreak is a textbook illustration of the unexpected and sudden reemergence of an infectious disease epidemic that is potentially highly lethal. It also demonstrates that the danger of a plague outbreak is not limited to the currently indexed natural foci.

Acknowledgments

We are grateful to Claire Préaud for the cartography and Christine Gregory, Suzy Lyons, and Stephen Martin for carefully reviewing the manuscript.

Dr Bertherat is a medical officer at the Department of Epidemic and Pandemic Alert and Response, Communicable Diseases, World Health Organization, Geneva. His activities focus on plague and epidemic meningitis.

References

1. World Health Organization. International meeting on preventing and controlling plague: the old calamity still has a future. Wkly Epidemiol Rec. 2006;81:278–84.
2. Misonne X. A natural focus of plague in Libya. Ann Soc Belg Med Trop. 1977;57:163–8.
3. Bulletin sanitaire. Gouvernorat General de l'Algerie. 1909–1941. p. 94–524.
4. Mafart B, Brisou P, Bertherat E. Epidémiologie et prise en charge des épidémies de peste en Méditerranée au cours de la Seconde Guerre Mondiale. Bull Soc Pathol Exot. 2004;97:306–10.
5. Pollitzer R. Plague. WHO Monograph Series. 1954;22:233–50.
6. Roux AH, Mercier C. Sur cinq cas de peste pulmonaire primitive dont trois suivis de guérison, observés à l'hôpital civil d'Oran. Bull Soc Pathol Exot. 1946;39:173–8.
7. Chanteau S, Rahalison L, Ralafiarisoa L, Foulon J, Ratsitorahina M, Ratsifasoamanana L, et al. A. Development and testing of a rapid diagnostic test for bubonic and pneumonic plague. Lancet. 2003;361:211–6.
8. Devignat R. Variétés de l'espèce *Pasteurella pestis*. nouvelle hypothèse. Bull World Health Organ. 1951;4:247–63.
9. Chanteau S, Rahalison L, Ratsitorahina M, Mahafaly M, Rasolomaharo M, Boisier P, et al. Early diagnosis of bubonic plague using F1 antigen capture ELISA assay and rapid immunogold dipstick. Int J Med Microbiol. 2000;290:279–83.
10. Raynaud L. Epidemie de peste à forme septicémique. Revue d'hygiène et police sanitaire. Aug. 1919.
11. Torrea G, Chenal-Francisque V, Leclercq A, Carniel E. Efficient tracing of global isolates of *Yersinia pestis* by restriction fragment length polymorphism analysis using three insertion sequences as probes. J Clin Microbiol. 2006;44:2084–92.
12. Baltazard M, Bahmanyar M, Mofidi C, Seydian B. Kurdistan plague focus [in undetermined language]. Bull World Health Organ. 1952;5:441.
13. Bitam I, Baziz B, Rolain JM, Belkaid M, Raoult D. Zoonotic focus of plague, Algeria. Emerg Infect Dis. 2006;12:1975–7.

Address for correspondence: Eric Bertherat, Communicable Diseases, EPR/ERI, World Health Organization, 20 av. Appia, CH-1211 Geneva 27, Switzerland; email: bertherate@who.int

"So many scientists think that once they figure it out, that's all they have to do, and writing it up is just a chore. I never saw it that way; part of the art of any kind of total scholarship is to say it well."

—Stephen Jay Gould

別紙様式第2-1

No. 21

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
				2007. 12. 17	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	Mead S, Joiner S, Desbruslais M, Beck JA, O'Donoghue M, Lantos P, Wadsworth JD, Collinge J. Arch Neurol. 2007 Dec;64(12):1780-4.	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

○英国の若年女性におけるクロイツフェルト・ヤコブ病、プリオンタンパク質遺伝子コドン129VV、および新規PrP^{Sc}型

背景:変異型クロイツフェルト・ヤコブ病(vCJD)は、ウシ海綿状脳症と因果関係のある後天性プリオン疾患であり、若い成人に多く発現する。調査した臨床例は全て、プリオンタンパク遺伝子(PRNP)のコドン129がメチオニンホモ接合体であり、典型的な神経病理所見を伴い、分子学的系統は典型的なPrP^{Sc}タイプ4であった。トランスジェニックマウスのモデル試験では、他のPRNP遺伝子型もウシ海綿状脳症に感染しやすいことが示されているが、特徴的な表現型を発現すると考えられる。

目的:PRNP コドン129がバリンホモ接合である非定型孤発型CJDの若年英国人女性の組織病理学的、分子学的検討。

デザイン:症例報告、剖検、分子学的解析。

設定・施設:neurology referral centerおよびMRC(医学研究審議会)プリオン部門の研究所。

結果:剖検所見は非定型孤発型CJDであり、灰白質と白質の変性が顕著で、プリオンタンパク質(PrP)の広域な沈着があった。解析用のリンパ網内系組織は得られなかった。小脳組織由来のPrP^{Sc}(PrPのスクレイピーアイソフォーム)の分子解析は、vCJDで見られるものと同等の新規PrP^{Sc}型を示した(PrP^{Sc}タイプ4)。しかし、金属イオンキレート剤EDTA存在下においてプロテアーゼ切断部位が変化したことにより、典型的なvCJD パターンと区別することができた。

結論:本患者に見られたプリオン系統の特徴を明らかにし、ウシ海綿状脳症との因果関係を検討するには、さらに試験が必要である。本症例は、PrP^{Sc}のプロテアーゼ切断パターンの金属イオン依存性を検討するため、EDTAによるプリオン疾患の分子解析の重要性を明らかにしている。

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
PRNP コドン129がバリンホモ接合である非定型孤発型CJDの若年英国人女性の症例報告である。	日本赤十字社は、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ヶ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1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。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。

3-1

OBSERVATION

Creutzfeldt-Jakob Disease, Prion Protein Gene Codon 129VV, and a Novel PrP^{Sc} Type in a Young British Woman

Simon Mead, PhD, MRCP; Susan Joiner, MSc; Melanie Desbruslais, BSc; Jonathan A. Beck, BSc; Michael O'Donoghue, PhD; Peter Lantos, FRCP; Jonathan D. F. Wadsworth, PhD; John Collinge, FRS

Background: Variant Creutzfeldt-Jakob disease (vCJD) is an acquired prion disease causally related to bovine spongiform encephalopathy that has occurred predominantly in young adults. All clinical cases studied have been methionine homozygotes at codon 129 of the prion protein gene (*PRNP*) with distinctive neuropathological findings and molecular strain type (PrP^{Sc} type 4). Modeling studies in transgenic mice suggest that other *PRNP* genotypes will also be susceptible to infection with bovine spongiform encephalopathy prions but may develop distinctive phenotypes.

Objective: To describe the histopathologic and molecular investigation in a young British woman with atypical sporadic CJD and valine homozygosity at *PRNP* codon 129.

Design: Case report, autopsy, and molecular analysis.

Setting: Specialist neurology referral center, together with the laboratory services of the MRC [Medical Research Council] Prion Unit.

Subject: Single hospitalized patient.

Main Outcome Measures: Autopsy findings and molecular investigation results.

Results: Autopsy findings were atypical of sporadic CJD, with marked gray and white matter degeneration and widespread prion protein (PrP) deposition. Lymphoreticular tissue was not available for analysis. Molecular analysis of PrP^{Sc} (the scrapie isoform of PrP) from cerebellar tissue demonstrated a novel PrP^{Sc} type similar to that seen in vCJD (PrP^{Sc} type 4). However, this could be distinguished from the typical vCJD pattern by an altered protease cleavage site in the presence of the metal ion chelator EDTA.

Conclusions: Further studies will be required to characterize the prion strain seen in this patient and to investigate its etiologic relationship with bovine spongiform encephalopathy. This case illustrates the importance of molecular analysis of prion disease, including the use of EDTA to investigate the metal dependence of protease cleavage patterns of PrP^{Sc}.

Arch Neurol. 2007;64(12):1780-1784

Author Affiliations: MRC [Medical Research Council] Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, London, England (Drs Mead, Wadsworth, and Collinge; Mss Joiner and Desbruslais; and Mr Beck); and Institute of Psychiatry, King's College London (Dr Lantos). Dr O'Donoghue is now with the Department of Clinical Neurology, Nottingham University Hospitals NHS [National Health Service] Trust, Nottingham, England.

THE ORIGINAL RECOGNITION of variant Creutzfeldt-Jakob disease (vCJD) was based on a case series of young patients with rapidly progressive dementia, a geographic and temporal association with bovine spongiform encephalopathy (BSE), and novel neuropathological findings consisting of abundant florid prion protein (PrP) plaques.[1] Molecular strain typing allowed identification of a unique type of PrP^{Sc} (the scrapie isoform of PrP) (type 4) in the brain that was distinct from those seen in classic (sporadic or iatrogenic) CJD and similar to that seen in BSE prion infection of cattle and other species.[2] Subsequent biological strain typing in both conventional and transgenic mice confirmed that vCJD and BSE were caused by the same prion strain.[3,4]

Variant CJD also differs markedly from classic CJD in having prominent and consistent involvement of lymphoreticular tissue, allowing its diagnosis by tonsil biopsy findings.[5-7] To date, more than 160 individuals have died of vCJD in the United Kingdom; the number infected by BSE prions and who may develop prion disease in the years ahead is unknown because human prion incubation periods may exceed 50 years.[8]

All clinical cases of vCJD studied have had a methionine-homozygous (MM) genotype at polymorphic codon 129 of the prion protein gene (*PRNP*).[9] The extension of BSE prion-related disease to individuals with valine-homozygous (VV) or heterozygous (MV) genotypes at *PRNP* codon 129 has been predicted by comparison with other acquired human prion diseases[10,11] and by transgenic mouse

models.[12-14] These models also predict that infection of VV and MV genotypes with BSE or vCJD prions may result in propagation of distinct prion strain types and that patients with VV or MV genotypes might present with clinical, pathological, and molecular phenotypes distinct from that of vCJD.[12-14]

To date, we know of no reported cases of clinical vCJD occurring in the VV or MV genotypes. However, PrP^{Sc} has been reported in lymphoid tissues, but not in the brain, of a patient with *PRNP* 129 MV who had received blood from a person with preclinical vCJD and who died of an unrelated cause.[15] In addition, abnormal PrP immunoreactivity has been reported in anonymous archived lymphoid tissue from 2 individuals with *PRNP* 129 VV.[16] It is unknown whether the individual with the MV genotype would have gone on (or if those with VV will go on) to develop clinical disease and, if so, whether the phenotype will fit the case definition of vCJD.

METHODS

Brain homogenates (10% w/v) were prepared in Dulbecco phosphate buffered saline lacking Ca^{2+} or Mg^{2+} ions. Aliquots were analyzed with or without proteinase K digestion (50 µg/mL final protease concentration, 1 hour, 37°C) by immunoblotting with anti–PrP monoclonal antibody 3F4[17] as described previously.[7,18] Metal ion–dependent conformations of PrP were determined as previously described.[19] Genomic DNA was extracted from peripheral blood, and the entire *PRNP* open reading frame was amplified by polymerase chain reaction and sequenced as described previously.[20]

REPORT OF A CASE

A 39-year-old woman presented to an optician in January 1999 with episodes of blurred vision and photophobia, but no abnormality was found. Two months later, she noted memory impairment, diplopia, dysarthria, and an unsteady gait of fluctuating severity. Five months after onset, the gait and limb ataxia had progressed, although walking was still possible, and the memory loss became more profound. The patient then developed paranoid ideation, aggression, restless nocturnal behavior, anorexia, and mood disturbance. By 5½ months after onset, she could not walk and was unsteady sitting, and limb movements were clumsy.

Examination showed dysarthria, broken pursuit eye movements without nystagmus, impaired upgaze, and stereotyped involuntary movements of the legs. However, limb power, vibration, proprioception, tendon reflexes, and plantar responses were normal. During the ensuing 4 weeks, speech ceased and incontinence and jerky involuntary limb movements became evident. Eight months after onset, the patient was mute but could follow some commands. She was able to visually fixate and follow moving objects but also had abnormal, spontaneous horizontal roving eye movements with a supranuclear vertical gaze palsy. Her face was impassive with occasional twitching movements, brisk facial reflexes, and trismus. There were prominent jerking movements of all limbs brought out by use; power was relatively preserved and the plantar responses were extensor.

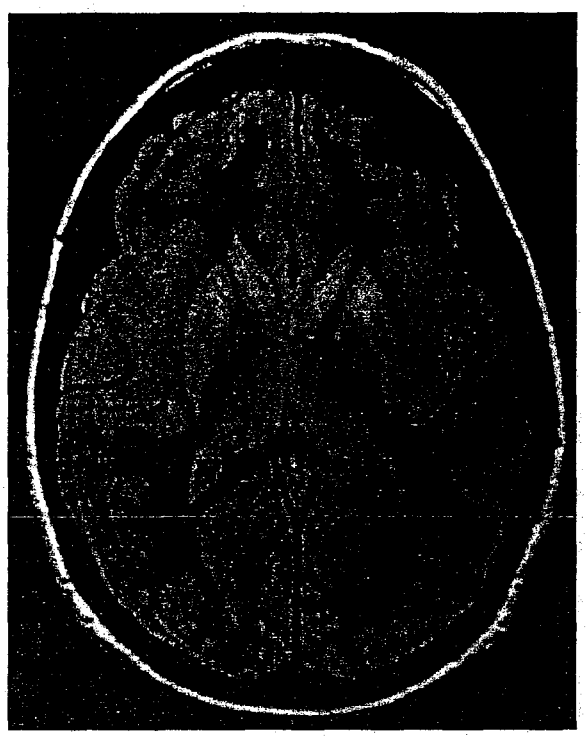

Figure 1. T2-weighted axial magnetic resonance image showing diffuse increased signal within both caudate nuclei and each putamen.

There was a strong family history of late-onset cerebellar ataxia consistent with autosomal dominant inheritance. A polyglutamine expansion in ataxin 3 associated with spinocerebellar ataxia type 3 was found in a symptomatic family member, but our patient did not share this expansion.

Normal results of the following investigations were found: complete blood cell count, erythrocyte sedimentation rate, C-reactive protein, electrolytes, liver function, thyroid function, enzyme-linked immunosorbent assay for syphilis, vitamin B_{12}, folate, ferritin, vitamin E, and serum ceruloplasmin. Tests for antinuclear, antineuronal, anti–Purkinje cell, and antiganglioside antibodies were negative. Nerve conduction studies showed no evidence of a peripheral neuropathy. The electroencephalogram 6 months after onset was reported as normal, but at 7 and 8 months electroencephalograms showed diffuse slow-wave activity, without epileptiform changes or periodic discharges typical of CJD. Cerebrospinal fluid examination showed a normal cell count, protein level, and glucose level, and oligoclonal immunoglobulin bands were absent. The protein S100b level of 4.39 ng/mL (reference cutoff, <0.38 ng/mL), neuron-specific enolase level of 98 ng/mL (reference cutoff, <20 ng/mL), and 14-3-3 protein were all abnormal values.

A magnetic resonance image of the brain (**Figure 1**) showed diffuse cerebellar atrophy and diffuse increased signal within both caudate nuclei and each putamen. Tonsil biopsy was not possible because of a previous tonsillectomy from which little tissue remained. Genetic testing for mutations associated with spinocerebellar ataxia 1, 2, 3, 6, and 7 and Friedreich ataxia gave negative re-

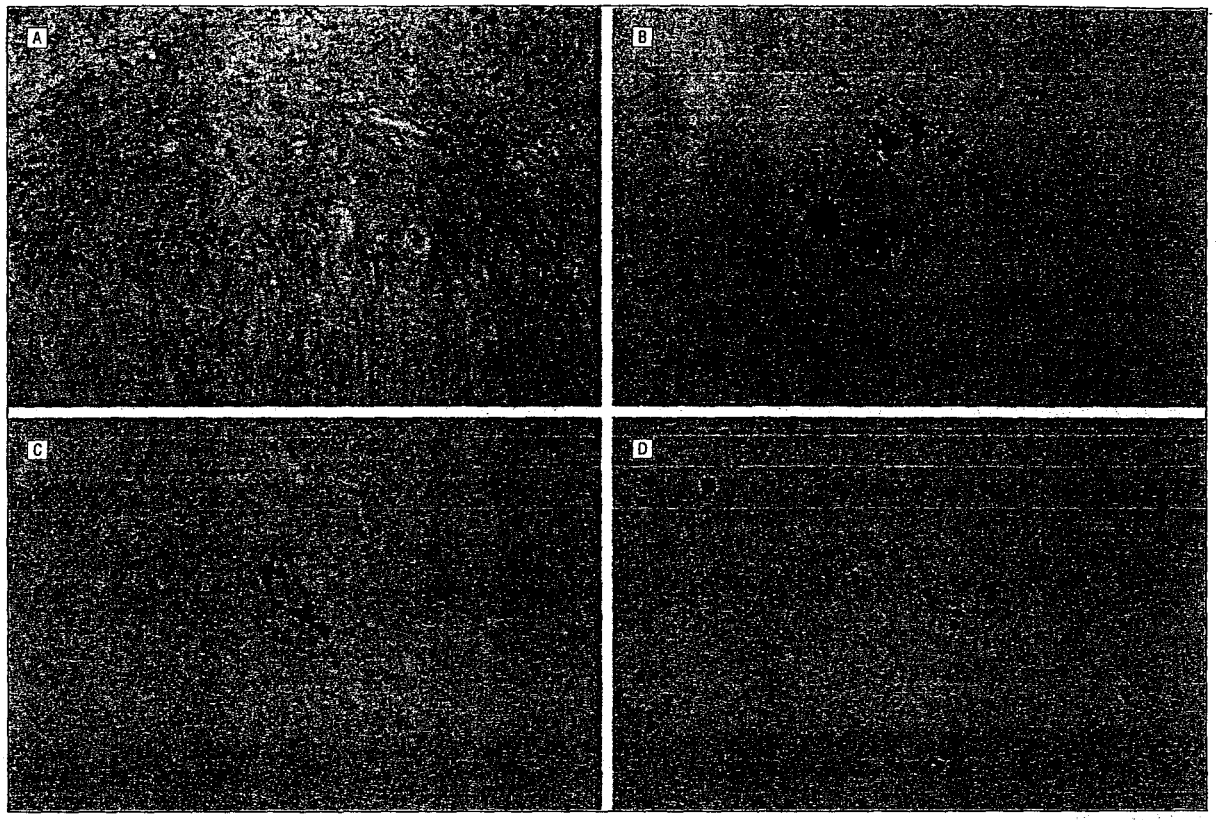

Figure 2. Immunohistochemical analysis of brain sections from the patient. A, Glial fibrillary acidic protein immunohistochemistry of the molecular and granule cell layers of the cerebellum showing neuronal loss and Bergmann astrocytosis (original magnification ×20). B, Granular prion protein staining in the cerebellum (original magnification ×40). C, Perineuronal prion protein staining in the temporal lobe (original magnification ×20). D, Prion protein plaques in the temporal lobe (original magnification ×20).

sults. Sequencing of the *PRNP* open reading frame was normal on 2 separate occasions. A polymerase chain reaction performed with primers designed to amplify the octapeptide repeat region of *PRNP* did not demonstrate an insertion mutation. The codon 129 polymorphism was homozygous for valine.

Fourteen months after onset, the patient died and an autopsy was performed.

AUTOPSY FINDINGS

Histopathologic examination was limited to the brain and spinal cord (**Figure 2**). The findings were atypical of sporadic CJD in the severity of white matter degeneration and the extensive nature of PrP deposition in the cortex and white matter. The frontal cortex showed extremely severe neuronal loss with striking astrocytosis and prominent spongiform vacuolation. There was severe overall loss of white matter, in places reminiscent of infarction. Deposition of PrP was extensive throughout the cortex and white matter. In places this was a diffuse punctate deposition similar to the recognized synaptic pattern. Occasionally, individual cells, mainly pyramidal neurons, were outlined by PrP deposition and had a fine granular intracellular deposition. More dense deposits, similar to plaques, were seen in the cortex. Also in the white matter, PrP deposits were seen ranging from a couple of micrometers to much larger plaquelike deposits, although these were not florid.

Temporal, parietal, and occipital lobes showed histologic features similar to those described in the frontal lobe, the occipital lobe being most severe. The hippocampus was relatively well preserved. In the caudate, putamen, and amygdala there was neuronal loss, astrocytosis, and microglial activation. The thalamus, midbrain, and substantia nigra showed mild to moderate spongiform change, neuronal loss, and astrocytosis with intraneuronal and extracellular punctate deposits. The pons and medulla were less severely affected than the midbrain with punctate PrP deposits. The cerebral peduncles were severely affected, with nearly complete loss of myelin. The cerebellum was very severely affected, with a dramatic loss of Purkinje and granule cells accompanied by vacuolation and astrocytosis. The cerebellar white matter showed severe white matter loss similar to incipient infarcts. Deposition of PrP in the cerebellum was marked with accumulation of punctate deposits resembling plaques, most commonly in the granule cell layer. In the white matter the deposits were denser still, occasionally plaquelike or forming irregular linear deposits.

PrP^{Sc} TYPING STUDIES

Western blot analysis was performed on fresh frozen cerebellar tissue from the patient. Identical results were ob-

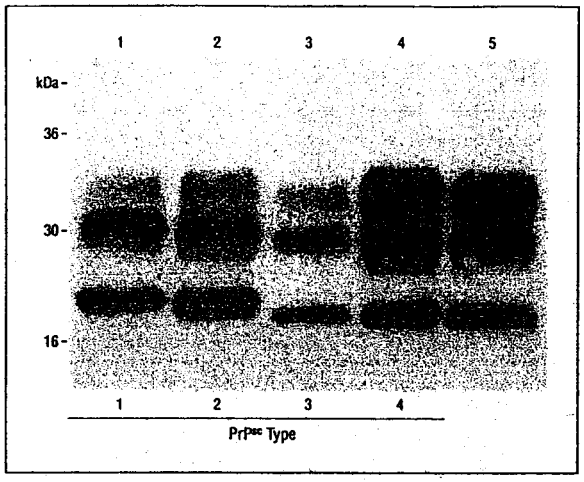

Figure 3. Immunoblotting of 10% brain homogenate after limited proteinase K digestion using anti–prion protein (PrP) monoclonal antibody 3F4. Lanes 1, 2, and 3 show 3 types of PrP^{Sc} (the scrapie isoform of PrP) seen in sporadic and iatrogenic cases of Creutzfeldt-Jakob disease; lane 4 shows PrP^{Sc} type 4, which is uniquely seen in brain tissue from patients with variant Creutzfeldt-Jakob disease.[21] Lane 5 shows PrP^{Sc} from the cerebellum of our patient demonstrating the same predominance of the high-molecular-mass diglycosylated PrP glycoform and a molecular mass of all PrP fragments similar to those of PrP^{Sc} type 4.

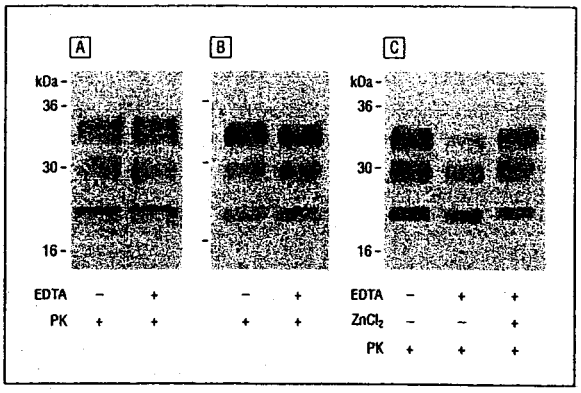

Figure 4. Immunoblotting of 10% brain homogenate after limited proteinase K (PK) digestion using anti–prion protein (PrP) monoclonal antibody 3F4. A, Cerebellum from a patient with variant Creutzfeldt-Jakob disease demonstrating a doublet of low-molecular-mass nonglycosylated bands of PrP^{Sc} (the scrapie isoform of PrP) with an identical pattern of PrP fragments observed after proteolysis in the presence of 25mM EDTA. B, Cerebellum from our patient demonstrating a doublet of low-molecular-mass nonglycosylated PrP^{Sc} bands. All bands migrate with lower apparent molecular mass following proteolysis in the presence of 25mM EDTA. C, Aliquots of cerebellum homogenate from our patient digested directly with proteinase K or after treatment with 25mM EDTA and sequential washing of insoluble pellets with *N*-ethyl morpholine buffer either lacking (–) or containing (+) 20μM zinc chloride ($ZnCl_2$).[19]

tained from separately analyzed tissue samples from opposite poles of the cerebellum. The glycoform ratio and fragment sizes resembled PrP^{Sc} type 4 seen in vCJD (**Figure 3**). The nonglycosylated band was seen as a doublet, as is seen for PrP^{Sc} in the cerebellum in vCJD (**Figure 4**). The effect of adding the metal ion chelator EDTA to the cerebellum homogenate before proteinase K cleavage was to reduce the apparent molecular weight of PrP^{Sc} fragments. This reflects the involvement of metal ions (most likely copper and zinc) in the conformation of PrP and determination of accessible protease cleavage sites.[19] This deduction was verified by showing that application of zinc ions to EDTA-treated samples before proteolysis resulted in preservation of the original PrP^{Sc} fragment size (Figure 4C). Although similar dependence on metal ions is observed for some PrP^{Sc} conformers associated with sporadic CJD,[19,21] this is not observed with PrP^{Sc} type 4 propagated in vCJD[19,21] (Figure 4). Therefore, these findings reflect a novel PrP^{Sc} type when compared with the diversity we and others have so far documented.[21,22]

COMMENT

Does the PrP^{Sc} typing suggest a BSE-related cause, or can our findings be accommodated by the spectrum seen in sporadic CJD cases worldwide? The molecular strain typing of the patient's brain material demonstrated a novel PrP^{Sc} type when compared with our archived cases.[21] There is as yet no internationally agreed-on classification of PrP^{Sc} type. Parchi and colleagues[23] identified 2 PrP^{Sc} types in sporadic CJD. However, Hill et al[21] described 3 PrP^{Sc} types associated with sporadic and iatrogenic CJD (types 1-3) and PrP^{Sc} type 4 associated with vCJD. The PrP^{Sc} type 5 has, to our knowledge, been observed only in mice expressing human PrP 129V inoculated with vCJD.[3,12] Hill et al[21] recently described a novel PrP^{Sc} type 6 in sporadic CJD.

The PrP^{Sc} type from our case has features similar to PrP^{Sc} type 4 (vCJD) in the predominance of the diglycosylated band; however, it is distinct from PrP^{Sc} type 4 in the dependence of the protease cleavage pattern of PrP^{Sc} on metal ions, suggesting a distinct PrP^{Sc} conformation. Unfortunately, only cerebellum was available for Western blotting in this case, although in vCJD cases from which whole brain was available we have not found evidence of any regional variation in PrP^{Sc} type. Others have reported coexistence of Gambetti PrP^{Sc} type 1 in the brain from patients with vCJD as a minority component.[24] It would also have been interesting to look for peripheral lymphoreticular PrP deposition because this is prominent in vCJD, but that tissue was not available for analysis. Transmission of BSE isolates to transgenic mice expressing human PrP 129 valine results in clinical prion disease with undetectable PrP^{Sc}; however, transmission of vCJD isolates to the same mice produces PrP^{Sc} type 5 that shares the same predominance of diglycosylated PrP^{Sc} to that of PrP^{Sc} type 4, and these data suggest that the molecular signature of BSE may be preserved after BSE transmission to *PRNP* codon 129 VV humans.[3,12] Transmission studies of the current case in transgenic mice are now being undertaken to investigate transmission characteristics.

