Emergence of multiple prion strains from single isolates of ovine scrapie

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The infectious agent associated with prion diseases such as ovine scrapie shows strain diversity. Ovine prion strains have typically been identified by their transmission properties in wild-type mice. However, strain typing of ovine scrapie isolates in wild-type mice may not reveal properties of the infectious prion agent as they exist in the original host. This could be circumvented if ovine scrapie isolates are passaged in ovine prion protein (PrP)-transgenic mice. This study used incubation time, lesion profile, immunohistochemistry of the disease-associated PrP (PrP Sc ) and molecular profile to compare the range of ovine prion strains that emerged from sheep scrapie isolates following serial passage in wild-type and ovine PrP transgenic mice. It was found that a diverse range of ovine prion strains emerged from homozygous ARQ and VRQ scrapie isolates passaged in wild-type and ovine PrP transgenic mice. However, strain-specific PrP Sc deposition and PrP27–30 molecular profile patterns were identified in ovine PrP transgenic mice that were not detected in wild-type mice. Significantly, it was established that the individual mouse brain selected for transmission during prion strain typing had a significant influence on strain definition. Serial passage of short- and long-incubation-time animals from the same group of scrapie-inoculated mice revealed different prion strain phenotypes. These observations are consistent with the possibility that some scrapie isolates contain more than one prion strain.

INTRODUCTION

Prion diseases, or transmissible spongiform encephalopathies, are infectious fatal neurodegenerative conditions of humans and various animal species. These diseases include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease in humans. Collectively, prion diseases may be inherited, arise sporadically or occur through environmental exposure to infectious prion material (Collinge, 2001). These different types of prion diseases appear to share the same pathogenic mechanism, which involves the misfolding and aggregation of the normal host prion protein (PrP C ) into an oligomeric β-sheet-rich isoform termed PrP Sc (Aguzzi & Polymenidou, 2004). Spongiform-like vacuolation, neuronal loss and PrP Sc deposition are commonly seen in these conditions, although clinical signs, distribution of neuronal loss and type of PrP Sc deposition vary among the different prion diseases.

The molecular nature of the infectious agent responsible for transmissible spongiform encephalopathies remains an enigma. The prion hypothesis proposes that the transmissible agent consists principally of proteinaceous material, which propagates by promoting the conversion of PrP C into the disease-associated isoform PrP Sc (Prusiner, 1982).

The existence of multiple strains of scrapie has questioned the validity of the prion hypothesis and has been used to support alternative views that suggest that the transmissible agent contains a nucleic acid-based informational molecule (Bruce & Dickinson, 1987; Chesebro, 1998; Farquhar et al., 1998). However, to date no prion disease-specific nucleic acid has been identified (Alper et al., 1966, 1967; Safar et al., 2005). In the context of the prion hypothesis, prion strain-specific information is encoded within the conformation of PrP Sc . There has been strong support for the prion hypothesis with the demonstration that prions can be generated from recombinant PrP in vitro that are infectious in vivo (Castilla et al., 2005; Colby et al., 2009, 2010; Deleault et al., 2007; Legname et al., 2004, 2005; Makarava et al., 2010; Saá et al., 2006; Wang et al., 2010; Weber et al., 2007).

Scrapie disease of sheep is the prototypic mammalian prion disease. Strain typing of ovine scrapie isolates by transmission in wild-type mice has reported a significant number of ovine prion strains and allowed the discrimination of ovine scrapie from bovine BSE (Bruce, 1993, 2003; Bruce et al., 2002). Typically, ovine prion strain identity is revealed after serial passage of scrapie isolates in a panel of conventional
mice (Bruce, 1993). This process has its limitations, as it is usually a single mouse brain that is serially passed from the original group of inoculated animals. Analysis of PrP^Sc deposition within individual mouse brains reveals heterogeneity within a group of inoculated animals that may collectively be scored as having one lesion profile (Beck et al., 2010a, b). The implication of this is that the individual mouse brain selected for serial passage will significantly influence the final prion strain definition of the isolate under study. Furthermore, strain typing of ovine scrapie isolates in wild-type mice may lead to the generation of new strain phenotypes as the original prions undergo ‘adaptation’ or ‘selection’ as a consequence of the infectious agent crossing the species barrier into a new host with a different PrP genotype (Dickinson, 1976; Prusiner, 2004). This may mean that serial passage of scrapie isolates in wild-type mice does not reveal properties of the infectious prion agent as they exist in the original host.

