Transmission of murine scrapie to P101L transgenic mice

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The PrP protein is central to the transmissible spongiform encephalopathies (TSEs), and the amino acid sequence of this protein in the host can influence both incubation time of disease and targeting of disease pathology. The N terminus of murine PrP has been proposed to be important in the replication of TSE agents, as mutations or deletions in that region can alter the efficiency of agent replication. To address this hypothesis and to investigate the mechanisms by which host PrP sequence controls the outcome of disease, we have assessed the influence of a single amino acid alteration in the N-terminal region of murine PrP (P101L) on the transmission of TSE agents between mice. Mice homozygous for the mutation (101LL) were inoculated with TSE strains 139A and 79A derived from mice carrying a Prnpα allele, and 79V and 301V derived from mice carrying a Prnpβ allele. Incubation times in 101LL mice were extended with all four strains of agent when compared with those in the corresponding mouse genotype from which the infectivity was derived. However, the degree to which the incubation period was increased showed considerable variation between each strain of agent. Moreover, the presence of this single amino acid alteration resulted in a 70 day reduction in incubation time of the 301V strain in Prnpα mice. The effect of the 101L mutation on murine scrapie incubation time appears therefore to be strain specific.

INTRODUCTION

The transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases which include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt–Jacob disease (CJD) in humans. These diseases are thought to be caused by the conversion of a normal cellular glycoprotein (PrP C) into an abnormal disease associated form, PrP Sc (Prusiner, 1996). As their name indicates, TSE diseases are transmissible, and many different TSE agents have been transmitted to and maintained in laboratory mice (Bruce, 1993; Bruce et al., 1993, 2002). Mouse-passaged TSE strains can be readily studied in the laboratory, and the identical genetic background of inbred strains of laboratory mice allows the direct comparison of different TSE agents in each individual strain of mouse (Bruce et al., 1991). The murine Prnp gene which encodes the PrP protein, appears to be the major but not the only factor controlling the incubation time of TSE disease in mice (Carlson et al., 1988, 1994; Lloyd et al., 2001; Manolakou et al., 2001; Stephenson et al., 2000). Mice that lack PrP protein (PrP null mice) do not develop disease when inoculated with TSE infectivity (Bueler et al., 1993; Manson et al., 1994a). Transgenic mice containing multiple copies of the Prnp gene which express elevated levels of PrP protein, show reduced incubation times with TSE infectivity when compared with wild-type mice (Scott et al., 1989; Westaway et al., 1991, 1994). This gene dosage effect is also evident in transgenic mice containing only one copy of the Prp gene, which express reduced levels of PrP protein and show prolonged TSE incubation times (Manson et al., 1994b). Different lines of inbred mice can show altered incubation times with the same TSE agent, and this has been linked to two naturally occurring polymorphisms found in the mouse Prp protein at amino acids 108 and 189 (Westaway et al., 1987). Mice encoding 108L/189T in Prp (such as C57BL and 129/Ola) contain the murine Prnpα gene, while mice encoding 108F/189V (such as VM) contain the murine Prnpβ gene. In general, strains passaged in Prnpα mice maintain shortened incubation times in Prnpα mice, but show prolonged incubation times in Prnpβ mice, and vice versa. However, some strains passaged in Prnpβ mice (e.g. 79V) do show shorter incubation times in Prnpα mice (Bruce, 1993). The different genetic backgrounds of the various inbred lines of mice complicated studies of the
effects of these polymorphisms. However, introduction of the 108F/189V polymorphisms into the Prnp allele of murine PrP by gene targeting (Prnp108F/189V) has established that it is indeed the 108/189 polymorphisms which exert the major control over scrapie incubation time in wild-type mice (Moore et al., 1998).

The influence of other amino acid substitutions in the murine Prnp gene on the incubation time of disease has been demonstrated previously. The substitution of leucine for proline at amino acid 101 (101L) in the murine Prnp allele by gene targeting (Prnp101L) has been shown to shorten TSE incubation times with P102L GSS from humans, 263K from hamsters and SSBP/1 from sheep when compared with wild-type (101P) mice. Conversely, a second human TSE agent, variant CJD (vCJD), displayed prolonged incubation times in Prnp101L (101LL) mice when compared with wild-type mice (Barron et al., 2001; Manson et al., 1999). Furthermore, when challenged with the mouse-passaged scrapie strains ME7 (derived from mice carrying the Prnp allele) and 22A (derived from mice carrying the Prnp allele), the 101LL mice also displayed increased incubation times when compared with mice homozygous for either Prnp or Prnp alleles (Barron et al., 2001; Manson et al., 1999). Transgenic mice overexpressing murine PrP containing a methionine substitution at position 108 have also been shown to produce a lengthening in scrapie incubation time compared with mice overexpressing the wild-type gene; however, the effect of mutation and transgene copy number cannot readily be separated in these experiments (Supattapone et al., 2001).