We have described a novel PrP^{Sc} type that would be designated type 7 by our classification. A firm connection between novel PrP^{Sc} types and BSE cannot be made on the basis of a single case, and it will be important to see whether other similar cases occur in the United Kingdom and other BSE-exposed countries but not elsewhere and to perform detailed transmission studies of prions from this patient into transgenic and conventional mice to compare with BSE-derived isolates from

cattle and other species. Two other cases of prion disease with valine homozygosity and atypical features have been reported in the United Kingdom and the Netherlands. One of these cases was atypical because of very young onset and a protracted psychiatric history[25]; the other was notable because certain clinical and molecular features of the case overlapped with those of vCJD, including Western blot analysis of autopsied brain showing a predominance of a diglycosylated PrP^{Sc} isoform.[26]

We recommend keeping an open mind about the etiology of such cases during the ensuing years. These cases emphasize the importance both of continued surveillance of prion disease and the further development and refinement of molecular classification of prion diseases of humans and animals. It will also be important to assess lymphoreticular involvement in subsequent cases either at diagnostic tonsil biopsy or at autopsy.

Accepted for Publication: February 22, 2006.
Correspondence: John Collinge, FRS, MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, England (j.collinge@prion.ucl.ac.uk).
Author Contributions: *Study concept and design:* Mead and Collinge. *Acquisition of data:* Mead, Joiner, Desbruslais, O'Donoghue, Lantos, Wadsworth, and Collinge. *Analysis and interpretation of data:* Mead, Joiner, Desbruslais, Beck, Wadsworth, and Collinge. *Drafting of the manuscript:* Mead, Desbruslais, Beck, Lantos, Wadsworth, and Collinge. *Critical revision of the manuscript for important intellectual content:* Mead, Joiner, O'Donoghue, Wadsworth, and Collinge. *Statistical analysis:* Mead. *Obtained funding:* Collinge. *Administrative, technical, and material support:* Desbruslais, Beck, Lantos, and Collinge. *Study supervision:* Wadsworth and Collinge.
Financial Disclosure: None reported.
Funding/Support: This work was funded by the MRC and undertaken at the University College London Hospitals and University College London, who received a proportion of funding from the Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme.
Additional Contributions: Ray Young assisted with figure design. The MRC London Neurodegenerative Diseases Brain Bank (Institute of Psychiatry) provided pathological material. We also acknowledge the many clinicians involved in the care of this patient.

REFERENCES

1. Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet.* 1996;347(9006):921-925.
2. Collinge J, Sidle KCL, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. *Nature.* 1996;383(6602):685-690.
3. Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J. The same prion strain causes vCJD and BSE. *Nature.* 1997;389(6650):448-450.
4. Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. *Nature.* 1997;389(6650):498-501.
5. Hill AF, Zeidler M, Ironside J, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet.* 1997;349(9045):99-100.
6. Hill AF, Butterworth RJ, Joiner S, et al. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet.* 1999;353(9148):183-189.
7. Wadsworth JDF, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant CJD using a highly sensitive immuno-blotting assay. *Lancet.* 2001;358(9277):171-180.
8. Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century—an acquired human prion disease with very long incubation periods. *Lancet.* 2006;367(9528):2068-2074.
9. Collinge J, Beck J, Campbell T, Estibeiro K, Will RG. Prion protein gene analysis in new variant cases of Creutzfeldt-Jakob disease. *Lancet.* 1996;348(9019):56.
10. Collinge J. Variant Creutzfeldt-Jakob disease. *Lancet.* 1999;354(9175):317-323.
11. Mead S, Stumpf MP, Whitfield J, et al. Balancing selection at the prion protein gene consistent with prehistoric kurulike epidemics. *Science.* 2003;300(5619):640-643.
12. Wadsworth JD, Asante EA, Desbruslais M, et al. Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science.* 2004;306(5702):1793-1796.
13. Bishop MT, Hart P, Aitchison L, et al. Predicting susceptibility and incubation time of human-to-human transmission of vCJD. *Lancet Neurol.* 2006;5(5):393-398.
14. Asante EA, Linehan JM, Gowland I, et al. Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. *Proc Natl Acad Sci U S A.* 2006;103(28):10759-10764.
15. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet.* 2004;364(9433):527-529.
16. Ironside JW, Bishop MT, Connolly K, et al. Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study [published correction appears in *BMJ.* 2006;333(7565):416]. *BMJ.* 2006;332(7551):1186-1188.
17. Kascsak RJ, Rubenstein R, Merz PA, et al. Mouse polyclonal and monoclonal antibody to scrapie-associated-fibril proteins. *J Virol.* 1987;61(12):3688-3693.
18. Hill AF, Joiner S, Beck JA, et al. Distinct glycoform ratios of protease resistant prion protein associated with *PRNP* point mutations. *Brain.* 2006;129(pt 3):676-685.
19. Wadsworth JDF, Hill AF, Joiner S, Jackson GS, Clarke AR, Collinge J. Strain-specific prion-protein conformation determined by metal ions. *Nat Cell Biol.* 1999;1(1):55-59.
20. Poulter M, Baker HF, Frith CD, et al. Inherited prion disease with 144 base pair gene insertion, I: genealogical and molecular studies. *Brain.* 1992;115:675-685.
21. Hill AF, Joiner S, Wadsworth JD, et al. Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain.* 2003;126(pt 6):1333-1346.
22. Parchi P, Giese A, Capellari S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol.* 1999;46(2):224-233.
23. Parchi P, Castellani R, Capellari S, et al. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. *Ann Neurol.* 1996;39(6):767-778.
24. Yull HM, Ritchie DL, Langeveld JP, et al. Detection of type 1 prion protein in variant Creutzfeldt-Jakob disease. *Am J Pathol.* 2006;168(1):151-157.
25. Hillier CE, Llewelyn JG, Neal JW, Ironside JW. Creutzfeldt-Jakob disease in a young person with valine homozygosity at codon 129: sporadic or variant? *J Neurol Neurosurg Psychiatry.* 2001;70(1):134-135.
26. Head MW, Tissingh G, Uitdehaag BMJ, et al. Sporadic Creutzfeldt-Jakob disease in a young Dutch valine homozygote: atypical molecular phenotype. *Ann Neurol.* 2001;50(2):258-261.

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 3-1

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 11 月 14 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Species barrier for chronic wasting disease by in vitro conversion of prion protein. Li, L. et al, Biochem. Biophys. Res. Com. 364(4), 796-800 (2007)	公表国 カナダ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
本稿の著者らは，慢性消耗性疾患（北米シカに影響を及ぼす伝染性海綿状脳症）は，in vitro アッセイにおいてある特定の条件下で種の壁をすり抜けて感染することを明らかにした。本アッセイは，異種動物からの正常な脳ホモジネート（正常 PrP^{C}）を基質として，エルク（ヨーロッパヘラジカ）の異常プリオンタンパク質（PrP^{Sc}）とともにインキュベートするものである。標準の条件（pH 7.4）下では，エルク（ヨーロッパヘラジカ）PrP^{Sc} は同種系列〔トナカイ，ムース（アメリカヘラジカ），カリブー及びエルク（ヨーロッパヘラジカ）〕の PrP^{C} をタンパク質分解酵素耐性アイソフォームへと変換させたが，異種 PrP^{C}（ヒト，マウス，ヒツジ，ウシ，ハムスター）については，PrP^{C} のタンパク質配列が全ての種で 90%以上保持されているにもかかわらずタンパク質分解酵素耐性アイソフォームへ変換されたものは僅かであった。しかしながら，低 pH（3.5）による部分変性の条件下では，PrP^{Sc} によるタンパク質分解酵素耐性アイソフォームへの変換は全ての種で劇的に増大した。これより，基質の部分変性によって構造上の変化が起こり，遠隔種間の種の壁を越えることが示唆される。	BYL-2008-0303

報告企業の意見	今後の対応
異常プリオン PrP^{Sc} によるアイソフォーム変換への感度および耐性は，基質である PrP^{C} の立体構造が重要であるとしているが，生物学的な関連性については疑問が残る。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

32

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Biochemical and Biophysical Research Communications 364 (2007) 796–800
BBRC
www.elsevier.com/locate/ybbrc

Species barriers for chronic wasting disease by *in vitro* conversion of prion protein

Li Li [a], Michael B. Coulthart [b], Aru Balachandran [c], Avi Chakrabartty [d], Neil R. Cashman [a,*]

[a] *Brain Research Centre, Division of Neurology, Department of Medicine, University of British Columbia and Vancouver Coastal Health, UBC Hospital, 2211 Wesbrook Mall, Vancouver, BC, Canada V6T 2B5*
[b] *Prion Diseases Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Man., Canada R3E 3R2*
[c] *National Reference Laboratory for Scrapie and CWD, Animal Diseases Research Institute, Canadian Food Inspection Agency, 3851 Fallowfield Road, Nepean, Ont., Canada K2H 8P9*
[d] *University Health Network, Department of Medical Biophysics, University of Toronto, Toronto, Ont., Canada M5G 1L7*

Received 6 October 2007
Available online 25 October 2007

Abstract

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy that can affect North American cervids (deer, elk, and moose). Using a novel *in vitro* conversion system based on incubation of prions with normal brain homogenates, we now report that PrP^{CWD} of elk can readily induce the conversion of normal cervid PrP (PrP^{C}) molecules to a protease-resistant form, but is less efficient in converting the PrP^{C} of other species, such as human, bovine, hamster, and mouse. However, when substrate brain homogenates are partially denatured by acidic conditions (pH 3.5), PrP^{CWD}-induced conversion can be greatly enhanced in all species. Our results demonstrate that PrP^{C} from cervids (including moose) can be efficiently converted to a protease-resistant form by incubation with elk CWD prions, presumably due to sequence and structural similarities between these species. Moreover, partial denaturation of substrate PrP^{C} can apparently overcome the structural barriers between more distant species.

Keywords: CWD; PrP^{C}; PrP^{Sc}; *In vitro* conversion; Species barrier

Chronic wasting disease (CWD) is a cervid form of transmissible spongiform encephalopathy (TSE) or prion disease. CWD's rapid spread from' Colorado to other states [1,2], to Canadian provinces (Alberta, Saskatchewan) [1] and to Korea [2,3] has raised concerns about its species tropism [4–6]. CWD has been transmitted to cattle via intracerebral inoculation [7], and to other animals, including ferrets, mink, and goats [8,9]. Reports documenting CWD prions in the muscle [10,11], blood, and saliva [12] of infected cervids, have heightened interest in the disease by public health agencies [13].

CWD and other TSEs are believed to be due to the template-directed accumulation of disease-associated prion protein, generically designated PrP^{Sc}. PrP^{C} in brain homogenates can be converted to a protease-resistant form by incubation with PrP^{Sc} "seeds" which are thought to recapitulate the template-directed misfolding of prion protein in disease [14,15], including protein misfolding cyclic amplification (PMCA) [15]. We have previously reported that partially denatured human brain PrP^{C} (which may mimic a PrP conversion intermediate [16]) is a superior substrate for templated *in vitro* conversion compared with untreated PrP^{C} in an incubation-shaking assay that does not utilize PMCA sonication [17].

Materials and methods

Reagents and antibodies. Proteinase K (PK) was purchased from Invitrogen. Mouse monoclonal antibody 6H4 was from Prionics Co. (Zürich, Switzerland). Horseradish peroxidase-conjugated sheep anti-

* Corresponding author. Fax: +1 604 822 7299.
E-mail address: Neil.Cashman@vch.ca (N.R. Cashman).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.10.087

mouse antibody was purchased from Amersham Biosciences. All other chemicals were purchased from Sigma unless specified otherwise.

Brain tissues and homogenate preparation. All brain samples were obtained from the disease control and surveillance programs of the Canadian Food Inspection Agency (CFIA) and were harvested within 24 h of death. The normal brain tissue was determined to be free of neurological disorders on the basis of neuropathological examination. The presence of PrP^{Sc} in brain tissue from an elk with clinical chronic wasting disease (CWD) was confirmed by immunohistochemistry and PK resistance on immunoblotting analysis. All tissues were frozen immediately after collection and stored at −80 °C. Ten percent (w/v) brain homogenates were prepared in lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, and 10 mM Tris–HCl, pH 7.5) as previously described [17].

Preparation of acid/GdnHCl-treated PrP^{C}. The preparation was followed as previously described [17], in brief, 100 µl of 10% brain homogenate was mixed with an equal volume of 3.0 M guanidine hydrochloride GdnHCl (final concentration of 1.5 M) in PBS at pH 7.4 or pH 3.5 adjusted with 1 M HCl, and incubated for 5 h at room temperature with shaking. After that, samples were precipitated with methanol and resuspended in 100 µl of PBS (pH 7.4) with 0.05% SDS, 0.5% Triton X-100.

In vitro conversion of acid/GdnHCl-treated PrP^{C}. In vitro conversion was performed in a 50 µl volume of the appropriate test substrate material (49 µl of normal brain homogenate + 1 µl CWD brain homogenate in a 1:50 dilution as the prion template). The sample was then incubated in a thermomixer at 37 °C for 12 h with shaking. After PK digestion and boiling in the loading buffer, the samples were subjected to SDS–PAGE and immunoblotting.

Proteinase K resistance and immunoblotting. To determine the PK-resistance of the PrP, 20 µl of the sample was incubated with PK at 100 µg/ml for 1 h at 37 °C, and the digestion reaction was terminated by addition of PMSF to 2 mM of final concentration. Proteins were separated by NuPAGE 4–12% pre-cast Bis–Tris gel (Invitrogen) and electrotransferred onto PVDF membranes. 6H4 was used as primary antibody (1:5000) and horseradish peroxidase-conjugated sheep anti-mouse IgG as secondary antibody. The proteins were visualized by enhanced chemiluminescence + Plus (ECL + Plus, Amersham Biosciences), the blots were scanned and were analyzed by Quantity One (Bio-Rad) software. At least eight experiments were performed on each species.

Results and discussion

Sequence alignment of prion protein

CWD appears to be freely transmitted among susceptible species of cervids by direct or environmentally medi-

A

```
          1                                                  50
Rangifer  MVKSHIGSWI LVLFVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN
Elk       MVKSHIGSWI LVLFVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN
Moose     MVKSHIGSWI LVLFVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN

          51                                                 100
Rangifer  RYPPQGGGGW GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GGWGQGGTHS
Elk       RYPPQGGGGW GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GGWGQGGAHS
Moose     RYPPQGGGGW GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GGWGQGGTHS

          101                                                150
Rangifer  QWNKPSKPKT NMKHVAGAAA AGAVVGGLGG YMLGSAMSRP LIHFGNDYED
Elk       QWNKPSKPKT NMKHVAGAAA AGAVVGGLGG YMLGSAMSRP LIHFGNDYED
Moose     QWNKPSKPKT NMKHVAGAAA AGAVVGGLGG YMLGSAMSRP LIHFGNDYED

          151                                                200
Rangifer  RYYRENMYRY PNQVYYRPVD QYNNQNTFVH DCVNITVKQH TVTTTTKGEN
Elk       RYYRENMYRY PNQVYYRPVD QYNNQNTFVH DCVNITVKQH TVTTTTKGEN
Moose     RYYRENMYRY PNQVYYRPVD QYNNQNTFVH DCVNITVKQH TVTTTTKGEN

          201                                                250
Rangifer  FTETDIKMME RVVEQMCITQ YQRESQAYYQ RGASVILFSS PPVILLISFL
Elk       FTETDIKMME RVVEQMCITQ YQRESQAYYQ RGASVILFSS PPVILLISFL
Moose     FTETDIKMME RVVEQMCITQ YQRESQAYYQ RGASVILFSS PPVILLISFL

          251  256
Rangifer  IFLIVG
Elk       IFLIVG
Moose     IFLIVG
```

Fig. 1. Prion protein amino acid sequence alignment. (A) Prion protein sequence alignment of caribou/reindeer (rangifer), elk and moose. Protein sequences of PrP^{C} in cervid group are highly conserved, except for one amino acid polymorphism boxed in grey. (B) Prion protein sequence alignment of elk and other species (hamster, human, mouse, bovine, and sheep). PrP is >90% conserved.

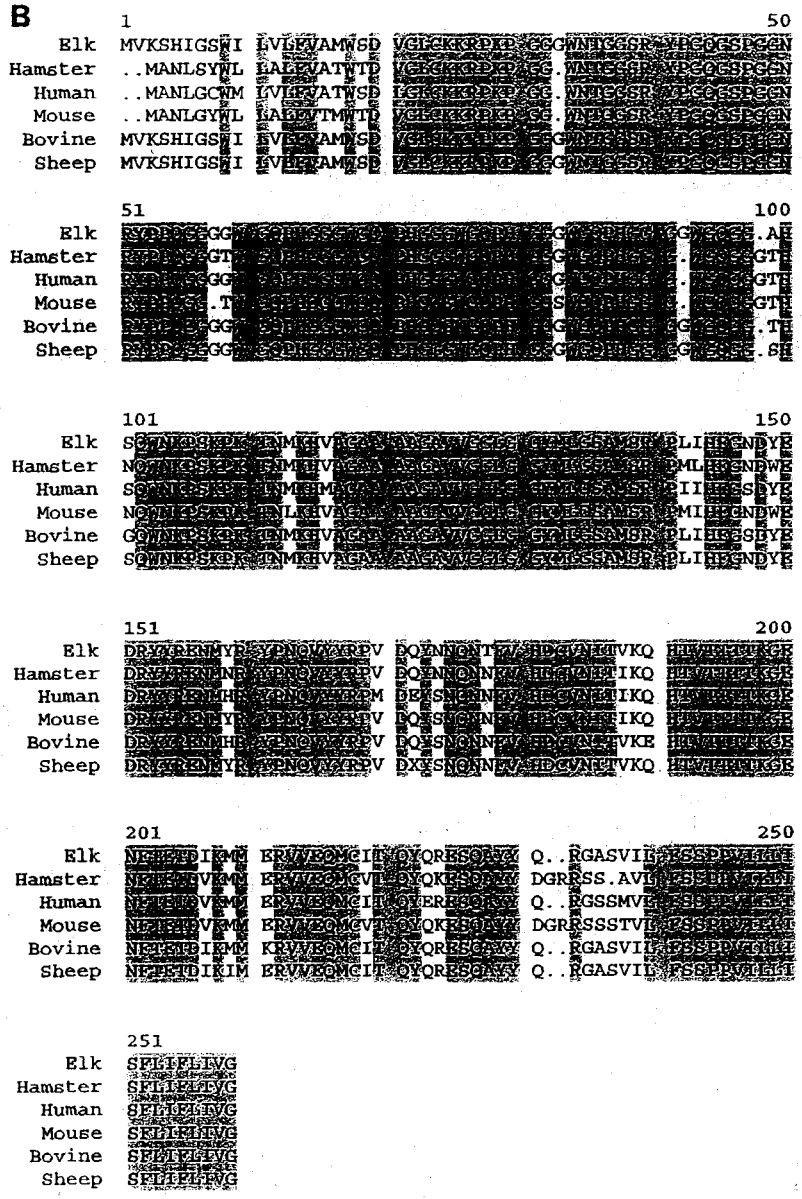

Fig. 1 (*continued*)

ated horizontal contact [5,9,18,19]. We aligned the amino acid sequences from species of cervid species which were used in the experiment: elk (*Cervus elaphus*; GenBank Accession No. CAA70902) reindeer/caribou, (*Rangifer tarandus*; GenBank Accession No. AAZ81477—reindeer is the European name for wild caribou), and moose (*Alces alces*; GenBank Accession No. AAZ81479) (Fig. 1A). The protein sequence of these three cervid species is highly conserved, with only one amino acid polymorphism reported in GenBank. We also aligned the amino acid sequences of elk with other species, such as hamster, human, mouse, bovine, and sheep, which reveals that the protein sequence of PrP^{C} is more than 90% conserved (Fig. 1B).

In vitro conversion of various species with CWD prion template

Normal brain homogenates from elk, reindeer, moose, caribou, human, hamster, mouse, bovine, and sheep, which were incubated with CWD-affected elk brain "seeds", were tested for conversion to a protease-resistant PrP isoform (Fig. 2) as previously described for human CJD *in vitro* conversion [17]. As a negative control, *Prnp* null mouse brain showed no signal corresponding to PK-resistant PrP^{Sc} (Fig. 2, K/O mouse bar). Partial denaturation of normal brain homogenates induced by exposure to low pH and guanidine enhanced *in vitro* conversion to PK-resistant PrP^{Sc} (Fig. 2) has been previously reported for the human

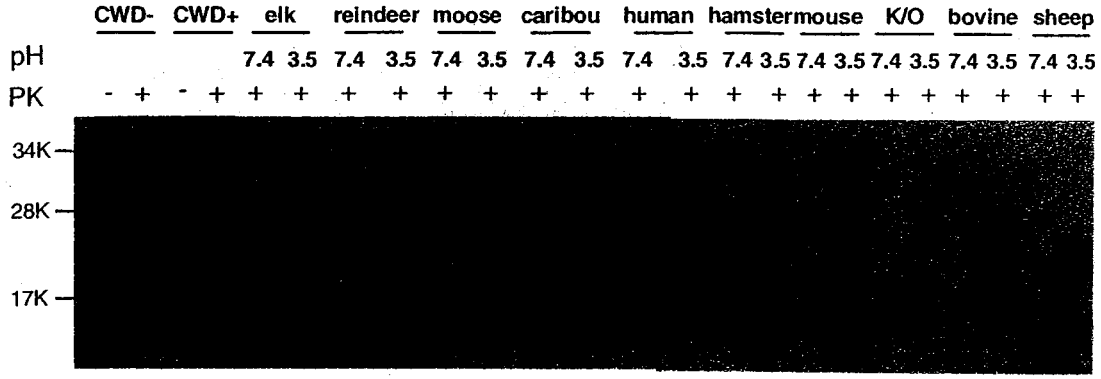

Fig. 2. *In vitro* conversion of treated PrP in the presence of PrP^{Sc} from CWD elk brain. Immunoblots of the PK-resistant PrP isoforms with 6H4 antibody. Samples were treated with GdnHCl and incubated in PBS (pH 7.4) with 0.05% SDS and 0.5% Triton X-100, at 37 °C for 12 h with shaking in the presence of trace amount of elk PrP^{Sc}. CWD−, normal elk brain homogenate as control; − and + indicates the PK treatment. CWD+, elk CWD brain homogenate as a control. The rests are the amplification of PrP^{Sc} in the different species, using elk CWD as seed, treated or untreated with acid (pH 7.4 or pH 3.5).

system [17]. All samples of normal brain contained PrP, which was sensitive to PK digestion (elk shown in Fig. 2, other species not shown). Five microliters of CWD brain homogenate was barely visible after PK digestion (Fig. 2), which was 25-fold greater than the dilution-adjusted CWD seed used in conversion system, excluding artifact from input PrP^{Sc}. Bands of the PK-resistant PrP^{Sc} form were present at ~21 kDa in all the species under acidic conditions (pH 3.5), except for the *Prnp* null mouse (Fig 2). However, PK-resistant PrP^{Sc} was poorly generated in some species in which the brain homogenates were treated under neutral conditions (pH 7.4), such as in human, hamster, mouse, bovine, and sheep. For homogenates treated at neutral pH (pH 7.4), the progression from most susceptible to least susceptible was: elk, reindeer > moose > caribou > hamster > human, bovine, sheep > mouse, with no detected conversion in *Prnp* null mouse brain.

PrP conversion efficiency enhancement by partial denaturation

Treatment of substrate brain with acidic pH (pH 3.5) enhanced PrP^{CWD}-induced conversion of all species, except *Prnp* null mice as expected (Fig. 3A). If the conversion of partially denatured PrP can be considered to be the maximum achievable conversion, the ratio of conversion of brain homogenates treated at pH 7.4 relative to pH 3.5 may provide a "conversion efficiency ratio" (CER) for that species. The comparative CER within different species is shown in Fig. 3B. Notably, some cervid species showed variability in crude conversion efficiency of native and denatured substrate, despite similar (or even identical) PrP amino acid sequences (e.g., caribou and reindeer). Although individual assays might vary for trivial reasons such as slightly differing concentration of brain homogenate, the adjusted CER seems to indicate all cervids display similar substrate conversion efficiency as expected from their evolutionary proximity. The CER analysis also

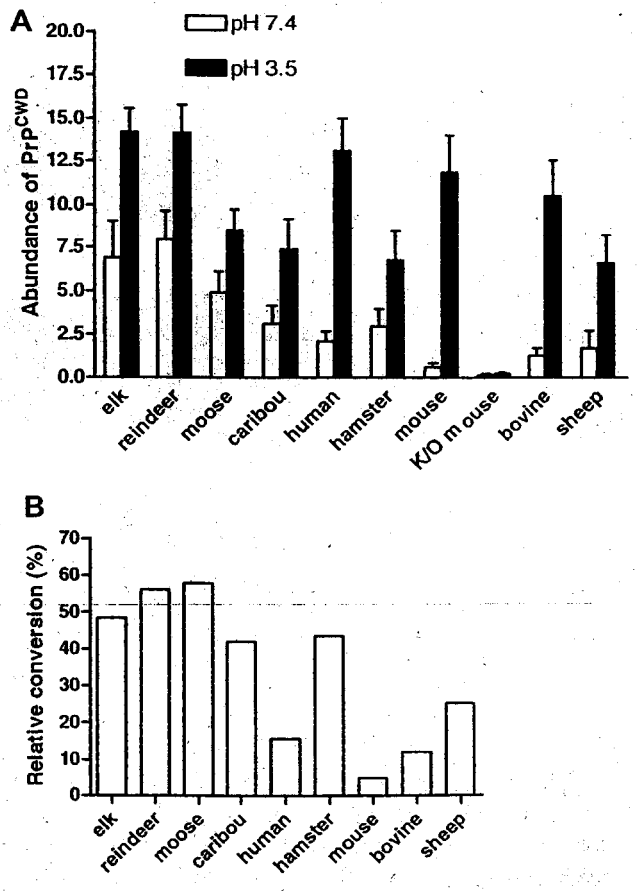

Fig. 3. (A) The immunoblots as in Fig. 2 were examined by densitometry to determine the ratio of neutral (pH 7.4) and acidic (pH 3.5) forms of PrP^{Sc} using Quantity One software (Bio-Rad). (B) Conservation efficiency ratio of native and denatured PrP substrate.

appears to show that hamster segregates with the cervids. Although Syrian hamsters were initially deemed resistant to CWD, a recent publication demonstrates that CWD can be transmitted and adapted to hamsters [20].

Measurement of species barriers by in vitro conversion assays

A number of studies have been published on the PrP^{Sc}-induced conversion of PrP^{C} [14,15,21–25]. However, in these assays require molecular cloning to obtain recombinant PrP of different species, derived from cells in culture that may not possess brain-specific PrP posttranslational modifications, and/or brain molecules which may facilitate PrP isoform conversion. Furthermore, it now appears that PMCA may trigger stochastic generation of PrP^{Sc} *de novo* [15], which may render this technique unsuitable for determining species barriers of prion infection.

Substrate denaturation and human health

We confirm with multiple species that acid/GdnHCl-treated brain PrP^{C} is a superior substrate for *in vitro* conversion than untreated PrP^{C}, possibly by overcoming conformational barriers in partial denaturation of substrate PrP^{C}. PrP conversion in scrapie-infected neuroblastoma cells is believed to occur in endosomes, a low-pH and reducing environment [26]. The non-ruminant stomach possesses a low pH lumen, and PrP^{C} is expressed in this organ [27]. Such acidic (denaturing) organ or cellular organellar environments might also promote CWD transmission to non-cervid species, including humans.

Acknowledgments

This work was supported by the Canadian Institutes of Health Research (Institute of Infection and Immunity, Safe Food and Water program) and PrioNet Canada.