Here, we performed serial transmission of homozygous ARQ or VRQ ovine scrapie isolates in wild-type and ovine PrP transgenic mice in order to investigate the range of ovine prion strains that emerge in these different hosts. In order to maximize the potential range of observable prion strains, we serially passaged short- and long-incubation-time mice from the original group inoculated with the scrapie isolate. We found that ovine PrP transgenic mice and wild-type mice can reveal diversity in ovine prion strains. However, prion strain properties, such as PrP^Sc deposition and PrP27–30 molecular profile, were identified in ovine PrP transgenic mice that were not detected in wild-type mice. Significantly, we established that the individual mouse brain selected for transmission during prion strain typing has a significant influence on strain definition. Serial passage of short- and long-incubation-time mice from the same group of inoculated animals revealed different prion strain phenotypes. Our observations are consistent with the possibility that some scrapie isolates contain more than one prion strain.

**RESULTS**

**Ovine prion strain typing in wild-type and ovine PrP transgenic mice**

Two homozygous ARQ and two homozygous VRQ scrapie isolates were previously subjected to an in-depth transmission study in C57BL/6 (murine Prnp^+/+), tg59 (ovine ARQ PrP) and tg338 (ovine VRQ PrP) mice. Following primary passage, these different genotypes of scrapie isolates displayed similar incubation periods of >350 days in wild-type and tg59 mice (Thackray et al., 2008). Facilitated transmission of the scrapie isolates occurred in tg338 mice with incubation times for VRQ/VRQ inocula reduced to 64 days and for ARQ/ARQ samples to ≤210 days (Thackray et al., 2008). Here, we reported the secondary and tertiary passages of these scrapie isolates in C57BL/6, tg59 and tg338 mice. For secondary passage in the same mouse line, mice that succumbed to prion disease with the shortest and longest incubation times were selected from each group of prion-inoculated animals at primary passage. For tertiary passage, the shortest-incubation-time mouse from the short-incubation-time groups and the longest-incubation-time mouse from the long-incubation-time groups were selected from secondary-passage mice. Collectively, incubation periods, lesion profiles (Fraser & Dickinson, 1968), the distribution of different PrP^Sc types in the brains of serially passaged mice (Beck et al., 2010a) and PrP27–30 profiles were used to allow strain identification in each mouse line.

**Strain phenotypes identified in C57BL/6 and ovine PrP transgenic mice**

Table 1 summaries the different prion strain phenotypes detected in C57BL/6 and ovine PrP transgenic mice for the four scrapie isolates subjected to serial passage in these mouse lines. Serial passage of the homozygous ARQ samples resulted in similar strain phenotypes in wild-type mice and different strain phenotypes in ovine PrP transgenic mice. Conversely, serial passage of the homozygous VRQ samples resulted in similar strain phenotypes in ovine PrP transgenic mice and different strain phenotypes in wild-type mice. The transmission properties of the different scrapie isolates passaged in C57BL/6 and ovine PrP transgenic mice are discussed in detail below.

**C57BL/6 mice**

Serial passage of the homozygous ARQ isolates in C57BL/6 mice resulted in prion disease incubation times of >329 days (Table 2) and a lesion profile typified by peak pathology scores in grey matter areas 1, 4 and 7 (see Fig. 1a, b). These features are characteristic of the ovine scrapie strain 87A (Bruce & Dickinson, 1987; Fraser & Dickinson, 1973) and were seen in short- and long-incubation-time mice for both ARQ/ARQ scrapie isolates (only SE1848/0007 is shown). The 87A lesion profile was not seen at primary passage for either of the ARQ/ARQ isolates in C57BL/6 mice.

