The elk PRNP codon 132 polymorphism controls cervid and scrapie prion propagation

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INTRODUCTION

The transmissible spongiform encephalopathies, a group of fatal incurable neurodegenerative conditions, include sheep scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) of deer and elk, and human Creutzfeldt–Jakob disease (CJD). The prevailing viewpoint attributes these disorders to prions, defined as subcellular proteinaceous infectious particles that lack informational nucleic acid (Prusiner, 1982). Considerable evidence now supports the once unorthodox hypothesis that prions are composed largely, if not entirely, of a pathogenic conformer of the prion protein (PrP), referred to as PrPSc, which, during disease, imposes its conformation on the normal host-encoded version of PrP, referred to as PrP0, resulting in the exponential accumulation of infectious PrPSc.

Prion diseases frequently occur as epidemics and their capacity to transmit between species is unpredictable. Of particular concern is the emergence of CWD, the only recognized prion disease of wild animals (Williams, 2005). In addition to its distribution in an increasingly wide geographical area of North America, outbreaks in South Korea resulted from importation of subclinically infected animals (Kim et al., 2005; Sohn et al., 2002). Its enigmatic origins, contagious transmission, uncertain strain prevalence and environmental persistence raise the possibility of uncontrolled prion dissemination and bring into question
the risk to other species of developing a CWD-related disease. 

Seminal studies in transgenic (Tg) mice (Prusiner et al., 1990; Scott et al., 1993) and cell-free systems (Kocisko et al., 1994) suggested that the sequences of PrPSc in the inoculum and PrPSc in the host should be isologous for optimal progression of disease. However, the rules governing PrP primary structure control over prion transmission appear not to apply in the case of bank voles, which are susceptible to prions from a number of different mammalian species (Nonno et al., 2006). Prion strain properties are an equally important consideration. Mammalian prion strains are classically defined by their incubation times in susceptible animals (Westaway et al., 2006). Prion strain properties are an equally important consideration. Mammalian prion strains are classically defined by their incubation times in susceptible animals and the profile of central nervous system (CNS) lesions. Since numerous studies indicate that strain diversity is encoded in the tertiary structure of PrPSc (Bessen & Marsh, 1994; Korth et al., 2003; Peretz et al., 2002; Scott et al., 2005; Telling et al., 1996), assessment of different PrPSc types according to the Western blot migration patterns of protease-resistant PrPSc glycoforms, the conformational stability of PrPSc and the neuroanatomic distribution of PrPSc are parameters that have also been used to characterize prion strains (Collinge et al., 1996b; Hecker et al., 1992; Hill et al., 1997; Peretz et al., 2002; Safar et al., 1998; Taraboulos et al., 1992). The unexpectedly wide host range of BSE prions illustrates the capacity of prion strains to cross species barriers and overcome the influence of PrP primary structure.

PrP polymorphisms influence prion pathogenesis in mice (Westaway et al., 1987), sheep (Hunter et al., 2000) and humans (Baker et al., 1991; Collinge et al., 1991; Palmer et al., 1991). The PRNP-coding sequence of elk is polymorphic at codon 132 encoding either methionine (M) or leucine (L) (O’Rourke et al., 1998; Schatzl et al., 1997). Residue 132 in elk is equivalent to the human PRNP codon M129 or valine (V) polymorphism, which is an important factor in sporadic, iatrogenic and familial human prion diseases (Baker et al., 1991; Collinge et al., 1991; Palmer et al., 1991) and plays an important role in controlling the propagation of human prion strains (Collinge et al., 1996b; Wadsworth et al., 1999). To date, all clinical cases of vCJD have only occurred in patients homozygous for M at codon 129 (Collinge et al., 1996a; Zeidler et al., 1997) and analyses in Tg mice confirmed that V129 protects against the development of vCJD (Wadsworth et al., 2004). Since oral transmission experiments in elk suggest that the 132 L allele may protect against CWD (Hamir et al., 2006), and because of the relationship between the cervid codon 132 and the human codon 129 polymorphism, we produced Tg mice expressing cervid PrP (CerPrP) with L at residue 132, referred to as Tg(CerPrP-L132) mice, to address the mechanism by which this polymorphism affects prion pathogenesis.