References

[1] Alberta Government (Department of Agriculture, Food and Rural Development, Chronic wasting disease (CWD) of elk and deer. Available from: <http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3594#occurred>, Ref Type: Internet Communication1-1-2006.

[2] Department of Agriculture – Animal and Plant Health Inspection Service. Chronic wasting disease herd certification program and interstate movement of farmed or captive deer, elk, and moose: final rule, Federal Register 71 (2006) 41681–41707.

[3] T.Y. Kim, H.J. Shon, Y.S. Joo, U.K. Mun, K.S. Kang, Y.S. Lee, Additional cases of chronic wasting disease in imported deer in Korea, J. Vet. Med. Sci. 67 (2005) 753–759.

[4] M.W. Miller, E.S. Williams, Prion disease: horizontal prion transmission in mule deer, Nature 425 (2003) 35–36.

[5] M.W. Miller, E.S. Williams, N.T. Hobbs, L.L. Wolfe, Environmental sources of prion transmission in mule deer, Emerg. Infect. Dis. 10 (2004) 1003–1006.

[6] E.S. Williams, Chronic wasting disease, Vet. Pathol. 42 (2005) 530–549.

[7] A.N. Hamir, R.A. Kunkle, J.M. Miller, J.C. Bartz, J.A. Richt, First and second cattle passage of transmissible mink encephalopathy by intracerebral inoculation, Vet. Pathol. 43 (2006) 118–126.

[8] J.C. Bartz, R.F. Marsh, D.I. McKenzie, J.M. Aiken, The host range of chronic wasting disease is altered on passage in ferrets, Virology 251 (1998) 297–301.

[9] E.S. Williams, Scrapie and chronic wasting disease, Clin. Lab. Med. 23 (2003) 139–159.

[10] R.C. Angers, S.R. Browning, T.S. Seward, C.J. Sigurdson, M.W. Miller, E.A. Hoover, G.C. Telling, Prions in skeletal muscle of deer with chronic wasting disease, Science (2006) 311:117.

[11] J.E. Jewell, M.M. Conner, L.L. Wolfe, M.W. Miller, E.S. Williams, Low frequency of PrP genotype 225SF among free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease, J. Gen. Virol. 86 (2005) 8–34.

[12] C.K. Mathiason, J.G. Powers, S.J. Dahmes, D.A. Osborn, K.V. Miller, R.J. Warren, G.L. Mason, S.A. Hays, J. Hayes-Klug, D.M. Seelig, M.A. Wild, L.L. Wolfe, T.R. Spraker, M.W. Miller, C.J. Sigurdson, G.C. Telling, E.A. Hoover, Infectious prions in the saliva and blood of deer with chronic wasting disease, Science 314 (2006) 133–136.

[13] E.D. Belay, L.B. Schonberger, The public health impact of prion diseases, Ann. Rev. Public Health 26 (2005) 191–212.

[14] D.A. Kocisko, J.H. Come, S.A. Priola, B. Chesebro, G.J. Raymond, P.T. Lansbury, B. Caughey, Cell-free formation of protease-resistant prion protein, Nature 370 (1994) 471–474.

[15] G.P. Saborio, B. Permanne, C. Soto, Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding, Nature 411 (2001) 810–813.

[16] S. Hornemann, R. Glockshuber, A scrapie-like unfolding intermediate of the prion protein domain PrP(121–231) induced by acidic pH, Proc. Natl. Acad. Sci. USA 95 (1998) 6010–6014.

[17] W.Q. Zou, N.R. Cashman, Acidic pH and detergents enhance in vitro conversion of human brain PrPC to a PrPSc-like form, J. Biol. Chem. 277 (2002) 43942–43947.

[18] C.J. Sigurdson, T.R. Spraker, M.W. Miller, B. Oesch, E.A. Hoover, PrP(CWD) in the myenteric plexus, vagosympathetic trunk and endocrine glands of deer with chronic wasting disease, J. Gen. Virol. 82 (2001) 10–34.

[19] E.S. Williams, M.W. Miller, Chronic wasting disease in deer and elk in North America, Rev. Sci. et Tech. 21 (2002) 305–316.

[20] G.J. Raymond, L.D. Raymond, K.D. Meade-White, A.G. Hughson, C. Favara, D. Gardner, E.S. Williams, M.W. Miller, R.E. Race, B. Caughey, Transmission and adaptation of chronic wasting disease to hamsters and transgenic mice: evidence for strains, J. Virol. 81 (2007) 4305–4314.

[21] A. Bossers, P.B.G.M. Belt, G.J. Raymond, B. Caughey, V.R. de, M.A. Smits, Scrapie susceptibility-linked polymorphisms modulate the in vitro conversion of sheep prion protein to protease-resistant forms, Proc. Natl. Acad. Sci. USA 94 (1997) 4931–4936.

[22] D.A. Kocisko, S.A. Priola, G.J. Raymond, B. Chesebro, P.T. Lansbury Jr., B. Caughey, Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier, Proc. Natl. Acad. Sci. USA 92 (1995) 3923–3927.

[23] A. Bossers, V.R. de, M.A. Smits, Susceptibility of sheep for scrapie as assessed by in vitro conversion of nine naturally occurring variants of PrP, J. Virol. 74 (2000) 1407–1414.

[24] G.J. Raymond, A. Bossers, L.D. Raymond, K.I. O'Rourke, L.E. McHolland, P.K. Bryant III, M.W. Miller, E.S. Williams, M. Smits, B. Caughey, Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease, EMBO J. 19 (2000) 4425–4430.

[25] G.J. Raymond, J. Hope, D.A. Kocisko, S.A. Priola, L.D. Raymond, A. Bossers, J. Ironside, R.G. Will, S.G. Chen, R.B. Petersen, P. Gambetti, R. Rubenstein, M.A. Smits, P.T. Lansbury Jr., B. Caughey, Molecular assessment of the potential transmissibilities of BSE and scrapie to humans, Nature 388 (1997) 285–288.

[26] L. Laszlo, J. Lowe, T. Self, N. Kenward, M. Landon, T. McBride, C. Farquhar, I. McConnell, J. Brown, J. Hope, Lysosomes as key organelles in the pathogenesis of prion encephalopathies, J. Pathol. 166 (1992) 333–341.

[27] Z. Marcos, K. Pfeifer, M.E. Bodegas, M.P. Sesma, L. Guembe, Cellular prion protein is expressed in a subset of neuroendocrine cells of the rat gastrointestinal tract, J. Histochem. Cytochem. 52 (2004) 1357–1365.

別紙様式第 2-1
番号 5

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2007 年 12 月 10 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理人免疫グロブリン ③人免疫グロブリン		研究報告の公表状況	Journal of Biological Chemistry 2007; 282(49): 35878-35886	公表国 イギリス	
販売名 （企業名）	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②ヴェノグロブリン-IH（ベネシス） ③グロブリン-Wf（ベネシス）					

研究報告の概要

ヒトおよび反芻動物における伝達性海綿状脳症(TSE)の診断は死後の脳組織中のプロテアーゼ抵抗性の宿主糖タンパク質 PrP の検出に依存している。この異常なアイソフォーム(PrP^{Sc})が組織中に存在することは TSE の感染性が存在することを示すものとされている。
本研究は、PrP^{Sc} のレベルが低いか、もしくは検出されない動物の TSE 疾患の臨床的および空胞化徴候を示す脳組織内に、高タイターの TSE 感染性が存在しうることを明確に示している。本研究は PrP^{Sc} のレベルと感染価との間の相関性に疑問を投げかけるものであり、プロテアーゼ K 抵抗性の PrP をほとんどもしくは全く含まない組織が感染源となりうること、および高タイターの TSE 感染性を有しうることを示すものである。
従って、プロテアーゼ抵抗性の PrP^{Sc} を感染性の唯一の尺度としてそれに依存することは、場合によっては診断しようとするサンプルの生物学的特性を著しく過小評価し、そのことによって TSE を防止し根絶しようとする努力を減弱させる可能性がある。

報告企業の意見	今後の対応
PrP^{Sc} のレベルが低いか、もしくは検出されない動物の TSE 疾患の臨床的および空胞化徴候を示す脳組織内に、高タイターの TSE 感染性が存在するとの報告である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 略
1) 略
2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 49, pp. 35878–35886, December 7, 2007

High Titers of Transmissible Spongiform Encephalopathy Infectivity Associated with Extremely Low Levels of PrP^{Sc} *in Vivo*[*] [S]

Received for publication, May 25, 2007, and in revised form, September 24, 2007 Published, JBC Papers in Press, October 8, 2007, DOI 10.1074/jbc.M704329200

Rona M. Barron[‡1,2], **Susan L. Campbell**[‡1,3], **Declan King**[‡], **Anne Bellon**[§], **Karen E. Chapman**[¶], **R. Anthony Williamson**[§], **and Jean C. Manson**[‡]

From the [‡]*Neuropathogenesis Unit, Roslin Institute, Ogston Building, West Mains Road, Edinburgh EH9 3JF, Scotland, United Kingdom, the* [§]*Department of Immunology, Scripps Research Institute, La Jolla, California 92037, and the* [¶]*Centre for Cardiovascular Sciences, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland, United Kingdom*

Diagnosis of transmissible spongiform encephalopathy (TSE) disease in humans and ruminants relies on the detection in post-mortem brain tissue of the protease-resistant form of the host glycoprotein PrP. The presence of this abnormal isoform (PrP^{Sc}) in tissues is taken as indicative of the presence of TSE infectivity. Here we demonstrate conclusively that high titers of TSE infectivity can be present in brain tissue of animals that show clinical and vacuolar signs of TSE disease but contain low or undetectable levels of PrP^{Sc}. This work questions the correlation between PrP^{Sc} level and the titer of infectivity and shows that tissues containing little or no proteinase K-resistant PrP can be infectious and harbor high titers of TSE infectivity. Reliance on protease-resistant PrP^{Sc} as a sole measure of infectivity may therefore in some instances significantly underestimate biological properties of diagnostic samples, thereby undermining efforts to contain and eradicate TSEs.

The transmissible spongiform encephalopathy (TSE)[4] diseases (also known as prion diseases) are infectious, fatal neurodegenerative diseases of animals, which include Creutzfeldt-Jacob disease (CJD) in humans and bovine spongiform encephalopathy (BSE) in cattle. The true identity of the infectious agent responsible for these diseases is not known. However, it has been proposed that TSE disease is caused by an abnormal form of the host glycoprotein, PrP (1). The abnormal, disease-associated form of the protein (PrP^{Sc}), is partially protease-resistant and detergent-insoluble unlike the normal cellular conformer (PrP^{C}), and is seen to accumulate in diseased tissues. The prion hypothesis predicts that PrP^{Sc} alone is the infectious agent of TSE and is able to induce the conversion of endogenous PrP^{C} into the abnormal form during disease (2).

Most human TSE diseases are familial or sporadic, but disease can also be acquired by surgical intervention (3) or blood transfusion from infected individuals (4–9), or possibly from the consumption of BSE-infected meat products, the presumed cause of variant CJD (vCJD) (10). The extent to which vCJD infection in particular is present in the United Kingdom population is unknown, but recent research has suggested there may be a higher rate of subclinical or preclinical vCJD than previously thought in different human PrP genotypes (7, 11–13). Although BSE is declining in the United Kingdom, cases have now been observed in cattle in countries that have not previously reported BSE. It is also unknown whether the agent responsible for BSE has re-entered the human food chain following transmission to sheep. For these reasons a high level of active and passive surveillance of ruminants is required at slaughter to monitor and prevent TSE-infected material from entering the human food chain. The introduction of ante-mortem surveillance in the human population is also critical to prevent the human-to-human transmission of vCJD by blood transfusion or surgical procedures. This will be of particular importance if subclinical disease proves to be a significant risk in vCJD transmission (12, 13).

Positive identification of TSE infectivity can only be demonstrated conclusively by transmission of disease to laboratory animals. Such assays are time-consuming, due to long incubation times, and expensive, and are therefore not suitable for the rapid diagnosis of all ante- or post-mortem samples. Current diagnostic tests instead rely on the detection of disease-associated PrP^{Sc} in samples taken from brain post-mortem. The development of ante-mortem diagnostic tests is also being based around more sensitive assays for PrP^{Sc}. Several diagnostic tests are available commercially, and most require proteinase K (PK) treatment of tissue homogenates to isolate disease-specific PK-resistant PrP^{Sc} (PrP-res). It has not yet been definitively proven that PrP^{Sc} is the TSE infectious agent, and whether it is present in all infected tissues. Studies using 263K hamster scrapie have shown a strong correlation between PrP-

* This work was supported by United Kingdom Department for Environment, Food, and Rural Affairs Grant SE1437. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3 and Table S1.

[1] Both authors contributed equally to this work.

[2] To whom correspondence should be addressed. Tel.: 44-131-667-5204; Fax: 44-131-668-3872; E-mail: rona.barron@bbsrc.ac.uk.

[3] Current address: Medical Research Council Clinical Sciences Centre, Imperial College, Hammersmith Campus, Du Cane Road, London W12 0NN, United Kingdom.

[4] The abbreviations used are: TSE, transmissible spongiform encephalopathy; CJD, Creutzfeldt-Jacob disease; vCJD, variant Creutzfeldt-Jacob disease; PK, proteinase K; GSS, Gerstmann Sträussler Scheinker; CDI, conformation-dependent immunoassay; IP, immunoprecipitation; IHC, immunohistochemistry; mAb, monoclonal antibody; BSE, bovine spongiform encephalopathy; PrP-res, PK-resistant PrP^{Sc}; $sPrP^{Sc}$, PK-sensitive form of PrP^{Sc}; ELISA, enzyme-linked immunosorbent assay; d/n, ratio of denatured to native signal; Wt, wild-type.

res and infectivity (2, 14, 15). However, other studies have demonstrated the transmission of disease from infected animals that appear to lack significant levels of PrP-res (16–19). In such cases it has been suggested that a PK-sensitive form of PrP^{Sc} ($sPrP^{Sc}$) may represent the infectious agent (20–22). Hence it is possible that infectivity may be associated with a specific isoform of abnormal PrP. The identification of this specific conformer is imperative for the future of TSE diagnosis. If present, large amounts of PrP^{Sc} may be a clear indication of the presence of infectivity in a tissue sample. However, if TSE infectivity does not always associate with high levels of PrP^{Sc}, current diagnostic methods may fail to identify all animals with TSE disease and may not provide a realistic estimate of the level of infectivity in an infected tissue. For the purposes of this study, PrP^{Sc} is used to define all abnormal forms of PrP, whereas PrP-res specifically defines PK-resistant PrP, and $sPrP^{Sc}$ defines PK-sensitive forms of PrP^{Sc}.

We have previously identified two mouse models of TSE disease (18, 19) that indicate that the association between PrP-res and infectivity is not as straightforward as predicted by the prion hypothesis. Unlike wild-type controls, transgenic mice homozygous for a targeted mutation at amino acid 101 (proline to leucine) in endogenous murine PrP (101LL) develop clinical TSE disease following inoculation with hamster 263K scrapie or human Gerstmann Sträussler Scheinker (GSS) P102L disease (patient shown to contain vacuolar pathology and PrP-res at post-mortem) (18, 19). Pathological analysis of brain tissue from these mice (101LL/GSS and 101LL/263K) showed TSE-associated vacuolization, and the disease could be further transmitted to 101LL mice with short incubation times of 100–160 days (18, 19). Such incubation times were indicative of a high titer of infectivity in the 101LL/GSS and 101LL/263K tissues, yet analysis by immunoblot revealed that most animals contained extremely low levels of PrP-res, and several contained no detectable PrP-res at all (18, 19). However, the presence of high titers of infectivity cannot be proven by a short disease incubation time. To establish the true relationship between PrP^{Sc} and infectivity we have now performed detailed and quantitative analyses of the disease in these mice. The ID_{50} (dilution at which 50% of the animals become infected) and titer of infectivity in several 101LL/GSS- and 101LL/263K-infected brains have been established by bioassay. Corresponding levels of PrP-res in the same tissues have also been established semi-quantitatively by immunoblot. These analyses have shown no relationship between infectivity titer and PrP-res level. Moreover no other disease-associated forms of PrP were detectable in these tissues. Thus within our model system there is a clear dissociation between titer of infectivity and level of PrP^{Sc}.

EXPERIMENTAL PROCEDURES

Transgenic Mouse Lines and Tissues—Inbred gene-targeted transgenic mouse line 101LL and the corresponding inbred 129/Ola wild-type control line have been described previously (18). 101LL/GSS tissues were produced by inoculation of 101LL transgenic mice with 1% brain homogenate prepared from the occipital cortex of a GSS P102L brain showing numerous multicentric plaques and abundant PrP-res by immunoblot. The individual was methionine 129 homozygous with a confirmed proline to leucine mutation at codon 102.[5] 101LL/263K tissues were produced by inoculation of 101LL transgenic mice with 1% brain homogenate from a 263K-infected hamster. Control tissues were produced by ME7 inoculation of 129/Ola wild-type mice and 101LL transgenic mice.

Preparation of Inocula—Separate inocula were prepared from the brains of two 101LL/GSS- and three 101LL/263K-infected mice with terminal TSE disease, which had been shown by immunohistochemical (IHC) analysis to contain extremely low levels of PrP deposition. Inocula were also prepared from brains of one wild-type and one 101LL mouse with terminal ME7 scrapie as controls. A 10% homogenate of each sample was prepared in sterile saline prior to use as an inoculum. This inoculum was then used to produce a series of 10-fold dilutions from 10^{-2} to 10^{-9} in sterile saline. Each dilution (20 μl) was inoculated intracerebrally under anesthesia into groups of 101LL mice for 101LL/ME7, 101LL/GSS, and 101LL/263K tissues, or wild-type 129/Ola mice for Wt/ME7 tissue. All experimental protocols were submitted to the Local Ethical Review Committee for approval before mice were inoculated. All experiments were performed under license and in accordance with the UK Home Office Regulations (Animals (Scientific Procedures) Act of 1986).

Scoring of Clinical TSE Disease—The presence of clinical TSE disease was assessed as described previously (23). Animals were scored for clinical disease without reference to the genotype of the mouse. Genotypes were confirmed for each animal by PCR analysis of tail DNA at the end of the experiment. Incubation times were calculated as the interval between inoculation and cull due to terminal TSE disease. Mice were killed by cervical dislocation at the terminal stage of disease, at termination of the experiment (between 500–700 days), or for welfare reasons due to intercurrent illness. The proportion of mice showing positive vacuolar pathology was calculated for each group, and the ID_{50} (dilution at which 50% of the mice became infected) was determined using the Karber method (24). This value was used to calculate the number of infectious units per gram wet weight of tissue (IU/g).

Genotyping of Mouse Tail DNA—A 2- to 3-cm portion of tail was removed post-mortem from each mouse. DNA was prepared, and the PrP genotype of each mouse was confirmed as described previously (18).

Immunoblot Analysis and Quantification of PrP-res—For immunoblot analysis, residual inocula (10% saline homogenate) were mixed with an equal volume of 2× Nonidet P-40 buffer (2% Nonidet P-40, 1% sodium deoxycholate, 300 mM NaCl, 100 mM Tris/HCl, pH 7.5) and further homogenized in a microcentrifuge tube using 20–30 strokes with a pre-cooled centrifuge tube pestle (Anachem). The homogenate was centrifuged at 11,000 × *g* for 10 min at 10 °C to remove cellular debris, and the supernatant stored in 50-μl aliquots at −70 °C. For quantification of PrP-res levels in each tissue, homogenates were digested with 20 μg/ml PK at 37 °C for 1 h. Digested homogenates were diluted to 1%, and 2-fold serial dilutions were prepared using PK-treated normal brain homogenate as

[5] J. W. Ironside and M. W. Head, personal communication.

the diluent to keep overall protein concentrations constant. Diluted samples were mixed with sample loading buffer and sample reducing agent (Invitrogen) and loaded across two 12% Tris/glycine polyacrylamide gels (Invitrogen) at concentrations ranging from 1 mg/ml to 3.9 μg/ml (200 μg to 0.8 μg of wet weight tissue equivalent). 50 ng of recombinant PrP was loaded onto each gel as an internal control. After separation, proteins were transferred onto a polyvinylidene difluoride membrane by electroblotting, and PrP was detected with mAb 8H4 (West Dura ECL substrate, Pierce). Monoclonal antibody 7A12 and polyclonal antibody 1B3 were also used to confirm the low PrP-res levels in 101LL/GSS and 101LL/263K tissues. Images were captured on both x-ray film and by a Kodak Digital Image Station 440. Experiments were repeated in duplicate or triplicate depending on sample availability.

Digital images of each gel were analyzed using Kodak ID software, and PrP-res levels were expressed as pixel intensities. Samples were normalized across the two blots and quantified using the recombinant PrP controls as standards. Each value was multiplied by the dilution factor, and an average was taken for all samples run per tissue to determine the level of PrP-res per gram wet weight brain tissue in each model. This value, combined with the titer of TSE infectivity measured in each tissue (IU/g) was used to calculate the number of molecules of PrP-res per infectious unit for each tissue as in Equations 1–3.

$$\text{Number of PrP-res molecules per g of tissue} = n \quad \text{(Eq. 1)}$$

$$n = [\text{PrP-res per g/Avagadro's number } (6.02 \times 10^{23})]/\text{molecular weight PrP } (30{,}000) \quad \text{(Eq. 2)}$$

$$\text{Number of molecules PrP-res per infectious unit} = n/\text{titer (IU/g)} \quad \text{(Eq. 3)}$$

Measurement of Alternative Forms of PrP—The PK resistance of PrP in all samples was analyzed by digestion with a range of PK concentrations. Individual 9-μl aliquots of each 5% Nonidet P-40 brain homogenate were incubated at 37 °C for 1 h with PK concentrations ranging from 1 to 20 μg/ml. The reaction was terminated by addition of phenylmethylsulfonyl fluoride to 1 mM, and samples were analyzed by SDS-PAGE and immunoblotting as described above.

For "cold PK" digestion, samples (10% homogenate) were incubated with 250 μg/ml PK on ice for 1 h. Digestion was stopped by the addition of phenylmethylsulfonyl fluoride to 1 mM. Samples were de-glycosylated with peptide *N*-glycosidase F (New England Biolabs) following the manufacturer's instructions and analyzed by SDS-PAGE and immunoblotting.

CDI Analysis—Samples were analyzed for the presence of PrP^{Sc} using conformation-dependent immunoassay (CDI) as described by Safar *et al.* (20). Briefly, abnormal PrP was precipitated from brain homogenates of 101LL/GSS, 101LL/263K, and 101LL/ME7 infected mice and uninfected 101LL mice using sodium phosphotungstate, and pellets were resuspended in either distilled water or 4 M guanidine hydrochloride to produce native and denatured samples. 4 M guanidine hydrochloride samples were further heat-denatured at 80 °C for 6 min. Samples were added to 96-well plates coated with mAb FH11, and PrP levels were detected using europium-labeled mAb 7A12 and a Victor 2 ELISA plate reader (PerkinElmer Life Sciences). The ratio of denatured to native signal (d/n) was calculated for each tissue to determine the presence of PrP^{Sc}.

Immunoprecipitation of PrP^{Sc}—Laterally bisected brain halves from 101LL transgenic mice were homogenized at 10% (w/v) in Tris-buffered saline and diluted to reach a concentration of 5% (w/v) in Tris-buffered saline containing 1% Triton. Homogenates were sonicated for three pulses of 4 s and clarified by centrifugation at 400 × *g* for 10 min at 4 °C. Phenylmethylsulfonyl fluoride was added to all samples to a concentration of 2 mM. Each sample was analyzed by dot blot to estimate the total PrP content. Briefly, brain homogenates were serially diluted (1:1) in Tris-buffered saline containing 1% Triton then denatured in Tris-SDS sample buffer at 100 °C for 5 min. Equivalent amounts of each sample were then deposited on a nitrocellulose membrane and left until dry. The membrane was probed with mAb 6H4 (Prionics) and a horseradish peroxidase-labeled anti-mouse secondary antibody (Pierce). The resulting signals were compared semi-quantitatively. These data were used to ensure equal PrP input into each individual immunoprecipitation (IP) reaction. For each IP reaction, the motif grafted antibodies or control antibodies were incubated at 10 μg/ml final concentration for 2 h at room temperature in a reaction mixture with 1% Triton. Rabbit anti-human antibodies (Jackson) coupled to magnetic Dynabeads (Dynal) were used to capture the PrP-specific antibodies as described (25, 26). Immunoblot membranes were probed with mAb 6H4 and developed using the ECL femtomolar kit (Pierce).

RESULTS

101LL Mice Infected with 263K and GSS P102L Show Little PrP Deposition in Brain—Brain tissue from 101LL transgenic mice, which showed TSE clinical signs and TSE-associated vacuolar pathology following inoculation with hamster 263K scrapie or human GSS P102L (18, 19), was screened for PrP deposition by IHC using anti-PrP mAb 6H4. As previously demonstrated, 101LL/GSS- and 101LL/263K-infected mice had low levels of PrP deposition in the brain, despite having confirmed TSE disease. Three 101LL/263K- and two 101LL/GSS-infected tissues, which showed extremely low PrP deposition in the brain, were selected for further analysis by bioassay (Fig. 1 and Table 1). In each case, PrP deposition was restricted to the thalamus and, in most cases, was only visible as small grainy deposits under high power microscopy (Fig. 1, *F–H*). Low or undetectable levels of PrP-res in each brain homogenate were confirmed by immunoblot following PK treatment of residual inoculum (Fig. 2).

High Levels of Infectivity Can Be Measured by Bioassay of 101LL/GSS and 101LL/263K Brain Tissue—Although short incubation times in mice can be indicative of high levels of TSE infectivity in an inoculum, the actual level can only be determined by establishing the ID_{50} (dilution at which 50% of the animals become infected) for the inoculum. Infectivity titers were therefore established for the five selected tissues: 101LL/263K(a), 101LL/263K(b), 101LL/263K(c), 101LL/GSS(d), and 101LL/GSS(e) (Table 1). It was considered extremely important in these experiments that, as far as possible, a single brain be

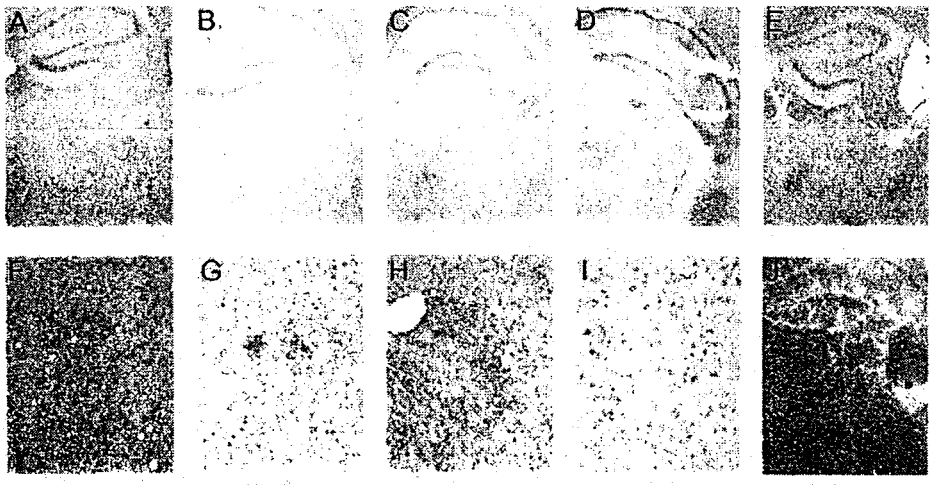

FIGURE 1. **Low levels of PrP deposition in 101LL/GSS- and 101LL/263K-infected brain.** Immunohistochemistry was performed on sections of brain from 101LL/263K- and 101LL/GSS-infected mice using mAb 6H4 to determine the levels of PrP deposition. ME7-infected control mouse brain was stained as control (*J*). Five brains shown in *A–E* (3× 101LL/263K and 2× 101LL/GSS) showing very low levels of deposition were selected for further analysis to quantify the levels of TSE infectivity and PrP^{Sc} in each tissue. Very low levels of PrP deposition were observed in brain tissue, which varied between each individual mouse brain. Deposition was mainly observed in the thalamus (*F–H*). Thalamus of an uninfected 101LL mouse is shown for background comparison (*I*). *A–E* and *J*, 4× magnification; *F–I*, 20× magnification. *A*, 101LL/263K(a); *B*, 101LL/263K(b); *C*, 101LL/263K(c); *D*, 101LL/GSS(d); *E*, 101LL/GSS(e); *F*, thalamus of 101LL/263K(a); *G*, thalamus of 101LL/263K(c); *H*, thalamus of 101LL/GSS(d); *I*, thalamus of 303-day-old uninfected 101LL mouse; and *J*, Wt/ME7 control.