Serial passage of the short-incubation-time VRQ/VRQ mice resulted in prion disease incubation times of ~160 days and a lesion profile at tertiary passage that was ME7-like (Bruce & Dickinson, 1987) in the case of SE1848/0005 (Fig. 1c), and 221C-like (Bruce et al., 2002) in the case of SE1848/0006 (data not shown). Serial passage of the long-incubation-time VRQ/VRQ-inoculated mice resulted in prion disease incubation times of >400 days and variable prion strain profiles that have not been described previously (Fig. 1d).

**tg338 mice**

Serial passage of the scrapie isolates in tg338 mice resulted in prion disease incubation times of 69–162 days (Table 2)
Table 1. Summary of prion strains isolated from ovine scrapie isolates

The different prion strain phenotypes identified by serial passage of homozygous ARQ or VRQ ovine scrapie isolates are shown. Short refers to short-incubation-time mice and long to long-incubation-time mice; 87A, ME7 and 221C are known mouse-adapted ovine prion strains; Apl59, G59, G338, G338ii, P338 and Pa59 refer to disease-associated PrP patterns in ovine PrP transgenic mice. NC, Non-classified prion strain phenotypes.

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<th>Isolate</th>
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<td></td>
<td>C57BL/6</td>
<td>tg59</td>
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<tr>
<td>ARQ/ARQ (SE1848/0007)</td>
<td>87A</td>
<td>Ap159</td>
</tr>
<tr>
<td>ARQ/ARQ (SE1848/0008)</td>
<td>87A</td>
<td>Pa59</td>
</tr>
<tr>
<td>VRQ/VRQ (SE1848/0005)</td>
<td>ME7</td>
<td>G59</td>
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<tr>
<td>VRQ/VRQ (SE1848/0006)</td>
<td>221C</td>
<td>G59</td>
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and lesion profiles typified by peak pathology scores in grey matter areas G1 and G4 (Fig. 2). Areas G6 and G7 were variable in the VRQ/VRQ inoculum (Fig. 2c, d) and the short-incubation-period mice from the ARQ/ARQ isolate SE1848/0007 (Fig. 2a). Serial passage from the long-incubation-period mice from isolate SE1848/0007 induced low lesion profile scores in brain areas G5–G9 (Fig. 2b). Whilst the lesion profiles in tg338 were similar for each of the different inocula, distinct PrPSc patterns were evident, as shown in Fig. 3.

In tg338 mice, three different PrPSc patterns were identified, designated granular (G338), granular ii (G338ii) and punctate (P338). G338 was characterized by fine granular deposits affecting mainly the neuropil (Fig. 3a, b). Weak or ill-defined intraneuronal deposits were observed primarily in the large neurons of the reticular formation and the cerebellar nuclei (Fig. 3i). Small aggregates in the medial habenular nucleus (Fig. 3a), often in close association with the nuclei of glial cells, were also characteristic of the G338 pattern. G338ii was the only pattern observed in all VRQ/VRQ bioassayed mice and short-incubation-time ARQ/ARQ SE1848/0007 mice. G338ii was defined by fine granular deposits of PrPSc, the intensity of which varied focally to give rise to denser granular deposits (Fig. 3d, e) and occasionally aggregates (Fig. 3f). The G338ii pattern emerged from the long-incubation-time SE1848/0007 mice. The P338 pattern was defined by punctate PrPSc deposits in the neuropil (Fig. 3g, h) and prominent intraneuronal deposits in neurons, mainly in midbrain, medulla and cerebellar nuclei (Fig. 3i). This pattern was detected in all subpassages from ARQ/ARQ isolate SE1848/0008. In all three patterns described above, PrPSc was generally restricted along the brainstem and the basal nuclei.

