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.,

The Journal of Biological Chemistry

Downloaded from www.jbc.org at MITSUBISHI PHARMA CO on December 12, 2007

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

The Journal of Biological Chemistry

Downloaded from www.jbc.org at MITSUBISHI PHARMA CO on December 12, 2007

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 イタリア	
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)					

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。

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

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。

JRC2008T-016

JOURNAL OF VIROLOGY, Apr. 2008, p. 3697–3701 Vol. 82, No. 7
0022-538X/08/$08.00+0 doi:10.1128/JVI.02561-07
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

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

Downloaded from jvi.asm.org at JAPANESE RED CROSS CENTRAL BLOOD INSTITUTE on April 28, 2008

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.

Downloaded from jvi.asm.org at JAPANESE RED CROSS CENTRAL BLOOD INSTITUTE on April 28, 2008