TABLE 1

Tissues selected for analysis

Details of clinical disease and vacuolar pathology in the five tissues selected for analysis. All mice showed positive clinical and vacuolar signs of TSE disease and low levels of PrP deposition.

Tissue used for titration	Clinical TSE	Vacuolar pathology	PrP deposition[a]	Incubation period	
				Primary[b]	Secondary[c]
				days ± S.E.	
101LL/263K(a)	Positive	Positive	+	385	109 ± 2
101LL/263K(b)	Positive	Positive	+/−	464	129 ± 2
101LL/263K(c)	Positive	Positive	+/−	534	262 ± 4
101LL/GSS(d)	Positive	Positive	+	259	154 ± 3
101LL/GSS(e)	Positive	Positive	+/−	252	123 ± 1

[a] Scoring of PrP deposition: +++, high; ++, medium; +, low; +/−, very small grainy deposits.
[b] Incubation time of each individual mouse on primary transmission of either 263K or P102L GSS.
[c] Incubation time of 101LL mice inoculated with 1% brain homogenate from each specific 101LL/263K- or 101LL/GSS-infected tissue. Transmission of disease on subpass to 101LL mice was 100% in each case.

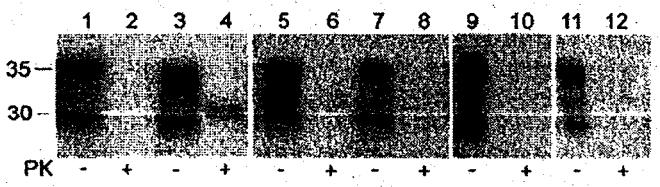

FIGURE 2. **Low or undetectable levels of PrP-res in 101LL/GSS- and 101LL/263K-infected brain.** Residual inoculum from the tissues selected for ID_{50} bioassay were analyzed by immunoblot following PK treatment to detect PrP-res. *Lanes 2, 4, 6, 8, 10,* and *12*, digested with PK at 20 μg/ml for 1 h at 37 °C; *lanes 1, 3, 5, 7, 9,* and *11*, no PK control; *lanes 1* and *2*, uninfected Wt 129/Ola mouse; *lanes 3* and *4*, 101LL/263K(a); *lanes 5* and *6*, 101LL/263K(b); *lanes 7* and *8*, 101LL/263K(c); *lanes 9* and *10*, 101LL/GSS(d); and *lanes 11* and *12*, 101LL/GSS(e). All samples were loaded at 10 mg/ml (w/v) wet weight tissue (200 μg per lane). Blots probed with mAb 8H4.

used for each series of procedures (ID_{50} determination, PK digestion, IHC, etc.). This allowed direct correlation to be made between the level of infectivity and PrP-res in each individual brain and avoided any variation that may occur between tissues, as is often observed on a primary transmission. Moreover this approach avoided the necessity of carrying out large numbers of titration experiments, which would have been both impractical and ethically unacceptable. Inocula were prepared from each individual tissue as 10% sterile saline homogenates and used to produce a series of 10-fold dilutions (10^{-2} to 10^{-9}) for inoculation. Wild-type control 129/Ola and transgenic 101LL mouse brains infected with the well characterized mouse scrapie strain ME7 (Wt/ME7 and 101LL/ME7, respectively) (18) were also assayed as controls. The seven samples were inoculated intracerebrally into groups of 129/Ola mice for Wt/ME7, and transgenic 101LL mice for all other samples. The percentage of mice that developed TSE pathology was calculated for each group in each dilution series, and the ID_{50} was determined using the Karber calculation (24). The numbers of infectious units per gram tissue (IU/g) for each individual mouse brain are shown in Table 2. Assuming a ±0.5 log error for each titer (24), all 101LL/GSS and 101LL/263K samples produced titers of infectivity ranging from ~10^7 to 10^9 IU/g. The highest titer ($10^{9.8}$) was identified in 101LL/GSS(d), however a titer of $10^{8.7}$ was also identified in 101LL/263K(a). Both of these brains showed low levels of PrP deposition by IHC, but titers were higher than that measured in control Wt/ME7 brain ($10^{8.5}$), which showed significantly more PrP deposition by IHC (Fig. 1). Titers in the other three tissues were similar ($10^{7.2}$ to $10^{7.5}$) and confirmed a high level of infectivity in the presence of extremely low or undetectable PrP deposition in the brain (Figs. 1 and 2). The results of the ID_{50} determination therefore prove the presence of high levels of infectivity in 101LL transgenic mice infected with P102L GSS or hamster 263K.

Little or No PrP-res Is Detected in Highly Infectious Tissue—IHC using anti-PrP monoclonal and polyclonal antibodies found little or no PrP deposition in brain tissue of 101LL/263K and 101LL/GSS infected mice (Fig. 1, and data not shown). However, IHC does not distinguish between different forms of PrP, therefore direct measurement of brain PrP-res levels was undertaken to determine the amount of PrP-res associated with titer of infectivity in each brain, listed in Table 1. Residual inoculum from each bioassay was mixed with detergent buffer and digested with PK (Fig. 2), and a 2-fold serial dilution from 1 mg/ml to 3.9 μg/ml (wet weight brain tissue) was analyzed by immunoblotting with mAb 8H4 (27). Recombinant PrP was loaded on each gel at 50 ng as an internal control. For the ME7-infected tissues, the limit of PrP-res detection was 15.6 μg/ml for Wt/ME7 homogenate and 31.3 μg/ml for 101LL/ME7 homogenate. Hence the same agent produced ~2-fold less PrP-

TABLE 2

Comparison of titer of infectivity and PrP-res level

PrP-res levels, quantified relative to recombinant PrP from digital immunoblot images, and infectivity titer, measured by ID_{50} bioassay. Detection limit of the immunoblot system was estimated to be equivalent to 25 μg of PrP-res/g wet weight brain.

Model	PrP-res	PrP-res	Titer[a]
	μg/g tissue[b]	% of ME7	IU/g tissue
Wt/ME7	1994	100	$10^{8.5}$
101LL/ME7	1040	52	$10^{7.8}$
101LL/263K(a)	498	25	$10^{8.7}$
101LL/263K(b)	<25	<1.3	$10^{7.3}$
101LL/263K(c)	<25	<1.3	$10^{7.5}$
101LL/GSS(d)	<25	<1.3	$10^{9.8}$
101LL/GSS(e)	<25	<1.3	$10^{7.2}$

[a] Titer of infectivity per gram of brain tissue as calculated from ID_{50} bioassay in mice using the Karber calculation.

[b] The actual amount of PrP-res quantified from the blots (0.5–2 mg/g) is higher than would be predicted for mouse tissue and may reflect the use of recombinant PrP for calibration, because this does not possess any post-translational modifications and may therefore display altered antibody affinity. However, this internal control acts to normalize each blot and, therefore, ensures that the relative proportions of PrP-res between each model are real, despite possible errors in the absolute quantification.

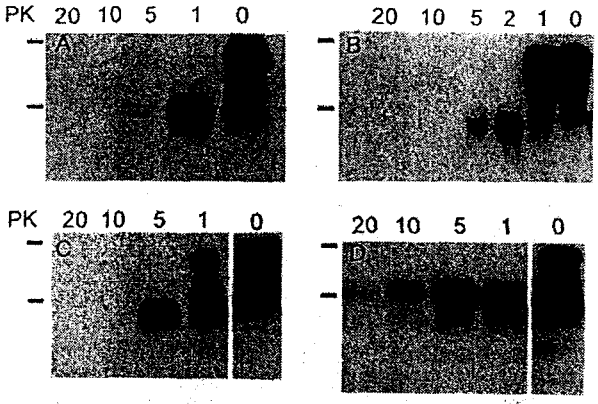

FIGURE 3. **PK resistance of PrP in 101LL/GSS and 101LL/263K brain tissue.** Brain homogenates in Nonidet P-40 lysis buffer were digested with varying concentrations of proteinase K at 37 °C for 1 h. Samples were subjected to SDS-PAGE and immunoblotting to determine the PK sensitivity of the PrP present in 101LL/GSS and 101LL/263K tissue. Representative images show: *A*, uninfected 101LL control mouse brain; *B*, uninfected Wt 129/Ola control mouse brain; *C*, 101LL/263K(b) mouse brain; and *D*, 101LL/263K(a) mouse brain. The PK concentration used for digestion is shown *above* each *lane* (micrograms/ml). Blots were probed with mAb 8H4. *Bars* indicate molecular mass markers of 36 and 30 kDa.

res in the 101LL transgenic mice compared with wild-type mice, although this was associated with a 0.7 log drop in titer (Table 2). In 101LL/263K(a) the limit of PrP-res detection was 62.5 μg/ml brain homogenate, which was approximately half the level in 101LL/ME7 and one quarter the level in Wt/ME7. For all other samples, no PrP-res was detectable in even the most concentrated (1 mg/ml) sample examined (Table 2, Fig. 2, and supplemental Fig. S1*A*). Digital imaging of immunoblots and quantitation of PrP-res relative to recombinant PrP control allowed the calculation of PrP^{Sc} concentration (mean PrP-res grams per gram wet weight of tissue) in each sample (Table 2). The level of sensitivity for the immunoblot, determined using recombinant PrP, was 5–10 ng, therefore the level of PrP-res in samples that showed no PK-resistant material must be below this threshold. Previous studies have shown that PrP-res from other well characterized rodent scrapie strains with titers ranging from $10^{5.5}$ to 10^{9} can be easily identified on immunoblot of 1% brain homogenate following PK treatment (supplemental Fig. S1*B*). These data would suggest that tissue containing titers of 10^{7} to 10^{9} IU/g should contain levels of PrP-res, which can be easily identified by immunoblot. However, for 101LL/GSS- and 101LL/263K-infected tissue this was clearly not the case. Although we cannot eliminate the possibility that PrP-res was indeed present below the threshold level of the immunoblot, a poor correlation between the level of infectivity and the amount of PrP-res in the brain is nevertheless clearly established. To confirm that the failure to detect PrP-res on these immunoblots was not simply a consequence of the loss of the monoclonal antibody epitope (8H4) duplicate blots were also probed with a second monoclonal antibody (7A12) and a polyclonal antibody (1B3), which detects multiple epitopes in PrP. These results confirmed the low PrP-res levels in 101LL/GSS and 101LL/263K tissues (data not shown). Although the combination of monoclonal and polyclonal antibodies used to examine these tissues makes it unlikely that a form of PrP-res exists that has not been detected in our immunoassays, this possibility has not been totally excluded and we continue to investigate these tissues with new antibodies.

Are Alternative Forms of PrP Associated with Infectivity?—Although PrP-res was present at low or undetectable levels in tissues from 101LL/GSS- and 101LL/263K-infected mice, it is possible that forms of PrP other than PrP-res may be infectious (28). Alternative forms of PrP such as transmembrane PrP (29, 30), cytoplasmic PrP (31, 32), and PrP with amino acid insertions or deletions (33–36) have been linked with disease. In addition, a PK-sensitive variant of PrP^{Sc}, $sPrP^{Sc}$, has been recently described (20–22) that may represent an intermediate in the refolding of PrP^{C} to PrP^{Sc} during the disease process and could therefore be associated with infectivity. To test whether $sPrP^{Sc}$ may account for the dissociation between PrP-res and infectivity in 101LL/263K and 101LL/GSS tissues we examined the protease resistance of PrP in such brains by digesting with a range of PK concentrations from 1 μg/ml to 20 μg/ml. Homogenates from Wt/ME7, 101LL/ME7, and uninfected 101LL and 129/Ola mice were also treated with varying PK concentrations as controls. Digestion was stopped by the addition of phenylmethylsulfonyl fluoride to 1 mM, and samples were analyzed by immunoblot (Fig. 3). In the positive controls (Wt/ME7 and 101LL/ME7) PrP-res was evident in all dilutions, with the PK-resistant core still visible after treatment with 20 μg/ml PK (data not shown). PrP in the uninfected controls was found to be sensitive to PK concentrations >5 μg/ml, and produced mildly PK-resistant fragments at PK concentrations of 2–5 μg/ml under the digestion conditions used here (Fig. 3). PrP in the 263K-infected 101LL brains showed variable PK resistance, in agreement with the level of PrP-res detectable in each homogenate. Thus, 101LL/263K(a) showed PrP-res at 20 μg/ml, but 101LL/263K(b) and -(c) showed a similar pattern of PK resistance to uninfected mice (Fig. 3). In addition, samples from both 101LL/GSS(d) and 101LL/GSS(e) showed a PK-sensitivity pattern identical to that of uninfected 101LL mice (data not shown).

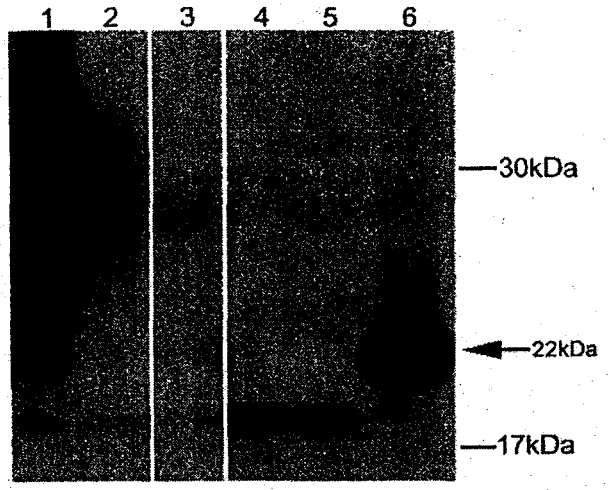

FIGURE 4. **Cold PK treatment of tissues from high titer/low PrP-res models.** 101LL/GSS and 101LL/263K tissues taken from the same transmission experiments as those shown in Table 1 were subjected to cold PK digestion on ice. Uninfected and Wt/ME7-infected brains were also digested as controls. *Lane 1*, undigested 101LL/GSS brain homogenate; *lane 2*, 101LL/263K(g); *lane 3*, 101LL/GSS(f); *lane 4*, 101LL uninfected control; *lane 5*, Wt129/Ola uninfected control; *lane 6*, Wt/ME7 infected control. *Lanes 2–6* were treated with 250 μg/ml PK on ice for 1 h and de-glycosylated with peptide *N*-glycosidase F. ME7 control was loaded at ~25% of the concentration of *lanes 2–5* to allow comparison. The blot was probed with mAb 7A12. The image has been cropped from a single blot to remove lanes with samples that are not relevant to this figure.

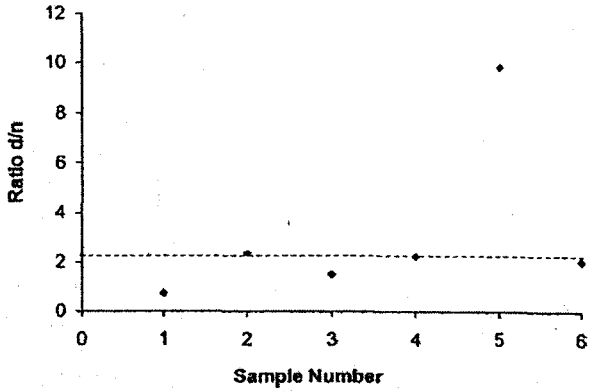

FIGURE 5. **CDI analysis of 101LL/GSS and 101LL/263K brain homogenate.** Samples of 101LL/GSS brain homogenate, 101LL/263K homogenate, and uninfected or ME7-infected controls were analyzed for the presence of PrP^{Sc} using a CDI. Samples were precipitated with sodium phosphotungstate, and pellets were resuspended in either distilled water or 4 M guanidinium-HCl to provide native and denatured samples. These were analyzed in a sandwich ELISA using mAb FH11 as capture and mAb 7A12 as detector. Ratio of denatured to native (*d/n*) signal plotted to show presence of PrP^{Sc}. *Sample 1*, 101LL/GSS(j); *sample 2*, 101LL/GSS(k); *sample 3*, 101LL/263K(m); *sample 4*, 101LL/263K(n); *sample 5*, 101LL/ME7; and *sample 6*, uninfected 101LL mouse. All samples were assayed in duplicate. *Dotted line* indicates cut-off value, which was calculated as the d/n ratio of the uninfected 101LL plus 10%.

The presence of $sPrP^{Sc}$ in brain tissue has also been demonstrated by performing cold PK digestion, *i.e.* PK digestion on ice (21, 22). $sPrP^{Sc}$ has been previously identified in samples that showed no PrP-res (using standard digestion conditions of 20 μg/ml for 1 h at 37 °C) by the presence of a 22-kDa band on immunoblot after digestion with PK on ice and subsequent de-glycosylation with peptide *N*-glycosidase F (21, 22). Although we aimed to perform all procedures on each individual mouse brain, the limited tissue size meant this was not possible for the cold PK analyses carried out here. However, cold PK digestion was performed on brain tissue taken from mice showing positive clinical and vacuolar signs of TSE, but low levels of PrP deposition in the same primary transmission experiments as those listed in Table 2 (details in supplemental Fig. S2 and Table S1). These tissues failed to demonstrate any marked increase in the 22-kDa PK-resistant PrP band after cold PK digestion (Fig. 4, *lanes 2* and *3*). When compared with the ME7 control (Fig. 4, *lane 6*, loaded at 25% concentration of *lanes 2–5*), the low levels of PrP apparent in *lanes 2* and *3* after digestion with PK on ice demonstrate that $sPrP^{Sc}$ cannot account for the high titer of infectivity in the 101LL/263K and 101LL/GSS models.

Although PrP^{Sc} is generally defined by its partial resistance to PK digestion, it can also be identified using immunoassays that exploit the differential binding of anti-PrP antibodies to PrP^{Sc} in the native and denatured state. Epitopes that are hidden in the native PrP^{Sc} conformation become exposed on denaturation in increasing concentrations of guanidine hydrochloride, leading to an increase in antibody binding. This observation is the basis of the CDI, where levels of PrP^{Sc} are calculated by measuring the ratio of the denatured to native signal (d/n ratio) in a sandwich ELISA (20, 37, 38). An increase in d/n ratio indicates the presence of PrP^{Sc}, which produces the increased signal obtained on denaturation of the sample. Because this assay does not use PK digestion to identify abnormal PrP, it can also be used to identify $sPrP^{Sc}$. To confirm the absence of large amounts of PrP-res or $sPrP^{Sc}$ in the models described here, CDI analysis was performed on tissues from 101LL/GSS- and 101LL/263K-infected mice. Tissue from animals detailed in Table 1 was not analyzed due to limited sample availability, but analysis was performed on tissues from 101LL/GSS- and 101LL/263K-infected mice with confirmed clinical and pathological TSE disease, but little or no PrP^{Sc} in the brain (supplemental Fig. S3 and Table S1). The d/n ratios obtained for all four infected animals ranged from 0.73 to 2.39, which were similar to or lower than the uninfected 101LL control (d/n ratio of 2.01). The 101LL/ME7 control gave a d/n ratio of 9.8 (Fig. 5). These data confirm the limited PK digestion studies, proving that no PrP^{Sc}-like conformers are present in 101LL/GSS- and 101LL/263K-infected tissues that could account for the observed titers of infectivity.

Immunoprecipitation Using PrP^{Sc}-specific Monoclonal Antibodies—Several mAbs have been generated that specifically bind PrP^{Sc} isoforms, but not PrP^{C}. These antibodies can therefore isolate PrP^{Sc} from non-PK-treated tissue homogenates by immunoprecipitation, ensuring that all abnormal PrP isoforms are identified. This technique has been used by others to demonstrate the presence of $sPrP^{Sc}$ in the brains of mice overexpressing 101L-PrP (22). Here, PrP^{Sc}-specific motif grafted mAbs 89–112 and 136–158 (25) were used to immunoprecipitate PrP from brain tissue homogenates of 101LL/GSS- and 101LL/263K-infected mice. Tissues analyzed were taken from mice showing positive clinical and vacuolar signs of TSE but low levels of PrP deposition in the same primary transmission experiments as those used to determine titer of infectivity in each model (details in supplemental Fig. S2 and Table S1). Positive control mAb D13 (which precipitates only the cellular form of PrP) and negative control mAb b12 were also

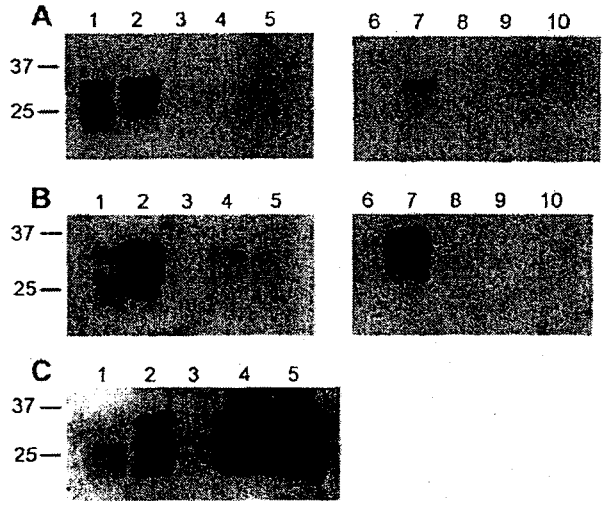

FIGURE 6. **Immunoprecipitation using PrP^{Sc}-specific monoclonal antibodies.** 101LL/GSS and 101LL/263K tissues taken from the same transmission experiments as those shown in Table 1 were subjected to IP using PrP^{Sc}-specific mAbs 89–112 and 136–158 to determine whether forms of PrP^{Sc}, which were sensitive to PK, were present in these tissues. mAb D13, which precipitates only cellular PrP, and mAb b12, which recognizes the HIV gp120 antigen, were used as IP controls. In *A*: *lanes 1–5*, 101LL/GSS(h); *lanes 6–10*, uninfected 101LL; in *B*: *lanes 1–5*, 101LL/263K(i); *lanes 6–10*, uninfected 101LL; in *C*: RML scrapie Wt control. *Lanes 1* and *6*, crude brain homogenate; *lanes 2* and *7*, IP with mAb D13 (positive control antibody); *lanes 3* and *8*, IP with mAb b12 (negative control antibody); *lanes 4* and *9*, IP with mAb 89–112; *lanes 5* and *10*, IP with mAb 136–158.

included in all experiments. For all 101LL/GSS and 101LL/263K tissues examined, extremely low levels of PrP^{Sc} were immunoprecipitated by both PrP^{Sc}-specific antibodies (Fig. 6). These levels were estimated by immunoblot to be 100- to 1000-fold less than those precipitated from control RML-infected mouse brain. Results from these immunoprecipitations therefore support our previous biochemical data, which show no evidence of PK-sensitive forms of PrP^{Sc} in brain tissue from 101LL/GSS- and 101LL/263K-infected mice.

DISCUSSION

PrP^{Sc} is thought to be the sole component of the prion, or TSE infectious agent. For this reason it has become the main target for TSE diagnostic assays, where identification of PrP^{Sc} in post-mortem brain tissue indicates a TSE-positive animal. However the relationship between PrP^{Sc} and TSE infectivity has not been definitively demonstrated, and concerns have been raised by earlier reports of disease transmission in the apparent absence of PrP-res (16, 18). In particular, 101LL gene-targeted transgenic mice inoculated with GSS P102L or 263K succumb to a disease, which is highly transmissible to both 101LL and wild-type mice but shows extremely low levels of PrP-res in the brain. Extended analyses of this model (described here) have now used quantitative assays to unequivocally demonstrate that titers of 10^7 to 10^9 IU/g can be present in brain tissue, which shows little or no abnormal PrP accumulation by standard immunoblot analysis, IHC, CDI, or immunoprecipitation. These titers are similar to or higher than those observed in our well characterized, high titer control strain ME7, but for 4 of 5 brains analyzed, PrP-res levels were below the limit of detection of our immunoblot assay (<1.3% of the amount of PrP-res in wild-type ME7 tissue). Previous studies have shown that PrP-res from other well characterized rodent scrapie strains with titers ranging from $10^{5.5}$ to 10^9 can be easily identified on immunoblot of 1% brain homogenate following PK treatment. Based on these previous data, it would be predicted that the tissues studied here should contain titers far below 10^5 IU/g tissue. However the transmission data clearly show that 101LL/GSS- and 101LL/263K-infected tissues contained high titers of infectivity, which exceed those measured in both 79V- and 22A-infected tissue (supplemental Fig. S1*B*). These data suggest that current diagnostic assay systems that rely on PrP^{Sc} detection might fail to identify some highly infectious tissues. To this end, tissues from 101LL/GSS- and 101LL/263K-infected mice are currently being assessed in several of these assay systems in our laboratory.

Several independent studies have previously shown that one TSE infectious unit is composed of ~10^5 PrP^{Sc} molecules (2, 14, 15). In contrast to these studies the data obtained from 101LL/GSS- and 101LL/263K-infected tissues indicate that the number of PrP^{Sc} molecules per unit of infectivity must display a wide range, with 101LL/GSS and 101LL/263K tissues showing between 10 to 1000 times fewer PrP-res molecules per unit infectivity than Wt/ME7. Alternatively, these data could indicate that only a very small proportion of PrP^{Sc} present in TSE-infected tissue is actually infectious. This lack of correlation between levels of PrP-res and infectivity does not support PrP-res as the infectious agent of TSE.

Because PrP-res does not appear to be a major component of infectivity in this study, it is possible that another form of PrP is responsible for disease in these mice. We have shown previously that 101LL mice can form PrP-res when inoculated with other rodent TSE strains (39); therefore, the lack of PrP^{Sc} in these models is not due to an inherent inability of 101L-PrP to convert to a protease-resistant isoform. In contrast to the gene-targeted transgenic 101LL mice described here, transgenic mice, which overexpress 101L-PrP at levels 8- to 16-fold higher than endogenous PrP, develop a spontaneous neurological disease that appears to be associated with a PK-sensitive form of PrP^{Sc} (21, 22). We have found no evidence of $sPrP^{Sc}$ in 101LL/GSS or 101LL/263K brain tissue by either limited PK digestion studies or CDI analysis. Additionally, motif-grafted mAbs 89–112 and 136–158, which specifically bind PrP^{Sc}, did not immunoprecipitate PK-sensitive forms of PrP^{Sc} from 101LL/GSS or 101LL/263K brain tissue, even though these mAbs have been shown to immunoprecipitate abnormal PK-sensitive PrP^{Sc} from 101L-overexpressing transgenic mice.[6] One possible reason for this discrepancy between models is that disease in 101LL/GSS and 101LL/263K mice is due to a TSE infection, which has been transmitted from a known infected source, and can be further passaged to both 101LL and wild-type 129/Ola mice (18, 19). In contrast the disease observed in transgenic mice overexpressing 101L PrP does not transmit to wild-type mice and only appears to accelerate the phenotype already present in mice expressing lower levels of the transgene (17, 22). This

[6] A. Bellon and R. A. Williamson, unpublished data.

suggests that $sPrP^{Sc}$ may instead be associated with overexpression or misfolding of 101L-PrP and not TSE. The species of abnormal PrP produced due to overexpression of 101L-PrP is therefore different from that produced by TSE infection. The nature of the infectious agent in the current study has yet to be established. We now aim to use this unique model to determine whether infectivity in these tissues is consistent with other abnormal conformations of PrP or with factors other than PrP.