Table 2. Incubation times for secondary and tertiary passages of ovine scrapie isolates in mice

Mice were inoculated with ovine scrapie isolates, monitored for clinical signs of mouse prion disease and euthanized at the point of neurological disease and dysfunction. Prion disease was confirmed by histopathology, immunohistochemistry for disease-associated PrP or Western blotting for proteinase K-resistant PrP27–30. The data shown are incubation times from inoculation to death for prion disease-positive mice in days (mean ± SD) and attack rate (in parentheses) for secondary- and tertiary-passage mouse groups. Short and long indicate passage of the shortest- and longest-incubation-time mouse, respectively. ND, Not done.

<table>
<thead>
<tr>
<th>Passage</th>
<th>Short</th>
<th>Long</th>
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<tr>
<td></td>
<td>C57BL/6</td>
<td>tg59</td>
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<tr>
<td></td>
<td>C57BL/6</td>
<td>tg59</td>
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<tr>
<td>Secondary passage</td>
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<tr>
<td>ARQ/ARQ (SE1848/0007)</td>
<td>348 ± 36 (19/20)</td>
<td>448 ± 63 (17/20)</td>
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<td>ARQ/ARQ (SE1848/0008)</td>
<td>482 ± 25 (19/19)</td>
<td>309 ± 43 (18/19)</td>
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<td>VRQ/VRQ (SE1848/0005)</td>
<td>167 ± 6 (20/20)</td>
<td>529 ± 74 (14/19)</td>
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<td>VRQ/VRQ (SE1848/0006)</td>
<td>167 ± 3 (19/19)</td>
<td>450 ± 37 (17/19)</td>
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<tr>
<td>Tertiary passage</td>
<td></td>
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<tr>
<td>ARQ/ARQ (SE1848/0007)</td>
<td>329 ± 33 (20/20)</td>
<td>438 ± 94 (17/18)</td>
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<td>ARQ/ARQ (SE1848/0008)</td>
<td>372 ± 20 (20/20)</td>
<td>290 ± 60 (19/19)</td>
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<tr>
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<td>156 ± 10 (20/20)</td>
<td>440 ± 52 (20/20)</td>
</tr>
<tr>
<td>VRQ/VRQ (SE1848/0006)</td>
<td>165 ± 1 (20/20)</td>
<td>503 ± 87 (14/17)</td>
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short- and long-incubation-time mice with characteristic peak scores in areas G1 and G5 (data not shown). The homozygous ARQ isolate SE1848/0007 induced peak scores in areas G1 and G4 in short- and long-incubation-period mice (data not shown) whilst inoculum SE1848/0008 induced peak scores in areas G3, G4 and G5 in short- and long-incubation-period mice (Fig. 4a and b, respectively).

In tg59 mice, three distinct PrPSc patterns were identified, designated granular (G59), aggregates and plaques (Apl59), and punctate and aggregates (Pa59) (Fig. 5). Pattern G59 defined by granular PrPSc deposits was observed in all affected neuroanatomical areas of all VRQ/VRQ bioassayed mice. These PrPSc deposits were more prominent in the cerebral cortex where their intensity increased multifocally (Fig. 5a) and in the thalamus (Fig. 5b) where they showed a relatively uniform distribution within the affected nuclei compared with other involved areas (Fig. 5c). The most prominent PrPSc deposits in Apl59 were fibrillar plaques (Fig. 5d) and amorphous aggregates, although granular deposits were also detected. Apl59 emerged through the bioassays of ARQ/ARQ isolate SE1848/0007. This pattern was most prominent in the thalamus/hypothalamus border (Fig. 5e) and throughout the midbrain (Fig. 5f). In pattern Pa59, the cortex (Fig. 5g) and the thalamus (Fig. 5h) were colonized by punctate deposits and small aggregates. In other affected areas, the labelling intensity was reduced (Fig. 5i) and punctate PrPSc deposits prevailed. Pa59 was the only pattern observed in bioassays of ARQ/ARQ isolate SE1848/0008.

