Transmission of scrapie and sheep-passaged bovine spongiform encephalopathy prions to transgenic mice expressing elk prion protein

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Chronic wasting disease (CWD) is a transmissible, fatal prion disease of cervids and is largely confined to North America. The origin of CWD continues to pose a conundrum: does the disease arise spontaneously or result from some other naturally occurring reservoir? To address whether prions from sheep might be able to cause disease in cervids, we inoculated mice expressing the elk prion protein (PrP) transgene [Tg(ElkPrP) mice] with two scrapie prion isolates. The SSBP/1 scrapie isolate transmitted disease to Tg(ElkPrP) mice with a median incubation time of 270 days, but a second isolate failed to produce neurological dysfunction in these mice. Although prions from cattle with bovine spongiform encephalopathy (BSE) did not transmit to the Tg(ElkPrP) mice, they did transmit after being passaged through sheep. In Tg(ElkPrP) mice, the sheep-passaged BSE prions exhibited an incubation time of approximately 300 days. SSBP/1 prions produced abundant deposits of the disease-causing PrP isoform, denoted PrPSc, in the cerebellum and pons of Tg(ElkPrP) mice, whereas PrPSc accumulation in Tg mice inoculated with sheep-passaged BSE prions was confined to the deep cerebellar nuclei, habenula and the brainstem. The susceptibility of 'cervidized' mice to 'ovinized' prions raises the question about why CWD has not been reported in other parts of the world where cervids and scrapie-infected sheep coexist.
particularly widespread, have so far proven unsuccessful (Conner et al., 2007; Samuel, 2007).

The risk that CWD poses to humans and other animals is not clear. Whilst many people hunt and consume venison, surveillance shows no evidence that CWD can transmit to humans (Belay et al., 2004; MaWhinney et al., 2006). Although studies have shown that CWD does not transmit to transgenic (Tg) mice expressing human PrP, the possible risk to humans cannot be eliminated fully (Kong et al.; Miller & Williams, 2003). In infected cervids, CWD prions have been detected in the nervous system, lymphoid system (including tonsils and Peyer’s patches) (Spraker et al., 2002), skeletal muscle (Angers et al., 2006), blood and saliva, but not in urine or faeces (Mathiasen et al., 2006). These observations make direct or indirect horizontal transmission through saliva a likely route.

It remains to be determined whether CWD originated in cervids as a spontaneous disease or was transmitted to cervids by infection from another species, as has been discussed for transmissible mink encephalopathy, which may have resulted from feeding downer cattle with BSE to mink (Marsh et al., 1991; Williams, 2005; Williams & Young, 1980, 1992). Sheep scrapie has been transmitted successfully to cattle and to Tg mice expressing bovine PrP (Foster et al., 1993; Scott et al., 2005). In one study, sheep scrapie was transmitted to three elk after intracerebral (i.c.) inoculation (Hamir et al., 2004). CWD transmitted poorly to sheep and more efficiently to cattle, but failed to transmit to Tg mice expressing ovine, bovine or human PrP (Hamir et al., 2006, 2007; Tamgune et al., 2006). We addressed whether prions causing scrapie in sheep and BSE in cattle could be transmitted to cervids by using a mouse model for CWD. Whilst the apparent incidence of BSE in North America is low (Richt et al., 2005), scrapie occurs relatively frequently; at least 1117 cases were diagnosed between 1947 and 1992 and, since the start of the regulatory scrapie slaughter surveillance programme in the USA in 2003, 359 scrapie cases from among 168 593 screened animals have been reported (USDA, 2008; Wineland et al., 1998).

To test whether scrapie or BSE prions could be transmitted to cervids, we i.c.-inoculated Tg mice expressing elk PrP, denoted Tg(ElkPrP) mice, with two scrapie isolates, mouse-adapted RML (Rocky Mountain Laboratory) prions, prions from a cow with BSE (O’Rourke et al., 1999) and sheep-passaged BSE prions. Tg(ElkPrP) mice express the most common ElkPrP sequence, with methionine at polymorphic residue 132. We found that the experimental SSBP/1 scrapie isolate and mouse-adapted RML prions transmitted to Tg(ElkPrP) mice, whereas the natural, North American scrapie isolate 027 did not. Most unexpectedly, BSE prions did not transmit to Tg(ElkPrP) mice, but experimentally sheep-passaged BSE prions did cause disease in approximately 300 days. Neuropathological changes in prion-diseased Tg(ElkPrP) mice inoculated with either scrapie or sheep-passaged BSE prions were distinguishable from those observed in the same line of Tg mice inoculated with CWD prions. Considering that some sheep or sheep-passaged prion strains can be transmitted to Tg(ElkPrP) mice, it is surprising that CWD cases have not been reported elsewhere in the world where cervids and scrapie-infected sheep coexist.