The models of disease described herein demonstrate the potential for the existence of high levels of TSE infectivity with undetectable PrP-res in natural disease. Indeed, increased surveillance and sensitivity of testing methods has identified a new TSE of sheep, termed atypical scrapie. These animals were identified as TSE infected by one PrP^{Sc}-specific diagnostic ELISA, but could not be confirmed by other methods (40, 41). Such cases are now only identifiable using assays that require low concentrations of PK, or no PK, in the assay procedure. It is unknown whether this is truly a new TSE of sheep, or whether it has been present in sheep for some time (42) but was not detected due to the reduced PK resistance of PrP^{Sc}. However, the disease has been shown to be highly transmissible to transgenic mice expressing ovine PrP (43), indicating the presence of substantial levels of infectivity. The results of our study raise concern over the suitability of PrP^{Sc} as a sole diagnostic marker of TSE disease. It is vital that markers of TSE infectivity other than PrP^{Sc} are identified and validated in models such as those we have described and characterized here. We anticipate that such research will lead to the development of more robust diagnostic assays for TSE disease, which will have important implications for both animal and human health.

Acknowledgments—We acknowledge Prof. D. W. Melton (University of Edinburgh, UK) for the production of the 101LL transgenic line; V. Thomson, S. Cumming, E. Murdoch, S. Dunlop, and K. Hogan for experimental setup and care and scoring of the animals; A. Coghill and S. Mack for histology processing and sectioning; A. Boyle and W.-G. Liu for vacuolar profiling; I. Sylvester (Institute for Animal Health, UK) for recombinant PrP; and M.-S. Sy (Case Western Reserve University, Cleveland, OH) for providing anti-PrP monoclonal antibodies 7A12 and 8H4.

REFERENCES

1. Prusiner, S. B. (1996) *Trends Biochem. Sci.* **21**, 482–487
2. Prusiner, S. B. (1982) *Science* **216**, 136–144
3. Brown, P., Preece, M., Brandel, J. P., Sato, T., McShane, L., Zerr, I., Fletcher, A., Will, R. G., Pocchiari, M., Cashman, N. R., D'Aignaux, J. H., Cervenakova, L., Fradkin, J., Schonberger, L. B., and Collins, S. J. (2000) *Neurology* **55**, 1075–1081
4. Houston, F., Foster, J. D., Chong, A., Hunter, N., and Bostock, C. J. (2000) *Lancet* **356**, 999–1000
5. Hunter, N., Foster, J., Chong, A., McCutcheon, S., Parnham, D., Eaton, S., MacKenzie, C., and Houston, F. (2002) *J. Gen. Virol.* **83**, 2897–2905
6. Llewelyn, C. A., Hewitt, P. E., Knight, R. S. G., Amar, K., Cousens, S., Mackenzie, J., and Will, R. G. (2004) *Lancet* **363**, 417–421
7. Peden, A. H., Head, M. W., Ritchie, D. L., Bell, J. E., and Ironside, J. W. (2004) *Lancet* **364**, 527–529
8. Pincock, S. (2004) *Br. Med. J.* **329**, 251
9. Wroe, S. J., Pal, S., Siddique, D., Hyare, H., Macfarlane, R., Joiner, S., Linehan, J. M., Brandner, S., Wadsworth, J. D. F., Hewitt, P., and Collinge, J. (2006) *The Lancet* **368**, 2061–2067
10. Bruce, M. E., Will, R. G., Ironside, J. W., McConnell, I., Drummond, D., Suttie, A., McCardle, L., Chree, A., Hope, J., Birkett, C., Cousens, S., Fraser, H., and Bostock, C. J. (1997) *Nature* **389**, 498–501
11. Hilton, D. A., Ghani, A. C., Conyers, L., Edwards, P., McCardle, L., Ritchie, D., Penney, M., Hegazy, D., and Ironside, J. W. (2004) *J. Pathol.* **203**, 733–739
12. Ironside, J., Bishop, M. T., Connolly, K., Hegazy, D., Lowrie, S., Le Grice, M., Ritchie, D., McCardle, L., and Hilton, D. A. (2006) *Br. Med. J.* **332**, 1164–1165
13. Bishop, M. T., Hart, P., Aitchison, L., Baybutt, H., Plinston, C., Thomson, V., Tuzi, N., Head, M., Ironside, J., Will, R., and Manson, J. (2006) *Lancet Neurol.* **5**, 393–398
14. McKinley, M. P., Bolton, D. C., and Prusiner, S. B. (1983) *Cell* **35**, 57–62
15. Beekes, M., Baldauf, E., and Diringer, H. (1996) *J. Gen. Virol.* **77**, 1925–1934
16. Lasmezas, C. I., Deslys, J., Robain, O., Jaegly, A., Beringue, V., Peyrin, J., Fournier, J., Hauw, J., Rossier, J., and Dormont, D. (1997) *Science* **275**, 402–405
17. Hsiao, K. K., Groth, D., Scott, M., Yang, S. L., Serban, H., Raff, D., Foster, D., Torchia, M., Dearmond, S. J., and Prusiner, S. B. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 9126–9130
18. Manson, J. C., Jamieson, E., Baybutt, H., Tuzi, N. L., Barron, R., McConnell, I., Somerville, R., Ironside, J., Will, R., Sy, M. S., Melton, D. W., Hope, J., and Bostock, C. (1999) *EMBO J.* **18**, 6855–6864
19. Barron, R. M., Thomson, V., Jamieson, E., Melton, D. W., Ironside, J., Will, R., and Manson, J. C. (2001) *EMBO J.* **20**, 5070–5078
20. Safar, J., Wille, H., Itri, V., Groth, D., Serban, H., Torchia, M., Cohen, F. E., and Prusiner, S. B. (1998) *Nat. Med.* **4**, 1157–1165
21. Tremblay, P., Ball, H. L., Kaneko, K., Groth, D., Hegde, R. S., Cohen, F. E., DeArmond, S. J., Prusiner, S. B., and Safar, J. G. (2004) *J. Virol.* **78**, 2088–2099
22. Nazor, K. E., Kuhn, F., Seward, T., Green, M., Zwald, D., Pürro, M., Schmid, J., Biffiger, K., Power, A. M., Oesch, B., Raeber, A., and Telling, G. (2005) *EMBO J.* **24**, 2472–2480
23. Dickinson, A. G., Meikle, V. M., and Fraser, H. (1968) *J. Comp. Pathol.* **78**, 293–299
24. Karber, G. (1931) *Arch. Exp. Pathol. Pharmacol.* **162**, 480–483
25. Moroncini, G., Kanu, N., Solforosi, L., Abalos, G., Telling, G. C., Head, M., Ironside, J., Brockes, J. P., Burton, D. R., and Williamson, R. A. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101**, 10404–10409
26. Solforosi, L., Bellon, A., Schaller, M., Cruite, J. T., Abalos, G. C., and Williamson, R. A. (2007) *J. Biol. Chem.* **282**, 7465–7471
27. Zanusso, G., Liu, D., Ferrari, S., Hegyi, I., Yin, X., Aguzzi, A., Hornemann, S., Liemann, S., Glockshuber, R., Manson, J. C., Brown, P., Petersen, R. B., Gambetti, P., and Sy, M.-S. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 8812–8816
28. Chiesa, R., and Harris, D. A. (2001) *Neurobiol. Dis.* **8**, 743–763
29. Hegde, R. S., Tremblay, P., Gorth, D., DeArmond, S. J., Prusiner, S. B., and Lingappa, V. R. (1999) *Nature* **402**, 822–826
30. Stewart, R. S., Piccardo, P., Ghetti, B., and Harris, D. A. (2005) *J. Neurosci.* **25**, 3469–3477
31. Ma, J., Wollmann, R., and Lindquist, S. (2002) *Science* **298**, 1781–1785
32. Ma, J., and Lindquist, S. (2002) *Science* **298**, 1785–1788
33. Chiesa, R., Piccardo, P., Ghetti, B., and Harris, D. A. (1998) *Neuron* **21**, 1339–1351
34. Chiesa, R., Drisaldi, B., Quaglio, E., Migheli, A., Piccardo, P., Ghetti, B., and Harris, D. A. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 5574–5579
35. Kitamoto, T., Iizuka, R., and Tateishi, J. (1993) *Biochem. Biophys. Res. Commun.* **192**, 525–531
36. Zanusso, G., Petersen, R. B., Jin, T. C., Jing, Y., Kanoush, R., Ferrari, S., Gambetti, P., and Singh, N. (1999) *J. Biol. Chem.* **274**, 23396–23404
37. Safar, J. G., Scott, M., Monaghan, J., Deering, C., Didorenko, S., Vergara, J., Ball, H., Legname, G., Leclerc, E., Solforosi, L., Serban, H., Groth, D., Burton, D. R., Prusiner, S. B., and Williamson, R. A. (2002) *Nat. Biotechnol.* **20**, 1147–1150
38. Safar, J. G., Geschwind, M. D., Deering, C., Didorenko, S., Sattavat, M.,

Sanchez, H., Serban, A., Vey, M., Baron, H., Giles, K., Miller, B. L., DeArmond, S. J., and Prusiner, S. B. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 3501–3506
39. Barron, R. M., Thomson, V., King, D., Shaw, J., Melton, D. W., and Manson, J. C. (2003) *J. Gen. Virol.* **84**, 3165–3172
40. Buschmann, A., Biacabe, A.-G., Ziegler, U., Bencsik, A., Madec, J.-Y., Erhardt, G., Luhken, G., Baron, T., and Groschup, M. H. (2004) *J. Virol. Methods* **117**, 27–36
41. Buschmann, A., Luhken, G., Schultz, J., Erhardt, G., and Groschup, M. H. (2004) *J. Gen. Virol.* **85**, 2727–2733
42. Bruce, M. E., Nonno, R., Foster, J., Goldmann, W., Di Bari, M., Esposito, E., Benestad, S. L., Hunter, N., and Agrimi, U. (2007) *Vet Rec.* **160**, 665–666
43. Le Dur, A., Beringue, V., Andreoletti, O., Reine, F., Lai, T. L., Baron, T., Bratberg, B., Vilotte, J.-L., Sarradin, P., Benestad, S. L., and Laude, H. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 16031–16036

別紙様式第2-1

No. 22

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
				2008. 3. 17	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Kong Q, Zheng M, Casalone C, Qing L, Huang S, Chakraborty B, Wang P, Chen F, Cali I, Corona C, Martucci F, Iulini B, Acutis P, Wang L, Liang J, Wang M, Li X, Monaco S, Zanusso G, Zou WQ, Caramelli M, Gambetti P. J Virol. 2008 Apr;82(7):3697-3701. Epub 2008 Jan 30.	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			イタリア

研究報告の概要

○非定型ウシ海綿状脳症プリオン株のヒト伝播リスクの評価
ウシのプリオン疾患である、ウシ海綿状脳症(BSE)は、BSE-Cというたった1つの株から発生したと広く考えられている。BSE-Cは、ヒトにおいて変異性クロイツフェルト・ヤコブ病と称される致死性プリオン疾患を引き起こす。2004年以降、ウシアミロイド海綿状脳症(BASE、またはBSE-Lとも呼ばれる)およびBSE-Hという2つの非定型BSE株が複数の国で発見された。これらのヒトにおける伝播性と表現型は不明である。我々は、ヒトプリオンタンパク発現トランスジェニック(Tg)マウスに2つのBASE株感染ウシ由来ホモジネートを接種することにより、BASE株の感染性とヒトの表現型を検討した。接種20～22ヶ月後に接種実施Tgマウスの60%が感染し、これはBSE-Cで報告された伝播率よりも高かった。BASE株感染Tgマウスの4分の1が脾臓に病原性プリオンタンパクアイソフォームの存在を示し(孤発性ヒトプリオン疾患によるプリオン感染Tgマウスではゼロ)、BASEプリオンが本質的にリンパ向性であることを示した。BASE株に感染したヒト化Tgマウスの脳の病原性プリオンタンパクアイソフォームは、元のウシBASEまたは散発性ヒトプリオン疾患由来のアイソフォームとは異なった。BASE株感染Tgマウスでは脳の海綿化がごくわずかで、潜伏期間が長いことが観察された。以上の結果は、ヒトにおいて、BASE株は、BSE株よりも感染性が強く、リンパ向性が高いことを示している。

報告企業の意見	今後の対応
非定型ウシ海綿状脳症プリオン株は、ヒトにおいて通常のBSE株よりも感染性が強く、リンパ向性が高いことが示されたとの報告である。	今後も引き続き、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。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-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

JOURNAL OF VIROLOGY, Apr. 2008, p. 3697–3701 Vol. 82, No. 7
0022-538X/08/$08.00+0 doi:10.1128/JVI.02561-07

Evaluation of the Human Transmission Risk of an Atypical Bovine Spongiform Encephalopathy Prion Strain[▽]

Qingzhong Kong,[1]* Mengjie Zheng,[1] Cristina Casalone,[2] Liuting Qing,[1] Shenghai Huang,[1]† Bikram Chakraborty,[1] Ping Wang,[1] Fusong Chen,[1] Ignazio Cali,[1] Cristiano Corona,[2] Francesca Martucci,[2] Barbara Iulini,[2] Pierluigi Acutis,[2] Lan Wang,[1] Jingjing Liang,[1] Meiling Wang,[1] Xinyi Li,[1] Salvatore Monaco,[3] Gianluigi Zanusso,[3] Wen-Quan Zou,[1] Maria Caramelli,[2] and Pierluigi Gambetti[1]*

Department of Pathology, Case Western Reserve University, Cleveland, Ohio 44106[1]; CEA, Istituto Zooprofilattico Sperimentale, 10154 Torino, Italy[2]; and Department of Neurological and Visual Sciences, University of Verona, 37134 Verona, Italy[3]

Received 30 November 2007/Accepted 16 January 2008

Bovine spongiform encephalopathy (BSE), the prion disease in cattle, was widely believed to be caused by only one strain, BSE-C. BSE-C causes the fatal prion disease named new variant Creutzfeldt-Jacob disease in humans. Two atypical BSE strains, bovine amyloidotic spongiform encephalopathy (BASE, also named BSE-L) and BSE-H, have been discovered in several countries since 2004; their transmissibility and phenotypes in humans are unknown. We investigated the infectivity and human phenotype of BASE strains by inoculating transgenic (Tg) mice expressing the human prion protein with brain homogenates from two BASE strain-infected cattle. Sixty percent of the inoculated Tg mice became infected after 20 to 22 months of incubation, a transmission rate higher than those reported for BSE-C. A quarter of BASE strain-infected Tg mice, but none of the Tg mice infected with prions causing a sporadic human prion disease, showed the presence of pathogenic prion protein isoforms in the spleen, indicating that the BASE prion is intrinsically lymphotropic. The pathological prion protein isoforms in BASE strain-infected humanized Tg mouse brains are different from those from the original cattle BASE or sporadic human prion disease. Minimal brain spongiosis and long incubation times are observed for the BASE strain-infected Tg mice. These results suggest that in humans, the BASE strain is a more virulent BSE strain and likely lymphotropic.

Overwhelming evidence indicates that bovine spongiform encephalopathy (BSE), a prion disease that has been detected in several hundred thousand cattle in the United Kingdom and many other countries since the 1980s, has been transmitted to humans through the consumption of prion-contaminated beef, causing a prion disease named variant Creutzfeldt-Jakob disease (vCJD) (5, 19, 24). Over 200 cases of vCJD have been reported around the world (19). In 2004, two types of bovine prion disease that differ from the original BSE, now named classical BSE (BSE-C), were reported (3, 8). The two atypical BSE types were associated with prion protein (PrP) scrapie isoforms (PrP^{Sc}) that after protease digestion, displayed distinct electrophoretic mobility or ratios of the PrP^{Sc} glycoforms different from those of BSE-C (3, 8). Currently, a total of at least 36 cases of these two atypical BSE types have been reported for cattle older than 8 years (5; M. Caramelli, unpublished data). The two atypical BSE types are identified as BSE-H and bovine amyloidotic spongiform encephalopathy (BASE, also named BSE-L); the "L" and "H" identify the higher and lower electrophoretic positions, respectively, of their protease-resistant PrP^{Sc} isoforms (7). The bovine phenotype and the PrP^{Sc} molecular features of BASE have previously been described in detail (8). The histopathology of BASE and the PrP immunostaining pattern of BASE strains are characterized by the presence of prion amyloid plaques and a more rostral distribution of the PrP^{Sc}, which at variance with BSE-C is present in the cerebral cortex, including the hippocampus, but is underrepresented in the brain stem (8). These phenotypic features and PrP^{Sc} characteristics resemble a subtype of sporadic Creutzfeldt-Jakob disease (sCJD) named sCJDMV2, which affects subjects who are methionine (M)/valine (V) heterozygous at codon 129 of the PrP gene, and it is associated with PrP^{Sc} identified as type 2 (15). This similarity has raised the question of whether sCJDMV2 is not sporadic but acquired from the consumption of BASE strain-contaminated meat (5, 8). To begin to investigate the transmissibility to humans and the "human" disease phenotype of BASE, including the involvement of the lymphoreticular system, we have inoculated brain homogenates from BASE-affected cattle to transgenic (Tg) mice expressing normal human PrP with Met at codon 129 (HuPrP-129M) in a mouse PrP-ablated background [Tg(HuPrP)] (13). The inoculated Tg mice were examined for attack rates and the disease phenotype, including the presence and characteristics of protease-resistant PrP^{Sc} in the brain and spleen and the histopathology, along with the PrP^{Sc} topography and pattern of deposition in the brain.

* Corresponding author. Mailing address: Department of Pathology, Case Western Reserve University, Cleveland, OH 44106. Phone for Pierluigi Gambetti: (216) 368-0586. Fax: (216) 368-2546. E-mail: pxg13@case.edu. Phone for Qingzhong Kong: (216) 368-1756. Fax: (216) 368-2546. E-mail: qxk2@case.edu.

† Present address: Department of Patient Education and Health Information, Cleveland Clinic Foundation, Cleveland, OH 44195.

▽ Published ahead of print on 30 January 2008.

MATERIALS AND METHODS

Transgenic mice. Transgenic mice expressing human PrP-129M [Tg(HuPrP)] were reported previously (13). The Tg40 line that expresses human PrP-129M at

the wild-type level in the mouse PrP-ablated background was used in this study. Intracerebral (i.c.) inoculation of Tg mice and the monitoring of symptoms were performed as described previously (13). The mice were sacrificed 2 or 3 days after the appearance of symptoms or at death, and the brains and spleens were taken. The brains were sliced sagittally, with half frozen for immunochemical studies and the other half either fixed in formalin for histological and immunohistochemical staining or frozen for histoblot analysis (see below). Total PrP as well as proteinase K (PK)-resistant PrP^{Sc} was determined by immunoblotting in sodium dodecyl sulfate (SDS)-polyacrylamide gels as described below. This study was conducted with approvals from the Institutional Review Board and the Institutional Animal Care and Use Committee.

Immunoblotting, histology, histoblotting, and immunohistochemistry. Frozen brain or spleen tissues were homogenized in 2 volumes of cold phosphate-buffered saline to obtain 33% (wt/vol) crude homogenate for storage in aliquots at −80°C. The frozen 33% crude homogenate was thawed at 4°C for 2 h and diluted to 10% (wt/vol) with the lysis buffer (final concentration, 100 mM Tris, 10 mM EDTA, 100 mM NaCl, 0.5% sodium deoxycholate, 1.0% NP-40, pH 8.0). After incubation at room temperature for 15 min, the 10% homogenate was subjected to sonication with the Ultrasonic Dismembrator 100 (Fisher Scientific) for 3 min. The sonicated 10% homogenate was treated with 100 μg/ml PK (Roche Diagnostics GmbH, Mannheim, Germany) for 30 min at 37°C and denatured by being boiled at 100°C for 10 min after being mixed with an equal volume of 2× sample buffer (200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie blue G-250, 2% β-mercaptoethanol). The enrichment of PrP^{Sc} by precipitation with sodium phosphotungstate (NaPTA) was performed virtually as previously reported (18), and special care and efforts were taken to ensure that the pellets were completely resuspended each time. Proteins were separated by precast 10 to 20% gradient Tris-Tricine gel (Bio-Rad), transferred to a polyvinylidene difluoride membrane, and subjected to Western blot analysis with monoclonal antibody (MAb) 8H4, 6H4, or 3F4 in conjunction with horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin G Fc antibody (GE Healthcare, Buckinghamshire, United Kingdom) as described previously (13). The blots were developed with the ECL Western blotting detection reagent (GE Healthcare Amersham, Buckinghamshire, United Kingdom) and exposed to X-ray films. The blots were digitized by scanning the film. To determine the precise molecular weights of the bands, the digitized blots were analyzed by image acquisition and analysis software (UVP, Upland, CA) that automatically detects the midpoint of the band and calculates the molecular weight based on the sizes of the unglycosylated PK-resistant PrP fragments of sCJDMM1 and sCJDMM2; the values were statistically analyzed by Matlab 7.0 software (MathWorks, Natick, MA). To determine the glycoform ratios of PK-resistant PrP^{Sc} fragments, each PrP band on the digitized blots was quantified with UN-SCAN-IT software (Silk Scientific, Orem, UT); the values from duplicate blots were analyzed with Excel software to calculate the averages and standard deviations and to create the column chart.

Histological staining with hematoxylin and eosin (H&E) and immunohistochemical staining with 3F4 were performed as reported previously (13). Histoblot analysis was performed mostly as described previously (20), with the following modifications: the cryosections were 12 μm thick, and the sections were treated with 100 μg/ml of proteinase K for 4 h at 37°C, incubated with monoclonal antibody 3F4 (1:10,000 dilution) overnight at 4°C, followed by incubation with alkaline phosphatase-conjugated goat anti-mouse secondary antibody (1:500; DAKO), and developed with BCIP (5-bromo-4-chloro-3-indolylphosphate)-nitroblue tetrazolium solutions (Sigma).

RESULTS

To assess the transmissibility of BASE in humans, two BASE isolates (8) were used to intracerebrally inoculate 30 Tg40 mice that express normal levels of human PrP-129M. More than half of the inoculated mice (18/30) became infected, as determined by the presence of protease-resistant PrP^{Sc}, with average incubation times of 649 ± 34 days for BASE isolate 1 and 595 ± 28 days for BASE isolate 2, respectively (Table 1). Ten of the 18 infected mice that could be examined showed clear clinical signs of disease (Table 1), including hunched backs, ruffled fur, lethargy, occasional wobbling, and rigid tails. These signs were best detected in the younger mice, because in mice older than 24 months, the signs became difficult to distinguish from aging-related changes.

TABLE 1. BASE transmission in Tg(HuPrP) mice

Inoculum	Attack rate as determined by:			Incubation time (days)
	Clinical signs	Presence of PrP^{Sc}	Spongiform degeneration	
BASE-1	4/15	9/15	1 (focal)/8	649 ± 34
BASE-2	6/15	9/15	1 (focal)/11	595 ± 28
sCJDMM1	10/10	9/10	4/4	263 ± 13[a]
sCJDMM2	9/9	9/9	7/7	267 ± 17

[a] Reported previously (13).

All the Tg40 mice were examined for the presence of PK-resistant PrP^{Sc} in the brain by immunoblot analysis both directly and after enrichment with NaPTA precipitation. Such immunoblot analysis with three monoclonal antibodies (3F4, 6H4, and 8H4) to various PrP regions (12, 14, 25) showed that all 18 BASE strain-infected Tg40 mice accumulated comparable amounts of PK-resistant PrP^{Sc} in the brain (Fig. 1A, Table 1, and data not shown). The electrophoretic mobility of PK-resistant PrP^{Sc} fragments from all the BASE strain-infected Tg40 mice was indistinguishable from that of the PK-resistant PrP^{Sc} present in either the BASE strain inoculum or sCJDMM2, which contains type 2 PrP^{Sc} (Fig. 1A). The PK-resistant PrP^{Sc} fragments associated with both the BASE strain-infected Tg40 mice and the BASE isolates migrated slightly faster than those of BSE-C as originally reported (8). Measurements with software that automatically calculates the midpoint of the bands revealed a difference of 0.29 ± 0.12 kDa in gel mobility between the unglycosylated PK-resistant PrP^{Sc} bands of the BASE strain (native as well as from the Tg40 mice) and BSE-C.

The glycoform ratio of PrP^{Sc} in isolates from the BASE strain-infected Tg40 mice was slightly different from that of the BASE isolates (Fig. 1B), and both were quite different from that of BSE-C (Fig. 1B). The monoglycosylated form was the most prominent species in the BASE strain inocula, where the glycoform ratio (diglycosylated-to-monoglycosylated-to-unglycosylated) is 32:41:27, whereas the diglycosylated form was slightly more intense than the monoglycosylated form in BASE strain-infected Tg40 mice, where the glycoform ratio is 44:39:17 (Fig. 1B). In contrast, the diglycosylated form accounted for over 70% of the total PrP^{Sc} in BSE-C (glycoform ratio of 72:20:8).

PrP^{Sc} in the spleen was also examined after NaPTA enrichment for all 30 BASE strain-inoculated Tg40 mice. PK-resistant PrP^{Sc} was readily detected in the spleens of four mice (Fig. 1C), all of which also contained PK-resistant PrP^{Sc} in the brain. The electrophoretic mobility of the spleen PrP^{Sc} was similar to that of the brain PrP^{Sc}. The glycoform ratio of the spleen PrP^{Sc} was different from that of the brain and was characterized by the prominence of the monoglycosylated and unglycosylated forms (Fig. 1C), but the glycoform ratio may have been affected by the NaPTA enrichment. In contrast, none of the nine Tg40 mice inoculated with sCJDMM1 had detectable PK-resistant PrP^{Sc} in the spleen after NaPTA enrichment (data not shown).

None of the 12 BASE strain-infected Tg40 mice examined showed prominent and consistent histopathological changes related to prion diseases (Fig. 2A). Focal, ambiguous spongiform degeneration was observed for two mice. No PrP amyloid plaques were observed in BASE strain-infected Tg40 mice.