**Strain-specific properties of ovine prion-induced PrP27–30**

We investigated the molecular profile of disease-associated PrP that accumulated in the brains of mice as a consequence of serial passage of the scrapie isolates. Mouse brains were subjected to proteinase K digestion and SDS-PAGE followed by Western blotting with anti-PrP mAb Sha31. Fig. 6 shows that scrapie-inoculated mice were characterized by the presence of significant levels of PrP27–30. The molecular mass profile of PrP27–30 in tg59 mice induced by SE1848/0007 (Fig. 6, lanes 1 and 2), SE1848/0005 (Fig. 6, lanes 5 and 6) and SE1848/0006 (Fig. 6, lanes 7 and 8) was similar, whereas the PrP27–30 induced by
SE1848/0008 (Fig. 6, lanes 3 and 4) was characterized by lower-molecular-mass PrP bands. In tg338 mice, the PrP27–30 induced by SE1848/0008 (Fig. 6, lanes 3 and 4) was also characterized by lower-molecular-mass PrP bands compared with that induced by all of the other inocula (Fig. 6, lanes 1, 2 and 5–8). These similarities and differences in molecular profile of PrP27–30 were also seen in the corresponding long-incubation-time tg59 and tg338 mice (data not shown). The PrP27–30 that was induced by the homozygous ARQ scrapie isolates in tg59 and tg338 mice was characterized by a predominance of diglycosylated PrP, whereas that induced by the homozygous VRQ isolates was characterized by equal amounts of mono- and diglycosylated bands. This difference in PrP27–30 glycoform profile was more evident in tg59 compared with tg338 scrapie-inoculated mice. These differences in PrP27–30 molecular mass profile and glycoform ratios were not evident in wild-type mice: either C57BL/6 (Prnp<sup>a</sup>; data shown in Fig. 6) or VM mice (Prnp<sup>b</sup>; data not shown). These trends in wild-type and ovine PrP transgenic mice were seen in the brains of primary and serially passaged mice.

**DISCUSSION**

Here, we compared the molecular and transmission properties of different genotypes of natural cases of ovine scrapie. Primary transmission studies revealed that, despite differences in the level of disease-associated PrP, these different genotypes of scrapie isolates displayed similar primary incubation periods of >350 days in conventional and tg59 mice, and facilitated transmission in tg338 mice with incubation times for VRQ/VRQ inocula reduced to 64 days and for ARQ/ARQ samples to <210 days (Thackray et al., 2008).

Serial passage of the scrapie isolates in mice allowed the emergence of distinct prion strain phenotypes. In C57BL/6 mice, the 87A prion strain was evident in short- and long-incubation-time serially passaged ARQ/ARQ-inoculated mice. In contrast to this relatively uniform strain identity for the ARQ/ARQ isolates, different strain phenotypes emerged from serially passaged VRQ/VRQ-inoculated C57BL/6 mice. The lesion profiles from short-incubation-time VRQ/VRQ-inoculated mice showed similarities to both ME7 and 221C profiles (Bruce et al., 2002; Bruce &
Dickinson, 1987). In contrast, the long-incubation-time-inoculated mice were characterized by relatively extended incubation periods and dissimilar lesion profiles. Collectively, these data showed that the different genotypes of scrapie isolates analysed here were characterized by the emergence of different prion strain phenotypes in C57BL/6 mice. The data

![Fig. 3. Immunohistochemistry of ovine scrapie-inoculated tg338 mouse brains. Characteristic features of the three PrPSc distribution patterns observed in tg338 mice inoculated with ovine scrapie isolates are shown. G338, G338ii and P338 denote granular, granular ii and punctate patterns, respectively. Bars, 50μm.](image)

![Fig. 4. Lesion profiles induced by transmission of ovine scrapie isolates in tg59 mice. Brains were harvested from mice that had developed prion disease and were subjected to neuropathological examination for the presence and severity of spongiform neurodegeneration. Representative lesion profiles for mice inoculated with ARQ/ARQ ovine scrapie isolate SE1848/0008 are shown. Short (a) and long (b) are the shortest- and the longest-incubation-time mice, respectively (see Methods). Red, primary passage; blue, secondary passage; black, tertiary passage.](image)
Fig. 5. Immunohistochemistry of ovine scrapie-inoculated tg59 mouse brains. Characteristic features of the three PrP<sub>Sc</sub> distribution patterns observed in tg59 mice inoculated with ovine scrapie isolates. G<sub>59</sub>, Apl<sub>59</sub> and Pa<sub>59</sub> denote granular, aggregates and plaques, and punctate and aggregates, respectively. Bars, 50 μm (a, d, g); 200 μm (b, e, h); 500 μm (c, f, i).

