Alteration of the biological and biochemical characteristics of bovine spongiform encephalopathy prions during interspecies transmission in transgenic mice models

Takashi Yokoyama, Kentaro Masujin, Yoshifumi Iwamaru, Morikazu Imamura and Shirou Mohri

Prion Disease Research Center, National Institute of Animal Health, Tsukuba, Ibaraki 305-0856, Japan

In the interspecies transmission of prions, the species barrier influences the susceptibility of the host. Bovine spongiform encephalopathy (BSE) prions affect a wide range of host species but do not affect hamsters. In order to study this species barrier, this study analysed the transmissibility of BSE prions to several lines of transgenic (Tg) mice, including those expressing mouse and hamster chimeric prion proteins (MH2M and MHM2 mice). BSE prions were transmitted to tga20, MHM2 and ICR mice, and the incubation period was approximately 400 days. Thus, these mice were classified as 'susceptible mice'. However, BSE prions were not transmitted to MH2M and TgHaNSE mice, and these mice were classified as 'resistant mice'. After the BSE prions were passaged once in wild-type mice, they could be transmitted to resistant mice. The characteristics of the accumulated abnormal isoform of PrP (PrP<sub>Sc</sub>) in susceptible and resistant mice were determined using Western blotting. A BSE-like glycoform pattern of PrP<sub>Sc</sub> was detected in all of the susceptible mice using two different antibodies that recognized either the N- or the C-terminal end of the 27–30 kDa protease-resistant fragment of PrP (PrP<sub>27–30</sub>) as the epitope. In contrast, proteinase digestion followed by deglycosylation analysis showed that, in addition to PrP<sub>27–30</sub>, truncated PrP<sub>Sc</sub> fragments existed in resistant mice. These mixed PrP<sub>Sc</sub> fragments may have resulted from the adaptation of resistant mice to BSE prions.

INTRODUCTION

Bovine spongiform encephalopathy (BSE) is a type of transmissible spongiform encephalopathy or prion disease (Prusiner, 1991). The host gene-encoded cellular isoform of prion protein (PrP<sup>C</sup>) and the conversion of PrP<sup>C</sup> to the abnormal isoform (PrP<sup>Sc</sup>) is the central event in prion pathogenesis. PrP<sup>Sc</sup> is the major component of the prion, if not the entire infectious agent. With interspecies transmission of prions, which means that the PrP<sub>Sc</sub> in the inoculum has a different amino acid sequence to the PrP<sup>C</sup> in the host, prolonged incubation periods and/or less efficient transmissibility have been observed, and this phenomenon is referred to as the ‘species barrier’ (Kimberlin, 1991). At the molecular level, species barriers can be explained, at least partly, by the dependence of PrP<sup>C</sup> formation on the PrP amino acid sequence homology.

Surprisingly, BSE prions affect a wide range of host species. In nature, BSE is transmitted to cattle (Wells & Wilesmith, 1995), several zoo ruminants (Kirkwood & Cunningham, 1994) and wild and domestic cats (Wyatt <i>et al.</i>, 1990, 1991). Experimentally, BSE has been transmitted to mice (Fraser <i>et al.</i>, 1992), sheep, goats (Foster <i>et al.</i>, 1993), minks (Robinson <i>et al.</i>, 1994), marmosets (Baker <i>et al.</i>, 1998), macaques (Lasmézas <i>et al.</i>, 1996) and lemurs (Bons <i>et al.</i>, 1999). Furthermore, BSE has been transmitted to humans, in whom it causes variant Creutzfeldt–Jakob disease (vCJD) (Collinge <i>et al.</i>, 1996; Hill <i>et al.</i>, 1997). However, BSE prions are not transmitted to hamsters. Mice and hamsters are good models for analysing the mechanisms underlying the species barrier to BSE prions.

Transgenic (Tg) mice that express heterologous PrP genes are useful tools for analysis of the species barrier. Eight amino acid differences are present between the mouse and hamster proteinase K (PK)-resistant 27–30 kDa core fragment of PrP<sup>Sc</sup> (PrP<sub>27–30</sub>). Studies using Tg mice have shown that the sequence of PrP influences the interspecies transmission of prions between mice and hamsters (Scott <i>et al.</i>, 1989, 1993). Transmission studies using mouse and hamster chimeric PrP-expressing mice (MHM2 and MH2M, respectively) have shown that the tertiary structure of the interacting sites of PrP<sup>C</sup> and PrP<sup>Sc</sup> may depend partially on the side chains of one or more of the amino acids at positions 138, 154 and 169 (Scott <i>et al.</i>, 1993).
Furthermore, in vitro analysis has shown that residue 155 in hamsters (equivalent to residue 154 in the mouse sequence) is necessary for the efficient formation of PK-resistant PrP (Priola et al., 2001). However, the precise mechanism underlying the species barrier has not yet been elucidated.