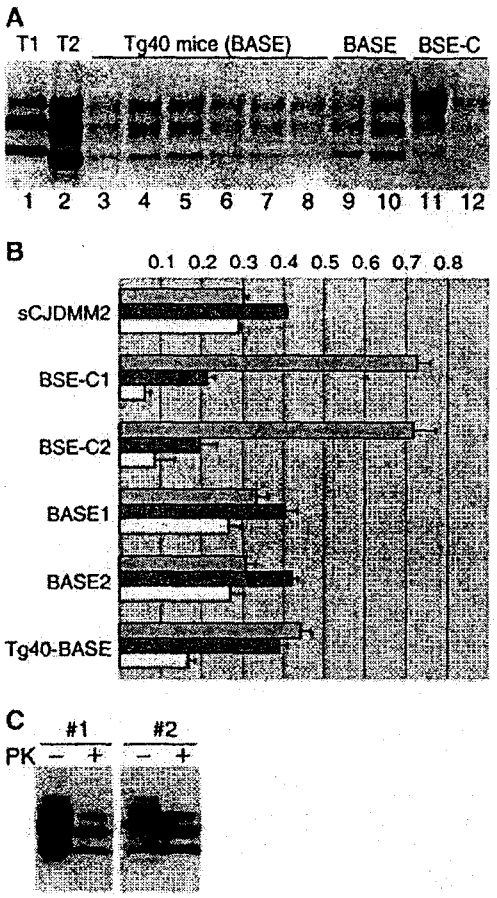

FIG. 1. Immunoblots and glycoform ratios of PK-resistant PrP^{Sc} from sCJD-affected, BASE strain-infected Tg(HuPrP) mice, the BASE strain inocula, and BSE-C and of PK-resistant PrP^{Sc} from the spleens of BASE strain-infected Tg(HuPrP) mice. (A) Immunoblot of PK-resistant PrP^{Sc} in the brain. Lanes 1 and 2, type 1 (sCJDMM1) (T1) and type 2 (sCJDMM2) (T2) sCJD, respectively; lanes 3 to 6, Tg(HuPrP) (Tg40) mice infected with BASE isolate 1 inoculum; lanes 7 to 8, Tg40 mice infected with BASE isolate 2 inoculum; lane 9, BASE isolate 1; lane 10, BASE isolate 2; lanes 11 and 12, two BSE-C isolates. All brain homogenates were treated with 100 μg/ml of PK for 30 min at 37°C and processed for immunoblot analysis with MAb 8H4. Five microliters of 10% brain homogenate was loaded for lanes 3 to 10. (B) Glycoform ratios of PK-resistant PrP^{Sc} in the brain. The upper (diglycosylated) (blue), middle (mostly monoglycosylated) (red), and lower (unglycosylated) (yellow) bands of PK-resistant PrP^{Sc} from BASE strain-infected Tg40 mice, the BASE strain, and BSE-C were quantified after optical scanning of duplicate immunoblots for panel A. Error bars indicate standard deviations. (C) PK-resistant PrP^{Sc} in the spleen. Ten milligrams of spleen tissue each from two of the BASE strain-infected Tg(HuPrP) (Tg40) mice (#1 and #2) was homogenized, PrP^{Sc} enriched by NaPTA precipitation, and either treated (+) or not treated (−) with 100 μg/ml of PK for 30 min at 37°C, followed by electrophoresis in a 10 to 20% Tris-Tricine SDS-polyacrylamide gradient gel and immunoblot analysis with MAb 8H4.

Histoblot analysis with MAb 3F4 showed a very distinct and selective distribution of PrP^{Sc} (Fig. 3A to D). Particular nuclei or groups of adjacent periventricular nuclei in the thalamus, hypothalamus, and brain stem were intensely immunostained for PrP^{Sc} (Fig. 3B to D). In contrast, PrP^{Sc} appeared to be overall less intense in the cerebral and cerebellar cortices (Fig. 3A to D). Immunohistochemical staining of paraffin-embedded brain tissue with 3F4 revealed PrP deposits in 5 of the 11 BASE strain-infected Tg40 mice examined. PrP^{Sc} deposits that stained intensely in the histoblots consisted of relatively large and well-circumscribed granules (Fig. 3E and G). Fine granular or small plaque-like aggregate patterns were occasionally seen in inferior regions of the cerebral cortex and in the thalamus (Fig. 3I and data not shown). In contrast, widespread, mostly fine-granular staining was detected in the cerebral cortex of symptomatic Tg40 mice inoculated with sCJDMM1 brain homogenate (Fig. 3J).

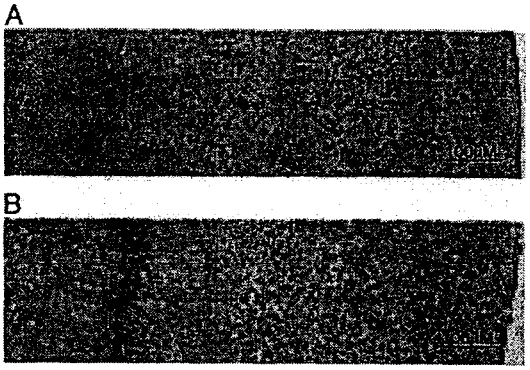

FIG. 2. Histopathology (with H&E) of BASE strain-infected and sCJDMM1-infected Tg(HuPrP) mice. (A) No consistent pathology was detected in the cerebral cortex as well as subcortical brain regions of symptomatic and immunoblot-positive BASE strain-infected Tg(HuPrP) (Tg40) mice. (B) In contrast, Tg40 mice inoculated with sCJDMM1 brain homogenate showed widespread spongiform degeneration.

The histopathological features of the BASE strain-inoculated Tg40 mice were quite different from those observed following inoculation with brain homogenates from the two forms of sCJD, sCJDMM1 and sCJDMM2. The sCJDMM1-inoculated Tg40 mice had widespread spongiform degeneration in the cerebrum (Fig. 2B) and moderate apoptosis of neuronal cells without spongiform degeneration in the cerebellum (13). Widespread spongiform degeneration was also seen in Tg40 mice inoculated with sCJDMM2 brain homogenate (data not shown).

DISCUSSION

We have shown that 60% of our Tg40 mice (in an inbred FVB background) that express normal levels of human PrP-129M became infected 20 to 22 months after i.c. inoculation with 0.3 mg of brain tissue from the two BASE isolates, suggesting a titer of approximately 3 50% infective dose units per milligram of brain tissue in the Tg40 line. An approximately 20% attack rate has been reported for the Tg650 line (in a mixed 129/Sv × C57BL/6 background) after i.c. inoculation with 2 mg brain tissues from BSE-C-infected cattle (2). It is noteworthy that the Tg650 mice express human PrP-129M at five to eight times the normal level, and high PrP levels are known to increase prion transmissibility (9, 17, 22). Inefficient BSE-C transmissions (0 to 30%) in Tg mouse lines of other genetic backgrounds expressing human PrP-129M at one or two times the normal level have also been reported by different groups (1, 4). Although it is difficult to compare results from different mouse lines, these findings suggest that the BASE strain has higher transmissibility than BSE-C does for human-

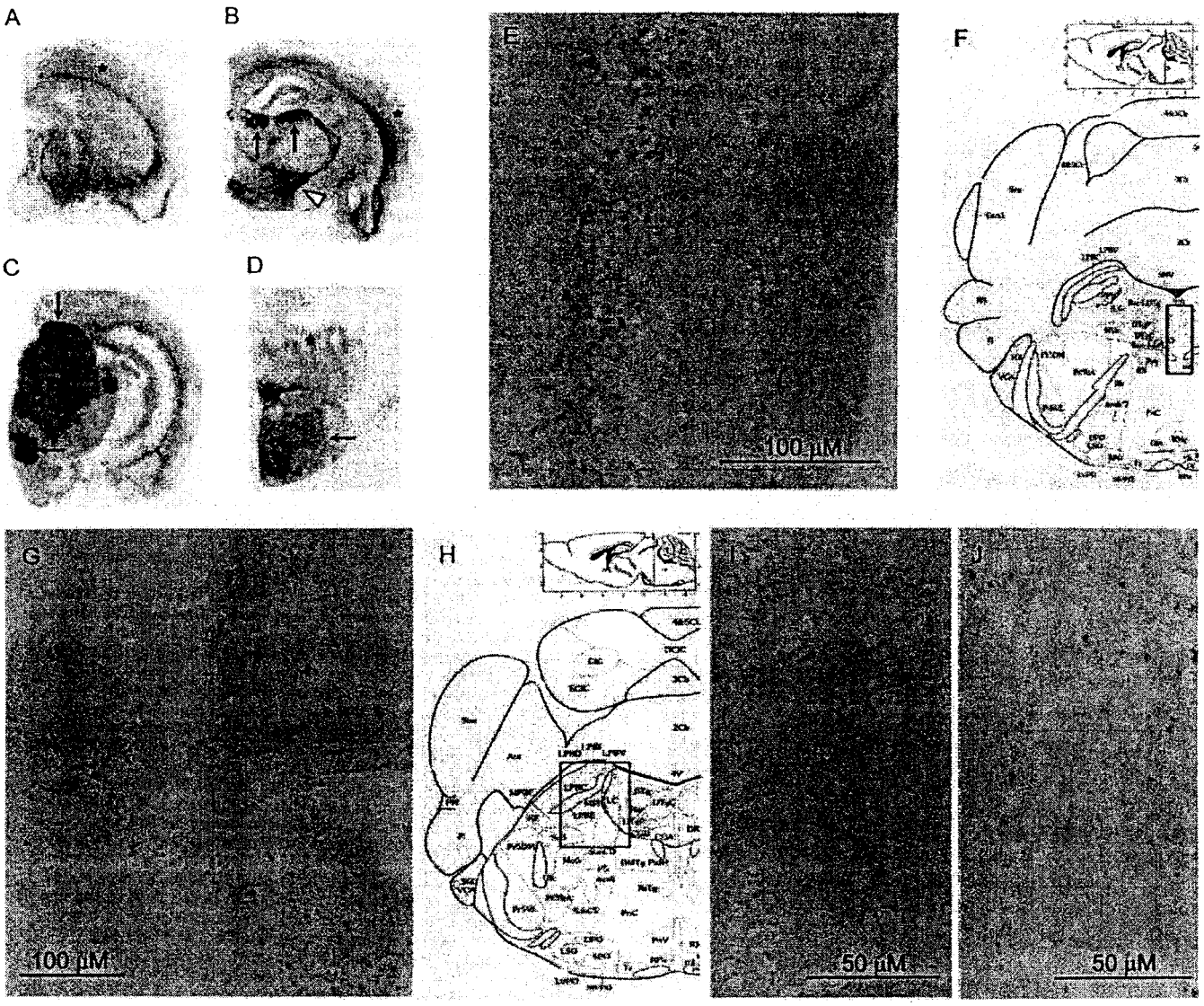

FIG. 3. Histoblot analysis and immunohistochemistry of BASE strain-infected and sCJDMM1-infected Tg(HuPrP) mice. (A to D) The histoblot analysis revealed preferential immunostaining of the PrP^{Sc} in the dorsal thalamic nuclei (arrows in panel B), along with hypothalamic nuclei (arrowhead) and brain stem nuclei (arrows in panels C and D), while PrP^{Sc} in the cerebral and cerebellar cortices (stars in panels A, B, and D) was mostly limited to the deep and inferior cortical regions. (E to J) The PrP immunostaining (E and G) of the intensely PrP-reactive brain stem nuclei in histoblot analysis (boxed regions in panels F and H) revealed coarse PrP granules, while the PrP immunostain in the cerebral cortex (I) was minimal and characterized mostly by a plaque-like pattern. In contrast, widespread fine-granular PrP immunostaining was observed in the cerebral cortex of symptomatic Tg40 mice following inoculation of sCJDMM1 brain homogenates (J). Monoclonal antibody 3F4 was used for all the staining.

ized Tg mice with PrP-129M and possibly for humans with PrP-129MM. The BASE strain also appears to be more virulent than BSE-C in bovinized Tg mice, since the incubation time for the BASE strain is 185 ± 12 days, whereas that for BSE-C is 230 ± 7 days (7). Nevertheless, compared with the 100% attack rate and incubation times of ~9 months for sCJDMM1 and sCJDMM2 in the Tg40 line (Table 1), the 60% attack rate and unusually long incubation times (20 to 22 months) for the BASE strain in the same Tg line suggest that the transmission barrier from the BASE strain to humans with PrP-129MM is still quite significant.

PK-resistant PrP^{Sc} was also detected in the spleen in 4 out of 18 BASE strain-infected Tg40 mice. In contrast, no spleen involvement could be demonstrated for the Tg40 mice following i.c. inoculation with human PrP^{Sc} from sCJDMM1. This is the first report of the presence of PrP^{Sc} in the spleens of humanized Tg mice after i.c. inoculation with a BSE strain, suggesting that the BASE strain, like BSE-C, where at least in vCJD-infected subjects PrP^{Sc} and prion infectivity have been detected in spleens and tonsils (6, 11), is intrinsically lymphotropic. Therefore, lymphoid tissues of BASE strain-infected individuals might also carry prion infectivity.

The gel mobility of the PK-resistant PrP^{Sc} recovered from the BASE strain-inoculated Tg40 mice was consistently slightly faster than the mobility of BSE-C, as originally reported for the BASE strain (8). The computed difference in gel mobilities

between BASE and BSE-C PrP^{Sc} is 0.29 ± 0.12 kDa, corresponding to 2 to 4 amino acid residues. In contrast, the gel mobilities of the PK-resistant PrP^{Sc} species from the BASE strain, BASE strain-infected Tg40 mice, and sCJDMM2, which was used as representative of human PrP^{Sc} of type 2, were indistinguishable. This finding suggests that the PK-resistant PrP^{Sc} electrophoretic heterogeneity between the BASE strain and BSE-C falls well within the 7-amino-acid variability of the N terminus (positions 92 to 99) that is consistently found in PK-resistant PrP^{Sc} of type 2 (16). Therefore, despite their minor but distinct variability in gel mobility, both the BASE strain and BSE-C PrP^{Sc} species appear to belong to the PrP^{Sc} of type 2. However, the PrP^{Sc} glycoform ratios of BASE strain-infected Tg40 mice and the BASE strain inocula display a small but statistically significant difference (Fig. 1). Therefore, PrP^{Sc} in BASE strain-infected human subjects may be expected to display a different glycoform ratio from that of the BASE strain. It is worth noting that the electrophoretic characteristics of the PK-resistant PrP^{Sc} of some human prion strains has been faithfully reproduced by our Tg40 line as well as by other humanized mouse lines (10, 13, 21).

Two distinct histopathological and PrP immunohistochemical phenotypes have been reported following BSE-C inoculation: one reproduced the distinctive features of vCJD with the "florid" plaques that intensely immunostained for PrP, and the other was reminiscent of sCJDMM1, with prominent spongiform degeneration and no plaque PrP immunostaining (1, 23). The brain histopathology, the PrP^{Sc} distribution, and the PrP immunostaining pattern of BASE strain-inoculated Tg40 mice were definitely distinct from such features described above (1, 23), further supporting the notion that BASE and classical BSE are associated with two distinct prion strains (8).

The relatively easy transmission of BASE to humanized Tg mice indicates that effective cattle prion surveillance should be maintained until the extent and origin of this and other atypical forms of BSE are fully understood.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grants (AG014359 [P.G.] from the National Institute of Aging and NS052319 [Q.K.] from the National Institute of Neurological Disorders and Stroke) and by an award to P.G. from the Charles S. Britton Fund.

We are grateful to Diane Kofsky, Phyliss Scalzo, Carrie Harris, and Kay Edmonds for their assistance.

REFERENCES

1. **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.
2. **Béringue, V., O. Andreoletti, A. Le Dur, R. Essalmani, J. L. Vilotte, C. Lacroux, F. Reine, L. Herzog, A. G. Biacabe, T. Baron, M. Caramelli, C. Casalone, and H. Laude.** 2007. A bovine prion acquires an epidemic bovine spongiform encephalopathy strain-like phenotype on interspecies transmission. J. Neurosci. 27:6965–6971.
3. **Biacabe, A. G., J. L. Laplanche, S. Ryder, and T. Baron.** 2004. Distinct molecular phenotypes in bovine prion diseases. EMBO Rep. 5:110–115.
4. **Bishop, M. T., P. Hart, L. Aitchison, H. N. Baybutt, C. Plinston, V. Thomson, N. L. Tuzi, M. W. Head, J. W. Ironside, R. G. Will, and J. C. Manson.** 2006. Predicting susceptibility and incubation time of human-to-human transmission of vCJD. Lancet Neurol. 5:393–398.
5. **Brown, P., L. M. McShane, G. Zanusso, and L. Detwiler.** 2006. On the question of sporadic or atypical bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Emerg. Infect. Dis. 12:1816–1821.
6. **Bruce, M. E., I. McConnell, R. G. Will, and J. W. Ironside.** 2001. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. Lancet 358:208–209.
7. **Buschmann, A., A. Gretzschel, A. G. Biacabe, K. Schiebel, C. Corona, C. Hoffmann, M. Eiden, T. Baron, C. Casalone, and M. H. Groschup.** 2006. Atypical BSE in Germany—proof of transmissibility and biochemical characterization. Vet. Microbiol. 117:103–116.
8. **Casalone, C., G. Zanusso, P. Acutis, S. Ferrari, L. Capucci, F. Tagliavini, S. Monaco, and M. Caramelli.** 2004. Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc. Natl. Acad. Sci. USA 101:3065–3070.
9. **Fischer, M., T. Rulicke, A. Raeber, A. Sailer, M. Moser, B. Oesch, S. Brandner, A. Aguzzi, and C. Weissmann.** 1996. Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. EMBO J. 15:1255–1264.
10. **Hill, A. F., M. Desbruslais, S. Joiner, K. C. Sidle, I. Gowland, J. Collinge, L. J. Doey, and P. Lantos.** 1997. The same prion strain causes vCJD and BSE. Nature 389:448–450.
11. **Hill, A. F., R. J. Butterworth, S. Joiner, G. Jackson, M. N. Rossor, D. J. Thomas, A. Frosh, N. Tolley, J. E. Bell, M. Spencer, A. King, S. Al-Sarraj, J. W. Ironside, P. L. Lantos, and J. Collinge.** 1999. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet 353:183–189.
12. **Kascsak, R. J., R. Rubenstein, P. A. Merz, M. Tonna-DeMasi, R. Fersko, R. I. Carp, H. M. Wisniewski, and H. Diringer.** 1987. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J. Virol. 61:3688–3693.
13. **Kong, Q., S. Huang, W. Zou, D. Vanegas, M. Wang, D. Wu, J. Yuan, H. Bai, M. Zheng, 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. 25:7944–7949.
14. **Korth, C., B. Stierli, P. Streit, M. Moser, O. Schaller, R. Fischer, W. Schulz-Schaeffer, H. Kretzschmar, A. Raeber, U. Braun, F. Ehrensperger, S. Hornemann, R. Glockshuber, R. Riek, M. Billeter, K. Wüthrich, and B. Oesch.** 1997. Prion (PrPSc)-specific epitope defined by a monoclonal antibody. Nature 390:74–77.
15. **Parchi, P., A. Giese, S. Capellari, P. Brown, W. Schulz-Schaeffer, O. Windl, I. Zerr, H. Budka, N. Kopp, P. Piccardo, S. Poser, A. Rojiani, N. Streichemberger, J. Julien, C. Vital, B. Ghetti, P. Gambetti, and H. Kretzschmar.** 1999. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann. Neurol. 46:224–233.
16. **Parchi, P., W. Zou, W. Wang, P. Brown, S. Capellari, B. Ghetti, N. Kopp, W. J. Schulz-Schaeffer, H. A. Kretzschmar, M. W. Head, J. W. Ironside, P. Gambetti, and S. G. Chen.** 2000. Genetic influence on the structural variations of the abnormal prion protein. Proc. Natl. Acad. Sci. USA 97:10168–10172.
17. **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.
18. **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.
19. **Seitz, R., F. von Auer, J. Blumel, R. Burger, A. Buschmann, K. Dietz, M. Heiden, W. E. Hitzler, H. Klamm, T. Kreil, H. Kretzschmar, M. Nübling, R. Offergeld, G. Pauli, V. Schottstedt, P. Volkers, and I. Zerr.** 2007. Impact of vCJD on blood supply. Biologicals 35:79–97.
20. **Taraboulos, A., K. Jendroska, D. Serban, S. L. Yang, S. J. DeArmond, and S. B. Prusiner.** 1992. Regional mapping of prion proteins in brain. Proc. Natl. Acad. Sci. USA 89:7620–7624.
21. **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.
22. **Tremblay, P., Z. Meiner, M. Galou, C. Heinrich, C. Petromilli, T. Lisse, J. Cayetano, M. Torchia, W. Mobley, H. Bujard, S. J. DeArmond, and S. B. Prusiner.** 1998. Doxycyline control of prion protein transgene expression modulates prion disease in mice. Proc. Natl. Acad. Sci. USA 95:12580–12585.
23. **Wadsworth, J. D., E. A. Asante, M. Desbruslais, J. M. Linehan, S. Joiner, I. Gowland, J. Welch, L. Stone, S. E. Lloyd, A. F. Hill, S. Brandner, and J. Collinge.** 2004. Human prion protein with valine 129 prevents expression of variant CJD phenotype. Science 306:1793–1796.
24. **Ward, H. J. T., D. Everington, S. N. Cousens, B. Smith-Bathgate, M. Leitch, S. Cooper, C. Heath, R. S. G. Knight, P. G. Smith, and R. G. Will.** 2006. Risk factors for variant Creutzfeldt-Jakob disease: a case-control study. Ann. Neurol. 59:111–120.
25. **Zanusso, G., D. Liu, S. Ferrari, L. Hegyi, X. Yin, A. Aguzzi, S. Hornemann, S. Liemann, R. Glockshuber, J. C. Manson, P. Brown, R. B. Petersen, P. Gambetti, and M. S. Sy.** 1998. Prion protein expression in different species: analysis with a panel of new MAbs. Proc. Natl. Acad. Sci. USA 95:8812–8816.

別紙様式第2-1

No. 24

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
				2008. 2. 18	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)		Tsukui K, Takata M, Tadokoro K. Microbiol Immunol. 2007;51(12):1221-31.	日本

研究報告の概要

○スクレイピー感染ハムスター血漿中のPrP^{res}様タンパクの糖鎖依存性凝集検出による伝達性海綿状脳症血液検査の可能性

感染組織に多量のPrP^{res}を含有することが知られている感染動物モデルにおいても、血中のPrP^{res}は(白血球を除き)めったに検出されない。血中PrP^{res}の検出が困難なのは、血中感染価が低いことを反映していると見られる。ここでは、新規酸性SDS沈殿法と高感度化学発光法を組み合わせて、プロテイナーゼK耐性3F4反応性タンパクが、スクレイピー感染ハムスターの血漿中からは検出されるが、疑似感染ハムスターでは検出されないことを示す。高感度化学発光法では、$1.4x10^{-9}$gの脳ホモジネート、及び$1.5x10^{-12}$g ($6.5x10^{-17}$mol)のrPrPを従来型のウエスタンブロットで検出した。スクレイピー感染ハムスターの血漿中の3F4反応性タンパクは複数の分子量からなるタンパクバンドとなり、二糖鎖PrP分子のバンドよりも高い位置に検出された。スクレイピー感染ハムスター脳ホモジネートと疑似感染ハムスター血漿を混合することにより、3F4反応性タンパクと類似する分子量の位置にバンドが形成された。混合前に、血漿または脳ホモジネート中のタンパクから予め糖鎖を除去することにより、上記の複数の3F4反応性タンパクは検出できなくなった。これらの結果から、血漿中においてPrP^{res}は他の血漿タンパクと糖鎖を通じて凝集しており、スクレイピー感染ハムスター血漿において検出可能となったことが示唆される。スクレイピー感染ハムスターの血漿中でPrP^{res}様タンパクと凝集している相手の血漿タンパクが何であるかはまだ不明であるが、それはプロテイナーゼKに抵抗性を持っていると思われる。

報告企業の意見	今後の対応
新規酸性SDS沈殿法と高感度化学発光法を組み合わせて、スクレイピー感染ハムスターの血漿中からプロテイナーゼK耐性3F4反応性タンパクを検出したとの報告である。	今後も引き続き、検査法の研究を進めるとともに、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。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-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

Microbiol. Immunol., 51(12), 1221–1231, 2007

Editor-Communicated Paper

A Potential Blood Test for Transmissible Spongiform Encephalopathies by Detecting Carbohydrate-Dependent Aggregates of PrPres-Like Proteins in Scrapie-Infected Hamster Plasma

Kazuo Tsukui*[,1], Masuhiro Takata[2], and Kenji Tadokoro[1]

[1]*Central Blood Institute, The Japanese Red Cross Society, Koto-ku, Tokyo 135–8521, Japan, and* [2]*Research Center for Prion Diseases, National Institute of Animal Health, Japan, Tsukuba-shi, Ibaraki 305–0856, Japan*

Communicated by Dr. Takashi Onodera: Received June 13, 2007. Accepted October 17, 2007

Abstract: **PrPres has rarely been detected in blood (except in leukocytes) even in diseased animal models that are known to contain a large amount of PrPres in infected tissues. It seems likely that PrPres detection in blood is difficult because of the low titer of infectious material within the blood. Here, we demonstrate the detection of proteinase K-resistant 3F4-reactive protein in the plasma of scrapie-infected hamsters but not in the plasma of mock-infected hamsters by partial purification using a novel method termed "acidic SDS precipitation," in conjunction with a highly sensitive chemiluminescence detection system used to show the presence of PrP at a concentration equivalent to 1.4×10^{-9} g of brain homogenate or 1.5×10^{-12} g (6.5×10^{-17} mol) of rPrP by conventional Western blotting. The 3F4-reactive proteins in scrapie-infected hamster plasma often resulted in multiple Mw protein bands occurring at higher Mw positions than the position of the di-glycosyl PrP molecule. Mixing scrapie-infected hamster brain homogenate with mock-infected hamster plasma resulted in the formation of similar Mw positions for multiple 3F4-reactive proteins. Predigestion of carbohydrate side chains from the proteins in the plasma or brain homogenate before mixing resulted in failure to obtain these multiple 3F4-reactive proteins. These observations indicate that PrPres aggregated with other proteins in the plasma through carbohydrate side chains and was successfully detected in the plasma of scrapie-infected hamsters. Counterparts in these aggregates with PrPres-like proteins in scHaPI are not known but any that exist should resist the PK digestion.**

Key words: **PrPres-like protein, Carbohydrate, Scrapie infection, Discrimination**

Transmissible spongiform encephalopathy (TSE) is a fatal infectious neurodegenerative disease. It is characterized pathologically by spongy deterioration of the central nervous system (CNS) and by the deposition of amyloid plaques composed of an abnormal isoform of the prion protein (PrP^{sc}) in infected tissues (1, 2, 19). An important biochemical property of PrP^{sc} is its partial resistance to protease digestion, which results in the formation of a β-sheet-rich isoform. This molecule has therefore also been called PrPres, and it has been considered a disease-specific entity associated with TSE (1, 2, 9). Although the vCJD epidemic in the U.K. is declining, expansion of the disease throughout continental Europe and in many other countries has raised concern all over the world (9, 10, 28). After the appearance of three cases of transfusion-related vCJD infection

**Address correspondence to* Dr. Kazuo Tsukui, 2–1–67 Tatsumi, Koto-ku, Tokyo 135–8521, Japan. Fax: +81–3–5534–7588. E-mail: k-tsukui@bs.jrc.or.jp

Abbreviations: 2× acidic saline, 0.02 M acetic acid containing 0.15 M NaCl and 10 mM EDTA-2Na; Brh, brain homogenates; CNS, central nervous system; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; mc, mock-infected; Pl, plasma; PMCA, protein misfolding cyclic amplification; PrP^{c}, normal prion protein; PrPres, proteinase K-resistant prion protein; PrP^{sc}, disease-associated prion protein; PTA, phosphotungstic acid; PVDF, polyvinylidene fluoride; RES, reticuloendothelial system; rPrP, recombinant hamster PrP(25-233); SB, super block; sc, scrapie-infected; TBST, Tris Buffered Saline containing 0.05% Tw20; TSE, transmissible spongiform encephalopathy.

in the U.K., transfusion-related iatrogenic expansion of vCJD between the asymptomatically infected donor and blood recipients has caused growing concern (13, 21, 26). In addition, the U.K. government has recently reported a fourth case of vCJD associated with a blood transfusion (HPA Press statement; 18 Jan. 2007, abbreviated in 13). For this reason, the aim of research into developing an antemortem test has changed from detecting infected persons in an endemic area to estimating the population size of infected persons within a more global area in order to prevent the iatrogenic infection by tainted blood (4, 8, 28). The most useful tissues for the diagnostic confirmation of vCJD in humans are CNS and reticulo-endothelial system (RES) tissue as well as the tonsils and appendix (14–16). The tonsils and appendix have been used successfully for the histopathological detection of PrPres in epidemiological studies of vCJD infection in the U.K.: an extremely high frequency of infection was detected compared to the frequency of so-called classical CJD (16, 17, 22). However, it is difficult to sample the tonsils and the appendix in living subjects. Therefore, the pathological diagnosis of TSE is currently made principally on the basis of postmortem preparations of CNS tissues, highlighting the need for the development of a more rapid diagnostic method using body fluids, especially blood (6, 27). For this purpose, several methods have been proposed and examined for prophylactic use (23, 30, 32). However, none of these methods has proved to be sufficient for the purposes (5, 7, 20, 29). To achieve this goal, several problems must be solved; solutions include using preparations with minimally invasive sampling techniques and establishing an effective and specific method for detecting the disease marker with sufficient sensitivity (5). The first problem can be solved by using body fluids such as blood or urine as test specimens. Therefore, the key is to develop a system with sufficient sensitivity to detect PrPres in blood or urine (5, 6, 20, 22, 29). The presence of PrPres in the urine of TSE-infected animals and humans has been reported previously (33). However, it has been suggested that this $uPrP^{sc}$ may be contaminated bacterial components in infected animal urine and not a marker of TSE (12). Blood has not been considered a highly infective source of classical CJD. The same was true for vCJD until the first victim of vCJD resulting from a blood transfusion was reported (21). This report was followed by reports of three more cases of possible transfusion-related transmission of vCJD (18, HPA Press statement; 18, Jan. 2007, 13). The development of a testing method using blood has therefore become a major goal of TSE research.