Fig. 6. Western blot analysis of PrP27–30 in secondary-passaged mouse brains. Mouse brain homogenates of secondary-passage short-incubation-time ovine scrapie isolates were prepared as described in Methods and analysed by SDS-PAGE and Western blotting using anti-PrP mAb Sha31. Molecular mass markers (in kDa) are shown on the left. The results from two representative mice per group are shown.
also strongly suggested that different prion strains had emerged from the same original scrapie isolate following serial transmission of brains from different incubation time primary-passaged wild-type mice.

A diverse range of prion strains also emerged when the scrapie isolates were passaged in ovine PrP transgenic mice. With the exception of the ARQ/ARQ isolate SE1848/0007, serial passage of the scrapie isolates in tg338 (ovine VRQ PrP) mice resulted in a fairly rapid stabilization for short- and long-incubation-time animals, which showed little variation in incubation time from that seen at primary passage (Thackray et al., 2008). This was not the case for ARQ/ARQ isolate SE1848/0007. In addition, all of the isolates induced similar lesion profiles in tg338 mice. We considered that different prion strains had been isolated or had emerged from the different ovine scrapie samples following passage in tg338 mice in view of their transmission properties and molecular profiles of associated PrPSc. This was most clearly shown in the secondary-passage short-incubation-time tg338 mice: isolates SE1848/0005, SE1848/0006 and SE1848/0007 were characterized by incubation times of ~85 days and associated with unglycosylated PrP27–30 of ~21 kDa, whereas isolate SE1848/0008 was characterized by tg338 mice with an extended incubation time of ~160 days and unglycosylated PrP27–30 of ~19 kDa. A similar trend was seen during serial passage of the scrapie isolates in tg59 mice where isolate SE1848/0008 induced a different lesion profile from that of all of the other isolates and unglycosylated PrP27–30 of ~19 kDa. The 19 kDa molecular mass form of unglycosylated PrP27–30 seen in SE1848/0008-inoculated tg59 and tg338 mice was not a characteristic feature of the original scrapie isolate. It remains to be established which form of PrPSc identified by immunohistochemistry in the brains of prion-inoculated tg338 and tg59 mice corresponds to the 21 or 19 kDa bands of PrP27–30 identified by Western blotting. All mice with secondary and tertiary tg338 passage incubation times of ~70–95 days were characterized by a fine granular pattern of PrPSc deposits, whereas SE1848/0008-inoculated mice displayed a punctate pattern, suggesting that these types of PrPSc correlate with the 21 and 19 kDa forms of unglycosylated PrP27–30, respectively. This is supported by the fact that SE1848/0008-inoculated tg59 mice displayed a punctate PrPSc pattern and a 19 kDa form of unglycosylated PrP27–30. These features of SE1848/0008 were not evident during serial transmission in wild-type mice, which suggests that ovine PrP transgenic mice allow the emergence of discriminatory properties of ovine prion strains not evident in conventional mice. Unglycosylated PrP27–30 protein bands of 19 and 21 kDa have been described in association with different strains of transmissible mink encephalopathy (Bartz et al., 2000; Bessen & Marsh, 1992) and different human prion diseases (Gambetti et al., 2003; Hill et al., 2003). This reinforces our view that different ovine prion strains were identified from the scrapie isolates in tg338 and tg59 mice, and may suggest that some commonality exists with respect to the prion strain-specific conformational forms of PrPSc in different species.