Here, we examined the species barrier to BSE prions using a chimeric PrP expression mouse model. MHM2 and MH2M mice (Scott et al., 1993) were inoculated intracerebrally with BSE prions. We found that aa 131–188 of PrP also contributed to the species barrier against BSE prions in hamsters. Following passage in mice, this barrier disappeared and the biological characteristics of the BSE prions changed; these prions could then affect MH2M mice. We analysed the characteristics of the accumulated PrPSc in the brains of these Tg mice. Accumulation of another truncated PrPSc was observed in MH2M mice, which were resistant to BSE prions of the primary passage.

### METHODS

#### Animals and prions

Three-week-old female ICR mice (SLC) were purchased and used as wild-type mice. Tg mice that expressed mouse and hamster chimeric PrP (MH2M and MHM2, respectively) were provided by Dr S. B. Prusiner (Scott et al., 1993). A comparison of the PrP amino acid sequence of these Tg mice is shown in Table 1. Amino acid substitutions of L108M and V111M are present in MHM2 mice. In addition to these amino acid substitutions, three other substitutions (I138M, Y154N and S169N) are present in MH2M mice. MHM2 and MH2M mice are susceptible to mouse-passaged and hamster-passaged prions, respectively (Scott et al., 1993; Yokoyama et al., 2007b) and these mice express different glycoforms of PrPSc (Yokoyama et al., 2007b). tga20 mice, which overexpress mouse PrP (Fischer et al., 1996), were purchased from the European Mutant Mouse Association (EMMA). TgHaNSE mice, which overexpress hamster PrP in their neurons, were provided by Dr B. Chesbro (Race et al., 1995). TgBoPrP mice, which overexpress cattle PrP, were provided by Dr S. B. Prusiner (Scott et al., 1997). The susceptibility of TgBoPrP mice to BSE has been confirmed previously (Yokoyama et al., 2007a). All of the Tg mice were maintained by crossing with PrPnull mice (Yokoyama et al., 2001) as a PrP-null background. Brain samples of BSE-affected cattle in Japan were used for the transmission study. Mouse-passaged BSE prions (Hayashi et al., 2005) were also used, along with the mouse-adapted scrapie Obihiro strain and hamster-adapted scrapie Sc237 strain (Yokoyama et al., 1995).

#### Incubation time assay

BSE and scrapie prions were transmitted to Tg mice as described previously (Yokoyama et al., 1995). An outline of the transmission studies is shown in Fig. 1. Brain homogenates were prepared using a Multi-Beads Shocker (Yasui Kikai). Mice were inoculated with 20 μl 10% homogenate (w/v) in sterile PBS. After inoculation, the clinical status of the mice was monitored daily to assess the onset of neurological signs. Diseased mice were sacrificed and subjected to examination for PrPSc, as described previously (Yokoyama et al., 2001).

#### Western blotting

PrPSc was extracted from the mice brains according to a previously described method (Yokoyama et al., 2001). The brain tissue (brain stem) was homogenized in buffer containing 100 mM NaCl and 50 mM Tris/HCl (pH 7.6). The homogenate was mixed with an equal volume of detergent buffer containing 4% Zwittergent 3-14, 1% Sarkosyl, 100 mM NaCl and 50 mM Tris/HCl (pH 7.6), and then incubated with 40 μg PK ml⁻¹ at 37°C for 30 min. PK digestion was terminated with 2 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (Pefablock; Roche Diagnostics). The sample was mixed with an equal volume of a gel-loading buffer containing 2% SDS and heated at 100°C for 6 min. The samples were separated by 12% SDS-PAGE and electrically blotted onto a PVDF membrane (Immobilon-P; Millipore). The blotted membrane was incubated with anti-PrP antibodies. Polyclonal antibody (pAb) B103 (Horiuchi et al., 1995) and monoclonal antibodies (mAbs) 4B1 (Kim et al., 2004), T2 (Hayashi et al., 2005) and 3F4 (Signet Laboratories) were used as primary antibodies. These antibodies recognized different epitopes: one located at the N terminus of PK-digested PrPSc (pAb B103 and mAb 3F4) and the others located at the C terminus of the globular domain of PrPSc (mAbs T2 and 4B1). mAbs T2 and 4B1 recognized the epitope located at aa 132–230 and 155–231 of PrP, respectively. Signals were detected using a chemiluminescent substrate (SuperSignal; Pierce Biotechnology).