**METHODS**

**Source of Tg mice.** All Tg mice originated from Zrchi/Prnp<sup>0/0</sup> mice, which do not express endogenous MoPrP (Büeler et al., 1992). Tg(ElkPrP<sup>+/+</sup>)12577, Tg(ElkPrP<sup>-/-</sup>)12584 and Tg(BoPrP<sup>+/+</sup>)14092 mice have been described previously (Safar et al., 2002; Tamguey et al., 2006). Tg(ElkPrP<sup>+/+</sup>) mice were bred to homozygosity from Tg(ElkPrP<sup>-/-</sup>) mice; based on the 3-fold PrP expression of Tg(ElkPrP<sup>-/-</sup>) mice, the complete sequences of the ORF construct were determined and archived by using Vector NTI Advance software (Invitrogen). Tg(ElkPrP<sup>+</span>)14882 mice were generated by using the cosHSA.Tet cosmid vector for transgene expression as described previously (Scott et al., 1992). Tg(ElkPrP<sup>+/+</sup>)12584 and Tg(OvPrP<sup>+/+</sup>)14882 mice were made respectively by intercrossing Tg(ElkPrP<sup>+/+</sup>)12584 and Tg(OvPrP<sup>+/+</sup>)<span>14882</span> mice. PrP<sup>+</span>-expression levels in the brains of Tg(ElkPrP<sup>+/+</span>) and Tg(OvPrP<sup>+/+</span>) mice were determined by dot-blot using serial dilutions of brain homogenate and compared with those of wt FVB mice (Scott et al., 1993). PrP was detected with the humanized recombinant fragment antibody (recFab) HuM-P linked to horseradish peroxidase (HRP) (Safar et al., 2002) in combination with the enhanced chemiluminescent detection system (Amersham Biosciences) (Scott et al., 1999). The recFab HuM-P was expressed and fermented in *Escherichia coli* 33B6 competent cells and purified as described previously (Peretz et al., 2001). Covalent linkage of recFab HuM-P to HRP was performed by Rockland Immunochemicals, Inc.

**Prion isolates and transmission studies.** The RML prion strain, which was derived from the Chandler sheep scrapie isolate passaged in Swiss CD1 mice (Charles River Laboratories) expressing mouse PrP-A, was originally provided by William Hadlow at the Rocky Mountain Laboratory (Hamilton, MT, USA) (Chandler, 1961). The experimental SSBP/1 sheep scrapie was obtained from Nora Hunter at the Roslin Institute, University of Edinburgh (Edinburgh, UK). It was derived from the brain homogenates of three sheep with scrapie and then passaged mainly through NPU Cheviot sheep (Dickinson & Outram, 1988). Transmission of SSBP/1 seems to be controlled by the PrP residues at codons 136, 154 and 171: sheep expressing VRQ/VRQ and VRQ/AXX are susceptible to subcutaneous infection with SSBP/1 prions, whereas those expressing AXX/AXX seem to be largely protected (Goldmann et al., 1994). BSE isolate PG31/90 was provided by John Wilesmith at the Central Veterinary Laboratory (Weybridge, UK). The natural, North American scrapie 027 isolate, derived from a scrapie-sick Suffolk sheep expressing PrP(AQ), was obtained from...
the USDA. Elk1, derived from elk case 03-12609, and WTD1, derived from white-tailed deer case 03-12473, were isolated at the Colorado Division of Wildlife, Wildlife Research Center (Fort Collins, CO, USA). Experimental isolate SA04, which was derived from a Romney sheep expressing PrP(ARQ) inoculated i.c. with BSE prions, was obtained from the Veterinary Laboratories Agency (New Haw, UK).

