L-Type Bovine Spongiform Encephalopathy in Genetically Susceptible and Resistant Sheep: Changes in Prion Strain or Phenotypic Plasticity of the Disease-Associated Prion Protein?

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Background. Sheep with prion protein (PrP) gene polymorphisms QQ171 and RQ171 were shown to be susceptible to the prion causing L-type bovine spongiform encephalopathy (L-BSE), although RQ171 sheep specifically propagated a distinctive prion molecular phenotype in their brains, characterized by a high molecular mass protease-resistant PrP fragment (HMM PrPres), distinct from L-BSE in QQ171 sheep.

Methods. The resulting infectious and biological properties of QQ171 and RQ171 ovine L-BSE prions were investigated in transgenic mice expressing either bovine or ovine PrP.

Results. In both mouse lines, ovine L-BSE transmitted similarly to cattle-derived L-BSE, with respect to survival periods, histopathology, and biochemical features of PrPres in the brain, as well as splenotropism, clearly differing from ovine classic BSE or from scrapie strain CH1641. Nevertheless and unexpectedly, HMM PrPres was found in the spleen of ovine PrP transgenic mice infected with L-BSE from RQ171 sheep at first passage, reminiscent, in lymphoid tissues only, of the distinct PrPres features found in RQ171 sheep brains.

Conclusions. The L-BSE agent differs from both ovine classic BSE or CH1641 scrapie maintaining its specific strain properties after passage in sheep, although striking PrPres molecular changes could be found in RQ171 sheep and in the spleen of ovine PrP transgenic mice.

Keywords. prion; L-BSE; BASE; BSE; CH1641; scrapie; strain.

Following its emergence in British cattle in the early 1980s, bovine spongiform encephalopathy (BSE) has been believed to be a uniform foodborne disease caused by a single transmissible agent. In contrast with scrapie in small ruminants, a major finding has been its remarkable stability after natural or experimental transmission in other species. Evidence includes the consistent molecular features of the protease-resistant form of the prion protein (PrPres) and similar biological features following transmission of the disease in wild-type and transgenic mouse models from BSE passaged in different species.

BSE has never been reported so far to occur naturally in sheep, although the disease was shown to be transmissible experimentally, including by the oral route [1]. The key molecular features of BSE can nevertheless be readily identified in sheep, although slight variations were observed during serial passages [2] or in sheep carrying the R171 allele of the prion-encoding PRNP gene [3, 4].

The BSE situation has changed significantly since 2004, however, with the identification of 2 atypical and rare diseases, known as H-type and L-type BSEs in relation to their higher or lower apparent molecular masses of PrPres compared to "classic" BSE (C-BSE), that have now been recognized worldwide [5–8] and are thought to represent sporadic cases of prion diseases in old cattle [9].

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Preliminary data on the L-BSE intracerebral challenge in sheep [10] and on the molecular characterization of sheep with L-BSE [11] have been previously reported, and the definitive results will be published elsewhere. In these studies we found that L-BSE transmission was readily achieved in sheep—not only in QQ171 sheep but also in sheep carrying an R171 allele (RQ171 sheep) known to confer high genetic resistance to C-BSE and classic scrapie [12]. Although L-BSE has been transmitted in 2 lines of ovine PrP transgenic mice [13, 14] and in sheep [10, 15], so far no study has examined the strain properties of this agent after passage in an ovinized host. This is a crucial issue because the strain properties of the L-BSE agent might undergo modification in sheep [13, 16–18].

In this study, we describe the biological characterization of the L-BSE agent in bovine and ovine PrP transgenic mice after passage by the intracerebral route in both QQ and RQ171 sheep.

MATERIALS AND METHODS

Ethics Statement
All mouse transmission experiments were performed in the biohazard prevention area (A3) of the ANSES-Lyon animal facilities, in accordance with the guidelines of the French Ethical Committee (decrees 87–848) and European Community Directive 86/609/EEC, with the relevant approval to carry out animal experiments (A 69 387 0801) by licensed individuals (LL 69 387 191) according to a protocol approved (Permit number 98) by the Committee on the Ethics of Animal Experiments (CREEA of the Région Rhône Alpes Auvergne).