METHODS

Production and characterization of Tg mice. Tg(CerPrP)1536 and Tg(CerPrP)1534 have been described previously (Browning et al., 2004). These lines were maintained in the hemizygous state by breeding with Prnp0/0 mice and are therefore referred to as Tg(CerPrP)1536 v/v− and Tg(CerPrP)1534 v/v−. To produce Tg mice expressing the L132 polymorphism, referred to as CerPrP-L132, we mutated residue 132 in the CerPrP-coding sequence, originally derived from mule deer PRNP (Browning et al., 2004) by PCR-induced oligonucleotide-mismatch mutagenesis. Prior to cloning into the cosSHa.Tet cosmid (Scott et al., 1992), the CerPrP-L132-coding sequence was verified. Transgene purification, Tg mouse production using FVB/Prnp0/0 mice (Lledo et al., 1996) and genotyping of Tg mice was accomplished using previously published methods (Browning et al., 2004). Estimates of the relative levels of PrP expression in the CNS of F1 offspring compared to the level of PrP in brain extracts from wild-type mice were determined by Western blotting and quantitative immuno-dot blotting by using anti-PrP 6H4 monoclonal antibody (mAb) (Korth et al., 1997) (Prionics AG). Immunoblots were developed using enhanced chemiluminescence (ECL), and exposed to X-ray film or analysed using a FLA-5000 scanner (Fuji). Tg(CerPrP-L132) lines were also maintained in the hemizygous state by breeding with Prnp0/0 mice.

Sources and preparation of brain homogenates. The naturally infected captive mule deer isolates D10 and D999, the 7378 elk isolate and the CWD pool have been described previously (Browning et al., 2004). The 132 M/M (isolate ID #99W12389), 132 M/L (isolate ID #03W3297) and 132 L/L (isolate ID #03W3355) CWD-infected elk of defined PrP genotypes were captive animals from the Wyoming Game and Fish Department’s Sybille Wildlife Research Unit (Supplementary Material available in JGV Online). The SSF(1) isolate originated as a homogenate of three natural scrapie brains subsequently passaged mostly through Cheviot sheep at the Neuropathogenesis Unit, Edinburgh, UK (Dickinson et al., 1989). Homogenates (10%, w/v) in PBS lacking calcium and magnesium ions, of cervid, sheep and mouse brain homogenates, were prepared by repeated extrusion through an 18-gauge followed by a 21-gauge syringe needle.

Determination of incubation periods. Groups of anaesthetized mice were inoculated intracerebrally with 30 μl 1% brain extracts prepared in PBS. Inoculated mice were diagnosed with prion disease following the progressive development of at least three signs including truncal ataxia, ‘plastic’ tail, loss of extensor reflex, difficulty righting and slowed movement. The time from inoculation to the onset of clinical signs is referred to as the incubation period.

Analysis of PrP in CNS. For PrP analysis in brain extracts, total protein content from 10% brain homogenates prepared in PBS was determined by bichinchoninic acid assay (Pierce Biotechnology). Brain extracts were either untreated or treated with 40 μg proteinase K (PK) ml−1 for 1 h at 37 °C in the presence of 2% Sarkosyl and the reaction was terminated with 4 mM PMSF. Proteins were separated by SDS-PAGE, electrophoretically transferred to PVDF-FL membranes (Millipore) that were probed with mAb 6H4 or the Hum-P anti-PrP recombinant Fab (Safar et al., 2002) followed by horseradish peroxidase-conjugated sheep anti-mouse IgG or goat anti-human secondary antibody, respectively, developed using ECL-plus detection (Amersham), and analysed using a FLA-5000 scanner (Fuji). In some cases mice exhibiting neurological dysfunction were humanely killed and their brains were immediately frozen on dry ice. Histoblots of 10 μm thick cryostat sections were generated and transferred to nitrocellulose as described previously (Taraboulos et al., 1992). Histoblots were immunostained with the Hum-P anti-PrP recombinant Fab followed by alkaline phosphatase-conjugated goat anti-human secondary antibody. PrP54 in brain homogenates of terminally sick mice was also analysed by a modified conformational stability assay (Peretz et al., 2002; Scott et al., 2005). Analysis of PrP in the brains of Tg mice by immunohistochemistry was performed as described previously (Muramoto et al., 1997) by using anti-PrP mAb
6H4 as the primary antibody and IgG1, biotinylated goat anti-mouse as the secondary antibody (Southern Biotech). Digitized images for figures were obtained by using a Nikon Eclipse E600 microscope equipped with a Nikon DMX 1200F digital camera, and Metamorph software to view and photograph slides.