I.c. transmissions of prion isolates to Tg mice were performed as described previously (Scott et al., 1997). For samples Elk1 and WTD1, 15% (w/v) brain homogenates were prepared in Ca2+- and Mg2+-free PBS (pH 7.4) by three 75 s cycles to disease onset of sick animals. Calculations were performed when an upper 95% c.i. could not be calculated by survival-analysis intervals (c.i.) were determined (Brookmeyer & Crowley, 1982). Median incubation periods were calculated by using the Kaplan–Meier function (Kaplan & Meier, 1958); mice with intercurrent illness or with 2 mM PMSF and samples were centrifuged at 100 000 g for 1 h at 4 °C. Pellets were resuspended in 10 mM Tris/HCl (pH 8.0), 0.15 M NaCl, 0.5% (w/v) NP-40, 0.5% (w/v) sodium deoxycholate. Prior to electrophoresis, equal volumes of 2× SDS sample buffer were added and the samples were boiled for 5 min (Laemmli, 1970). SDS gel electrophoresis and Western blotting were performed by using Novex 12% or 4–12% Tris–glycine gels and the iBlot dry-blotting system (Invitrogen). PrP was detected with the recFab HuM-P bound covalently to HRP and developed with the enhanced chemiluminescent detection system (Amersham Biosciences). To determine the glycoform ratios of Western blot signals on exposed and developed film, we scanned the film using an Epson Perfection 4990 Photo, then quantified the signals by using AlphaEase FC software, version 6.0.0 (Alpha Innotech Corp.).

Conformational-stability assay. A series of concentrations of guanidine hydrochloride (GdnHCl) was added to samples of 10% brain homogenate to obtain 2.5% brain-homogenate samples in the presence of 0–6 M GdnHCl. Samples were incubated for 2 h at 22 °C and then adjusted to 0.4 M GdnHCl and a total volume of 1.2 ml by addition of Tris buffer composed of 10 mM Tris–HCl (pH 8.0), 150 mM NaCl, 0.5% Nonidet P-40 and 0.5% deoxycholate. Samples were digested with 20 µg PK ml−1 for 1 h at 37 °C. Reactions were terminated by the addition of PMSF (final concentration, 1 mM) and subsequently centrifuged in an Optima Max-E Ultracentrifuge (Beckman) for 1 h at 100 000 g. From each sample, the supernatant was decanted and the pellet was solubilized in 40 µl Tris buffer and 40 µl 2× SDS sample buffer. SDS gel electrophoresis and Western blot analyses were performed as described above.

Neuropathology. Brains were removed rapidly from euthanized animals and either immersion-fixed in 10% buffered formalin or frozen on dry ice for neuropathological analysis. For evaluation of neurodegeneration, paraffin-embedded brains sections (8 µm) were stained with haematoxylin and eosin (H&E).

For immunohistochemistry, PrPsc was detected on formalin-fixed, paraffin-embedded tissue sections by the hydrolytic autoclaving method and with recFab HuM-P against PrP (Muramoto et al., 1992). For histoblots, frozen coronal sections (10 µm) were blotted onto nitrocellulose membranes and processed for immunohistochemistry using recFab HuM-P directed against PrP (Taraboulos et al., 1992). For evaluation of reactive astrocytic gliosis, we used a rabbit antisera to glial fibrillary acidic protein (GFAP; Dako) with peroxidase immunostaining. For immunohistochemistry, PrPsc was detected on formalin-fixed, paraffin-embedded tissue sections by the hydrolytic autoclaving method and with recFab HuM-P against PrP (Muramoto et al., 1992). For histoblots, frozen coronal sections (10 µm) were blotted onto nitrocellulose membranes and processed for immunohistochemistry using recFab HuM-P directed against PrP (Muramoto et al., 1992). For evaluation of reactive astrocytic gliosis, we used a rabbit antisera to glial fibrillary acidic protein (GFAP; Dako) with peroxidase immunohistochemistry (Muramoto et al., 1997). Vacuolation scores, which are semiquantitative estimates of an area of a brain region occupied by vacuoles, were determined by a single rater (S. J. D.) using two or three mouse brains for each inoculation (Carlson et al., 1994).

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Tg line</th>
<th>Incubation time (days)</th>
<th>No. prion-diseased animals/ no. inoculated animals*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elk1</td>
<td>Tg(ElkPrP+/+0)12577</td>
<td>186</td>
<td>54–203</td>
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<tr>
<td>Elk1</td>
<td>Tg(ElkPrP+/+0)12584</td>
<td>125</td>
<td>116–132</td>
</tr>
<tr>
<td>Elk1→Tg(ElkPrP+/+0)12584</td>
<td>Tg(ElkPrP+/+0)12584</td>
<td>109</td>
<td>106–126</td>
</tr>
<tr>
<td>WTD1</td>
<td>Tg(OvPrP+/+0)14882</td>
<td>&gt;601</td>
<td>–</td>
</tr>
<tr>
<td>WTD1</td>
<td>Tg(OvPrP+/+0)14882</td>
<td>&gt;544</td>
<td>–</td>
</tr>
<tr>
<td>RML</td>
<td>Tg(ElkPrP+/+0)12577</td>
<td>398</td>
<td>280–508</td>
</tr>
<tr>
<td>RML→Tg(ElkPrP+/+0)12577</td>
<td>Tg(ElkPrP+/+0)12584</td>
<td>169</td>
<td>169–179</td>
</tr>
<tr>
<td>RML→Tg(ElkPrP+/+0)12577</td>
<td>Tg(ElkPrP+/+0)12584</td>
<td>193</td>
<td>187–202</td>
</tr>
</tbody>
</table>