#### Band profile of PK-digested PrPSc

For band analysis, the relative quantities of the three PrPSc bands were measured using Fluorochem software (Alpha-Innotech) after background subtraction. For band profile analysis, only samples within the linear range, i.e. those with unsaturated signal intensities, were used. All values were calculated as the mean ± s of at least three independent determinations.

---

**Table 1.** Comparison of the PrP amino acid sequences of MHM2 and MH2M mice

The PrP amino acid sequence at residues 108, 111, 138, 154 and 169 is shown. Residue numbers correspond to those in murine PrP (Westaway et al., 1987).

<table>
<thead>
<tr>
<th>Species</th>
<th>108</th>
<th>111</th>
<th>138</th>
<th>154</th>
<th>169</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>L</td>
<td>V</td>
<td>I</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>MHM2</td>
<td>M</td>
<td>M</td>
<td>I</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>MH2M</td>
<td>M</td>
<td>M</td>
<td>L</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hamster</td>
<td>M</td>
<td>M</td>
<td>L</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Cattle</td>
<td>M</td>
<td>V</td>
<td>I</td>
<td>H</td>
<td>S</td>
</tr>
</tbody>
</table>

---

**Fig. 1.** Summary of the transmission experiment. BSE prions or mouse-passaged BSE prions (BSE/ICR) were inoculated intracerebrally into ICR, tga20, MHM2, MH2M and TgHaNSE mice. The clinical status of the mice was monitored. TgBoPrP-passaged BSE prions (BSE/TgBoPrP) were also inoculated into the same set of mice. 1st, 2nd and 3rd indicates the passage history of the BSE prions.
**Peptide N-glycosidase F digestion.** The PK-digested brain samples (10% brain homogenate; NP-40 lysis buffer) were denatured in glycoprotein-denaturing buffer (0.5% SDS, 1% β-mercaptoethanol; New England Biolabs) at 100°C for 10 min prior to incubation with peptide N-glycosidase F (PNGase F; New England Biolabs) and G7 reaction buffer (50 mM NaPO4; New England Biolabs) at 37°C for 2–4 h. The reaction was terminated by SDS denaturation.

**RESULTS**

**Transmissibility of mouse-passaged BSE prions**

Next, the transmissibility of wild-type mouse-passaged BSE prions was examined using the same set of Tg mice as in the primary transmission experiment. The MH2M and TgHaNSE mice showed clinical signs 212.8 and 153.1 days after inoculation, respectively (Table 2). The presence of PrPSc in their brains also supported the transmissibility (Fig. 2b). Mouse-passaged BSE prions were transmissible to all of the examined mice, including the MH2M and TgHaNSE mice. Subsequently, the mouse-passaged BSE prions were also used to challenge the same set of mice. In the wild-type mice, the incubation period of BSE prions was shortened, depending on the passage history. These Tg mice were also inoculated with secondary mouse-passaged BSE prions in order to monitor the transition of biological characteristics. Although the incubation periods in MH2M and TgHaNSE mice in the second and third passages were unstable, MH2M and tga20 mice showed similar incubation periods in both the second and third passages (Table 2). The susceptibility of Tg mice to mouse-passaged BSE prions differed from that to the original BSE prions. Moreover, mouse-passaged BSE prions showed different biological characteristics from those in the first passage.