The mechanism by which these polymorphisms and mutations in murine PrP control incubation time has not yet been established. Amino acids 101 and 108 are located in the N-terminal region of PrP (amino acids 21–121), which has been described in NMR structural studies as having no discernible secondary structure (Donne et al., 1997; Hornemann et al., 1997; Zahn et al., 2000). However, despite the apparent lack of structure, this region of PrP contains the octapeptide repeat sequences which have been shown to bind copper, and therefore may influence both the structure and the function of the normal protein (Brown et al., 1997; Brown & Harris, 2002). Deletion studies have shown that removing sections of the N-terminal region can cause increased incubation times and reduced efficiency of disease propagation in mice (Fleischig et al., 2000; Supattapone et al., 2001), and can reduce efficiency of PrP conversion in cell-free conversion assays (Lawson et al., 2001). Due to the close proximity of amino acids 101 and 108, it is possible that similar mechanisms are operating by which changes at these positions in murine PrP control scrapie incubation time in mice. In order to further investigate the role of this region of PrP in influencing TSE disease transmission, we have inoculated 101LL mice with four mouse-passaged TSE strains derived from both Prnp and Prnp mice, and compared the disease profile of 101LL mice with that of Prnp and Prnp mice of the same genetic background.

METHODS

Preparation of inoculum. Each inoculum was prepared from one mouse brain with terminal TSE disease (139A, 79A, 79V and 301V mouse scrapie). 139A and 79A were prepared from C57BL mice (Prnp), and 79V and 301V were prepared from VM mice (Prnp). A 1% homogenate of each sample was prepared in normal saline prior to use as an inoculum. Groups of wild-type, FV/FV (Moore et al., 1998) and 101LL transgenic mice (Manson et al., 1999) were inoculated intracerebrally with 20 μl of inoculum under fluorohane anaesthesia.

Scoring of clinical TSE disease. The presence of clinical TSE disease was assessed as described (Dickinson et al., 1968). Incubation times were calculated as the interval between inoculation and cull due to terminal TSE disease. Mice were killed by cervical dislocation at the terminal stage of disease, at termination of the experiment (between 600–700 days), or due to intercurrent illness. Half brains were fixed in 10% formal saline for 48 h, followed by decontamination in 98% formic acid for 1 h. The remaining half brain was frozen at −70 °C for Western blot analysis. Fixed brain tissue was dehydrated in alcohol and impregnated in wax during a 7 h automated processing cycle. Sections were cut coronally at four levels and mounted on Superfrost slides.

Lesion profiles. Sections were haematoxylin & eosin stained and scored for vacuolar degeneration on a scale of 0 to 5 in nine standard grey matter areas and three standard white matter areas as described previously (Fraser & Dickinson, 1967). Mean scores were produced from a minimum of six mice and plotted with standard error of mean (SEM) against scoring areas to give a lesion profile for each strain with each agent.

Western blot analysis. For detection of PrPsc in transgenic and wild-type mice, 10% (w/v) homogenates of frozen brain tissue were prepared in NP40 buffer [0.5% (w/v) NP40, 0.5% (w/v) sodium deoxycholate, 150 mM NaCl, 50 mM Tris/HCl pH 7.5]. Homogenates were centrifuged at 10 000 g for 15 min at 4 °C to remove cellular debris. Clarified supernatants (100 mg wet weight ml−1) were incubated with or without Proteinase K at 20 μg ml−1 for 1 h at 37 °C, and the reaction terminated by addition of PMSF. Samples were mixed with 2x sample buffer (Novex, Invitrogen) at 10 mg ml−1 final concentration and incubated at 90 °C for 20 min. Total protein estimates were not performed, as the effect of the mutation on PrPsc levels was unknown. Samples were therefore loaded as wet weight tissue equivalents. Twenty μl of each sample (approximately 200 μg wet weight tissue) was separated on 12% Novex Tris/glycine acrylamide gels (Invitrogen). Proteins were transferred onto PVDF membrane by electroblotting, and incubated for 2 h at room temperature with mAbs 8H4 or 7A12 (Zanusso et al., 1998) at 50 ng μl−1 concentration. Proteins were visualized with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse secondary antibody (Jackson ImmunoResearch), and a chemiluminescence detection kit (Roche Diagnostics). Membranes were exposed to X-ray film for periods ranging from 10 s to 10 min.

Genotyping. All P101L transgenic mice were genotyped after termination of experiments to confirm genotype. A 765 bp fragment containing the Prnp ORF was generated using a 5’ primer (5’-ATGGCGAACCTTGGCTACTGGCTG-3’; position 107–130, GenBank acc. no. M18070) and a 3’ primer (5’-TCATCCCGACGTACGAGG- GTAGT-3’; position 871–848, GenBank acc. no. M18070). Cycle conditions were: 94 °C for 3 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 62 °C and 1 min at 72 °C. This was followed by a final 10 min at 72 °C (Biomatix Triblock). The presence or absence of a Dde site within the PCR product provided a marker for the codon 101P–L alteration.
RESULTS AND DISCUSSION

The 101L mutation alters murine scrapie incubation times

Transgenic mice homozygous for the 101 leucine mutation (101LL) and wild-type mice (101PP) were inoculated with infectivity from three sources of murine scrapie (139A, 79A, 79V) and one source of mouse-passaged BSE (301V). TSE strains 139A and 79A were both derived from mice homozygous for Prnp\textsuperscript{a}, whereas 79V and 301V were derived from mice homozygous for Prnp\textsuperscript{b}. The incubation times obtained for each transmission are shown in Table 1 and Fig. 1. The analysis of these results was complex due to transmission of TSE strains derived from both Prnp\textsuperscript{a} and Prnp\textsuperscript{b} mice. Incubation times produced by Prnp\textsuperscript{a}-derived strains in 101LL mice could be directly compared with the homologous transmission in Prnp\textsuperscript{a} mice, as the 101L mutation was introduced into the Prnp\textsuperscript{a} gene by gene targeting. This comparison would reveal the specific effect of the 101L mutation on TSE disease progression. In addition, comparison of 101LL mice with Prnp\textsuperscript{a} mice (which also contain the mutations in the Prnp\textsuperscript{a} gene) would demonstrate whether the 101L and the 108F/189V mutations influenced incubation time in a similar manner. However with Prnp\textsuperscript{b}-derived strains, the effects seen in 101LL mice were most likely due to the combined presence of 108L/189T and 101L. The interpretation of the Prnp\textsuperscript{b} strain data therefore depends on whether the 101LL incubation times are compared with the homologous strain transmission to Prnp\textsuperscript{b} mice, or the heterologous strain transmission to Prnp\textsuperscript{b} mice. Comparison of 101LL mice with Prnp\textsuperscript{b} mice shows the effect of these three amino acid changes in host PrP (108, 189 and 101) on transmission of homologous strains of agent, whereas comparison with Prnp\textsuperscript{b} mice examines the precise effect of the 101L mutation on the transmission of non-homologous strains of agent. The following interpretation of the results aims to address both of these points.

When inoculated with 139A and 79A, 101LL mice produced extended incubation times which were approximately double those obtained in Prnp\textsuperscript{a} mice. This observation was similar to that obtained previously with ME7 (Manson et al., 1999). In order to assess whether this mechanism of control on incubation time could be similar to that of the 108F/189V polymorphisms, we compared the incubation times of these agents in Prnp\textsuperscript{a\textsubscript{108F/189V}} mice, which are the same genetic background as 101LL mice. The results showed that 79A and 139A incubation times were increased in both 101LL and Prnp\textsuperscript{a\textsubscript{108F/189V}} mice when compared with Prnp\textsuperscript{a} mice (Table 1, Fig. 1). However, the degree to which incubation times were extended varied with the strain of agent. With the 79A strain of agent the 108F/189V alterations lead to a more prolonged incubation time than the 101L mutation, whereas the opposite was true for the 139A strain. This suggested that the interaction with each strain of agent and control of incubation time differs with 108F/189V and 101L.

Table 1. Transmission of murine scrapie strains to Prnp\textsuperscript{a\textsubscript{101L}} and Prnp\textsuperscript{a\textsubscript{108F/189V}} mice

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mouse line</th>
<th>Challenge</th>
<th>Amino acid differences\textsuperscript{*}</th>
<th>Incubation time (days ± SEM)</th>
</tr>
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<tbody>
<tr>
<td>ME7</td>
<td>Prnp\textsuperscript{a}</td>
<td>Homologous</td>
<td>None</td>
<td>161 ± 2\textsuperscript{+}</td>
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<tr>
<td></td>
<td>Prnp\textsuperscript{a\textsubscript{101L}}</td>
<td>Heterologous</td>
<td>101</td>
<td>338 ± 8\textsuperscript{+}</td>
</tr>
<tr>
<td></td>
<td>Prnp\textsuperscript{a\textsubscript{108F/189V}}</td>
<td>Heterologous</td>
<td>108/189</td>
<td>273 ± 3</td>
</tr>
<tr>
<td>139A</td>
<td>Prnp\textsuperscript{a}</td>
<td>Homologous</td>
<td>None</td>
<td>147 ± 2</td>
</tr>
<tr>
<td></td>
<td>Prnp\textsuperscript{a\textsubscript{101L}}</td>
<td>Heterologous</td>
<td>101</td>
<td>306 ± 7</td>
</tr>
<tr>
<td></td>
<td>Prnp\textsuperscript{a\textsubscript{108F/189V}}</td>
<td>Heterologous</td>
<td>108/189</td>
<td>240 ± 2</td>
</tr>
<tr>
<td>79A</td>
<td>Prnp\textsuperscript{a}</td>
<td>Homologous</td>
<td>None</td>
<td>139 ± 2</td>
</tr>
<tr>
<td></td>
<td>Prnp\textsuperscript{a\textsubscript{101L}}</td>
<td>Heterologous</td>
<td>101</td>
<td>298 ± 3</td>
</tr>
<tr>
<td></td>
<td>Prnp\textsuperscript{a\textsubscript{108F/189V}}</td>
<td>Heterologous</td>
<td>108/189</td>
<td>382 ± 12</td>
</tr>
<tr>
<td>22A</td>
<td>Prnp\textsuperscript{a}</td>
<td>Heterologous</td>
<td>108/189</td>
<td>493 ± 6\textsuperscript{+}</td>
</tr>
<tr>
<td></td>
<td>Prnp\textsuperscript{a\textsubscript{101L}}</td>
<td>Heterologous</td>
<td>101/108/189</td>
<td>527 ± 28\textsuperscript{+}</td>
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<td>Prnp\textsuperscript{a\textsubscript{108F/189V}}</td>
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<td>None</td>
<td>227 ± 3</td>
</tr>
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<td>79V</td>
<td>Prnp\textsuperscript{a}</td>
<td>Heterologous</td>
<td>108/189</td>
<td>234 ± 6</td>
</tr>
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<td></td>
<td>Prnp\textsuperscript{a\textsubscript{101L}}</td>
<td>Heterologous</td>
<td>101/108/189</td>
<td>377 ± 10</td>
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<tr>
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<td>301V</td>
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<td>108/189</td>
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<td>101/108/189</td>
<td>181 ± 1</td>
</tr>
<tr>
<td></td>
<td>Prnp\textsuperscript{a\textsubscript{108F/189V}}</td>
<td>Heterologous</td>
<td>None</td>
<td>134 ± 1</td>
</tr>
</tbody>
</table>

\textsuperscript{*}PrP amino acid positions which differ in host and source of infectivity.

\textsuperscript{+}Data shown from Manson et al. (1999).

\textsuperscript{+}Data shown from Barron et al. (2001).
For the strains derived from mice homozygous for the Prnp<sup>b</sup> allele the situation was more complex. Incubation times of 79V are reduced in Prnp<sup>a</sup> mice (234 days) compared with the expected homologous transmission in VM (Prnp<sup>b</sup>) mice of 270 days (Bruce, 1993) (data not available from transmission of 79V to Prnp<sup>a</sup>(108F/189V) mice). However, the incubation time in 101LL mice was found to be extended by over 100 days with respect to both Prnp<sup>a</sup> and Prnp<sup>b</sup> genotypes (377 days). With 301V, incubation times in both Prnp<sup>a</sup> and 101LL mice were extended with respect to Prnp<sup>a</sup>(108F/189V) mice, but the degree to which the incubation times were increased was greater in Prnp<sup>a</sup> than in 101LL mice (70 day difference). These results also differed from those obtained previously with 22A (also Prnp<sup>b</sup> derived) in which incubation times of Prnp<sup>a</sup> and 101LL mice were increased by approximately the same degree compared with Prnp<sup>a</sup>(108F/189V) mice (Table 1, Fig. 1). However, in transmissions of Prnp<sup>a</sup> strains of agent to 101LL mice, the observed effects are due to three amino acid changes in host PrP (108, 189 and 101). To truly examine the effect of the 101L mutation on Prnp<sup>b</sup> strains of agent to 101LL mice, the possibility of combinatorial or compensatory mechanisms of the 101L and 108L alterations cannot be ignored during infection with Prnp<sup>b</sup> strains (Vorberg et al., 2003). However, the effect of Prnp<sup>b</sup> strains in Prnp<sup>a</sup> and 101LL mice (which differ by only one amino acid) was unpredictable. While the 79V strain showed a further lengthening of the Prnp<sup>a</sup> incubation time in the presence of the 101L mutation, a reduction in the Prnp<sup>a</sup> incubation time was observed in 101LL mice inoculated with the 301V strain. These results, particularly those produced with the 301V strain, again indicate that the control of incubation time by the 101L mutation is different from that of the 108/189 polymorphisms. While the present results are unable to separate the effect of 108F from that of 189V we are currently addressing this issue in two further lines of gene-targeted mice homozygous for either Prnp<sup>a</sup>108F/189T or Prnp<sup>a</sup>108L/189V alleles.

**101L mutation can affect PrP<sup>Sc</sup> production**

It had been observed previously that brain tissue from 101LL mice infected with ME7 contained lower levels of protease-resistant PrP at the terminal stage of disease than wild-type mice (Manson et al., 1999). To determine whether the 101L mutation in the host PrP gene influenced the level of protease-resistant PrP produced by other strains of mouse scrapie, brain homogenates were prepared from 101LL and wild-type Prnp<sup>a</sup> mice infected with the Prnp<sup>a</sup> strains 139A and 79A, and the Prnp<sup>b</sup> strains 22A, 79V and 301V, and analysed by Western blotting with mAbs 7A12 and 8H4. Preliminary analysis focused on three mice of each genotype with each strain of agent, to assess whether levels of PrP<sup>Sc</sup> in the brain were consistent, or varied between animals. For all transmissions, similar levels of PrP<sup>Sc</sup> were observed between three mice in each group, except for the transmission of 22A to 101LL mice (data not shown). Comparison of 101LL and Prnp<sup>a</sup> mice inoculated with Prnp<sup>b</sup> strains showed that heterologous transmissions to 101LL mice produced levels of PrP<sup>Sc</sup> identical to those seen in the homologous transmission to Prnp<sup>a</sup> mice, despite the difference in incubation times (Table 1 and Fig. 2). Similarly, transmission of 79V and 301V produced the same PrP<sup>Sc</sup> levels in 101LL and
Targeting of vacuolation

The effect of the 101L mutation in host PrP on targeting of pathology was assessed by comparing the degree of vacuolation in mice carrying the 101L mutation with that in mice carrying wild-type 101P PrP (Prnp<sup>a</sup>). The degree of vacuolation in specific brain areas is characteristic of individual mouse-passaged scrapie strains. Vacuolation is scored on a scale of 0 to 5 in nine grey matter and three white matter areas, and average scores (± SEM) plotted to give a lesion profile, which is essentially a fingerprint for each strain (Fraser & Dickinson, 1967). Lesion profiles were calculated from wet weight of tissue. Previous analysis were produced for each strain of mouse scrapie, comparing mean vacuolation scores from 101LL and Prnp<sup>a</sup> mice displaying both clinical and pathological signs of TSE disease (Fig. 3). The heterologous transmission to 101LL mice of Prnp<sup>a</sup>-derived strains 139A and 79A produced significant variation in the targeting of vacuolation when compared with the corresponding homologous transmission in Prnp<sup>a</sup> mice. The 139A and 79A Prnp<sup>a</sup>-derived mouse scrapie strains share a common lineage, and produce identical lesion profiles in wild-type mice of both Prnp genotypes. These strains can, however, be distinguished by their incubation times in Prnp<sup>a</sup> mice (Table 1, Fig. 1). Both of these strains displayed increased levels of vacuolation in areas 1 (dorsal medulla), 3 (superior colliculus), 4 (hypothalamus) and 7 (septum), but decreased levels of vacuolation in the white matter regions (1* cerebellar white matter, 2* midbrain white matter, 3* cerebral peduncle) of 101LL mice. The change in vacuolar profile and incubation time of these two related strains in 101LL mice appears identical (Figs 1, 3; Table 1), indicating a similar interaction between each strain and the 101L mutation in PrP.

Heterologous challenges with Prnp<sup>b</sup>-derived strains (79V, 22A and 301V) produced less variability between Prnp<sup>a</sup> and 101LL mice, despite differing from the source of infectivity by two and three PrP amino acids respectively. Identical lesion profiles were obtained in 101LL and Prnp<sup>a</sup> mice challenged with 301V, and although 79V showed reduced levels of vacuolation in areas 6 to 9 (hippocampus, septum, cerebral cortex and forebrain cerebral cortex) of the 101LL brain, the overall pattern was the same as in Prnp<sup>a</sup> mice. The 22A strain showed slightly more variability due to altered levels of vacuolation in areas 4 (hypothalamus), 7 (septum) and 8 (cerebral cortex), but all other areas of grey and white matter showed identical targeting of vacuolation in both lines of mice. The 101L mutation therefore exerts a strain-specific effect on vacuolar targeting ranging from little or no alteration with heterologous challenges differing at three amino acids (108L, 189T and 101L), to major alterations with heterologous challenges differing at only one amino acid (101L), which was also observed previously with ME7 (Manson et al., 1999).

Conclusions

Transmission of TSE disease is thought to be most efficient when identical PrP sequences are present in both the donor and recipient of infectivity. The differences in PrP sequence...
between species are thought to reduce the efficiency of conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>, and result in low transmission frequencies and long incubation periods. However, TSE infectivity from human, hamster and sheep brain tissue has previously been transmitted to 101LL mice with reduced incubation times compared with wild-type mice, despite the lack of PrP sequence identity between species (Barron et al., 2001; Manson et al., 1999). The PrP sequence of C57BL mice (<i>Prnp<sup>a</sup></i>) in which ME7, 139A and 79A are propagated differs from that of 101LL mice by only one amino acid (101P), yet incubation times in 101LL mice are 2-fold greater than in wild-type mice. The PrP sequence of VM mice (<i>Prnp<sup>b</sup></i>) in which 79V, 301V and 22A are propagated differs from 101LL mice at three residues (101P, 108F and 189V); however, the effect is in this case far less predictable. With <i>Prnp<sup>b</sup></i>-derived strains, incubation times observed in 101LL mice were also extended when compared with <i>Prnp<sup>b</sup></i> mice, but were found to be both shorter (301V) and longer (79V and 22A) when compared with the <i>Prnp<sup>a</sup></i> mice. Thus within the mouse species the presence of the 101L alteration prolongs the scrapie incubation time normally observed in the donor genotype. However for agents from other species, the effect of the 101L mutation appears in most cases to be to facilitate the transmission of disease, perhaps indicating

**Fig. 3.** The extent of vacuolar change in the brain was scored on a scale of 0–5 in nine areas of grey matter and three areas of white matter, and mean scores (calculated from a minimum of six animals) are shown graphically (error bars represent ± SEM). Grey matter scoring areas: 1, dorsal medulla; 2, cerebellar cortex; 3, superior colliculus; 4, hypothalamus; 5, medial thalamus; 6, hippocampus; 7, septum; 8, cerebral cortex; 9, forebrain cerebral cortex. White matter scoring areas: 1*, cerebellar white matter; 2*, midbrain white matter; 3*, cerebral peduncle. Separate profiles are shown for each mouse TSE strain: (a) 79A, (b) 139A, (c) 79V, (d) 301V, (e) 22A. ▲, 101PP mice, ■, 101LL mice.
that the 101L mutation allows more efficient adaptation of an agent in mice.

The effect of the 101L mutation on murine scrapie incubation times largely parallels the effect of the 108/189 polymorphisms in murine PrP, as incubation times are extended in 101LL mice compared with the homologous transmission in either Prnpa or Prnpb mice. However, the degree to which incubation times are affected by 101L is distinct from 108/189, and is also very unpredictable with Prnpb-derived strains. Specific differences between 101L and 108/189 are also evident with some Prnpa-derived strains. The related strains 139A and 79A produce almost identical incubation times and lesion profiles in Prnpa mice, but can be distinguished by their incubation times in Prnpb and Prnpa (108S/189V) mice, where the 79A incubation time is approximately 140 days longer than 139A (Table 1, Fig. 1). On inoculation of 101LL mice, the 139A and 79A strains of agent produced almost identical incubation times and alterations in lesion profile (Table 1; Figs 1 and 3), proving that the 101L mutation alters the disease profile of these agents in a manner that is indeed distinct from that of 108/189. Future experiments involving mice expressing Prnpa (108S/189V) will allow us to examine the influence of L108F alone on TSE disease progression, and to directly compare the effect of the L108F and P101L alterations. These experiments may reveal how mutations in this unstructured N-terminal region of PrP can have dramatic effects on disease phenotype.

Other mutations in the N-terminal region of murine PrP have also been shown to affect incubation times of murine scrapie strains. The introduction of methionine at positions 108 and 111 in murine PrP (to create the epitope for mAb 3F4) increased the incubation times of four mouse-passaged scrapie strains in transgenic mice overexpressing Prnpa (108M/111M) compared with mice overexpressing Prnpa (Supattapone et al., 2001). The mutations caused prolonged incubation times (ranging from 1.6- to 2.8-fold) for each of the scrapie strains investigated, independent of the Prnp genotype from which the strain was derived. Although the precise effects of the 108M/111M mutations are difficult to quantify in these experiments due to the differences in Prnp gene copy number and genetic background between control and mutant mice, the results suggested that mutations in this region of PrP may extend TSE incubation times with all mouse-passaged TSE isolates. However, in this experiment incubation times with 301V were extended in Prnpa (108M/111M) mice compared with the transgenic mice overexpressing Prnpa. This was not observed in 101LL mice, where a shortening of 301V incubation time occurred with respect to the wild-type Prnpa mice. These observations indicate that the effect on incubation time of introducing the 3F4 epitope is distinct from that caused by the presence of 101L in murine PrP.

The shorter incubation time of 301V (Prnpb mouse-passaged BSE) in 101LL mice, when compared with the wild-type Prnpa mice, is in complete contrast with previous experiments which have shown longer incubation times in 101LL mice inoculated with vCJD than in 101PP mice (Barron et al., 2001). Strain characteristics of this particular TSE agent have been show to remain remarkably consistent after transmission through several different species (Bruce et al., 1997). However, vCJD (which is thought to be human-passaged BSE) produced increased incubation times and altered lesion profiles in 101LL mice compared with wild-type mice (Barron et al., 2001), while BSE transmitted through a Prnpb mouse (301V) produced shortened incubation times (Table 1, Fig. 1) and unaltered lesion profiles in 101LL mice compared with wild-type mice (Fig. 3). Adaptation to mouse with this strain of agent therefore increases the efficiency of transmission to 101LL mice, even in the presence of three polymorphisms in murine PrP, and this may in some way be related to why this human TSE agent alone transmits efficiently to wild-type mice (Bruce et al., 1997; Tateishi, 1996). These issues are currently being addressed by transmitting and subpassaging human TSE isolates in P101L mice (unpublished data).

The interaction between different strains of TSE agent and host PrP is a complex process, and alterations in the host PrP protein can have a dramatic effect on TSE disease progression. If the infectious process is dependent on interaction between PrP molecules, it is unclear why in 101LL mice the Prnpb-derived strains behave in a more unpredictable manner than the Prnpa-derived strains. It may be due to the presence of three amino acid changes between the 101LL host and the Prnpb line in which the agent was propagated. It is also possible that Prnpb strains have more diverse PrPSc conformations than the Prnpa strains we have examined. Alternatively, a direct interaction between PrPSc and PrPSc may not define the infectious process. Other molecules may be involved in this process, and their interaction with PrPSc may define the importance of PrP sequence in transmission of disease (Kaneko et al., 1997; Telling et al., 1995). We now aim to further define the nature of the interaction between the N terminus of host PrP and the infectious agent through the study of the other mutations in this region of PrP.

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