**RESULTS**

CWD prions fail to induce disease in Tg(CerPrP-L132) mice

We have previously shown that prion transmission from CWD-affected deer and elk to Tg(CerPrP)1536+/− and Tg(CerPrP)1534+/− mice was characterized by 100% attack rates and mean incubation times ranging from ~225 to 270 days (Browning et al., 2004) (Table 1). Second passage of mule deer isolates D10 and Db99, and elk isolate 7378 in Tg(CerPrP)1536+/− mice resulted in modest reductions in mean incubation times (Table 1). While these slight reductions may result from differences in CWD prion titres in the cervid and Tg mouse brains and/or from CWD prion strain adaptation, these data confirm that Tg(CerPrP)1536+/− mice are completely permissive for CWD prions.

![Table 1. Transmission properties of CWD prions](image)

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Inoculum genotype</th>
<th>Mean ± SEM incubation time (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tg1973+/−</td>
<td>Tg1970+/−</td>
</tr>
<tr>
<td>Mule deer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CWD pool</td>
<td>M/M</td>
<td>&gt;597 (0/6)</td>
</tr>
<tr>
<td>Db99</td>
<td>M/M</td>
<td>&gt;597 (0/6)</td>
</tr>
<tr>
<td>Db99 (p2)</td>
<td>M/M</td>
<td>–</td>
</tr>
<tr>
<td>D10</td>
<td>M/M</td>
<td>&gt;485 (0/7)</td>
</tr>
<tr>
<td>D10 (p2)</td>
<td>M/M</td>
<td>–</td>
</tr>
<tr>
<td>Elk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7378</td>
<td>M/M</td>
<td>&gt;597 (0/5)</td>
</tr>
<tr>
<td>7378 (p2)</td>
<td>M/M</td>
<td>–</td>
</tr>
<tr>
<td>99W12389</td>
<td>M/M</td>
<td>&gt;597 (0/6)</td>
</tr>
<tr>
<td>03W3297</td>
<td>M/L</td>
<td>&gt;597 (0/6)</td>
</tr>
<tr>
<td>03W3355†</td>
<td>L/L</td>
<td>&gt;604 (0/5)</td>
</tr>
<tr>
<td>03W3355†(p2)</td>
<td>M132</td>
<td>&gt;450 (0/8)</td>
</tr>
<tr>
<td>03W3355II</td>
<td>L/L</td>
<td>&gt;602 (0/6)</td>
</tr>
<tr>
<td>03W3355II(p2)</td>
<td>M132</td>
<td>&gt;450 (0/8)</td>
</tr>
<tr>
<td>PBS</td>
<td>–</td>
<td>&gt;595 (0/4)</td>
</tr>
</tbody>
</table>

*The number of mice developing prion disease divided by the original number of inoculated mice is shown in parentheses.
†Previously reported (Browning et al., 2004).
§Brain homogenate.
$Prion disease could only be confirmed in the number of animals represented by the numerator because of intercurrent illnesses in aged mice, which necessitated that mice be humanely euthanized. These illnesses included lung infection and serious dermatitis. In one case an animal was found dead, the animal being apparently cannibalized by cage mate(s). In no case did any intercurrent disease appear to be related to the overexpression of prion protein in Tg(CerPrP)1536+/− mice.
||Retropharangeal lymph node homogenate.

Since these Tg(CerPrP) mice encode CerPrP(C)-M132, we produced Tg(CerPrP-L132) mice to address the effects of the codon 132 PRNP polymorphism. CWD prions that transmitted to Tg(CerPrP)1536+/− and Tg(CerPrP)1534+/− mice uniformly failed to cause disease in Tg(CerPrP-L132)1973+/− and Tg(CerPrP-L132)1973+/− mice up to 600 days post-inoculation (Table 1), after which time asymptomatic-inoculated mice were humanely killed.

Any comparison between the susceptibility of Tg(CerPrP) and Tg(CerPrP-L132) mice must take into account the level of transgene expression. Generally, the level of transgene expression is inversely related to the length of the incubation time (Prusiner et al., 1990). The level of expression in Tg(CerPrP-L132)1970+/− and Tg(CerPrP-L132)1973+/− mice was approximately four-fold higher than the level of PrP(C) in the brains of wild-type FVB mice, while levels of expression in Tg(CerPrP)1536+/− and Tg(CerPrP)1534+/− mice were previously estimated to be approximately five- and threefold higher (Browning et al., 2004). Examples of PrP(C) expression in Tg(CerPrP)1536+/− and Tg(CerPrP-L132)1973+/− mice are shown in Supplementary Fig. S1(a) (available in JGV Online). Since Tg(CerPrP-L132)1973+/− and Tg(CerPrP-L132)1970+/−...
mice express transgene-encoded PrP at levels in the same range as Tg(CerPrP)1534+/− and Tg(CerPrP)1536+/−, and all mice are syngeneic except for the codon 132 polymorphism, we conclude that the differences in susceptibility between the Tg(CerPrP) and Tg(CerPrP-L132) lines are most likely the effect of the 132 polymorphism.

The resistance of animals expressing CerPrP<sup>C</sup>-L132 to CWD is not due to amino acid mismatches at residue 132

DNA sequence analysis of PRNP alleles confirmed that the D10, Db99 and 7378 isolates were from cervids that were homozygous M/M at codon 132 (Table 1). Since disease transmission only occurred in Tg(CerPrP)1536+/− or Tg(CerPrP)1534+/− mice that express CerPrP<sup>M</sup>–M132, we reasoned that the resistance of Tg(CerPrP-L132)1973+/− and Tg(CerPrP-L132)1970+/− mice to these CWD prion isolates might be the direct result of amino acid mismatches at residue 132 between CerPrP<sup>S</sup>–PrP<sup>C</sup>-M132 in the inocula and CerPrP<sup>C</sup>-L132 expressed in the recipient, resulting in inefficient conversion of PrP<sup>C</sup> to PrP<sup>S</sup>. We therefore compared the susceptibility of Tg(CerPrP-L132)1973+/− and Tg(CerPrP)1536+/− mice with prions from CWD-affected elk of different PRNP codon 132 genotypes, namely homozygous 132 M/M (isolate ID# 99W12389), homozygous 132 L/L (isolate ID# 03W3355) or heterozygous 132 M/L (isolate ID# 03W3297). Tg(CerPrP-L132)1973+/− mice remained resistant to disease for up to 60 days following inoculation with all three isolates (Table 1), demonstrating that matching amino acids at residue 132 between CerPrP<sup>S</sup> and CerPrP<sup>C</sup> of the recipient was not sufficient to facilitate CWD prion propagation in Tg(CerPrP-L132)1973+/− mice. Tg(CerPrP)1536+/− mice were susceptible to CWD prions from the 132 L/L 03W3355 elk isolate but with mean incubation times that were ~230–280 days longer, depending on whether inocula originated from brain or retropharyngeal lymph node, than the incubation time of CWD prions from 132 M/M elk (Table 1). Serial passage of infectivity from diseased Tg(CerPrP)1536+/− mice inoculated with prions from the 132 L/L isolate to additional Tg(CerPrP)1536+/− mice resulted in greatly abridged mean incubation times in the range of 240–260 days (Table 1). In contrast, at the time of writing, the same CWD prions had failed to induce disease upon serial passage to Tg(CerPrP-L132)1973+/− mice (Table 1). The ~330 days mean incubation time following primary passage of CWD prions from the 132 M/L isolate was intermediate between the 132 M/M CWD isolates and the 132 L/L isolate (Table 1).

