Characterization of two distinct prion strains derived from bovine spongiform encephalopathy transmissions to inbred mice

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Distinct prion strains can be distinguished by differences in incubation period, neuropathology and biochemical properties of disease-associated prion protein (PrPSc) in inoculated mice. Reliable comparisons of mouse prion strain properties can only be achieved after passage in genetically identical mice, as host prion protein sequence and genetic background are known to modulate prion disease phenotypes. While multiple prion strains have been identified in sheep scrapie and Creutzfeldt–Jakob disease, bovine spongiform encephalopathy (BSE) is thought to be caused by a single prion strain. Primary passage of BSE prions to different lines of inbred mice resulted in the propagation of two distinct PrPSc types, suggesting that two prion strains may have been isolated. To investigate this further, these isolates were subpassaged in a single line of inbred mice (SJL) and it was confirmed that two distinct prion strains had been identified. MRC1 was characterized by a short incubation time (110 ± 3 days), a mono-glycosylated-dominant PrPSc type and a generalized diffuse pattern of PrP-immunoreactive deposits, while MRC2 displayed a much longer incubation time (155 ± 1 days), a di-glycosylated-dominant PrPSc type and a distinct pattern of PrP-immunoreactive deposits and neuronal loss. These data indicate a crucial involvement of the host genome in modulating prion strain selection and propagation in mice. It is possible that multiple disease phenotypes may also be possible in BSE prion infection in humans and other animals.

INTRODUCTION

Prion diseases or transmissible spongiform encephalopathies are a group of fatal neurodegenerative disorders that include Creutzfeldt–Jakob disease (CJD) in humans and scrapie and bovine spongiform encephalopathy (BSE) in animals. Prion diseases are characterized by their prolonged incubation periods and distinctive neuropathology, which includes an accumulation in affected brains of an abnormal isomer (PrPSc) of host-encoded cellular prion protein (PrPc). The conversion of PrPc to PrPSc involves conformation change resulting in increased β-sheet secondary structure (Pan et al., 1993) and is associated with detergent insolubility and the acquisition of partial resistance to protease digestion (Meyer et al., 1986). According to the protein-only hypothesis (Griffith, 1967), PrPSc is the principal, if not sole, component of the infectious agent (Prusiner, 1991).