Transmissible Spongiform Encephalopathy Isolates
The transmissible spongiform encephalopathy (TSE) brain inocula used in this study were derived from both cattle and sheep. Except for the 4 experimental L-BSE isolates transmitted in sheep (see below in this paragraph), they had been previously characterized [14, 19, 20] and included 2 French natural bovine isolates of C-BSE (01-2281) and L-BSE (02-2528), an experimental isolate of C-BSE in QQ171 sheep [21], and the unusual CH1641 experimental scrapie isolate serially passaged in QQ171 sheep (kindly provided by the Institute for Animal Health, Edinburgh, United Kingdom).

Sheep L-BSE brain isolates were obtained after intracerebral inoculation of QQ171 and RQ171 Sarda sheep with a bovine L-BSE Italian field isolate [10, 11]. All sheep isolates were homozygous for alanine at codon 136.

Mouse Transmission Studies
Six-week-old TgOvPrP4 or BoPrP-Tg110 mice were inoculated intracerebrally with 20 µL of 10% (wt/vol) brain homogenates in 5% sterile glucose. Serial passages were performed by intracerebral inoculation of 1% (wt/vol) brain homogenates from PrPres-positive mice. At the terminal stage of the disease, mice were killed and their brains and spleens were removed and either frozen for PrPres analyses by Western blotting or fixed in buffered 4% paraformaldehyde for histopathological studies.

Western Blot Analysis
PrPres extractions from brain and spleen tissues using ultracentrifugation and Western blot analyses have been described previously [20, 22]. PrPres was detected by using monoclonal antibodies 12B2, P4, SHa31, or SAF84, which recognize epitopes 93-WQGGG-97, 93-WQGGGSH-99, 148-YEDRYYRE-155, and 167-RPVDQY-172 of the ovine PrP sequence, respectively.

Histopathology
Brain slices were immunostained for disease-associated prion protein (PrPd) as previously described with pretreatments designed to enhance PrPd detection using SAF84 antibody [23]. Amyloid deposits were examined on brain sections stained with Congo red [24] under polarized light.

To study in situ PrPres neuropeptide, the paraffin-embedded tissue blot (PET-blot) method was applied as previously described [25]. The monoclonal antibody used was either SAF84 (1/2500) or SHa31 (1/1000) and NBT/BCIP substrate chromogen was used to visualize the reaction product (dark-blue deposits).

PrPres/PrPd distribution and types were analyzed within 4 brain levels of coronal sections, available after macroscopic trimming prepared with respect to original lesion profile studies’ procedure [26]. It allows assessing main brain regions from the most rostral part of the brain till the brainstem.

RESULTS

PrPres Molecular Features Differ Between L-BSE in RQ171 and QQ171 Sheep
L-BSE was transmitted in QQ171 and RQ171 sheep by intracerebral inoculation [10]. The overall results of this experiment will be published elsewhere. Transmission of the disease was confirmed by Western blot identification of PrPres in all sheep examined. The PrPres pattern in all QQ171 sheep was highly reminiscent of the original cattle L-BSE inoculum, characterized by a low apparent molecular mass, poorly labeled by N-terminal antibodies (such as 12B2 or P4) [10]. In contrast, PrPres in RQ171 sheep was characterized by a “scrapie-like” labeling with the monoclonal antibody P4, although the apparent molecular weight varied between individual sheep and, in some cases, was similar to that in QQ171 sheep infected with L-BSE [11].

On this basis, 2 QQ171 and 2 RQ171 sheep were selected for biological characterization. The PrPres Western blot profiles for the brains of these 4 sheep infected with L-BSE were compared with those associated with C-BSE in sheep and CH1641 experimental scrapie (Figure 1). Both QQ171 L-BSE–infected
sheep accumulated PrPres fragments of low apparent molecular mass, which were similar to those found in ovine C-BSE and CH1641 scrapie. Ovine C-BSE could, however, be clearly distinguished from both L-BSE in QQ171 sheep and CH1641 scrapie by the higher proportions of the di-glycosylated PrPres species (>65% vs 50%–55%; Figure 1A). L-BSE in QQ171 sheep could only be differentiated from CH1641 (Figure 1C) by the absence of an additional C-terminal PrPres, which was abundant in CH1641 scrapie. They otherwise shared the same PrPres molecular masses and glycoform proportions (Figure 1A and 1B).