**Transmissibility of TgBoPrP-passaged BSE prions**

We examined the host range of TgBoPrP-passaged BSE prions. BSE prions were inoculated into TgBoPrP mice and the diseased mice brain homogenate was inoculated intracerebrally into TgBoPrP, ICR, tga20, MHM2, MH2M and TgHaNSE mice. The transmissibility of TgBoPrP-passaged BSE (BSE/TgBoPrP) resembled that of BSE: the prions of both could be transmitted to ICR, TgBoPrP, MHM2 and tga20 mice but not to MH2M and TgHaNSE mice (Table 2). The incubation periods of TgBoPrP mice inoculated with BSE and BSE/TgBoPrP were similar. In contrast, those of ICR, tga20 and MHM2 mice were unstable, MHM2 and tga20 mice showed longer incubation periods than those in the first passage.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Incubation period with mouse or hamster adapted prions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obihiro/ICR</td>
</tr>
<tr>
<td>BSE (1st)</td>
<td>150.0 ± 2.5</td>
</tr>
<tr>
<td>BSE/ICR (2nd)</td>
<td>159.1 ± 7.4</td>
</tr>
<tr>
<td>BSE/ICR/ICR (3rd)</td>
<td>187.4 ± 7.5</td>
</tr>
<tr>
<td>BSE/TgBoPrP (2nd)*</td>
<td>78.3 ± 2.9</td>
</tr>
<tr>
<td>ICR</td>
<td>ND</td>
</tr>
<tr>
<td>MHM2</td>
<td>ND</td>
</tr>
<tr>
<td>MH2M</td>
<td>ND</td>
</tr>
<tr>
<td>Tga20</td>
<td>ND</td>
</tr>
<tr>
<td>TgHaNSE</td>
<td>ND</td>
</tr>
<tr>
<td>TgBoPrP</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Diseased brain homogenate from the first passage of TgBoPrP-passaged BSE (BSE/TgBoPrP) was used to infect mice.†Mice and hamsters showed no clinical signs 600 days after inoculation. PrPSc was negative in these rodent brains.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Incubation periods of BSE prions in Tg mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICR</td>
<td>408.6 ± 28.2, 196.8 ± 8.4, 173.6 ± 7.9, 347.2 ± 55.9</td>
</tr>
<tr>
<td>MHM2</td>
<td>399.6 ± 56.7, 127.7 ± 4.3, 123.8 ± 2.4, 220.2 ± 20.7</td>
</tr>
<tr>
<td>MH2M</td>
<td>&gt;600†, 212.8 ± 16.4, 185.0 ± 3.4, 348.6 ± 57.9</td>
</tr>
<tr>
<td>Tga20</td>
<td>456.5 ± 49.5, 122.3 ± 11.3, 123.6 ± 2.4, 600†</td>
</tr>
<tr>
<td>TgHaNSE</td>
<td>&gt;600†, 153.1 ± 1.1, 164.4 ± 6.8, 600†</td>
</tr>
<tr>
<td>TgBoPrP</td>
<td>223.5 ± 13.5</td>
</tr>
</tbody>
</table>

Brain homogenate (10%) was inoculated intracerebrally into five to seven mice. PrPSc was detected in all of the diseased mice. Results are shown as mean ± SD (days). The passage number of the inoculum is indicated in parentheses. ND, Not done.
inoculated with BSE/TgBoPrP were shortened compared with inoculation with BSE. In particular, the incubation period in MHM2 mice was considerably shortened to 222.0 days (Table 2).

Molecular properties of PrPSc from BSE prion-infected Tg mice

All of the diseased mice harboured PrPSc in their brains. The typical three PrPSc bands were detected with pAb B103 analysis (Fig. 2a). Glycoform analysis revealed dominant high-molecular-mass (di-glycosylated) PrPSc in all of the Tg mice (Fig. 3a). In contrast, a different banding pattern was observed with the use of antibodies against the globular domain of PrPSc (mAbs T2 and 44B1) from the mouse-passaged-BSE-affected MH2M and TgHaNSE mice (second passage) (Fig. 2b). The PrPSc bands from the BSE/ICR-passaged MH2M and TgHaNSE mice appeared smeared between the high- and medium-molecular-mass bands, with a long exposure period required for detection. The amount of high-molecular-mass PrPSc was decreased, whilst the amounts of medium- and low-molecular-mass PrPSc were increased (Fig. 3b). The different PrPSc band patterns were not obtained using pAb B103 and mAb T2 in other mice (ICR, tga20 and MHM2) (Figs 2b and 3b). Furthermore, BSE/ICR/ICR-passaged ICR, tga20, MH2M and TgHaNSE mice (third passage) showed different PrPSc band ratios in the mAb T2 analysis (Fig. 3b) and mAb 44B1 analysis (data not shown).