More than one prion strain appeared to be present in isolate SE1848/0007 with the emergence at tertiary passage of short- and long-incubation-time tg338 mice (~70 and ~120 days, respectively), with each group of animals characterized by a distinct distribution of PrPSc in the brain. Similarly, transmission of the VRQ/VRQ isolates in C57BL/6 mice resulted in distinct short- and long-incubation-time mice, with distinct lesion profiles, indicative of the emergence of different prion strains. At the present time, we cannot distinguish whether these apparently different prion strains have emerged from a single strain or whether co-infection of ovine prion strains existed within the original inoculum. The conformational selection model (Collinge & Clarke, 2007), in the context of the prion hypothesis (Prusiner, 1982), provides the basis of a molecular explanation for the emergence of multiple PrPSc types and potentially different prion strains, or variants of the same strain, from the same scrapie inoculum. This model suggests that disease-associated PrP connected with a particular prion strain comprises a range of PrPSc conformers, within a particular PrP amino acid sequence, and that each conformer represents a variant or ‘substrain’ of the identified strain. The ability to detect the different PrPSc conformers will be dependent on the detection system used. Western blotting may only be capable of detecting the major PrPSc conformers of a particular prion strain (Polymenidou et al., 2005), whilst immunohistochemistry, which is considerably more sensitive, may be capable of detecting a greater range of PrPSc conformers, assuming that particular PrPSc conformations correspond to different PrPSc patterns. The predominance of different PrPSc conformers in individual mice will depend on their metabolic stabilities and hence level, or titre, in the original inoculum, which subsequently determines their emergence, and therefore the appearance of different strains, upon transmission to a new host. Prion strains have been shown to be heterogeneous comprising variants, and selective pressures result in the emergence of ‘substrains’ from a single population of prions (Li et al., 2010). Furthermore, studies with recombinant PrP have shown that amyloid fibrils are not composed of a uniform structure and that conformationally different amyloid-like structures can form within the same amino acid sequence (Makarava & Baskakov, 2008; Makarava et al., 2009). It has been shown that amyloid structures are capable of substantial conformational change even within individual fibres. Such observations help to explain the emergence of apparently different strains from the same inoculum and the ‘mutation’ of strain 87A into ME7 (Bruce & Dickinson, 1987).

Our studies reported here extend the observations we have made previously on the primary passage of these four ovine scrapie isolates (Thackray et al., 2008). The serial transmission studies reported here for the same scrapie isolates have shown that different genotypes of scrapie
isolates induce different prion strains in wild-type mice and that the same isolates induce a range of prion strains in ovine PrP transgenic mice. Although the range of lesion profiles induced by scrapie isolates in ovine PrP transgenic mice was less diverse than that seen in C57BL/6 mice, differences in the pattern of disease-associated PrP assessed by immunohistochemistry allowed the identification of different prion strain phenotypes. Significantly, we have shown that the individual mouse brain selected for transmission during prion strain typing has a significant influence on strain definition. Serial passage of short- and long-incubation-time mice from the same group of inoculated animals revealed different prion strain phenotypes. Our data also provide evidence for the emergence of different prion strains from single ovine scrapie isolates, which in some cases appear to correlate with the presence of distinct conformers of PrP

Western blot detection of PrP27–30. Murine brain homogenates were prepared by two cycles of homogenization in PBS in a Bio-Rad TeSeE Precess 24 homogenizer and enrichment for disease-associated PrP with sodium phosphotungstic acid performed as described previously (Thackray et al., 2007), except that C57BL/6 samples were treated with 32 μg proteinase K ml−1 and the tg338 and tg59 samples were treated with 64 μg proteinase K ml−1 (final concentrations). All tracks were loaded with comparable amounts of sample and analysed by SDS-PAGE and Western blotting as described previously (Thackray et al., 2008), except that the blots were probed with mAb Sha31 (Féraudet et al., 2005) diluted 1:1000. mAb Sha31 reacts with the epitope YEDRYRE (aa 145–152, human PrP numbering).

Acknowledgements

This work was supported by funds from Defra. L. H. was in receipt of a Defra PhD studentship. We thank INRA Jouy-en-Josas for supply of the tg338 mouse line. We thank Thierry Baron for supply of the tg59 mouse line. mAb Sha31 was a generous gift from Dr Jacques Grassi, CEA/Saclay, Gil sur Yvette, France. We thank support staff in the Departments of Histology, Neuropathology and the Animal Services Unit of the Veterinary Laboratories Agency, Weybridge, UK, for their skilled sample preparation and handling.