Multiple prion strains have been described that are distinguished by their incubation periods and patterns of neuropathology when passaged in inbred lines of mice, and these distinctive phenotypes are preserved on multiple passage in the same host (for review, see Bruce et al., 1992). The existence of prion strains challenges the protein-only hypothesis of prion propagation. However, it is clear that prion strains are associated with biochemical differences in PrPSc itself including differences in conformation (Hill et al., 1997, 2003; Bessen & Marsh, 1992, 1994; Collinge et al., 1996; Telling et al., 1996; Wadsworth et al., 1999), glycosylation (Hill et al., 1997, 2003; Collinge et al., 1996) and overall protease resistance (Kuczius & Groschup, 1999). That these strain-associated biochemical differences in PrPSc fragment sizes and glycoform ratios following proteolysis can be transmitted to PrP in an experimental host argues that they may be responsible for encoding strain diversity. While the precise nature of the molecular basis of prion strain diversity is unclear, that prion strains may be distinguished by the differing molecular mass of fragments following partial proteinase K digestion and by differing ratios of di-, mono- and unglycosylated PrPSc is clear. Using this approach, we described four common PrPSc types in humans (Collinge et al., 1996; Wadsworth et al., 1999; Hill et al., 2003). PrPSc types 1–3 are seen in sporadic and iatrogenic CJD, while type 4 PrPSc is exclusively associated with variant CJD (vCJD) and is associated with a di-glycosylated-dominant PrPSc.
pattern on a Western blot (Collinge et al., 1996; Hill et al., 2003). This characteristic glycoform ratio is also seen in BSE-infected cattle brain and these observations, together with bioassay data from wild-type and transgenic mice and non-human primates, have proved critical in establishing a link between BSE and vCJD (Hill et al., 1997; Bruce et al., 1997; Lasmezas et al., 1996; Collinge et al., 1996; Asante et al., 2002). This characteristic molecular signature of BSE-derived prion isolates is seen in all UK cattle BSE cases examined and, together with biological strain typing studies in inbred and transgenic mice, suggests that BSE is caused by a single strain of agent (Hill et al., 1997; Bruce et al., 1994, 2002; Collinge et al., 1996; Kuczies & Groschup, 1999; and our unpublished data). This molecular pattern, in addition to biological characteristics, is also maintained on transmission to other hosts such as domestic cat, sheep, macaque and other exotic animals, either by natural exposure or by experimental transmission (Bruce et al., 1994; Fraser et al., 1994; Collinge et al., 1996; Hill et al., 1998; Lasmezas et al., 2001). Recent data from French and Italian screening programmes suggest that more than one strain of BSE may exist in cattle (Biacabe et al., 2004; Casalone et al., 2004). We have also shown that on BSE transmission to a line of transgenic mice expressing only human PrP Met-129, Tg(HuPrP129M+/+ Prp0/0)-35, two distinct molecular phenotypes can be produced: one that mirrors the vCJD phenotype with type 4 PrPSc and an additional molecular phenotype that is indistinguishable from that of sporadic CJD with PrPSc type 2 (Asante et al., 2002). These transgenic mice were mixed on a generated genetic background, and one possibility was that the different patterns were determined by background effects in individual mice (Asante et al., 2002). This interpretation was supported by the demonstration that vCJD and BSE prions on primary passage to a series of inbred lines of mice were also able to produce two distinct molecular phenotypes on Western blotting, dependent only on the genetic background of the mice (Asante et al., 2002). These data argued that two distinct strains had been propagated from cattle BSE. However, since the parameters that distinguish prion strains (incubation time, neuropathology and PrPSc type) are also known to be modulated by host genetic background, these cannot be adequately compared after primary passage in different lines of inbred mice (Bruce, 1993; Moore et al., 1998; Somerville, 1999). We therefore subpassaged these mouse-adapted BSE prions in the same inbred mouse line to determine whether distinctive biological characteristics resulted, indicative of different strains, and whether these correlated with the different PrPSc types propagated in the animals.

METHODS

Transmission studies. SJL/OlaHsd (SJL) and C57BL/6J/OlaHsd (C57BL/6) mice were obtained from Harlan UK Ltd (Bicester, UK). BSE tissues were collected under strict aseptic conditions, using sterile instrumentation specifically for transmission studies, by the UK Central Veterinary Laboratory (now the Veterinary Laboratories Agency). 1783 is derived from a single natural BSE-affected cow brainstem and 1038 is derived from a pool of five natural BSE-affected brainstems. DNA sequence analysis of the bovine Prnp gene from 1783 showed that this animal was homozygous for the polymorphic repeat sequence (R6/R6). These inocula have all been previously used in transmission studies (Hill et al., 1997; Collinge et al., 1995, 1996; Asante et al., 2002).

For second passage to SJL mice, a single brain from a terminally sick mouse from the primary passage to SJL group (1783) was used to produce inoculum I1590 [cattle BSE (1783)→SJL mice (11590)→SJL mice]. For second passage to C57BL/6 mice, a single brain from sub-clinically infected mouse from the primary passage to C57BL/6 group (1038) was used to produce inoculum I656 [cattle BSE (1038)→C57BL/6 mice (1656)→C57BL/6 mice].

For third passage, the SJL-passaged inoculum was generated from a single mouse brain from the SJL second passage group (I1891) and was used to inoculate a group of SJL mice [cattle BSE (1783)→SJL (11590)→SJL (I1891)→SJL mice]. To generate the C57BL/6-passaged BSE inoculum, 11 mouse brains from the second passage were pooled to generate a larger volume of homogenate (I874). The inoculum was generated in this way as it was originally intended for use in a survey of incubation times in inbred lines and for use in a large mapping study to identify genes that influence prion disease incubation time (Lloyd et al., 2002). This pool (I874) was used to inoculate SJL mice [cattle BSE (I038)→C57BL/6 (1656)→C57BL/6 (I874)→SJL mice].