Western blot analysis showed different banding patterns for PrPSc in BSE-affected MH2M and TgHaNSE mice. The molecular mass of PK-digested PrPSc was analysed. After PNGase F digestion, the core of the PK-digested PrPSc detected using pAb B103 was similar among all of the BSE-
affected Tg mice. In contrast, the core detected using mAb 44B1 was different in the MH2M mice (Fig. 4a). The cores in the ICR, tga20 and MHM2 mice converged to one band. However, those in the MH2M mice converged to two bands, which were about 19 and 17.5 kDa, respectively (Fig. 4a). The core fragment of PrPSc from MHM2 and MH2M mice passed two and three times was analysed. As shown in Fig. 4(b), a distinct additional band was observed in MH2M mice, but not in MHM2 mice, regardless of the passage history. A similar result was also obtained by mAb T2 analysis (data not shown).

**Characteristics of PrPSc in TgBoPrP-passaged BSE-affected mice**

The glycoform pattern and molecular mass of PrPSc accumulated in the brains of BSE- and BSE/TgBoPrP-inoculated mice were analysed. The band pattern of PrPSc in BSE/TgBoPrP-affected mice resembled that of PrPSc in BSE-affected mice, as determined using mAb 44B1 (Fig. 5).

**DISCUSSION**

It is known that BSE prions can be transmitted to a wide range of species. The occurrence of vCJD has highlighted the interspecies transmission of BSE. No successful transmission of BSE prions was observed in MH2M and TgHaNSE mice in the primary passage, so they were classified as resistant mice. In contrast, tga20, MHM2 and wild-type mice were susceptible to BSE prions (Table 2). The PrP amino acid sequence comparison between MH2M and MHM2 mice showed that three amino acid substituitions located at aa 131–188 of PrP might contribute to the species barrier against BSE in hamsters. Residue 154 determines the efficiency of PrPSc formation induced by hamster PrPC and is associated with the species barrier between mice and hamsters (Priola et al., 2001). There was no commonality among these three amino acid substituitions with regard to the cattle PrP sequence (Table 1). It is well known that susceptibility to scrapie in sheep is determined by residue 171 of ovine PrP (Hunter et al., 1997), and that codon 129 in humans is associated with susceptibility to vCJD (Hill et al., 1997). Residue 142 in goat PrP is associated with resistance to both scrapie and BSE (Goldmann et al., 1996). The barrier to interspecies prion transmission can be enhanced by mismatches in key amino acid residues, but the most influential residues are not always the same in every species (Moore et al., 2005).
Interestingly, BSE prions that were passaged once in wild-type mice could be transmitted to resistant mice. This implies that passage of BSE prions in different species alters their biological characteristics. It has been reported that the virulence of BSE prions is enhanced on crossing the species barrier (Espinosa et al., 2007). We also confirmed that the range of host species susceptible to prion strains may not be constant; rather, it may easily change via interspecies transmission. This means that the risk analysis of newly emerging prion diseases should be carefully considered.

The incubation periods of BSE prions in wild-type mice were decreased depending on the number of passages (Table 2), and complete adaptation of BSE prions to wild-type mice required four passages. The shortened incubation periods were approximately 150 days long (data not shown). In contrast, the incubation periods in tga20 mice in the second (122.3 days) and third (123.6 days) mouse passages of BSE prions were similar (Table 2). MHM2 mice also showed similar incubation periods between BSE/ICR (second passage) and BSE/ICR/ICR (third passage) mice (Table 2). This result indicated that overexpression of PrP$^{C}$ during the serial passages may overcome the barrier to prion adaptation. However, no significant difference was found among the incubation periods for ICR, tga20 and MHM2 mice in the first passage (Table 2). This suggested that the overexpression of heterologous PrP could not overcome the barrier to interspecies transmission in the primary passage experiment. The prion adaptation process for overcoming the species barrier might comprise several steps— one of these is associated with PrP$^{C}$, whilst the other is not. Differences in the primary PrP sequence constitute part of the species barrier and these are not affected by heterologous PrP$^{C}$ overexpression, which overcomes the other element of the barrier.

To confirm the existence of related host factors other than PrP$^{C}$, the biological characteristics of BSE/TgBoPrP prions were analysed. The susceptibility of Tg mice to BSE/TgBoPrP was similar to that to BSE prions. However, the incubation period in BSE/TgBoPrP-inoculated MHM2 mice was significantly shortened and was equal to that in TgBoPrP mice (222 days) (Table 2). The incubation time assay using a set of Tg mice could be utilized for monitoring the alterations in prion characteristics. Our result may indicate that the biological characteristics of BSE prions are influenced by unidentified characteristics other than those of the host PrP$^{C}$, or that the PrP$^{Sc}$ of MHM2 mice conforms most closely to cattle PrP$^{C}$ in Tg mice and that this similarity is related to the conversion efficiency of PrP$^{Sc}$ from BSE prions. Further studies should be conducted to address these issues.