References


Methods

Mouse bioassay. Cerebral cortex from ARQ/ARQ (n=2, SE1848/0007 and SE1848/0008) and VRQ/VRQ (n=2, SE1848/0005 and SE1848/0006) confirmed ovine scrapie cases (each from a different farm) was passaged in C57BL/6 wild-type mice, and the ovine PrP transgenic mice tg59 (Cordier et al., 2001) and tg338 (Vilotte et al., 2001) (n=10 or 20 for each mouse line), as described previously (Thackray et al., 2008). For secondary passage, 1% (w/v) brain homogenate was prepared in normal saline from the shortest- and longest-incubation-time mouse that was clinically positive for prion disease and scored positive for brain vacuolation) at primary passage and administered intracranially into recipient mice. At tertiary passage, brain homogenate of the shortest-incubation-time mouse (that was clinically positive for prion disease and scored positive for brain vacuolation) at primary passage and administered intracranially into recipient mice. Incubation period was measured as the time between inoculation and death for prion-positive mice. Attack rate was calculated as the number of mice diagnosed as positive divided by the number surviving after the time of earliest diagnosis of a scrapie-positive animal in that mouse strain. All regulated procedures involving experimental animals were carried out under a Project and Personal licence authority issued in accordance with The Animals (Scientific Procedures) Act 1986.

Histopathology and immunohistochemistry. Serially passaged mouse brains were sectioned parasagittally, and a rostrolateral portion was removed and stored frozen, whilst the remainder was fixed in 10% formal saline. Histopathology and PrP immunohistochemistry were performed as described previously (Beck et al., 2010a; Fraser & Dickinson, 1968). The data shown are mean lesion profile scores (five or more brains examined) for the following areas of the brain: grey (G) matter, G1, dorsal medulla nuclei; G2, cerebellar cortex of the folia, including the granular layer, adjacent to the fourth ventricle; G3, cortex of the superior colliculus; G4, hypothalamus; G5, thalamus; G6, hippocampus; G7, septal nuclei of the paraterminal body; G8, cerebral cortex (at the level of G4 and G5); and G9, cerebral cortex (at the level of G7); and for white (W) matter, W1, cerebellar peduncles; W2, white matter in lateral tegmentum; and W3, cerebellar peduncle.

In this project, we have explored the diversity of ovine prion strains and the influence of strain definition. Our study highlights the possibility that BSE could co-exist with scrapie, as sheep are susceptible to both diseases. The results show that the same isolates induce a range of prion strains in wild-type mice and that the same isolates induce a range of prion strains in ovine PrP transgenic mice. Although the range of lesion profiles induced by scrapie isolates in ovine PrP transgenic mice was less diverse than that seen in C57BL/6 mice, differences in the pattern of disease-associated PrP assessed by immunohistochemistry allowed the identification of different prion strain phenotypes. Significantly, we have shown that the individual mouse brain selected for transmission during prion strain typing has a significant influence on strain definition. Serial passage of short- and long-incubation-time mice from the same group of inoculated animals revealed different prion strain phenotypes. Our data also provide evidence for the emergence of different prion strains from single ovine scrapie isolates, which in some cases appear to correlate with the presence of distinct conformers of PrP.

ACKNOWLEDGEMENTS

This work was supported by funds from Defra. L. H. was in receipt of a Defra PhD studentship. We thank INRA Jouy-en-Josas for supply of the tg338 mouse line. We thank Thierry Baron for supply of the tg59 mouse line. mAb Sha31 was a generous gift from Dr Jacques Grassi, CEA/Saclay, Gil sur Yvette, France. We thank support staff in the Departments of Histology, Neuropathology and the Animal Services Unit of the Veterinary Laboratories Agency, Weybridge, UK, for their skilled sample preparation and handling.