However, L-BSE in RQ171 sheep showed an alternative, high molecular mass PrPres fragment (denoted HMM PrPres) of 20–21 kDa, in variable proportions depending on the isolate (Figure 1A–C). As shown after PNGase deglycosylation (Figure 1B and 1C), in one isolate the HMM PrPres fragment was almost the only one detectable, whereas in the other isolate the HMM PrPres fragment was associated with a low molecular mass fragment (18–19 kDa), similar to that found in QQ171 sheep, in roughly equivalent proportions. This HMM PrPres was specifically labeled by the 12B2 antibody (Figure 1D). In addition, a faint labeling was detected as a double band by the SAF84 antibody when high PrPres loads were analyzed from RQ171 sheep (Figure 1C), but these had a higher apparent molecular mass and were substantially less abundant than the C-terminal PrPres fragment identified as a single band at approximately 14 kDa in CH1641 scrapie.

Thus, the PrPres molecular features differed strikingly between RQ171 and QQ171 sheep infected with L-BSE, and the Western blot profile in RQ171 sheep was quite distinct from ovine C-BSE but similar, in 1 of the 2 RQ171 sheep, to that found in most natural scrapie cases [27].

**Ovine L-BSE Shows Unaltered Strain-Specific Properties in Bovine PrP Transgenic Mice**

Transgenic mice overexpressing bovine PrP on a murine PrP knockout background (BoPrP-Tg110) [28] were inoculated intracerebrally with TSE brain homogenates originating from both cattle and sheep (Table 1).

All TSE sources transmitted readily at first passage with a 100% attack rate. C-BSE transmission, from both cattle and sheep, resulted in a consistent survival time of 279 days postinoculation (d.p.i.), which was not reduced upon the second passage of ovine C-BSE (mean of 275 ± 20 d.p.i.). Whereas transmission of L-BSE from cattle resulted in a mean survival time of 225 ± 35 and 224 ± 28 d.p.i. at first and second passage, respectively, ovine L-BSE isolates showed mean survival times of 237 ± 9 and 247 ± 19 d.p.i from QQ and RQ171 sheep, respectively, then of 228 ± 26 d.p.i at the second passage from a QQ171 sheep. Thus, compared to C-BSE, the L-BSE source was associated with a shorter survival period, whereas the CH1641 scrapie was the fastest prion source in this model with a survival time of 185 ± 31 d.p.i. at first passage.

The biochemical features of PrPres in the brains of bovine PrP transgenic mice were then analyzed by Western blotting (Figure 2). A low molecular mass PrPres fragment was detected...
in all prion sources in the mouse brains. No labeling was observed when N-terminal monoclonal antibodies (P4 or 12B2) were used (data not shown). However, in C-BSE, the PrPres molecular mass appeared slightly higher (approximately 0.3 kDa) than in L-BSE and CH1641, which were indistinguishable (Figure 2C). Also, PrPres in C-BSE infected mice was highly diglycosylated (around 70%), whereas the proportions of di- and mono-glycosylated PrPres in L-BSE and CH1641 were roughly the same (around 40%) using an antibody with the same regional specificity such as SHa31 (Figure 2A and Supplementary Figure 1A). Only mice inoculated with CH1641 scrapie had an approximately 14 kDa C-terminal PrPres fragment in their brains (Figure 2B and 2C), specifically detected by SAF84 antibody, which was again the only detectable molecular difference between L-BSE and CH1641 scrapie. Importantly, no molecular differences were found in bovine PrP transgenic mice inoculated with L-BSE from either RQ171 or QQ171 sheep. Finally, only mice infected with C-BSE, from cattle or sheep, consistently showed PrPres accumulation in their spleens (Table 1 and Figure 2D).

