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

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Received 9 December 2003
Accepted 27 April 2004

Distinct prion strains can be distinguished by differences in incubation period, neuropathology and biochemical properties of disease-associated prion protein ($PrP^{Sc}$) 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 $PrP^{Sc}$ 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 $PrP^{Sc}$ 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 $PrP^{Sc}$ 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 ($PrP^{Sc}$) of host-encoded cellular prion protein ($PrP^{c}$). The conversion of $PrP^{c}$ to $PrP^{Sc}$ involves conformation change resulting in increased $\beta$-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), $PrP^{Sc}$ 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 $PrP^{Sc}$ 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 $PrP^{Sc}$ 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 $PrP^{Sc}$ is clear. Using this approach, we described four common $PrP^{Sc}$ types in humans (Collinge *et al.*, 1996; Wadsworth *et al.*, 1999; Hill *et al.*, 2003). $PrP^{Sc}$ types 1–3 are seen in sporadic and iatrogenic CJD, while type 4 $PrP^{Sc}$ is exclusively associated with variant CJD (vCJD) and is associated with a di-glycosylated-dominant $PrP^{Sc}$

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; Lasmézas *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; Kuczius & Groschup, 1999; and our unpublished data). This molecular pattern, in additional 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$^{+/+}$ *Prnp*$^{0/0}$)-35, two distinct molecular phenotypes can be produced: one that mirrors the vCJD phenotype with type 4 PrP$^{Sc}$ and an additional molecular phenotype that is indistinguishable from that of sporadic CJD with PrP$^{Sc}$ type 2 (Asante *et al.*, 2002). These transgenic mice were generated on a mixed 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 PrP$^{Sc}$ 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 PrP$^{Sc}$ types propagated in the animals.

## METHODS

**Transmission studies.** SJL/OlaHsd (SJL) and C57BL/6JOlaHsd (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). I783 is derived from a single natural BSE-affected cow brainstem and I038 is derived from a pool of five natural BSE-affected brainstems. DNA sequence analysis of the bovine *Prnp* gene from I783 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 (I783) was used to produce inoculum I1590 [cattle BSE (I783)→SJL mice (I1590)→SJL mice]. For second passage to C57BL/6 mice, a single brain from a subclinically infected mouse from the primary passage to C57BL/6 group (I038) was used to produce inoculum I656 [cattle BSE (I038)→C57BL/6 mice (I656)→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 (I783)→SJL (I1590)→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 (I656)→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/$O_2$ 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 chemifluorescent substrate (AttoPhos; Promega) and visualized on a Storm 840 PhosphorImager (Molecular Dynamics). Quantification of PrP$^{Sc}$ glycoforms was performed using ImageQuaNT software (Molecular Dynamics). Sodium phosphotungstic acid pre-concentration of PrP$^{Sc}$ 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 gliosis was detected with anti-glial fibrillary acidic protein rabbit polyclonal antiserum (diluted 1 : 1000; Dako), using an automated immunostaining system (www.ventanamed.com). Sections of brains were examined by the same

**Table 1.** BSE transmissions to SJL and C57BL/6 mice

| Inoculation | Incubation time (days ± SEM) | Clinical signs (no./total) |
|---|---|---|
| **Primary passage** | | |
| Cattle BSE (I783)→SJL mice* | 196 ± 13 | 25/40 |
| Cattle BSE (I783)→C57BL/6 mice* | 710 ± 15 | 6/25 |
| Cattle BSE (I038)→C57BL/6 mice | > 839 | 0/12 |
| **Second passage** | | |
| BSE (I783)→SJL (I1590)→SJL | 125 ± 3 | 8/8 |
| BSE (I038)→C57BL/6 (I656)→C57BL/6 | 189 + 2 | 11/11† |
| **Third passage** | | |
| BSE→SJL→SJL (I1891) →SJL | 110 ± 3 | 8/8 |
| BSE→C57BL/6→C57BL/6 (I874) →SJL | 155 ± 1 | 36/36‡ |

*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.

person, who was blind to the identity of the animal and genotype. Sections were scored for spongiosis, neuronal loss, gliosis and $PrP^{Sc}$ deposition. Photographs were taken on an ImageView digital camera (www.soft-imaging.de) and composed with Adobe Photoshop.