*Excludes animals that died from intercurrent disease.
†Data from Tamgüney et al. (2006).
RESULTS

Construction of Tg(ElkPrP) and Tg(OvPrP) mice

Tg(ElkPrP)12584 mice were bred on the Prnp<sup>0/0</sup> background and express the most common elk Prnp allele that encodes methionine at residue 132 (GenPept/NCBI accession no. AAF80282). Tg(ElkPrP<sup>+/0</sup>)12577 and Tg(ElkPrP<sup>++/+</sup>)12584 mice express ElkPrP at 2- and 6-fold levels, respectively, compared with PrP in wt mice (see Supplementary Table S1, available in JGV Online). Uninoculated Tg(ElkPrP) mice did not exhibit abnormal signs or behaviours, and remained healthy for >600 days (Supplementary Table S1). Tg(OvPrP)14882 mice that express ovine PrP with VRQ at polymorphic codons 136, 154 and 171, respectively (GenPept/NCBI accession no. CAA04276), on the Prnp<sup>0/0</sup> background were obtained by using the cosSHa.Tet vector and bred to homozygosity for the transgene (Scott <i>et al.</i>, 1992). Hemizygous Tg(OvPrP<sup>+/0</sup>)14882 mice and homozygous Tg(OvPrP<sup>++/+</sup>)-
Uninoculated Tg(OvPrP)14882 mice remained healthy respectively, compared with PrP levels in wt mice. Tg(ElkPrP)12584 mice express OvPrP at levels 0.7- and 1.4-fold, Tg(ElkPrP) mice to different lines of Tg(ElkPrP) and Tg(OvPrP) mice. (a) Transmission of the Elk1 isolate to Tg(ElkPrP+/−)12577 mice (grey solid line) and Tg(ElkPrP+/+)12584 mice (black solid line) had median incubation times of 186 and 125 days, respectively. Upon second passage to Tg(ElkPrP+/+)12584 mice, incubation times were reduced to 109 days (dashed line). (b) Transmission of scrapie SSBP/1 prions to Tg(ElkPrP+/−)12577 (grey solid line) and Tg(ElkPrP+/+)12584 mice (black solid line) resulted in clinical symptoms in 270 and 200 days, respectively. SSBP/1 prions passedaged to Tg(ElkPrP+/−)12577 and then to Tg(ElkPrP+/+)12584 mice resulted in neurological symptoms in 147 days (dashed line). Scrapie 027 isolate did not transmit to either Tg(ElkPrP+/−)12577 or Tg(ElkPrP+/+)12584 mice (dashed/dotted line). (c) Transmission of RML prions to Tg(ElkPrP+/−)12577 mice resulted in disease in 398 days (grey solid line). Upon second passage using two different samples to Tg(ElkPrP+/−)12584 mice (dashed lines), median incubation times were reduced to 193 and 169 days. (d) BSE prions that transmitted to Tg(BoPrP+/+)4092 mice in 237 days (grey solid line) did not transmit to Tg(ElkPrP+/+)12584 mice (dashed/dotted line), whereas sheep-passaged BSE prions (isolate SA04) transmitted to nine of ten Tg(ElkPrP+/−)12584 mice (black solid line) with a median incubation time of 300 days. (e) Scapie prions transmitted successfully to Tg(OvPrP) mice: SSBP/1 (grey solid line) and scrapie 027 (grey dashed line) to Tg(OvPrP) mice; SSBP/1 (black solid line), SSBP/1 passaged first in Tg(ElkPrP+/−)12577 mice (dashed/dotted line) and scrapie 027 (black dashed line) to Tg(OvPrP+/+)14882 mice.