All inocula were prepared by homogenizing brain samples (1 % w/v in PBS) using disposable equipment for each inoculum in a microbiological containment level 3 laboratory and inoculations were performed within a class I microbiological safety cabinet. All mice were uniquely identified with a subcutaneous transponder tag. Disposable cages were used throughout and lids and water bottles were also uniquely tagged.

Mice were anaesthetized with halothane/O2 and inoculated intracerebrally into the right parietal lobe with 30 μl inoculum. Incubation time was defined as the number of days from inoculation to the onset of clinical signs. This was assessed by daily examination for neurological signs of disease. Criteria for clinical diagnosis of prion disease were as described (Carlson et al., 1986). Animals were killed as soon as clinical scrapie was confirmed or if showing signs of distress.

Western blotting. Brain homogenates (10 % w/v in PBS) were prepared, proteinase K-digested (100 μg proteinase K ml−1 for 1 h at 37 °C) and Western-blotted as described previously (Wadsworth et al., 2001). Blots were probed with a biotinylated anti-PrP monoclonal antibody ICSM-35 (Asante et al., 2002) in conjunction with an avidin–biotin–alkaline phosphatase conjugate (Dako) and developed in chemiluminescent substrate (CDP-Star; Tropix Inc.). For quantification and analysis of PrP glycoforms, blots were developed in chemiluminescent substrate (AttoPhos; Promega) and visualized on a Storm 840 apparatus (Molecular Dynamics). Quantification of PrPSc glycoforms was performed using ImageQuaNT software (Molecular Dynamics). Sodium phosphotungstic acid pre-concentration of PrPSc was performed as described previously (Wadsworth et al., 2001).

Neuropathology and immunohistochemistry. Mouse brains were fixed in 10 % buffered formol-saline, immersed in 98 % formic acid for 1 h, formalin post-fixed and paraffin wax-embedded. Serial sections of 4 μm nominal thickness were pre-treated with Tris/citrate/EDTA buffer (1:3 mM EDTA, 2:1 mM Tris, 1:1 mM citrate, pH 7.8) for antigen retrieval. PrP deposition was visualized using ICSM-35 as the primary antibody (diluted 1:3000) and glosis was detected with anti-glia fibrillary acidic protein rabbit polyclonal antisera (diluted 1:1000; Dako), using an automated immunostaining system (www. ventana.com). Sections of brains were examined by the same...
Table 1. BSE transmissions to SJL and C57BL/6 mice

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>Incubation time (days ± SEM)</th>
<th>Clinical signs (no./total)</th>
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<tr>
<td><strong>Primary passage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle BSE (I783) → SJL mice*</td>
<td>196 ± 13</td>
<td>25/40</td>
</tr>
<tr>
<td>Cattle BSE (I783) → C57BL/6 mice*</td>
<td>710 ± 15</td>
<td>6/25</td>
</tr>
<tr>
<td>Cattle BSE (I038) → C57BL/6 mice</td>
<td>&gt; 839</td>
<td>0/12</td>
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<tr>
<td><strong>Second passage</strong></td>
<td></td>
<td></td>
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<tr>
<td>BSE (I783) → SJL (I1590) → SJL</td>
<td>125 ± 3</td>
<td>8/8</td>
</tr>
<tr>
<td>BSE (I038) → C57BL/6 (I656) → C57BL/6</td>
<td>189 ± 2</td>
<td>11/11†</td>
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<tr>
<td><strong>Third passage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSE → SJL → SJL (I1891) → SJL</td>
<td>110 ± 3</td>
<td>8/8</td>
</tr>
<tr>
<td>BSE → C57BL/6 → C57BL/6 (I874) → SJL</td>
<td>155 ± 1</td>
<td>36/36‡</td>
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*Asante et al. (2002).
†An additional two animals were found dead at 174 and 168 days. No samples were available for Western blotting or histology.
‡An additional two animals were found dead at 150 and 173 days. No samples were available for Western blotting or histology.

RESULTS

Primary passage of BSE to SJL and C57BL/6 mice

As part of a study to map prion disease incubation time genes (Lloyd et al., 2001), we inoculated a range of inbred mouse lines with BSE prions (isolate I783). Coding polymorphisms of the mouse prion protein gene (Prnp) are known to influence incubation time (Westaway et al., 1987; Moore et al., 1998) and therefore all mice tested were Prnp<sup>a</sup> (Leu-108, Thr-189). While BSE transmits readily to wild-type mice, there is nevertheless a substantial transmission barrier (Wells et al., 1998; Wadsworth et al., 2001), which results in long and variable incubation times and an incomplete attack rate on primary passage (Hill et al., 1997; Collinge et al., 1997; Asante et al., 2002) (Table 1) compared with the passage of mouse adapted-prions in mice. Primary passage of BSE prions in mice usually results in a di-glycosylated-dominant PrP<sup>Sc</sup> pattern on a Western blot that closely resembles the PrP<sup>Sc</sup> types seen in cattle BSE and human vCJD (type 4) (Hill et al., 1997; Somerville et al., 1997; Collinge et al., 1996). Most of the inbred lines tested (C57BL/6/OlaHsd, FVB/NHsd, NZW/OlaHsd, SM/J and SWR/OlaHsd) corresponded to this pattern (Fig. 1 and unpublished data; Asante et al., 2002). However, two strains (SJL/OlaHsd and RIIIIS/J) produced an alternative PrP<sup>Sc</sup> type where the fragment sizes appeared the same but the glycoform ratios were different such that the mono-glycosylated glycoform was dominant (Fig. 1; Asante et al., 2002). Passage of cattle BSE (I783) in C57BL/6 mice gave, as reported previously, a prolonged incubation time (710 ± 15 days); however, passage of cattle BSE (I783) in SJL mice gave a very much shorter incubation time (196 ± 13 days) (Table 1; Asante et al., 2002). Neuropathological findings were unremarkable with only diffuse PrP staining in both inbred mouse lines (Asante et al., 2002). We also inoculated C57BL/6 mice with another cattle BSE inoculum (I038) (Collinge et al., 1996). On Western blots, both I783 and I038 show the same di-glycosylated-dominant PrP<sup>Sc</sup> pattern (Fig. 1a), which has,

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Fig. 1. Western blots of proteinase K-treated brain homogenates from cattle BSE and BSE transmission and subpassage in inbred mice. Western blots were analysed by high-sensitivity ECL using biotinylated anti-PrP monoclonal antibody ICSM-35. All lanes show PrP<sup>Sc</sup> present in 5 or 10 μl 10% brain homogenate with the exception of lane 2 of (a), and lane 1 of (b), which show PrP<sup>Sc</sup> derived from 100 μl 10% brain homogenate following pre-concentration with sodium phosphotungstic acid. (a) Cattle BSE isolates. Lane 1, isolate I038; lane 2, isolate I783. (b) Primary transmission of BSE prions to inbred mice. Lane 1, transmission of BSE (isolate I038) to C57BL/6 mice; lanes 2 and 3, transmission of BSE (isolate I783) to C57BL/6 mice (lane 2) or SJL mice (lane 3). (c) Secondary passage of BSE prions in inbred mice. Lane 1, secondary passage of BSE (isolate I038) in C57BL/6 mice; lane 2, secondary passage of BSE (isolate I783) in SJL mice. (d) Third passage of BSE in inbred mice. Lane 1, BSE (isolate I038) was passaged twice in C57BL/6 mice and then passaged in SJL mice; lane 2, BSE (isolate I783) was passaged three times in SJL mice.
to the best of our knowledge, been observed in all UK BSE cattle brain isolates reported to date. C57BL/6 mice inoculated with I038 did not show clinical signs of disease (0/12) at >839 days (Table 1; Collinge et al., 1996). However, I038 has been used in many transmissions in our laboratory and has transmitted efficiently with a consistent di-glycosylated-dominant PrPSc pattern on Western blots following transmission to various lines of inbred mice (Fig. 1b; Hill et al., 1997; Collinge et al., 1995, 1996).

To determine whether the two different PrPSc types corresponded to distinguishable prion strains, we completed additional passages so that they could be compared on the same host genetic background.

**Second passage of BSE to SJL and C57BL/6 mice**

On second passage to either SJL or C57BL/6 mice, incubation times for both groups were substantially reduced, with a 100% attack rate (Table 1), showing the expected adaptation on second passage to the mouse. It was not appropriate to compare the incubation times between inocula in this passage because of host genetic background effects. On Western blotting, the PrPSc type 'bred true', maintaining the pattern seen on primary passage, with the SJL-derived strain giving a mono-glycosylated-dominant pattern and the C57BL/6-derived strain showing a di-glycosylated-dominant pattern (Fig. 1c).

**Third passage of BSE to SJL and C57BL/6 mice**

All third passages were carried out in SJL mice and were therefore appropriate for comparison of all criteria of prion strains: incubation time, PrPSc type and neuropathology. The incubation time on third passage was reduced for both the SJL- and C57BL/6-derived BSE, suggesting further adaptation to mouse (Table 1). The incubation times of 110 ± 3 days for SJL-derived BSE and 155 ± 1 days for C57BL/6-derived BSE were highly significantly different (P<0.0001, Mann–Whitney test). As inoculation was intracerebral with brain homogenate from terminally affected animals, there should be no titre effects, also evidenced by the remarkably consistent incubation periods, and there are no genetic background effects since all transmissions were to inbred SJL mice. These data are therefore consistent with propagation of two distinct prion strains in these SJL mice.

Brains from each of these groups were examined by Western blotting. The PrPSc type observed on primary and secondary passage was faithfully maintained on third passage. The SJL-passaged BSE showed a mono-glycosylated-dominant pattern and the C57BL/6-passaged BSE showed a di-glycosylated-dominant pattern (Fig. 1 and Fig. 2) on further subpassage in SJL.

Further evidence that we have isolated two distinct mouse prion strains from cattle BSE came from neuropathological studies of SJL brains from both groups. For the C57BL/6-derived BSE (group 1) and the SJL-derived BSE (group 2), five and seven individual brains were examined, respectively. Findings were consistent within each group. The level and distribution of spongiosis (vacuolation) was similar for both groups (Fig. 3a and b, and Fig. 4a and b). Spongiosis was most prominent in the cortex, hippocampus and thalamus with some involvement of the brain stem and basal ganglia, while the cerebellum was spared. However, consistent differences were observed with regard to neuronal cell loss in the hippocampus where the granule cell layer of the CA1 region of the dentate gyrus showed significant loss of cells with the C57BL/6-derived inoculum but remained intact with the SJL-derived BSE (Fig. 3a and b). Patterns of PrPSc deposition were strikingly different between the two groups (Fig. 3c–f, Fig. 4c and d). For the C57BL/6-derived BSE (group 1), hippocampus, thalamus and brain stem were all heavily involved but less affected in SJL-derived BSE (group 2). In group 1, the cortex had a strongly pronounced ‘ribbon’ of PrPSc, which followed the cortical lamination, but the rest of the cortex was mainly negative. However, in group 2, the cortex had a uniform distribution of diffuse staining for PrPSc (Fig. 3c and d; Fig. 4c and d). Many plaques were found in the corpus callosum and on the surface of the brain in group 1, but very few were seen in group 2 (Fig. 3c and d; Fig. 4c and d). Differences were also observed in the cerebellum, which was only slightly involved in group 1, with occasional plaques found in the granular layer. However, in group 2, the cerebellum exhibited a uniform diffuse staining in both the molecular and granular layer with no plaques (Fig. 3e and f).

**DISCUSSION**

Several strains of sheep scrapie and human CJD have been described, yet BSE in the UK is thought to be caused by a single prion strain (Bruce et al., 1994, 1997; Collinge et al.,...
However, recent data from French and Italian cattle suggest that more than one strain of BSE may exist (Biacabe et al., 2004; Casalone et al., 2004). Strain typing may be carried out in many ways (Safar et al., 1998; Peretz et al., 2002) but strains were originally defined and classified by their characteristics on passage to particular inbred lines of mice based on incubation time and patterns of neuropathological targeting, and more recently have been distinguished by PrPSc type on Western blots. A mouse prion strain difference should only be assessed in the same line of inbred mouse, as genetic background is known to modulate all the defining features of a prion strain (Bruce, 1993; Moore et al., 1998). Based on these criteria, we have isolated two distinct strains from cattle BSE, which we have designated MRC1 and MRC2, respectively. MRC1 showed a mono-glycosylated-dominant PrPSc type on Western blots. It was derived from a primary BSE passage in SJL/OlaHsd mice and had a relatively short incubation time and a

**Fig. 3.** Neuropathological analysis of brain from SJL mice inoculated with BSE following two passages in C57BL/6 (a, c, e) or SJL (b, d, f) mice. (a, b) Haematoxylin and eosin (H&E)-stained sections of the hippocampus showing spongiform neurodegeneration. Arrowheads in (a) indicate neuronal loss. (c, d) PrP immunohistochemistry showing the distinct laminar distribution pattern of abnormal PrP in the cortex of C57BL/6→C57BL/6→SJL-passaged BSE (c), while the PrP immunoreactivity in SJL→SJL→SJL-passaged BSE is uniformly distributed (d). The cerebellum of the former group (e) mainly shows plaques and very little diffuse staining, while the latter group (f) predominantly shows diffuse staining and no plaques. Bars, 450 μm (a–d), 110 μm (e, f).
generalized diffuse pattern of PrP immunostaining in the brain. MRC2 shows a di-glycosylated-dominant PrP<sup>Sc</sup> type on Western blots, was derived from a primary BSE passage in C57BL/6J/OlaHsd mice and had a relatively long incubation time and showed a distinctive pattern of PrP immunoreactivity and neuronal loss.

SJL mice were able to support both the MRC1 and MRC2 strain patterns. However, it will be important to establish whether these strains are stable on further passage in SJL and other strains of mice. Both C57BL/6 and SJL mice share the same PrP amino acid sequence and therefore the strain selection must be a feature of other genetic loci. Within the limits of resolution available from Western blotting, the fragment sizes following proteinase K digestion were the same for the PrP<sup>Sc</sup> associated with both strains, raising the possibility that the differences related to glycosylation and not to gross conformational differences, at least as differentiated by proteinase K digestion. Additional studies will be required to characterize precisely the conformation and physico-chemical properties of PrP<sup>Sc</sup> associated with these prion strains.

Although recent reports suggest that alternative strains of BSE can be found in cattle, previous studies have suggested that BSE is caused by a single strain of agent (Bruce <i>et al</i>, 1994; Biacabe <i>et al</i>, 2004; Casalone <i>et al</i>, 2004). 301V and 301C represent previously reported independent BSE strains; however, these were propagated in different strains of mice, which not only had very different genetic backgrounds (VM/Dk and C57) but also had two amino acid coding differences in PrP and therefore their ‘strain’ characteristics cannot be directly compared. It is possible that MRC2 represents the same strain as 301C; however, formal comparisons will be required to investigate this.

The strain characteristics associated with MRC1 resemble those seen with passage of Chandler/RML scrapie in these strains of mice with respect to incubation time (122 ± 1 days), histology and PrP<sup>Sc</sup> type on Western blotting (unpublished data). We are confident that our findings are not the result of contamination with mouse scrapie as these transmissions were performed in accordance with rigorous biosafety protocols for preparation of inocula, inoculations and care of mice. Disposable equipment was used for each inoculum and each mouse was identified with a unique transponder. In addition, the MRC1 strain was also seen on primary passage to RIIS mice and also on primary passage of vCJD to SJL mice (Asante <i>et al</i>, 2002). vCJD is caused by a BSE-like prion strain (Hill <i>et al</i>, 1997; Bruce <i>et al</i>, 1997; Collinge <i>et al</i>, 1996); therefore, it is not surprising that the same phenomenon is seen with both vCJD and BSE prions in the same strains of mice. Further transmission studies are under way to characterize these vCJD-derived mouse strains. A similar bifurcation of the BSE strain characteristics was also observed with independent BSE inocula in transgenic mice expressing human PrP with methionine at codon 129, Tg[HuPrP<sup>129M<sup>+/−</sup>Prnp<sup>0/0</sup>]-35 (Asante <i>et al</i>, 2002).

MRC2 and MRC1 were derived from different sources of BSE prions, I038 and I783, respectively. I038 originated from a pool of five infected cow brainstems. However, our extensive transmission studies with these isolates excluded the pooling of material from five cows as the source of the strain variation that we observe in these transmissions. I038 gave the ‘classical’ BSE signature and the novel phenotype was derived from I783, which originated from a single cow brain. I783 has been used for transmissions in several inbred lines of mice in our laboratory and consistently produces the MRC2 strain (as determined by Western blotting) in C57BL/6J/OlaHsd, FVB/NHsd, NZW/OlaHsd, SM/J and SWR/OlaHsd mice. However, it also consistently produces the MRC1 strain (as determined by Western blotting) in SJL/OlaHsd and RIIS/J mice (Fig. 1b, lane 3 and Asante <i>et al</i>, 2002). DNA sequence analysis of the bovine Prnp gene in I783 confirmed that the animal was homozygous for PrP amino acids, thereby excluding PrP heterozygosity as the source of the strain bifurcation. I038 and other independent BSE inocula have also been shown to produce two ‘strains’ in Tg[HuPrP<sup>129M<sup>+/−</sup>Prnp<sup>0/0</sup>]-35 transgenic mice, which are on a mixed genetic background. We therefore believe that the prion strain selection or
‘mutation’ is a feature of the genetic background of the mice and studies are under way to identify the genes involved. Although recent data suggest that more than one strain of BSE may exist in cattle (Biacabe et al., 2004; Casalone et al., 2004), in this study, both 1038 and 1783 appeared to be the same single strain. However, Western blotting was unable to determine whether a mixed population of strains already existed in either of these brains or whether the alternative strains were generated de novo in the mice. The inbred lines studied to date represent only a small proportion of the allelic variation that exists in the mouse genome; therefore, the study of other strains of mice, particularly those derived from unrelated strains such as trapped wild mice, may reveal additional BSE-derived strains.

The human population exposed to BSE has a much more diverse genetic background than can be observed using laboratory strains of inbred mice. Therefore, it is possible that BSE infection may be revealed not only as the type 4 PrPSc and associated vCJD clinicopathological phenotype that BSE infection may be revealed not only as the type 4 PrPSc and associated vCJD clinicopathological phenotype seen to date (Collinge et al., 1996; Hill et al., 2003), but in future, may also present with alternative PrPSc types, incubation times and neuropathology that may not be distinguishable from sporadic CJD (Hill et al., 1997; Asante et al., 2002) or represent additional novel phenotypes (Collinge, 1999). BSE may also have infected sheep flocks in the UK and, given the genetic diversity of sheep breeds, it is also possible that in some breeds BSE propagates as a strain type that is indistinguishable from natural sheep scrapie.

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