## 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 $Prnp^{a}$ (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; 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 $PrP^{Sc}$ pattern on a Western blot that closely resembles the $PrP^{Sc}$ 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/6JOlaHsd, 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 RIIIS/J) produced an alternative $PrP^{Sc}$ 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 $PrP^{Sc}$ pattern (Fig. 1a), which has,

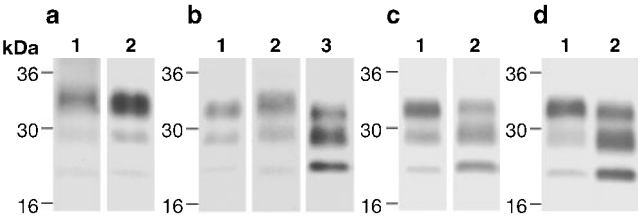


**Fig. 1.** Western blots of proteinase K-treated brain homogenates from cattle BSE and BSE transmission and subpassage in inbred mice. Western blots were analysed by high-sensitivity ECL using biotinylated anti-PrP monoclonal antibody ICSM-35. All lanes show $PrP^{Sc}$ present in 5 or 10 μl 10 % brain homogenate with the exception of lane 2 of (a), and lane 1 of (b), which show $PrP^{Sc}$ 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 $PrP^{Sc}$ 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 $PrP^{Sc}$ 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 $PrP^{Sc}$ 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, $PrP^{Sc}$ 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 $110 \pm 3$ days for SJL-derived BSE and $155 \pm 1$ days for C57BL/6-derived BSE were highly significantly different ($P < 0{\cdot}0001$, 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 $PrP^{Sc}$ 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),

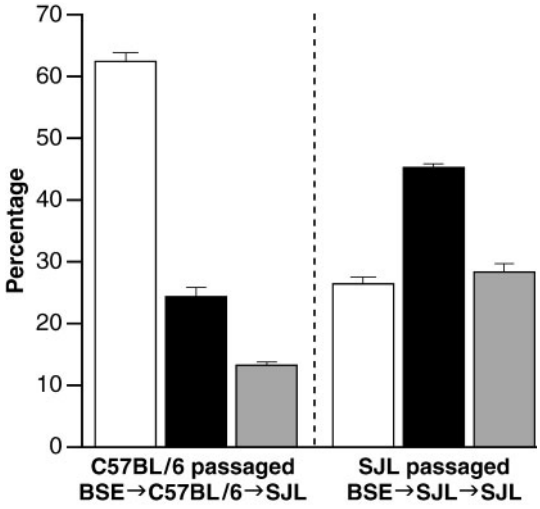


**Fig. 2.** Bar graph showing relative proportions of di- (white), mono- (black) and unglycosylated (grey) PrP following partial digestion with proteinase K. Data are plotted as mean ± SEM ($n = 5$).

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 $PrP^{Sc}$ 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 $PrP^{Sc}$, 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 $PrP^{Sc}$ (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.*,