Here we show the successful detection (using a highly sensitive chemiluminescence immunoblotting system) of a PrPres-like protein molecule in plasma collected from scrapie-infected (sc) but not from mock-infected (mc) hamsters. Although the infectivity of this molecule has not been tested, and the immunoblot pattern of the anti-PrP reactive protein in plasma (scHaPl) was somewhat different from that of the brain homogenate (scHaBrh) in scrapie-infected hamsters, the specific reactivity of these proteins to anti-PrP mAb, the demonstration of carbohydrate side chain-mediated association between PrPres and plasma proteins, and the removal of the carbohydrate chain resulted in the appearance of similar Mw proteins in scBrh and scPl firmly support the conjecture that the extra Mw proteins observed in the trial were the aggregates of PrPres and some plasma proteins.

Materials and Methods

Enzymes, monoclonal antibodies (mAb) and recombinant hamster PrP peptide. Proteinase K (PK: 40.0 mAnson units/mg protein) was purchased from Merck Co. (Rahway, N.J., U.S.A.). Peptide *N*-glycosidase F (PNGaseF, 25,000 units/mg protein) was purchased from Roche Diagnostics Co., Ltd.

The anti-PrP mAbs 3F4 (Signet, Mass., U.S.A.) and 6H4 (Prionics AG, Zürich, Switzerland) were stored in aliquots at −80 C until use. mAb 5C8-113 was prepared by immunizing PrP knockout mice with bovine recombinant PrP (Prionics AG); screening was conducted using the same molecule. TA180 and TA181 were provided by Medical Biological Laboratory (MBL) and were prepared by immunizing conventional Balb/c mice with synthetic peptides of the hamster PrP sequence CERYYRE or CAVVGGLGGYML conjugated with keyhole limpet hemocyanin (KLH), respectively, then screened by the same peptides without KLH and conjugated with an ELISA plate. The epitope sites of the mAbs were 150–152 and 163–165 for TA180, and 129–131 for TA181. The epitope site of 5C8-113 has not yet been determined but is possibly an unknown conformation-dependent site. Anti-HIV P24 mAb (7A8.1; CHEMICON) was kindly donated by Dr. Iwakura of the Institute of Medical Sciences, Tokyo University and was used as a negative control for anti-PrP mAb reactions. Hamster recombinant PrP(25-233) (abbreviated rPrP hereafter) was purchased from Alicon AG (Switzerland).

Material from scrapie-infected and mock-infected hamsters. Twelve Syrian golden hamsters were inoculated with scrapie (Sc237)-infected hamster brain homogenate intra-cerebrally. Six hamsters were similarly inoculated with uninfected normal hamster brain

homogenate and were used as mock-infected hamsters. Hamsters from the two groups were anesthetized with ether at the terminal stage of disease among animals in the scrapie-infected group (approximately 50–70 days after inoculation) and after the same time interval among animals in the mock-infected group. Blood was collected from the animals with ACD containing 10 mM EDTA as an anticoagulant. Blood samples from scrapie-infected and mock-infected hamsters was centrifuged at low speed and the plasma fractions were collected (scPl and mcPl, respectively). Both scPl and mcPl were processed similarly thereafter. Brains were removed from the terminal-stage infected hamsters or the mock-infected hamsters and homogenized in TBS containing 0.5% NP40, 0.5% DOC and a protease inhibitor cocktail (Sigma) using a closed system homogenizer. These brain homogenates were then adjusted to a concentration of 10% with the above-mentioned buffer (scBrh^{crude} or mcBrh^{crude}, respectively). scBrh^{crude} or mcBrh^{crude} were centrifuged at low speed to remove insoluble materials, and the supernatant fractions (scBrh or mcBrh) were processed as described below.

Enzyme treatment. The plasma or brain homogenates were diluted 4-fold with TBS containing 10 mM EDTA and digested with PK (50 μg/ml) at 37 C for 60 min. These reactions were stopped by adding 1 mM Pefablock. The digestion step was omitted in a set of controls. The samples were then treated with 3% SDS and 50 mM DTT in TBS before being inactivated at 100 C for 10 min and stored at −80 C in small aliquots.

Acidic SDS precipitation. Stored preparations were inoculated with equal volumes of 0.02 M acetic acid containing 0.15 M NaCl and 10 mM EDTA-2Na (2× acidic saline) at 10 C, followed by centrifugation at 15,000 rpm for 10 min. The resulting precipitates were dispersed in Tris Buffered Saline (TBS) with 5 mM EDTA and inoculated with equal amounts of 2× acidic saline again. After further centrifugation, the resulting precipitates were rinsed with a 5-fold volume of methanol, then dissolved in Laemmli's SDS sample buffer and analyzed thereafter.

Immunoblot detection of PrP-like proteins. SDS-PAGE was carried out on a 15% gel using Laemmli's conventional buffer system. The electrophoresed proteins were transferred onto polyvinylidene fluoride (PVDF) membranes using a semidry system. After Western blotting, the membranes were blocked with Super Block (SB; PIERCE, Rockford, Ill., U.S.A.) for 1 hr at room temperature then overnight at 4 C. The blocked membranes were first washed three times with TBS containing 0.05% Tween 20 (TBST), then incubated with an anti-PrP monoclonal antibody (mAb; 3F4, 6H4 or similar), in SB containing 10% Block Ace (Dainippon Pharmaceutical Co., Ltd.) and 0.01% BSA for 1 hr at room temperature then overnight at 4 C thereafter. For maximum detection of protein signals, the blotted membranes were incubated overnight at 4 C. After incubation, the membranes were washed five times with TBST, incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (HRPGAM) in TBST cotaining 10% Block Ace and 0.1% BSA for 1 hr as a secondary antibody, washed five more times with TBST and incubated with a chemiluminescence substrate (Super Signal West Femto Maximum Sensitivity Substrates: SSWF; PIERCE). To obtain optimum chemiluminescence signals, HRPGAM was used at a concentration of 2 ng/ml according to the manufacturers instruction manual and chemiluminescence signals for antibody-reactive bands were detected using an LAS3000 image analyzer (Fuji Film, Tokyo).

Results

Sensitivity of Detection Systems (Fig. 1)

To determine the limits of the detection system, rPrP, 10% homogenates of sc- or mcBrh (crude or low-speed centrifugation supernatant) and PK predigested sc- or mcBrh were used. They were abbreviated as sc- or mcBrh^{crude}, sc- or mcBrh^{sup} and sc- or mc BrhPK$_{50}$, respectively. In these experiments, the amount of PrP was indicated as brain equivalent (panel A) or brain protein (panel B) to enable convenient comparison between the equivalence to brain amount and brain protein. Protein amounts in the brain were determined before PK digestion. In panel A, 1.5×10^{-12} g (6.5×10^{-17} mol) for rPrP and PrPres in 1.4×10^{-9} g brain equivalent were detected. About 1/3 (equivalent to 2.25×10^{-6} g brain protein) of the PrP molecule in scBrh, and none of those in mcBrh looked like the PK-resistant molecule (PrPres; panel B). PrPres in scBrh was shifted from 30–32 kDa and 27–28 kDa before PK treatment to 25 and 20 kDa positions after PK treatment, respectively. As the total amount of PrPres plus PrPc in scBrh looked 3-fold larger than the amount of PrP in mcBrh, synthesis of the PrP was enhanced by scrapie infection in hamsters.

Discrimination of Scrapie Infection from Mock Infection by Plasma

When scrapie-infected or mock-infected Brh and plasma were pretreated with PK and subjected to immunoblot analysis, sc and mcBrh were easily discriminated by the PK treatment but sc and mcPl were not discriminated by the enzyme treatment (panel A). In mcPl, similar 3F4-reactive proteins were also

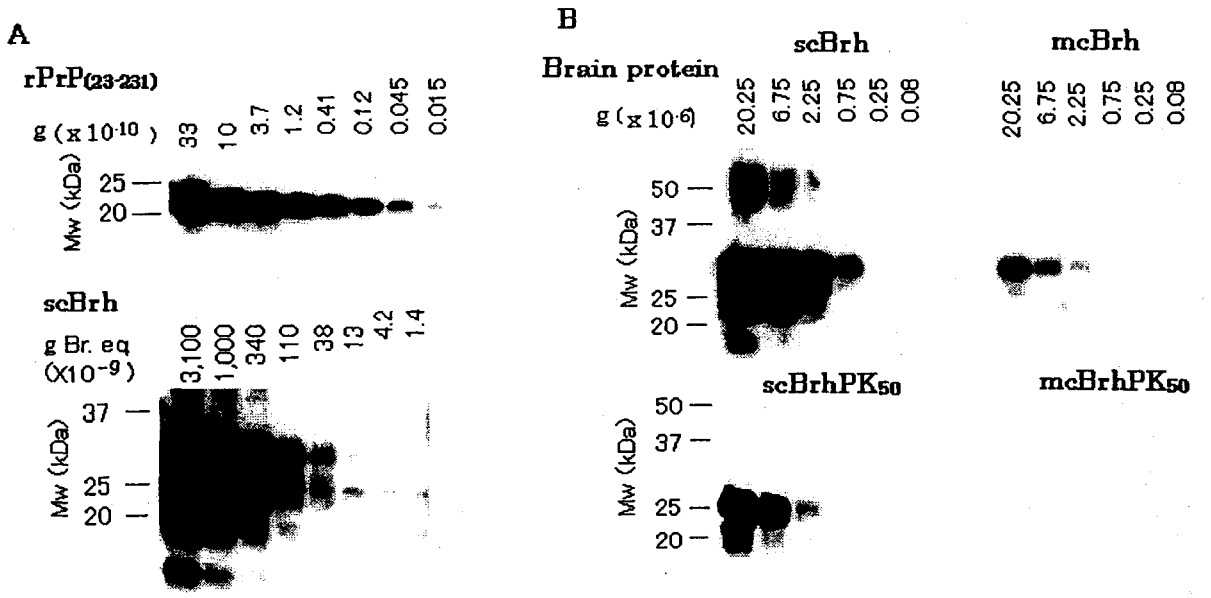

Fig. 1. Sensitivity and specificity determinations of immunoblot systems. Panel A: Sensitivity of used chemiluminescence immunoblots was determined using rPrP(25-233) (upper) or scBrh^{crude} (lower). These were diluted in the serial threefold manner as described in "Materials and Methods." Thereafter, each diluted preparation was subjected to chemiluminescence immunoblot detection. The amounts of each preparation used per lane are indicated in the figure as g ($\times 10^{-10}$) for rPrP(23-231) or g Br. eq. ($\times 10^{-9}$) for scBrh. Panel B: Specific detection of PrP in scBrh and mcBrh by chemiluminescence immunoblotting was indicated. scBrh or mcBrh (upper) or their PK-treated preparations (lower) were processed to serial threefold dilution series and subjected to chemiluminescence immunoblotting. PrP in each preparation was indicated per brain protein. Protein bands in scBrh showed the mixture of PrPres and PrPc. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

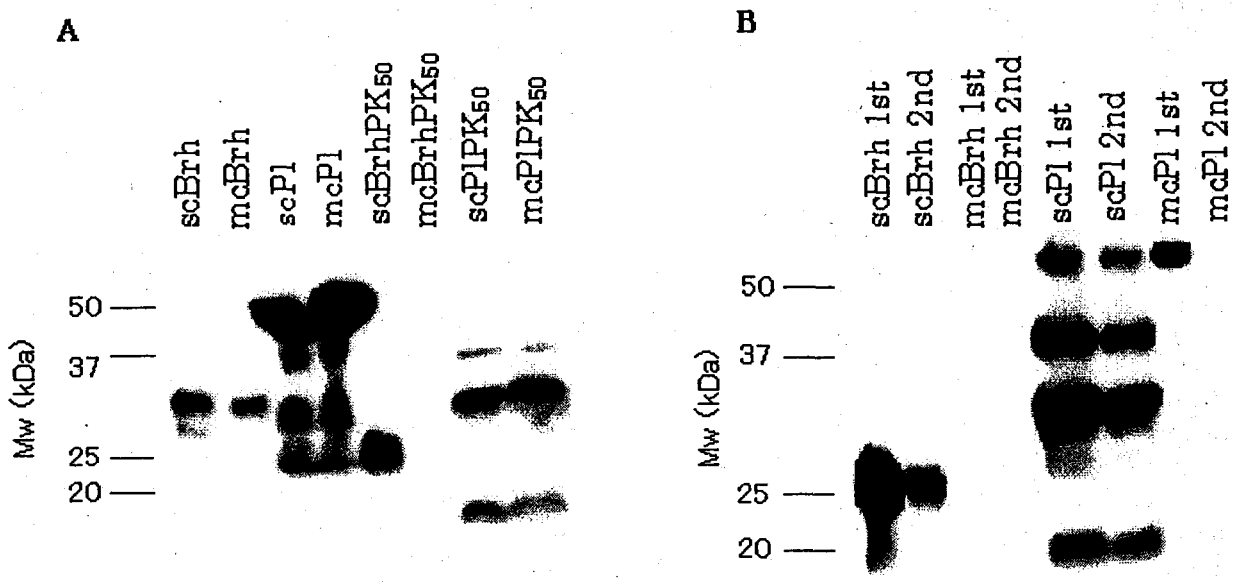

Fig. 2. Acidic SDS precipitation to discriminate scrapie infection and mock infection by their plasma. Panel A: scBrh, mcBrh, scPl and mcPl were processed and subjected to or not subjected to PK treatment. A chemiluminescence immunoblot analysis of PrP-like proteins was then performed using 3F4 primary and HRPGAM secondary antibodies. The PK treatment discriminated scBrh and mcBrh but did not discriminate between scPl and mcPl. Panel B: PK-pretreated scBrh, mcBRh, scPl and mcPl were subjected to acidic SDS precipitation condition, then analyzed by chemiluminescence immunoblotting. Acidic SDS precipitation condition was repeated twice (indicated as 1st and 2nd in the panel). This acidic SDS precipitation clearly discriminated scPl and mcPl as well as scBrh and mcBrh. The high MW protein band observed in the 1st precipitated fraction of mcPl was diminished in the 2nd precipitation. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

observed as in scPl. This observation was extremely different between the brain homogenate and plasma. From this observation, the presence of PK-resistant PrP molecules (PrPres-like molecules) in both sc- and mcPl was suspected (Fig. 2, panel A). These PrPres-like molecules in both plasma types have multiple inconstant Mw in experiments. Two patterns were often observed in 3F4-dependent immunoblot analysis; one was a 32 kDa major band with a 20 kDa minor band as well as 18, 25, 37 kDa faint bands (panel A; lane 7, 8), and the other was 20, 32, 40 and greater than 50 kDa dense bands as well as 27 kDa faint bands (Fig. 2, panel B; lane 5, 6).

The ability of the test to discriminate between scPl and mcPl was examined by acidic SDS precipitation (Fig. 2, panel B). PK-treated scBrh and mcBrh as well as scPl and mcPl preparations were subjected to acidic SDS precipitation condition (acidic SDS ppt) and analyzed with our immunoblotting system. In this experiment, precipitation was performed twice to ensure maximum removal of SDS soluble proteins. By this procedure, scPl and mcPl were clearly discriminated in the first precipitation and the higher Mw band that remained after the first precipitation was removed almost completely by the second precipitation. Discrimination between scPl and mcPl by acidic SDS precipitation was further confirmed by an experiment using 12 scPl and 6 mcPl samples (Fig. 3), but precipitation was only carried out once in this experiment. As shown in this figure, all 12 scPl samples showed the 3F4-reactive proteins but 4 mcPl samples did not. The mcPl of No. 1 and No. 6 showed weak 3F4-reactive bands. These observations confirm that scPl and mcPl can be successfully discriminated using the acidic SDS precipitation but that precipitation should be repeated twice. Weak bands observed in the mcPl No. 1 and No. 6 were expected to disappear by performing one more acidic SDS precipitation procedure.

Effect of Deglycosylation

It is known that three Mw species of PrP, di-, mono- and none-glycosylated molecules, exist in the brain and deglycosylation of the molecules causes the three protein species to accumulate into a single Mw. So, in order to determine whether deglycosylation affects the formation of multiple Mw protein bands in sc or mcPl, PK-treated sc and mcPl were deglycosylated or further processed using the acidic SDS precipitation procedure then compared to similarly processed scBrh. As shown in Fig. 4, 20–27 kDa proteins in scBrh and 19–50 kDa multiple Mw proteins in scPl and mcPl were detected following PK treatment (step 1). With deglycosylation of scBrh by PNGase F treatment, large amounts of 18 kDa protein appeared as was expected. Deglycosylation of scPl and mcPl resulted in 18 kDa proteins appearing but multiple higher Mw protein bands remained (step 2). After acidic SDS precipitation of these PK digested and deglycosylated materials, the multiple higher Mw protein bands in scPl disappeared, whereas a small amount of discrete 18 kDa protein bands remained in scBrh and scPl. These protein bands were not detected following similar treatment of mcPl (step 3). A long period of exposure (10 min) was necessary to obtain the protein signals described from step 3 of the experiment because the PrP-like proteins were difficult to detect after the deglycosylation step of the

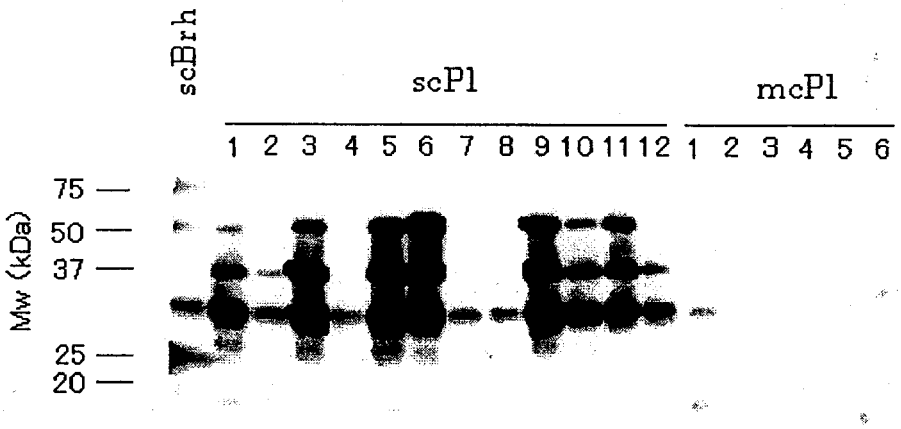

Fig. 3. Discrimination of PK-treated scPl and mcPl by acidic precipitation. Twelve preparations of scPl and 6 preparations of mcPl were pretreated with PK (50 μg/ml). scBrh was similarly treated before processing. After the pretreatment, the scPl and mcPl as well as scBrh were processed to the acidic SDS precipitation stage and analyzed by the normal immunoblot systems as described in "Materials and Methods." Anti-PrP mAb 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively, for the immunoblot analysis.

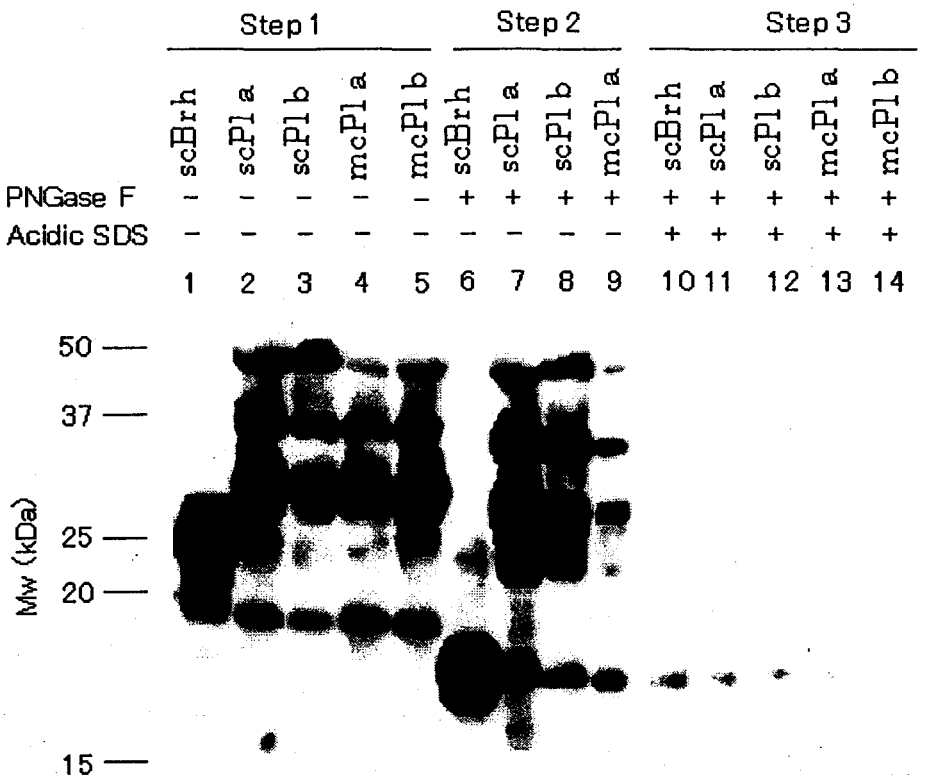

Fig. 4. Disappearance of extra Mw protein bands by digestion of carbohydrate side chains followed by acidic SDS precipitation. scBrh, two preparations of scPl and two preparations of mcPl were treated with PK (step 1, lanes 1–5). The PK-treated preparations were digested by PNGase F to remove the carbohydrate side chain on the protein molecules (step 2, lanes 6–9) then processed finally to the acidic SDS precipitation stage (step 3, lanes 10–14). Lanes were: Brain homogenate: 1, 6, 10; scPl: 2, 3, 7, 8, 11, 12; and mcPl: 4, 5, 9, 13, 14. The immunoblot pattern of each preparation during the three steps was determined. For the immunoblot analysis, 3F4 mAb and HRPGAM were used as the primary and secondary antibodies, respectively. Preparations treated with PNGase F or acidic SDS precipitation are indicated as (+) and untreated or unprocessed preparations are indicated as (−) in the figure. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

acidic SDS precipitation protocol. These observations suggested that carbohydrate side chains were involved in the formation of the multiple higher Mw protein bands (Fig. 4).

Appearance of Multiple Higher Mw Proteins by Mixing PK-Treated scBrh and PK-Treated or PK-Untreated mcPl

PK-treated Brh was mixed with PK-treated or PK-untreated mcPl then processed to the acidic SDS precipitation stage. These mixed preparations were compared with preparations of unmixed components in immunoblot analysis. Mixing of the PK-treated scBrh with PK-treated or PK-untreated mcPl resulted in the formation of higher Mw multiple protein bands as observed in scPl. Mixing with PK-treated mcPl seemed to show more discrete bands than mixing with PK-untreated mcPl. In PK-treated mcHaBrh, PK-treated or PK-untreated mcPl, these higher Mw protein bands were not observed. These immunoblot results suggested that the multiple Mw 3F4-reactive proteins were newly formed by the association between PrPres in scBrh and some PK-resistant plasma proteins in mcPl (Fig. 5).

Effect of Deglycosylation for the Association of PrPres in scBrh and PK-Resistant Protein in Plasma

As the deglycosylation of scPl resulted in failure to form the multiple higher Mw proteins but resulted in the appearance of a discrete 18 kDa band. As the Mw of which is similarly to the deglycosylated PrPres in scBrh, the possible involvement of saccharide chains was suspected for the formation of multiple extra Mw protein bands. To confirm this possibility, PK-pretreated scBrh and mcPl were deglycosylated by PNGase F or left untouched. After mixing the two preparations in the combination indicated in Fig. 6, acidic SDS precipitation was performed thereafter. As 3% SDS in the stored plasma or brain homogenates inhibits deglyco-

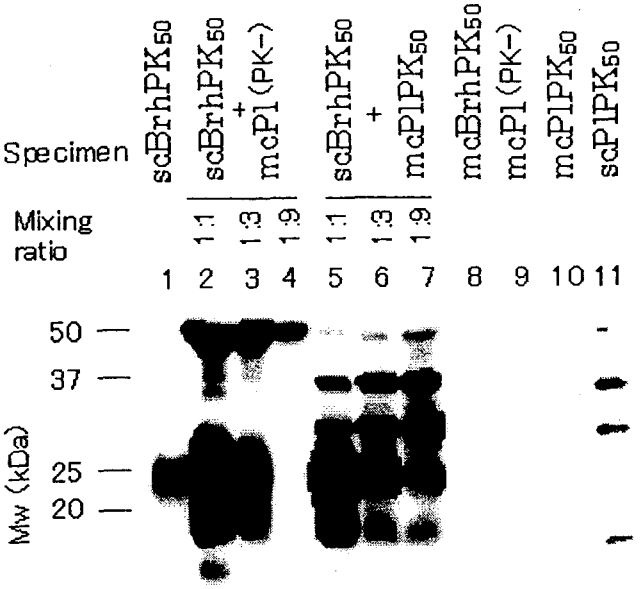

Fig. 5. Appearance of extra Mw proteins by mixing scBrh and mcPl after the acidic SDS precipitation. Proteinase K-pretreated scBrh was mixed with PK-treated or untreated mcPl and diluted to 1:3 or 1:9 in the presence of the PK-treated or untreated mcPl preparations. Then the mixed and unmixed preparations were processed to the acidic SDS precipitation stage. These processed preparations were compared by immunoblot analysis using 3F4 mAb and HRPGAM as the primary and secondary antibodies, respectively. Lanes: 1: PK treated scBrh; 2–4: PK-treated scBrh was mixed with an equal amount of PK-untreated mcPl (lane 2), diluted to 1:3 (lane 3), diluted to 1:9 (lane 4); 5–7: PK-treated scBrh was mixed with an equal amount of PK-treated mcPl (lane 5), diluted to 1:3 (lane 6), diluted to 1:9 (lane 7); 8: PK-treated mcBrh; 9: PK-untreated mcPl; 10: PK-treated mcPl; 11: PK-treated scPl. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

sylation reaction by PNGase F, the preparations for deglycosylation were diluted 30-fold before the reaction. After the deglycosylation, proteins in the reaction mixture of PNGase F treatment were precipitated by methanol and dissolved again to their original volumes with a primary buffer system that contained 3% SDS before mixing. Acidic SDS precipitation after the mixing of these deglycosylated preparations resulted in the appearance of an 18 kDa discrete band with a similar Mw to the deglycosylated PrPres in scBrh. Deglycosylation of brain proteins as well as of plasma proteins separately failed to form the higher Mw multiple protein bands. Mixing of PNGase F-treated scBrh and PNGase F-untreated mcPl formed a somewhat large amount of discrete 18 kDa proteins (lane 5).