The PrP$^{Sc}$ glycoform is utilized for prion strain classification (Collinge et al., 1996). PrP$^{Sc}$ in all of the Tg mice showed a BSE-like glycoform pattern with an antibody (pAb B103) that recognized the N-terminal end of PrP$_{27-30}$ as the epitope (Fig. 2a). PrP$^{Sc}$ in the susceptible mice also showed a BSE-like glycoform pattern with a different antibody (mAb T2) that recognized the C-terminal end of PrP$_{27-30}$ (Fig. 3b). In contrast, the use of mAb T2 revealed a different PrP$^{Sc}$ band pattern in MH2M and TgHaNSE mice at the second passage. Deglycosylation analysis showed that this banding pattern was not derived from different glycoform modifications, but demonstrated the presence of truncated PrP fragments in MH2M mice (Fig. 4). Our results showed the accumulation of another PK-resistant PrP$^{Sc}$ fragment in the resistant mice. The N-terminal end of PK-digested PrP$^{Sc}$ of mouse-passaged BSE prions converged to N96 in the susceptible mice (Hayashi et al., 2005). At the third passage, mAb T2 analysis of ICR and tga20 mice also showed different band patterns. However, the PrP$^{Sc}$ of these mice converged to one band with PNGase F treatment (data not shown). The different PrP$^{Sc}$ band patterns of ICR and tga20 mice at the third passage were caused by different glycoform modifications and were not due to the presence of mixed PrP$^{Sc}$ bands.

It has been reported that the shift in the size of PK-digested PrP$^{Sc}$ occurs during the cross-sequence transmission of sporadic CJD to Tg mice (Kobayashi et al., 2007), mice inoculated with vCJD prions (Hill et al., 1997) and mice inoculated with hamster Sc237 prions (Hill et al., 2000). The altered size of the PrP$^{Sc}$ of mouse-passaged Sc237 reverted to its original size following transmission to hamsters (Hill et al., 2000). In this study, we detected other truncated PrP$^{Sc}$ fragments in the BSE prion-infected resistant animal. The adaptation to the new host PrP$^{C}$ and/or selection of a PrP$^{Sc}$ subpopulation from heterogeneous PrP$^{Sc}$ may result in the emergence of a new prion strain.

Recently, the occurrence of atypical BSE with different PrP$^{Sc}$ characteristics has been reported (Biacabe et al., 2004; Casalone et al., 2004; Yamakawa et al., 2003). Atypical BSE has been classified into L type and H type according to the molecular mass of the unglycosylated PrP$^{Sc}$ (Buschmann et al., 2006). The altered N-terminal end of PK-digested PrP$^{Sc}$ may influence the biological phenotype of atypical BSE. The appearance of two non-glycosylated fragments has been reported in H-type BSE, which is thought to indicate the strain-dependent nature of this molecular phenomenon (Jacobs et al., 2007). The truncated PrP$^{Sc}$ detected in resistant mice may be a good model to generate different conformations of PrP$^{Sc}$. The pathogenicity of truncated PrP$^{Sc}$ fragments should be studied to elucidate the origin of different PrP$^{Sc}$ phenotypes.

ACKNOWLEDGEMENTS

We thank Dr Motohiro Horiuchi for providing mAb 44B1 and pAb B103. We would also like to thank Dr Morikazu Shinagawa for his encouragement; Ms Hiroko K. Hayashi, Kini Shimada, Naoko Tabeta and Shuko Kodani for their technical assistance; Ms Junko Yamada for her general assistance; and Ms Che Jing Zh and the animal laboratory staff at the National Institute of Animal Health for maintaining the mouse colony. This study was supported in part by a...
grant for BSE research from the Ministry of Health, Welfare and Labour of Japan; a Grant-in-Aid from the Bovine Spongiform Encephalopathy Control Project of the Ministry of Agriculture, Forestry and Fisheries of Japan; and a grant from the Special Coordination Funds for Strategic Cooperation to Control Emerging and Re-emerging Infections from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES