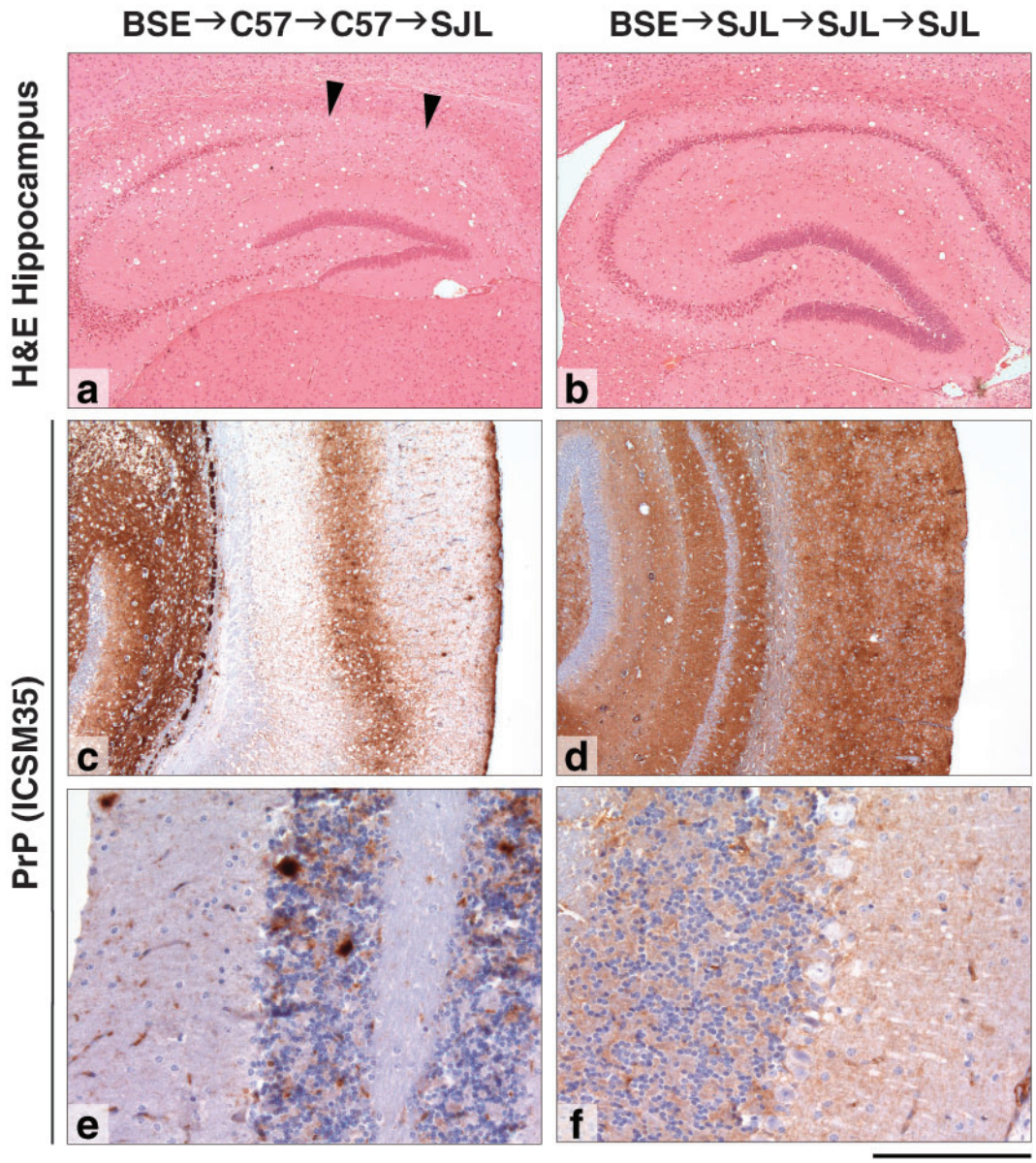


**Fig. 3.** Neuropathological analysis of brain from SJL mice inoculated with BSE following two passages in C57BL/6 (a, c, e) or SJL (b, d, f) mice. (a, b) Haematoxylin and eosin (H&E)-stained sections of the hippocampus showing spongiform neurodegeneration. Arrowheads in (a) indicate neuronal loss. (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).

1996; Hill *et al.*, 2003). 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 $PrP^{Sc}$ 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 $PrP^{Sc}$ 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

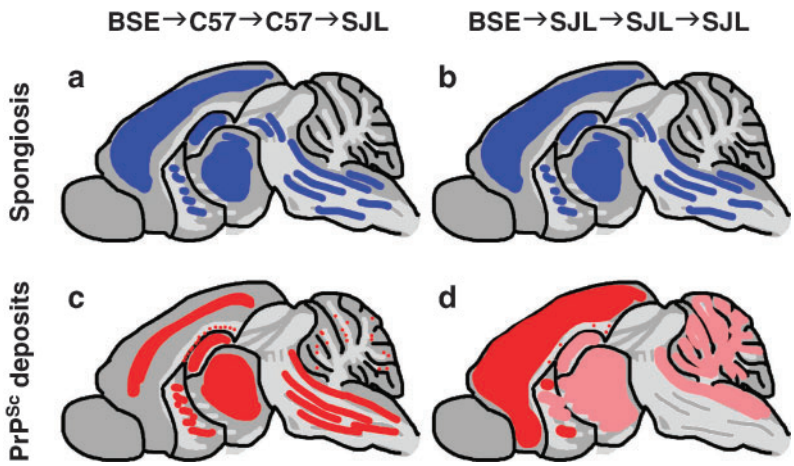


**Fig. 4.** Schematic representation of spongiosis (blue, a, b) and $PrP^{Sc}$ deposition (red, c, d) in the brain for SJL mice inoculated with BSE following two passages in C57BL/6 (a, c) or SJL (b, d) mice. For $PrP^{Sc}$ deposition, red represents areas of intense staining and pink represents areas of lighter staining.

generalized diffuse pattern of PrP immunostaining in the brain. MRC2 shows a di-glycosylated-dominant $PrP^{Sc}$ type on Western blots, was derived from a primary BSE passage in C57BL/6JOlaHsd 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 $PrP^{Sc}$ 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 $PrP^{Sc}$ 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 *et al.*, 1994; Biacabe *et al.*, 2004; Casalone *et al.*, 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 ($122 \pm 1$ days), histology and $PrP^{Sc}$ 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 RIIIS mice and also on primary passage of vCJD to SJL mice (Asante *et al.*, 2002). vCJD is caused by a BSE-like prion strain (Hill *et al.*, 1997; Bruce *et al.*, 1997; Collinge *et al.*, 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(HuPrP129M$^{+/+}$*Prnp*$^{0/0}$)-35 (Asante *et al.*, 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/6JOlaHsd, 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 RIIIS/J mice (Fig. 1b, lane 3 and Asante *et al.*, 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(HuPrP129M$^{+/+}$*Prnp*$^{0/0}$)-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 I038 and I783 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 $PrP^{Sc}$ and associated vCJD clinicopathological phenotype seen to date (Collinge *et al.*, 1996; Hill *et al.*, 2003), but in future, may also present with alternative $PrP^{Sc}$ 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.

## ACKNOWLEDGEMENTS

We thank R. Bradley, D. Matthews, S. A. C. Hawkins and colleagues at the UK Veterinary Laboratories Agency for providing BSE-infected material. We also thank Catherine O'Malley, Paul Hudson and Judy Beake for preparation of histological slides, Dave Moore and team for animal care, and Ray Young for preparation of figures. This work was supported by the Medical Research Council, UK.

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