**Table 2. Transmission of scrapie and BSE prions to Tg(ElkPrP) and Tg(OvPrP) mice**

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Tg line</th>
<th>Incubation time (days)</th>
<th>No. prion-diseased animals/no. inoculated animals*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBP/1</td>
<td>Tg(ElkPrP+/−)12577</td>
<td>270 197–448</td>
<td>10/11</td>
</tr>
<tr>
<td>SSBP/1</td>
<td>Tg(ElkPrP+/+)12584</td>
<td>200 175–237</td>
<td>12/12</td>
</tr>
<tr>
<td>SSBP/1</td>
<td>Tg(ElkPrP+/−)12577</td>
<td>147 125–152</td>
<td>7/7</td>
</tr>
<tr>
<td>SSBP/1</td>
<td>Tg(OvPrP+/−)14882</td>
<td>75 67–82</td>
<td>8/8</td>
</tr>
<tr>
<td>SSBP/1</td>
<td>Tg(OvPrP+/+)14882</td>
<td>76 57–81</td>
<td>8/8</td>
</tr>
<tr>
<td>SSBP/1→Tg(ElkPrP+/−)12577</td>
<td>Tg(OvPrP+/−)14882</td>
<td>95 88–96</td>
<td>11/11</td>
</tr>
<tr>
<td>Scapie 027</td>
<td>Tg(ElkPrP+/−)12577</td>
<td>&gt;606 –</td>
<td>0/7</td>
</tr>
<tr>
<td>Scapie 027</td>
<td>Tg(ElkPrP+/+)12584</td>
<td>&gt;606 –</td>
<td>0/8</td>
</tr>
<tr>
<td>Scapie 027</td>
<td>Tg(OvPrP+/−)14882</td>
<td>413 358–444</td>
<td>6/6</td>
</tr>
<tr>
<td>Scapie 027</td>
<td>Tg(OvPrP+/+)14882</td>
<td>249 222–307</td>
<td>5/5</td>
</tr>
<tr>
<td>BSE PG31/90</td>
<td>Tg(ElkPrP+/−)12577</td>
<td>&gt;605 –</td>
<td>0/10</td>
</tr>
<tr>
<td>SA04</td>
<td>Tg(OvPrP+/+)12584</td>
<td>300 272–431</td>
<td>9/10</td>
</tr>
<tr>
<td>BSE PG31/90†</td>
<td>Tg(BoPrP+/−)4092</td>
<td>237 216–258</td>
<td>9/9</td>
</tr>
</tbody>
</table>

*Excludes animals that died from intercurrent disease. †Data from Scott et al. (2005).

14882 mice express OvPrP at levels 0.7- and 1.4-fold, respectively, compared with PrP levels in wt mice. Uninoculated Tg(OvPrP)14882 mice remained healthy for >600 days (Supplementary Table S1). Thus, these Tg mouse lines tolerate the expression of cervid or ovine PrP without recognizable illness.

**Transmission of CWD and scrapie prions to Tg(ElkPrP) and Tg(OvPrP) mice**

I.c. transmission of the CWD isolate Elk1 to Tg(ElkPrP+/+)12577 mice resulted in disease in approximately 186 days (Tamgüney et al., 2006) and to Tg(ElkPrP+/+)12584 mice in 125 days (Table 1; Fig. 1a). A second passage in Tg(ElkPrP+/+)12584 mice reduced incubation times to a median of 109 days. Tg(ElkPrP) mice were also susceptible to the experimental SSBP/1 scrapie isolate (Table 2; Fig. 1b) and to RML prions (Table 1, Fig. 1c), which is a rodent-adapted scrapie strain. Transmission of SSBP/1 prions to Tg(ElkPrP+/+)12577 mice and Tg(ElkPrP+/+)12584 resulted in median incubation periods of 270 and 200 days, respectively (Table 2). Brain homogenates from prion-diseased Tg(ElkPrP+/+)12577 mice were prepared and injected into Tg(ElkPrP+/+)12584 mice, which resulted in disease in 147 days. RML prions transmitted to Tg(ElkPrP+/+)12577 mice in 398 days on first passage, which was shortened to 169 and 193 days on second passage to Tg(ElkPrP+/+)12584 mice using two different brain-homogenate samples. In contrast to SSBP/1 and RML prions, the natural scrapie 027 isolate and the BSE isolate PG31/90 did not transmit to Tg(ElkPrP) mice (Table 2; Fig. 1b, d). BSE prions that caused disease in Tg(BoPrP+/+)4092 mouse...
Biochemical analysis of PrPSc

We measured the electrophoretic mobility of PK-digested PrPSc (Fig. 2) and quantified the un-, mono- and diglycosylated PrPSc bands by densitometric analysis (see Supplementary Fig. S1, available in JGV Online). Whilst all scrapie isolates had unglycosylated PrPSc moieties with a slightly higher electrophoretic mobility than those of CWD (Fig. 2a), the mobility of the unglycosylated band became similar to that of CWD prions after transmission to Tg(ElkPrP) mice (Fig. 2b, d). Glycosylation ratios of SSBP/1 and RML prions were slightly different from those of CWD in Tg(ElkPrP) mice after first passage, but resembled those of CWD prions after second passage (Supplementary Fig. S1). In contrast to the scrapie isolates, the electrophoretic mobility, glycosylation profile and conformational stability of sheep-passaged BSE prions were different from those of CWD prions in Tg(ElkPrP) mice after first passage, but resembled those of CWD prions after second passage (Supplementary Fig. S1).

To characterize further some prion isolates, we measured their conformational stability before and after transmission to Tg mice (see Supplementary Table S2, available in JGV Online). Due to the limited amount of the sample, we could not determine the conformational stability of the SA04 isolate before transmission. The stability is expressed as the GdnHCl concentration required to denature half of PrPSc (Peretz et al., 2002). Serial passage of SSBP/1 to Tg(ElkPrP+/+)12584 mice and then to Tg(ElkPrP+/+)12584 resulted in unaltered [GdnHCl]50 values of 2.1–2.2 M. In comparison, serial passage of RML to
Tg(ElkPrP+/−)12577 mice and then to Tg(ElkPrP+/+)12584 reduced the [GdnHCl]50 values from 1.8 to 1.1 M (Supplementary Table S2). For SSBP/1 prions in Tg(OvPrP) mice, all stabilities were reduced to 1.6–1.7 M. In contrast, Elk1 prions had comparable stabilities of 2.1–2.3 M before and after transmission to Tg(ElkPrP) mice (Supplementary Table S2).

Neuropathology in Tg mice

Neuropathological analysis revealed that Elk1, SSBP/1, RML and SA04 produced distinct disease phenotypes. Vacuolation profiles (Bruce & Fraser, 1991; Fraser & Dickinson, 1973) were different for each prion isolate (Fig. 3). In Tg(ElkPrP) mice, CWD Elk1 prions produced greater vacuolation scores than scrapie SSBP/1 prions in most brain regions (Fig. 3a, b). Passage of SSBP/1 resulted in different vacuolation profiles depending on the host animal: Tg(ElkPrP) mice had substantially more vacuolation in the hypothalamus than Tg(OvPrP) mice upon first passage (Fig. 3b, c). Tg(ElkPrP) mice had the most widespread neuroanatomical distribution of vacuolation after infection with RML prions compared with the other inocula (Fig. 3d; Supplementary Fig. S2). Finally, SA04 produced a unique histogram in Tg(ElkPrP) mice, with vacuolation localized in the brainstem and absent from all other brain regions (Fig. 3e). Unlike the Elk1 CWD isolate (Tamgüney et al., 2006), SSBP/1 scrapie prions and SA04 sheep-passaged BSE prions did not give rise to PrP plaques in infected mouse brains.

Immunohistochemistry of brain sections revealed vacuolation, astrocytic gliosis and PrPSc accumulation resulting from each inoculum (Figs 4–6; Supplementary Fig. S2). After first and second passages of Elk1 prions to Tg(ElkPrP+/−)12584 mice, vacuolation was confined largely to the CA2 and CA3 regions of the hippocampus (Fig. 4a, e). Reactive astrocytic gliosis was more intense in the CA2–CA3 regions, but less abundant in the CA1 regions, in both passages (Fig. 4b, f). However, histoblots showed no protease-resistant PrPSc in the hippocampus (Fig. 4c, g), but relatively intense PrPSc accumulation in the dorsal and medial tegmentum of the pons (Fig. 4d, h) of Tg(ElkPrP+/+)12584 mice. Passage of SSBP/1 prions in both Tg(ElkPrP)12577 and Tg(OvPrP)14882 mice resulted in minimal vacuolation (Fig. 5a, i, m) and relatively little protease-resistant PrPSc in the hippocampus and thalamus (Fig. 5c, g, k, o), but abundant PrPSc accumulation in the cerebellum and pons (Fig. 5d, h, l, p). Intense reactive gliosis was noted in all mice inoculated with SSBP/1 prions (Fig. 5b, f, j, n). These results suggest that pathological changes, such as vacuolation and astrocytic gliosis, are not