Comparative PrPres brain mapping based on PET-blot analyses revealed similar distributions in bovine PrP transgenic mice inoculated with cattle or sheep C-BSE (Figure 3 and Supplementary Figure 1A). Only mice inoculated with CH1641 scrapie had an approximately 14 kDa C-terminal PrPres fragment in their brains (Figure 2B and 2C), specifically detected by SAF84 antibody, which was again the only detectable molecular difference between L-BSE and CH1641 scrapie. Importantly, no molecular differences were found in bovine PrP transgenic mice inoculated with L-BSE from either RQ171 or QQ171 sheep. Finally, only mice infected with C-BSE, from cattle or sheep, consistently showed PrPres accumulation in their spleens (Table 1 and Figure 2D).

Table 1. Summary of Transmission Data in Bovine Prion Protein Transgenic Mice

<table>
<thead>
<tr>
<th>TSE Inoculum</th>
<th>Passage</th>
<th>Survival Period, d.p.i., Mean ± SD</th>
<th>Brain PrPd Positive</th>
<th>Spleen PrPres Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>From cattle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-BSE</td>
<td>P1</td>
<td>279 ± 17</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td>L-BSE</td>
<td>P1</td>
<td>225 ± 35</td>
<td>11/11</td>
<td>0/11</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>224 ± 28</td>
<td>12/12</td>
<td>0/12</td>
</tr>
<tr>
<td>Passaged in sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-BSE QQ171</td>
<td>P1</td>
<td>279 ± 29</td>
<td>9/9</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>275 ± 20</td>
<td>7/7</td>
<td>7/7</td>
</tr>
<tr>
<td>L-BSE QQ171 #1</td>
<td>P1</td>
<td>237 ± 9</td>
<td>13/13</td>
<td>0/11</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>228 ± 26</td>
<td>9/9</td>
<td>0/9</td>
</tr>
<tr>
<td>L-BSE RQ171 #1</td>
<td>P1</td>
<td>247 ± 19</td>
<td>9/9</td>
<td>0/9</td>
</tr>
<tr>
<td>CH1641 QQ171</td>
<td>P1</td>
<td>185 ± 31</td>
<td>10/10</td>
<td>0/9</td>
</tr>
</tbody>
</table>

Abbreviations: C-BSE, classic bovine spongiform encephalopathy; d.p.i., days postinoculation; L-BSE, L-type bovine spongiform encephalopathy; PrPres, protease-resistant prion protein; PrPd, disease-associated prion protein; TSE, transmissible spongiform encephalopathy.

Figure 2. Western blot molecular typing of protease-resistant prion protein (PrPres) in brain and spleen of bovine PrP transgenic mice at first passage. PrPres was detected with SHa31 (A and D) or SAF84 (B and C) antibody, before (A, B, and D) or after (C) PNGase deglycosylation. Bars to the left of each panel indicate the 29.0 and 20.1 kDa (A, B, and D) or the 20.1 and 14.3 kDa (C) molecular mass markers. The same quantities of brain were loaded (A and B) shown after the same exposure time using the VersaDoc system. Abbreviations: Bov, bovine; BSE, bovine spongiform encephalopathy; C, classic; L, L-type; Ov, ovine.

were also visible as isolated dots on the PET-blot membranes. In contrast, bovine PrP transgenic mice inoculated with L-BSE from cattle or sheep showed highly comparable PrPres deposition patterns that clearly differed from those of C-BSE. Immunohistochemical analysis also revealed that plaque-type deposits were absent. These characteristics showed similarities with CH1641 scrapie transmitted to bovine PrP transgenic mice (Supplementary Figure 2).

Taken together, our findings clearly showed, after a backpassage of ovine L-BSE in a transgenic mouse model expressing
the PrP of the natural host of the disease, that the characteristic features distinguishing L-BSE from C-BSE were maintained.

Features of L-BSE From Both RQ171 and QQ171 Sheep Are Maintained in the Brains of Q171 PrP Ovine Transgenic Mice
Following serial passages in TgOvPrP4 mice, the C-BSE strain consistently produced longer survival times than L-BSE, irrespective of its bovine or ovine origin (Table 2). At the second or third passage, the mean survival times were always >350 d.p.i. or <250 d.p.i. for C-BSE and L-BSE, respectively. In this regard, L-BSE again shared the closest similarities with CH1641 scrapie (220 ± 31 d.p.i. at second passage).

The molecular features of PrPres in the brain of TgOvPrP4 mice were analyzed by Western blot (Figure 4). They included (1) a similar, low molecular mass, PrPres fragment in L-BSE, C-BSE, and CH1641 in contrast to the high molecular mass in

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**Figure 3.** Distribution and types of protease-resistant prion protein (PrPres)/disease-associated prion protein (PrPd) deposition in the brain of bovine PrP transgenic mice. Paraffin-embedded tissue (PET) blots (SAF84 antibody) of brain coronal sections (diencephalon level) from mice infected with bovine and ovine classic (C-BSE) and L-type bovine spongiform encephalopathy (L-BSE) sources are shown. They allow comparing the PrPres accumulation in critical brain regions such as cerebral cortex, hippocampus, thalamus, and hypothalamus. Immunohistochemical analyses (SAF84 antibody) show that C-BSE is typically associated with a granular type of PrPd deposition together with aggregates including plaques, also visible as isolated dots on PET-blots. In contrast, for L-BSE–infected mice, PrPd is detected as a low level, granular type of deposition, without any plaque-type deposits. Abbreviations: Bov, bovine; BSE, bovine spongiform encephalopathy; C, classic; L, L-type; Ov, ovine.
mice infected with a classic scrapie control (SSBP/1); (2) the characteristic and abundant approximately 14 kDa PrPres fragment detected with SAF84 antibody only in mice infected with CH1641 scrapie; and (3) the different ratios of the PrPres glycosylated species, with C-BSE and SSBP/1 scrapie being clearly distinguishable (Figure 4 and Supplementary Figure 1). PET-blot analyses showed highly comparable PrPres deposition patterns during 2 passages in the spleens of TgOvPrP4 mice infected with CH1641 scrapie at first and second passages. Examination of the PrPres in the spleen of L-BSE-infected mice revealed unexpected features. Indeed, in contrast to results obtained in bovine PrP transgenic mice, L-BSE appeared to be lymphotropic in ovine PrP transgenic mice (Table 2). Whereas L-BSE from cattle required 2 passages to become lymphotropic in TgOvPrP4 mice (Table 2), and this was still the case during serial passages. In contrast, PrPres was undetectable in the spleen of TgOvPrP4 mice infected with CH1641 scrapie at first and second passages. Western blot profiles of PrPres could thus be compared during 2 passages in the spleens of TgOvPrP4 mice infected with QQ171 and RQ171 scrapie at first and second passages. The only TgOvPrP4 mice that did not show HMM PrPres in their spleens (2/16) were inoculated with either RQ171 or QQ171 sheep isolates.

Discussion
The research described in the present paper was initially triggered by the unexpected finding that L-BSE acquired phenotypic features similar to those of C-BSE after experimental inoculation of sheep with C-BSE, L-BSE, and CH1641 TSE agents. We were again unable to find any significant difference between the brains of mice inoculated with either RQ171 or QQ171 sheep isolates.

Splenic PrPres Features at First Passage in Ovine PrP Transgenic Mice Reflect the Genotype-Dependent PrPres Variations Found in the Brains of Sheep

PrPres was consistently detected in the spleen of TgOvPrP4 mice infected with C-BSE at first passage, from cattle or sheep (Table 2), and this was still the case during serial passages. In contrast, PrPres was undetectable in the spleen of TgOvPrP4 mice infected with CH1641 scrapie at first and second passages. The spleens of mice infected with L-BSE. Granular labeling was particularly observed in the cerebral cortex, hippocampus, thalamus, mesencephalon, and brain stem that were affected in a similar way (Supplementary Figure 3).

Our findings in TgOvPrP4 transgenic mice thus confirmed previous data obtained in bovine PrP transgenic mice, with clear phenotypic differences between each of ovine L-BSE, C-BSE, and CH1641 TSE agents. We were again unable to find any significant difference between the brains of mice inoculated with either RQ171 or QQ171 sheep isolates.

Table 2. Summary of Transmission Data in Ovine Prion Protein Transgenic Mice

<table>
<thead>
<tr>
<th>TSE Inoculum</th>
<th>Passage</th>
<th>Survival Period, d.p.i., mean ± SD</th>
<th>Brain PrPd Positive</th>
<th>Spleen PrPres Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>From cattle</td>
<td></td>
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</tr>
<tr>
<td>C-BSE</td>
<td>P1</td>
<td>421 ± 48</td>
<td>10/10</td>
<td>5/5</td>
</tr>
<tr>
<td>L-BSE</td>
<td>P1</td>
<td>627 ± 74</td>
<td>9/10</td>
<td>0/5</td>
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<tr>
<td>C-BSE</td>
<td>P2</td>
<td>354 ± 40</td>
<td>10/10</td>
<td>5/5</td>
</tr>
<tr>
<td>L-BSE</td>
<td>P2</td>
<td>202 ± 26</td>
<td>9/9</td>
<td>3/5</td>
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<tr>
<td>C-BSE</td>
<td>P3</td>
<td>414 ± 61</td>
<td>8/8</td>
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<tr>
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<td>P3</td>
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<td>11/11</td>
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<td>Passed in sheep</td>
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<td>10/12</td>
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<td>P1</td>
<td>245 ± 17</td>
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<td>0/7</td>
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<td>11/12</td>
<td>5/6</td>
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<td>CH1641 QQ171</td>
<td>P2</td>
<td>220 ± 31</td>
<td>11/11</td>
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Abbreviations: C-BSE, classic bovine spongiform encephalopathy; d.p.i., days postinoculation; L-BSE, L-type bovine spongiform encephalopathy; PrPres, protease-resistant prion protein; PrPd, disease-associated prion protein; TSE, transmissible spongiform encephalopathy.
transmission in an ovine PrP transgenic mouse model expressing the ovine V136R154Q171 prion [13]. This led the authors to discuss the possibility that the (still unknown) origin of the C-BSE agent in cattle could have resulted from the recycling of L-BSE following passage in sheep. We therefore addressed this question by studying the biological properties of the L-BSE agent in sheep experimentally infected with L-BSE using 2 transgenic mouse models that had previously been described to maintain clear molecular, histopathological, and biological differences between C-BSE and L-BSE after transmission of the disease from bovine brain [14, 20, 30].

In both models that expressed either the bovine or the ovine (A136R154Q171) prion protein (Tg110 and TgOvPrP4 mouse lines, respectively), our data clearly did not indicate any change reminiscent of C-BSE in mice infected with AA136 QQ171 and AA136 RQ171 ovine L-BSE. This indeed showed (1) for both mouse lines, survival periods shorter than those of C-BSE and similar to those of L-BSE; (2) for both mouse lines, molecular and pathological phenotypes in their brains similar to L-BSE and clearly distinct from C-BSE; and (3) lack of lymphotropism in bovine PrP transgenic mice. It should be noted that both transgenic models are susceptible to both L-BSE and C-BSE, with shorter survival periods for L-BSE, although differences are smaller in the bovine PrP transgenic mouse line (differences of <50 days between the mean survival times of L-BSE and C-BSE from sheep). However, our data do not imply that the C-BSE agent was isolated from L-BSE–infected sheep but instead suggest that the L-BSE strain is preserved after passage in sheep. Interestingly, the L-BSE phenotype was also recovered from RQ171 sheep, despite the distinct molecular phenotypes observed in these inocula. In addition, the molecular analyses confirmed our earlier observations that the presence of an additional C-terminal PrPres fragment was the most reliable molecular feature distinguishing CH1641 scrapie from L-BSE [20], further demonstrated here in the comparison of CH1641 scrapie with ovine isolates of L-BSE and also confirmed following transmission of CH1641 scrapie in bovine PrP transgenic mice.

Studies of lymphotropism in the TgOvPrP4 transgenic mouse model had already revealed unexpected findings, first because PrPres could be detected in the spleen of mice infected with C-BSE or scrapie, even though we were unable to identify any detectable level of prion protein or mRNA by analyses of

Figure 4. Western blot molecular typing of protease-resistant prion protein (PrPres) in the brains and spleens of ovine PrP transgenic mice. PrPres was detected with SHa31 (A and D), SAF84 (B and C), or P4 (E) antibody, after PNGase deglycosylation (C), from the brain (second passage; A–Q) or the spleen (first [P1] or second [P2] passages; D and E). Bars to the left of the panels indicate the 29.0 and 20.1 kDa (A–B and D–E) or the 20.1 and 14.3 kDa (C) molecular mass markers. The same quantities of brain were loaded (A and B) shown after the same exposure time using the VersaDoc system. Abbreviations: Bov, bovine; BSE, bovine spongiform encephalopathy; C, classic; L, L-type; Ov, ovine.
We recently reported that a TSE agent could acquire lymphotropism in this model during a second passage from a case of human sporadic Creutzfeldt-Jakob disease, whereas PrPres was undetectable in the spleen at first passage [29]. A similar observation was made in the current study where PrPres was consistently detected in the spleens of TgOvPrP4 mice at first passage of L-BSE from sheep, but had not been detected from a bovine L-BSE isolate [22].

Most importantly, detailed characterization of PrPres by Western blot analysis revealed strikingly variable molecular features, which differ between RQ171 and QQ171 sheep [11], and were also observed in the 4 sheep examined here. Whereas the PrPres in the 2 QQ171 sheep was of low apparent molecular mass, as in both C-BSE and L-BSE [5], a PrPres fragment with high molecular mass (HMM PrPres) was readily identified in the 2 RQ171 sheep brains and was the only detectable form of...
PrP in 1 of the 2 sheep. Slight variations in PrPres size had already been reported in RR171 sheep experimentally infected with C-BSE, but did not prevent recognition of BSE in these sheep [3, 4]. This new finding in L-BSE could have public health implications as current measures for the surveillance of TSEs in small ruminants in Europe involve the characterization of PrPres in TSE-affected animals to identify those that could be infected with BSE [27, 33], a strategy that allowed the identification of C-BSE in 2 goats, but until now not in sheep [34, 35].

Whereas molecular and neuropathological features were indistinguishable and typical of L-BSE in the brains of TgOvPrP4 mice infected with isolates from both QQ171 and RQ171 sheep, characterization of the PrPres in the spleen revealed important differences at first passage. Indeed, HMM PrPres was demonstrated in the spleens of mice infected from RQ171 sheep at first passage, whereas PrPres from QQ171 sheep was of low molecular mass. Accordingly, this HMM PrPres was strongly labeled by the P4 monoclonal antibody. This finding further emphasizes possible tissue-specific differences in the propagation of the TSE agents between brain and lymphoid tissues [22, 36]. One hypothesis is that the presence of HMM PrPres could reflect the presence of a minor strain present in the bovine brain and favored in the brain of sheep coexpressing the R171 ovine prion protein. Ovine PrP could be expressed at low levels in the spleen of the transgenic mice [22], possibly in a particular subset of cell types, and this might be more permissive to the propagation of a minor strain associated with HMM PrPres. Alternatively, following the discovery of unexpected L-BSE changes that have already been found, with phenotypic features similar to C-BSE in an ovine transgenic mouse model expressing the VRQ prion protein [13] without subsequent strain changes [37], our data could suggest a phenotypic or conformational plasticity of the disease-associated form of the prion protein in L-BSE, in either RQ171 sheep brain or in TgOvPrP4 spleen that might dictate an unusual conformation of the prion protein. This concept has been recently proposed to explain the occurrence of some reversible variations of strain-specific features in response to changes in the replication environment [38].

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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