Discussion

For antemortem diagnostic tests, body fluids such as blood or urine may be the most convenient specimens. The infectivity of blood in TSE-infected animals has already been determined to be 10–30 ID_{50}/ml (5). For this reason, the detection sensitivity of PrPres in blood is required to be in the order of ng/ml to test for TSE as has been mentioned elsewhere. Immunoblotting systems cannot detect such a low level of PrPres even in the blood of experimentally infected animals, so more sensitive methods to detect lower concentrations of PrP molecules need to be developed for antemortem diagnostic tests using blood or other body fluids. Various trials by several investigators have attempted to solve this extremely difficult problem (7, 19, 20, 30). In these studies, capillary electrophoresis analysis using a fluorescence-labeled synthetic PrP peptide, a combination of conformation-dependent PTA precipitation and ELISA, PCR of synthetic RNA conjugated with anti-PrP mAb and *in vitro* multiplication of abnormal PrP isoform (Protein Misfolding Cyclic Amplification; PMCA) have been suggested (3, 20, 29, 30). The PMCA method was shown to detect the presence of PrPres in scrapie-infected pre-mortem hamster blood using the buffy coat lysate (29). However, because these methods are complex and require a long time to obtain final results, their use in blood screening may be restricted. On the other hand, the common immunoblotting system used after PK treatment is excellent for detecting PrPres in the CNS or in other disease-affected tissues of infected animals. However, the usual immunoblot detection is less sensitive than the methods mentioned above. Therefore, a method that uses the common immunoblotting system would be the first choice for an antemortem test if its detection sensitivity could be enormously enhanced. It is suspected that the detection of PrPres molecules in blood is made more difficult by contamination from a large amount of protein, and so a method that will selectively concentrate the PrPres in blood to allow detection is therefore required. We tried to use the common immunoblotting systems in combination with a selective concentration method for PrPres-like protein aggregates and a highly sensitive chemiluminescence method. Using this combination, we successfully showed the presence of PrPres-like proteins in the scPl by means of reactivity to several anti-PrP mAbs, and by the similarity of Mw with the PrPres in infected hamster brains after deglycosylation. Moreover, carbohydrate may cause the PrPres-plasma protein aggregation and form the multiple Mw 3F4-reactive PrP-like proteins. PrP is a membrane protein and is known to aggregate frequently, especially after conversion to its disease-associated abnormal isoform. For this reason, detection of these aggregates is also the optimal way to develop an assay

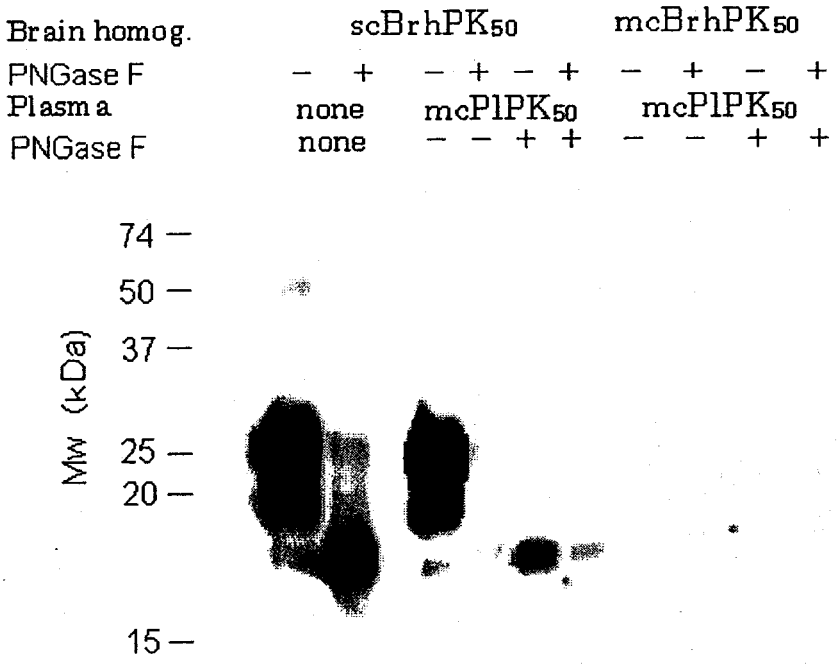

Fig. 6. Inability to form aggregate between scBrh and mcPl by digestion of carbohydrate before mixing. PK-treated scBrh, mcBrh and mcPl were further treated or not treated with PNGase F to digest the carbohydrate side chains on the proteins. These pretreated preparations were mixed with each other as indicated in the figure, and processed to the acidic SDS precipitation stage after mixing. Lanes: 1: scBrh (PNGase F−), 2: scBrh (PNGase F+); 3: scBrh (PNGase F−) mixed with mcPl (PNGase F−); 4: scBrh (PNGase F−) mixed with mcPl (PNGase F+); 5: scBrh (PNGase F+) mixed with mcPl (PNGase F−); 6: scBrh (PNGase F+) mixed with mcPl (PNGase F+); 7: mcBrh (PNGase F−) mixed with mcPl (PNGase F−); 8: mcBrh (PNGase F−) mixed with mcPl (PNGase F+); 9: mcBrh (PNGase F+) mixed with mcPl (PNGase F−); 10: mcBrh (PNGase F+) mixed with mcPl (PNGase F+), in which (PNGase F+) and (PNGase F−) mean digested or non-digested with PNGase F before mixing, respectively. 3F4 and HRPGAM were used as the primary and secondary antibodies, respectively.

method when using blood. However, previous tests for evaluating the sensitivity of detection systems using PrP molecules have frequently failed, presumably due to the tendency of the PrP molecule to form aggregates. We therefore evaluated the sensitivity of the detection system using SDS sample buffer which contained 0.1% BSA for the dilution buffer and by boiling the preparation throughout the serial dilution steps. This method allowed us to obtain a proper dispersion of the PrP aggregate in the test preparation and we successfully showed that the endpoint of the detection system was 1.5×10^{-12} g (6.05×10^{-17} mol) or more of rPrP and PrPres in 1.4×10^{-9} g brain equivalent of scHaBrh. As the scBrh has an infectivity titer of 10^{-7}–10^{-9} ID_{50}/ml, this chemiluminescence system can detect PrPres corresponding to 1 ID_{50}/ml or more, which is sufficiently greater than the value required to detect PrPres in blood (Fig. 1). We therefore decided to use this chemiluminescence system to detect PrPres in scPl. This system also allowed us to determine the detection limit of PrP protein in the brain (Fig. 1B).

Adding the acidic SDS precipitation stage to the protocol enabled successful discrimination of scPl and mcPl. The acidic SDS condition may selectively target aggregated PrP molecules, suggesting that PrP molecules in mcPl may not be aggregated. This observation is reasonable in that one of the main differences between PrPres and PrPc may be whether they exist in an aggregated form or not. Some investigators have tried to obtain PrPres in blood in an aggregated form (7). The aggregation of PrPres is thought to be a result of the more hydrophobic nature of the PrPres molecule than that of PrPc (24). However, the phenomenon observed here clearly suggests that an important factor for aggregation may be the presence of a carbohydrate side chain on both PrPres and plasma proteins rather than the hydrophobic nature of the PrPres. Carbohydrate has often been described as the outfitter for glycosylation and function (25).

The types of protein that aggregate with the PrPres-like molecules are not known. Some plasma proteins are known to associate with the PrP, but it is possible that the PrP molecules in hamster plasma may also be a candidate for these plasma proteins (11, 31, 34). PK-

resistant PrP molecules have recently been reported in uninfected human brains as well as in uninfected mouse and hamster brains and have been labeled a silent prion. PK-resistant protein in mcPl, which is able to aggregate with PrPres could be the silent prion in hamster plasma (34). Weakly observed 3F4-reactive protein bands in mcPl suggest the existence of the silent prion in plasma (Fig. 3).

In the lanes of scPl-2, -4, -7, -8, discrete bands in the Mw 32 kDa region were observed without other bands. As the band of this Mw region was weakly observed in mcPl-1 and scPl-6, it is somewhat difficult to decide the positive expression of 3F4-reactive protein for scPl-2, -4, -7 and -8. However, intensities of these signals in the scPl preparations were obviously strong compared to the signals in mcPl preparations. Thus it may be difficult to decide positive or not positive by performing acidic precipitation just one time. It is reasonable that an individual animal does not express the 3F4-reactive protein similarly in a time dependent manner and expression strength. Therefore, if blood testing is introduced, the plasma preparation should be processed twice with this acidic SDS precipitation, and the test should be conducted several times at different times.

Here we showed the successful discrimination of scrapie-infected and mock-infected hamsters by their plasma preparations using a novel combination method termed acidic SDS precipitation along with a highly sensitive chemiluminescence immunoblot system. In the immunoblots of PK-treated plasma preparations, multiple protein bands at Mw higher than the 25 kDa position were observed. These protein bands were observed in both scPl and mcPl after PK treatment. As these proteins were 3F4-reactive as well as PK-resistant, they were very likely to be PrPres molecules. However, observations showing multiple bands of higher than 25 kDa in Mw in mcPl as well as in scPl were very different from the electrophoresis pattern of scBrh. These differences between plasma and Brh have to be explained if the multiple PrPres-like proteins in plasma are aggregates of PrPres and some other plasma protein. This is similar with an observation in which the C-terminal domain of a recombinant mouse PrP peptide was aggregated spontaneously even in SDS sample buffer (24). Differences of electrophoresis patterns in Fig. 5, lanes 5–7 and Fig. 6, lane 3 or Fig. 2B, lane 5, 6 in spite of the same processing protocol may explain in which aggregation counterparts with PrPres in these plasma preparations may not be the same molecule, in preparation. After the PK treatment, an enormous amount of partial peptides was distributed in the broad Mw region if total protein was stained on WB membrane. This means that multiple partial peptides which possessing carbohydrate chains may have the potential to become the counterpart of these aggregates. We could not control the combination of the molecules. A deglycosylation experiment using both scPl and scBrh solved this question. After deglycosylation and acidic SDS precipitation, both scBrh and scPl showed a single discrete protein band at the 18 kDa Mw position. This observation strongly suggests that the carbohydrate side chain might be an important factor in the aggregation of the PrPres-like protein with some other proteins. From these observations, one of the components required to form aggregates must be the PrPres molecule but the other component need not be another PrPres molecule. That is, both self aggregation as well as aggregation of multiple hetero molecules could be resulted in the formation of the multiple Mw protein bands. Although dense bands at 25 kDa was observed in the scBrh and mcPl mixing (Fig. 5, lanes 5–7), the bands were obscure in scPl (Fig. 2, lanes 5, 6). This discrepancies between the preparations may conjectured by the differences of PrPres and plasma protein ratio. In Fig. 5, lanes 5–7, larger amount of scBrh compared to mcPl showed pattern more similar to that of scBrh, larger amount of mcPl showed more discrete band pattern after the PK treatment, in reverse. In this observation, 20 and 25 kDa protein bands were decreasing gradually along with mcPl was increasing. Therefore, it is conjectured that the 20 and 25 kDa proteins were not observed if less amounts of PrPres existed in scPl as observed in Fig. 2B, lane 5, 6.

PrPres was also found in uninfected human brains and labeled a silent prion (34). Similar molecules are likely to be present in non-infected hamsters and in mouse brains as well. If the silent prion in hamster and mouse brains is also exist in plasma, the PK-resistant 3F4-reactive proteins observed in mcPl in this experiment may be the candidate in hamster plasma. The silent prions in hamster plasma could aggregate with themselves or with other proteins to form the multiple higher Mw proteins in mcPl as well as scPl. But if the silent prion exists in mcPl, it must be discriminated through the blood tests. The acidic SDS precipitation process reported here may be useful for such trials.

So, as the PrPres molecules in hamster, 25 kDa, 20 kDa and 18 kDa proteins correspond to the di-, mono and no carbohydrate molecules, respectively. Multiple higher Mw protein bands were presumably aggregates with PrPres and other plasma proteins. The phenomenon that Mw of these aggregates were not found within a constant range indicated that counterparts of presumable PrPres might not be the specialized molecules in preparations; the silent prion may be included within these inconsistent molecules. Furthermore, we could

not control these combinations. The biological meaning of these aggregations is not known.

The observations from this experiment show that acidic SDS precipitation of plasma preparations enables discrimination between scrapie-infected and mock-infected hamsters and may be an extremely important finding for the developing of an antemortem blood test to diagnose TSE. The question as to why the silent prion is not precipitated by the acidic precipitation if it exists in mcPI remains to be answered.

We gratefully acknowledge Dr. Professor Takashi Onodera, Department of Molecular Immunology, Agricultural and Life Sciences, Tokyo University, for his great support and encouragement, Dr. Yokoyama, Research Center for Prion Diseases, National Institute of Animal Health, for his assistance to use infected hamster materials and Dr. Iwakura, Institute for medical Science, Tokyo University, for his kind gift of anti-HIV P24 mAb TA8.1 and for many useful discussions. We also acknowledge Dr. Yuasa, a member of the Administrative Committee of the Japanese Red Cross Society, Dr. Okazaki, vice director, and Dr. Nishimura, one of our scientific colleagues in the institute, for their useful discussions and encouragement. We would also like to thank all our colleagues of the Japanese Red Cross Society for their encouragement and support.

References

1) Agguzi, A., Montrasio, F., and Kaeser, P.S. 2001. Prions: health scares and biological challenge. Nat. Rev. Mol. Cell Biol. **2**: 118–126.
2) Agguzi, A., and Polimenidou, M. 2004. Mammalian prion biology: one century of evolving concepts. Cell **116**: 313–327.
3) Bellon, A., Sayfert-Brandt, W., Lang, W., Baron, H., Gröner, A., and Vey, M. 2003. Improved conformation-dependent immunoassay: suitability for human prion detection with enhanced sensitivity. J. Gen. Virol. **84**: 1921–1925.
4) Beley, E.D., and Schonberger, L.B. 2005. The public health impact of prion diseases. Annu. Rev. Public Health **26**: 191–212.
5) Brown, P. 2005. Blood infectivity, processing and screening tests in transmissible spongiform encephalopathy. Vox Sang. **89**: 63–70.
6) Brown, P., Cerevenáková, L., and Diringer, H. 2001. Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease. J. Lab. Clin. Med. **137**: 5–13.
7) Chang, B., Chen, X., Yin, S., Pan, T., Zhang, H., Wong, P., Kang, S.C., Yan, H., Li, C., Wolf, L., Miller, M.W., Wisniewski, T., Greene, M.I., and Sy, M.S. 2007. Test for detection of disease-associated prion aggregate in the blood of infected but asymptomatic animals. Clin. Vac. Immunol. **14**: 36–43.
8) Clerke, P., and Ghani, A.C. 2005. Projections of the future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider genetic susceptibility. J. R. Soc. Interface **2**: 19–31.
9) Collins, S.J., Lawson, V.A., and Masters, C.L. 2004. Transmissible spongiform encephalopathies. Lancet **363**: 51–61.
10) Farrugia, A. 2006. Globalization and blood safety. ISBT Science Series **1**: 25–32.
11) Fischer, M.B., Roeckl, C., Patrizek, P., Schwarz, H.P., and Agguzi, A. 2000. Binding of disease-associated prion protein to plasminogen. Nature **408**: 479–483.
12) Furukawa, H., Doh-ura, K., Okuwaki, R., Shirabe, S., Yamamoto, K., Udono, H., Ito, T., Katamine, S., and Niwa, M. 2004. A pitfall in diagnosis of human prion diseases using detection of protease-resistant prion protein in urine. Contamination with bacterial outer membrane proteins. J. Biol. Chem. **279**: 23661–23667.
13) Hewitt, P.E., Llewlyn, C.A., MacKenzie, J., and Will, R.G. 2006. Creutzfeldt-Jakob disease and blood transfusion: results of the UK transfusion medicine epidemiological review study. Vox Sang. **91**: 221–230.
14) Hill, A.F., Butterworth, R.J., Joiner, S., Jackson, G., Rossor, M.N., Thomas, D.J., Frosh, A., Tolley, N., Bell, J.E., Spencer, M., King, A., Al-Sarraj, S., Ironside, J.W., Lantos, P.L., and Collinge, J. 1999. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet **353**: 183–189.
15) Hilton, D.A., Fathers, E., Edwards, P., Ironside, J.W., and Zajicek, J. 1998. Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. Lancet **352**: 703–704.
16) Hilton, D.A., Ghani, A.C., Conyers, L., Edwards, P., McCardle, L., Penny, M., Richie, D., and Ironside, J.W. 2002. Accumulation of prion protein in tonsil and appendix: review of tissue samples. Brit. Med. J. **325**: 633–634.
17) Hilton, D.A., Gahni, A.C., Conyers, L., Edwards, P., McCardle, L., Ritchie, D., Penny, M., Hegazy, D., and Ironside, J.W. 2004. Prevalence of lymphoreticular prion accumulation in UK tissue samples. J. Pathol. **203**: 732–739.
18) Ironside, J.W. 2006. Human prion diseases: biology and transmission by blood. ISBT Science Series **1**: 15–20.
19) Johnson, R.T., and Gibbs, C.J. 1994. Creutzfeldt-Jakob diseases and related transmissible spongiform encephalopathies. New Engl. J. Med. **339**: 1994–2004.
20) Laurenco, P.C., Scherr, M.J., MacGregor, I., Will, R.G., Ironside, J.W., and Head, M.W. 2006. Application of an immunocapillary electrophoresis assay to the detection of abnormal prion protein in brain, spleen and blood specimens from patients with variant Creutzfeldt-Jakob disease. J. Gen. Virol. **87**: 3119–3124.
21) Llewlyn, C.A., Hewitt, P.E., Knight, R.S.G., Amar, K., Cousens, S., MacKenzie, J., and Will, R.G. 2004. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet **363**: 417–427.
22) MacGregor, I. 2001. Prion protein and developments in its detection. Transfusion Med. **11**: 3–14.
23) MacGregor, I., Hope, J., Barnard, G., Kirby, L., Drummond, O., Pepper, D., Hornsey, V., Barclay, R., Bessos, H., Turner, M., and Prowse, C. 1999. Application of a time-resolved fluoroimmunoassay for the analysis of normal prion protein in human blood and its components. Vox Sang. **77**: 88–96.

24) Martins, S.M., Frosoni, D.J., Martinez, A.M.B., DeFelice, F.G., and Ferreira, S.T. 2006. Formation of soluble oligomers and amyloid fibrils with physical properties of PrPsc from the C-terminal domain of recombinant murine prion protein, mPrP(121–231). J. Biol. Chem. **281**: 26121–26128.
25) Mitra, N., Siha, S., Ramya, T.N., and Surolia, A. 2006. N-linked oligosaccharides are outfitters for glycoprotein folding form and function. Trends Biochem. Sci. **31**: 156–163.
26) Peden, A.H., Head, M.W., Ritchie, D.L., Bell, J.E., and Ironside, J.W. 2004. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet **363**: 417–427.
27) Prowse, C. 2006. Controlling the blood-born spread of human prion disease. ISBT Science Series **1**: 21–24.
28) Rickets, M.M. 2004. Public health and the BSE epidemic. Curr. Top. Microbiol. Immunol. **284**: 99–119.
29) Saa, P., Castilla, J., and Soto, C. 2006. Presymptomatic detection of prion proteins in blood. Science **313**: 92–94.
30) Saborio, G.P., Permanne, B., and Soto, C. 2001. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature **411**: 810–813.
31) Safar, J.G., Will, H., Geschwind, M.D., Deering, C., Latawiec, D., Serban, A., King, D.J., Legname, G., Weisgraber, K.H., Mahley, R.W., Miller, B., DeArmond, S.J., and Prusiner, S.B. 2006. Human prions and plasma lipoproteins. PNAS **103**: 11312–11317.
32) Schmerr, M.J., Jenny, A.L., Bulgin, M.S., Miller, J.M., Hamir, A.N., Cutlip, R.C., and Goodwin, K.R. 1999. Use of capillary electrophoresis and fluorescence labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. J. Chromatogr. **A853**: 207–214.
33) Shaked, G.M., Shaked, Y., Kariv-Inbal, Z., Harimi, M., Avraham, I., and Gabizon, R. 2001. A protease resistant prion protein isoform is present in urine of animals and humans affected with prion diseases. J. Biol. Chem. **276**: 31479–31482.
34) Yuan, J., Xiano, X., McGeehan, J., Dong, Z., Cali, I., Fujioka, H., Kong, Q., Kneal, G., Gambetti, P., and Zou, W-Q. 2006. Insoluble aggregates and protease-resistant conformers of prion protein in uninfected human brains. J. Biol. Chem. **281**: 34846–34858.

別紙様式第2-1

No. 24

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
				2008. 1. 11	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		公表国
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)		ProMED 20080107-0087, 2008 Jan 7. 情報源:[1]UK National CJD Surveillance Unit, monthly statistics, 2007, 2008 Jan 7.	英国

研究報告の概要

○プリオン病最新情報
[1]英国CJDサーベイランスユニット—月次統計と2007年の合計
月次CJD統計—2008年1月7日時点
以下の数字は英国CJDサーベイランスユニットに報告されたCJD疑い症例数及び確定・可能性例の死亡数である。
内訳は以下の通り: vCJD患者:vCJD確定例における死亡患者:114名。vCJD可能性例における死亡患者(神経病理学的に未確定):48名。vCJD可能性例における死亡患者(神経病理学的診断を保留):1名。死亡患者総数:163名。vCJD患者-存命中:3名。vCJD確定例または可能性例総数:166名。2007年12月の月例統計以来、新たにvCJDと診断された患者はないが、存命中の患者数は1名減少した。このデータは英国におけるvCJD流行は減少しつつあるとする見解に一致する。死亡患者数のピークは2000年の28名であり、その後2001年に20名、2002年に17名、2003年に18名、2004年に9名、2005年に5名、2006年に5名、2007年に5名と減少している。
2007年における全ての型のCJD症例の報告数は111名であった。死亡例は47名が孤発性CJD、2名が医原性CJD、4名が家族性CJD、1名がGSS、5名がvCJDだった。

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
2008年1月7日の時点で、英国CJDサーベイランスユニットに報告されたvCJD確定例または可能性例総数は166名、2007年中の死亡患者数は5名であり、英国におけるvCJD流行は減少しつつあるとする見解に一致するとの報告である。なお、2007年1月の同報告ではvCJD確定例または可能性例総数165名、死亡患者総数158名であったことから、2007年中のvCJD新規発症患者は1名、死亡患者は5名である。	日本赤十字社は、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ヶ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1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。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。

Back

Archive Number 20080107.0087
Published Date 07-JAN-2008
Subject PRO/AH/EDR> Prion disease update 2008 (02)

PRION DISEASE UPDATE 2008 (02)
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[With the continuing decline of the number of cases of variant Creutzfeldt-Jacob disease (abbreviated previously as vCJD or CJD (new var.) in ProMED-mail) in the human population, it has been decided to broaden the scope of the occasional ProMED-mail reports to include other prion-related diseases. Data on vCJD cases from any part of the world are now included in these updates where appropriate, and other forms of CJD (sporadic, iatrogenic, familial, and GSS (Gerstmann-Straussler-Scheinker disease) are included also when they have some relevance to the incidence and etiology of vCJD. - Mod.CP]

In this update:
[1] UK: National CJD Surveillance Unit -- Monthly statistics & 2007 totals
[2] UK - New vCJD type
[3], [4], [5] vCJD in vitro assays

******
[1] UK: National CJD Surveillance Unit -- Monthly statistics & 2007 totals
Date: Mon 7 Jan 2008
Source: UK National CJD Surveillance Unit, monthly statistics, 2007 [edited]
<http://www.cjd.ed.ac.uk/figures.htm>

Monthly Creutzfeldt-Jakob disease statistics -- as of 7 Jan 2008

These following figures show the number of suspect cases of CJD referred to the CJD surveillance unit in Edinburgh and the number of deaths of definite and probable variant Creutzfeldt-Jakob disease [abbreviated in ProMED-mail as CJD (new var.) or vCJD], the form of the disease thought to be linked to BSE (bovine spongiform encephalopathy).

Definite and probable vCJD cases in the UK as of 7 Jan 2008

Summary of vCJD cases -- deaths

Deaths from definite vCJD (confirmed): 114
Deaths from probable vCJD (without neuropathological confirmation): 48
Deaths from probable vCJD (neuropathological confirmation pending): 1
Number of deaths from definite or probable vCJD (as above): 163

Summary of vCJD cases -- alive

Number of probable vCJD cases still alive: 3

Total

Number of definite or probable vCJD (dead and alive): 166

These data indicate that there have been no new cases diagnosed during the past month, but the number of patients alive has decreased

by one.

These data are still consistent with the view that the vCJD outbreak in the UK is in decline (although the incidence curve may be developing a tail). The peak number of deaths was 28 in the year 2000, followed by 20 in 2001, 17 in 2002, 18 in 2003, 9 in 2004, 5 in 2005, 5 in 2006, and 5 in 2007.

Totals for all types of CJD cases in the year 2007

As of 31 Dec 2007 in the UK in the year 2007, there were 111 referrals, 47 deaths from sporadic CJD, 2 deaths from iatrogenic CJD, 4 deaths from familial CJD, one from GSS, and 5 deaths from vCJD.

--
Communicated by:
ProMED-mail <promed@promedmail.org>

******
[2] UK - New vCJD type
Date: Mon 7 Jan 2008
Source: Arch Neurol. 2007 Dec; 64(12):1780-4 [edited]
<http://archneur.ama-assn.org/cgi/content/abstract/64/12/1780>

[Prion disease update 2008 (01) contained brief press reports of the identification of a new form of vCJD in a young female patient, homozygote V/V at codon 129 of the PrPSc gene. The Abstract of the scientific paper describing this observation is reproduced below. - Mod.CP]

Creutzfeldt-Jakob disease, prion protein gene codon 129V/, and a novel PrPSc type in a young British woman
---
By Mead S, Joiner S, Desbruslais M, Beck JA, O'Donoghue M, Lantos P, Wadsworth JD, Collinge J. MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, Queen Square, London, UK.

Background
Variant Creutzfeldt-Jakob disease (vCJD) is an acquired prion disease causally related to bovine spongiform encephalopathy that has occurred predominantly in young adults. All clinical cases studied have been methionine homozygotes at codon 129 of the prion protein gene (PRNP) with distinctive neuropathological findings and molecular strain type (PrPSc type 4). Modeling studies in transgenic mice suggest that other PRNP genotypes will also be susceptible to infection with bovine spongiform encephalopathy prions but may develop distinctive phenotypes.

Objective
To describe the histopathologic and molecular investigation in a young British woman with atypical sporadic CJD and valine homozygosity at PRNP codon 129.

Design
Case report, autopsy, and molecular analysis.

Setting
Specialist neurology referral center, together with the laboratory services of the MRC [Medical Research Council] Prion Unit.

Subject
Single hospitalized patient.

Main Outcome Measures
Autopsy findings and molecular investigation results.

Results
Autopsy findings were atypical of sporadic CJD, with marked gray and white matter degeneration and widespread prion protein (PrP) deposition. Lymphoreticular tissue was not available for analysis. Molecular analysis of PrPSc (the scrapie isoform of PrP) from cerebellar tissue demonstrated a novel PrPSc type similar to that