Fig. 3. Vacuolation histograms of prions in different lines of Tg(ElkPrP) and Tg(OvPrP) mice. Passage of: (a) Elk1 to Tg(ElkPrP+/+)12584 mice on first (■) and second (○) passage; (b) SSBP/1 to Tg(ElkPrP+/+)12584 mice (▲), to Tg(ElkPrP+/+)12577 mice first (■) and then to Tg(ElkPrP+/+)12584 mice (○); (c) SSBP/1 prions to Tg(OvPrP+/+)14882 mice (▲), to Tg(OvPrP+/+)14882 mice (○), to Tg(ElkPrP+/+)12577 first and then to Tg(OvPrP+/+)14882 mice (▲); (d) RML to Tg(ElkPrP+/+)12577 mice first (■) and then to Tg(ElkPrP+/+)12584 mice (○); and (e) SA04 to Tg(ElkPrP+/+)12584 mice (▲). Nc, Neocortex; Hp, hippocampus; Th, thalamus; Hy, hypothalamus; Cd, caudate nucleus; BS, brainstem (pons and/or midbrain); Cbm, cerebellar cortex molecular layer; Cbg, cerebellar cortex granule cell layer; WM, white matter.
always directly proportional to the levels of protease-resistant PrPSc in our 'cervidized' mice.

Sheep-passaged BSE prions produced a unique phenotype in Tg(ElkPrP+/+)12584 mice. In the hippocampus or thalamus, vacuolation, reactive astrocytic gliosis and PrPSc accumulation were absent (Fig. 6a, b, e). The neuropathological changes were confined largely to the brainstem, deep cerebellar nuclei and habenula (Fig. 6c, d, f, g).

DISCUSSION

We successfully transmitted the experimental sheep scrapie isolate SSBP/1, mouse-passaged RML prions and the experimentally sheep-passaged BSE isolate SA04 to Tg(ElkPrP) mice. Whilst mouse-passaged RML prions transmitted to Tg(ElkPrP) mice, their host range may differ from the original strain. Scrapie 027, the only North American scrapie isolate that we used, and BSE prions had no transmissions within the time frame observed, although we did not look for subclinical disease.

The reasons why SSBP/1 prions transmit to Tg(ElkPrP) mice whereas scrapie 027 prions do not are unclear. A number of factors can account for the marked differences between the transmissibility of prion strains among species, such as amino acid sequence, conformation and titre (Safar et al., 1998; Scott et al., 1989). Notably, scrapie 027 required approximately 249 days to transmit disease to Tg(OvPrP+/+) mice, whereas SSBP/1 caused neurological dysfunction in only about 76 days (Table 2), which may be due to differences in the prion strain, the amino acid sequence, the titre or a combination of these factors. Transmission is most efficient when the infecting PrPSc and the host PrPC share the same sequence. Among sheep, PrP residues 136, 154 and 171 (sheep numbering) govern susceptibility to scrapie (reviewed by Hunter, 2007). Sheep expressing VRQ/VRQ and VRQ/AXX are susceptible to subcutaneous infection with SSBP/1 prions, whereas those expressing AXX/AXX seem to be largely protected (Goldmann et al., 1994). Although Tg(ElkPrP) mice express ARQ at the corresponding residues, scrapie 027 of the same ARQ genotype did not transmit disease to these mice. Furthermore, SSBP/1 prions that transmitted poorly to sheep expressing PrP(ARQ) transmitted fully to Tg(ElkPrP) mice (Goldmann et al., 1996). Our results indicate that, for the two prion isolates that we tested from sheep, transmission to cervids is probably not controlled by A or V at residue 136, but possibly by PrPSc conformation.

Our inability to transmit BSE prions to Tg(ElkPrP) mice contrasts with the results of a recent report showing transmission of BSE prions to six European red deer (Cervus elaphus elaphus) with incubation periods of

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**Fig. 4.** Neuropathology resulting from the first (a–d) and second (e–h) passages of Elk1 prions into Tg(ElkPrP+/+)12584 mice. (a, e) H&E stain shows that vacuolation is largely confined to the CA2 and CA3 regions of the hippocampus. (b, f) GFAP immunostain shows little reactive astrocytic gliosis. Histoblots of coronal brain sections reveal little protease-resistant PrPSc accumulation through the dorsal hippocampus and thalamus (c, g), but abundant PrPSc in the cerebellum and pons (d, h). Bars, 50 μm (e) [magnification is the same for (a)]; 100 μm (f) [magnification is the same for (b)].
Fig. 5. SSBP1 prions transmitted first to Tg(ElkPrP<sup>+/+</sup>)12577 mice (a–d), then passed serially both to Tg(ElkPrP<sup>+/+</sup>) 12584 mice (e–h) and to Tg(OvPrP<sup>++/++</sup>)14882 mice (m–p), result in a brainstem-dominant prion disease. Primary passage of SSBP/1 to Tg(OvPrP<sup>++/++</sup>)14882 mice (i–l) also showed similar pathological changes. In Tg(ElkPrP) mice, vacuolation is not seen in the pontine tegmentum on first passage (a), but is present in the grey matter neuropil on second passage (e). In Tg(OvPrP<sup>++/++</sup>)14882 mice, very minimal vacuolation is observed in the hippocampal CA1–CA3 regions (i, m). Severe reactive astrocytic gliosis is observed in the pontine tegmentum of Tg(ElkPrP) mice (b, f) and in the hippocampus of Tg(OvPrP<sup>++/++</sup>)-14882 mice (j, n). Histoblots of coronal sections reveal little protease-resistant PrP<sup>Sc</sup> in the hippocampus and thalamus (c, g, k, o), but abundant PrP<sup>Sc</sup> deposition in the deep cerebellar nuclei and pontine tegmentum (d, h, i, p). Bars, 50 μm (a) [magnification is the same for (b), (e), (f), (i) and (m)]; 100 μm (n) [magnification is the same for (j)].
794–1290 days (Dagleish et al., 2008). In accord with our findings, BSE prions in bovine brain homogenates were unable to convert cervid PrPC into protease-resistant PrPSc, as measured by a protease-protection assay (Raymond et al., 2000). In our studies, sheep-passaged BSE prions transmitted to Tg(ElkPrP) mice with a median incubation time of 300 days, suggesting that a change in the primary structure of the infectious prions from BoPrP to OvPrP(ARQ) abrogated the species barrier for transmission. It is also conceivable that the strain characteristics of BSE prions changed after passage in sheep, which enabled transmission to Tg(ElkPrP) mice. This hypothesis is supported by the observation that scrapie 027 did not transmit to Tg mice expressing ovine PrP if the primary infection was of sheep scrapie origin. However, CWD prions did not transmit to Tg(OvPrP) mice, as shown in a previous study (Tamgüney et al., 2006) and again here. Furthermore, many of the experimentally adapted scrapie isolates are derived from passage of the SSBP/1 isolate through various hosts, suggesting that this experimental scrapie isolate may have an unusually broad host range (Kimberlin et al., 1989).

On the other hand, the biological properties of prion strains may change on passage in new hosts (Kimberlin et al., 1987). Although SSBP/1 and CWD prions presented with different vacuolation profiles in Tg(ElkPrP) mice after i.c. inoculation, the electrophoretic profile and conformational stability of serially passaged SSBP/1 are similar to those of CWD prions. It is conceivable that a particular scrapie strain might not remain stable upon passage to cervids, and that the resulting CWD prions may have properties different from those of the infecting prions. Furthermore, the i.c. transmission of prions in our laboratory studies may influence the strain characteristics differently from natural, oral infection of cervids in the wild.

While this manuscript was in preparation, another group reported the transmission of SSBP/1 prions to Tg(CerPrP) mice expressing a Prnp allele common to mule deer and white-tailed deer, which encodes glutamine at codon 226 (Green et al., 2008). Our Tg(ElkPrP) mice express glutamate...
at codon 226. The Tg(CerPrP) mice in that study had similar incubation times for SSBP/1 and CWD prions upon first passage, whereas our Tg(ElkPrP) mice showed shorter incubation times (of 70–90 days) with CWD prions. This difference may be due to the higher sensitivity of our mice to CWD prions and/or due to the polymorphism at codon 226.

It is surprising that, despite the coexistence of sheep and cervids in many areas of the world where scrapie is endemic, CWD is restricted to North America. This raises the possibility of the existence of a particular, as-yet-unidentified North American scrapie strain that may cause CWD (Hourrigan et al., 1979; Williams, 2005). While we think it unlikely that sheep-passaged BSE or SSBP/1 could be the cause of CWD, the transmission of sheep-derived prions to Tg mice expressing ElkPrP provides experimental evidence to support the hypothesis that sheep-derived prions may cause natural prion disease in cervids. It remains to be determined whether transmitting larger numbers of natural North American scrapie isolates from sheep with different genotypes, especially from areas with high incidence rates of CWD such as Colorado, Wyoming and Nebraska, by i.c. and oral routes to cervids or mouse models of CWD will identify the origin of CWD.

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REFERENCES