Subclinical CWD prion accumulation in Tg(CerPrP-L132)1973+/− mice

Western blot analysis showing lower levels of CerPrP<sup>S</sup> in the 132 M/L and 132 L/L inocula compared with the 132 M/M 99W12389 elk isolate [Supplementary Fig. S2(a) available in JGV Online] suggested that the longer incubation times in Tg(CerPrP)1536+/− mice of CWD prions from elk expressing the L132 allele were likely an effect of lower CWD prion titres. To address whether Tg(CerPrP-L132)1973+/− mice accumulated reduced CWD titres we asked whether low amounts of CerPrP<sup>S</sup>–L132 were present in the brains of asymptomatic Tg(CerPrP-L132)1973+/− mice. While the brains of sick Tg(CerPrP)1536+/− mice inoculated with CWD prions from all three isolates contained abundant protease-resistant CerPrP<sup>S</sup>–M132, CerPrP<sup>S</sup>–L132 was not detected in our initial analyses of Tg(CerPrP-L132)1973+/− mice (Fig. 1a), consistent with the absence of clinical signs in these mice. Examination of larger quantities of brain materials from all recipients in these three transmission studies revealed the presence of low, but detectable levels of protease-resistant CerPrP<sup>S</sup>–L132 in Tg(CerPrP-L132)1973+/− mice inoculated with the 132 M/M 99W12389 elk isolate, indicating subclinical accumulation of CWD cervid prions consisting of CerPrP<sup>S</sup>–L132 (Fig. 1b), but not in the brains of Tg(CerPrP-L132)1973+/− mice inoculated with the lower titre 132 M/L or 132 L/L inocula (data not shown).

Sheep scrapie prions overcome the inhibitory effects of the CerPrP-L132 allele

As part of a larger study involving Tg modelling of prion species barriers, we also tested the susceptibility of Tg(CerPrP)1536+/− and Tg(CerPrP-L132)1973+/− mice to scrapie prions from the brains of diseased sheep. Primary transmission of the sheep scrapie isolate SSBP/1 (Dickinson et al., 1989) efficiently induced disease in both Tg(CerPrP)1536+/− and Tg(CerPrP-L132)1973+/− mice. Surprisingly, the mean incubation time of SSBP/1 scrapie prions in Tg(CerPrP)1536+/− mice was comparable to that registered for primary transmissions of CWD prions from deer and 132 M/M elk (Tables 1 and 2), suggesting that Tg(CerPrP)1536+/− mice were equally susceptible to CWD and scrapie prions. While reductions in the mean incubation times of mule deer or elk CWD prions were not observed following serial passage in Tg(CerPrP)1536+/− mice (Table 1), serial passage of SSBP/1 prions from diseased Tg(CerPrP)1536+/− or Tg(CerPrP-L132)1973+/− mice resulted in reduced incubation times in Tg mice of either genotype, an effect indicative of prion adaptation following passage through a species barrier. Protease-resistant CerPrP<sup>S</sup> was present in the brains of Tg(CerPrP)1536+/− and Tg(CerPrP-L132)1973+/− mice inoculated with SSBP/1 scrapie prions (Fig. 2) with a subtle, but discernible shift in the molecular mass of PrP27-30 registered on adaptation of SSBP/1 prions in Tg(CerPrP-L132)1973+/− mice.

The ability of prions to overcome the inhibitory effects of the CerPrP-L132 allele are strain dependent

The close association of codon 129 genotypes and PrP<sup>S</sup> types in previous studies indicated that polymorphism at codon 129 plays an important role in the propagation of
human prion strains (Collinge et al., 1996b; Wadsworth et al., 1999). We therefore measured various strain-related parameters to ascertain whether the differential susceptibility of Tg(CerPrP)1536+/− and Tg(CerPrP-L132)1973+/− mice to CWD and SSBP/1 scrapie prions correlated with differences in the strain properties of those prions.

Since previous studies showed the unfolding characteristics of PrPSc to be a sensitive and quantitative means of assessing strain-dependent differences in PrPSc conformation (Peretz et al., 2002; Scott et al., 2005), we measured the conformational stability of CerPrPSc in brain extracts of sick Tg(CerPrP)1536+/− mice infected with CWD or SSBP/1 prions from the M/M elk isolate. Samples were either treated (+) or not treated with PK (−). In the case of untreated samples, 50 μg total protein was loaded, while 250 μg total protein was digested with PK and loaded. The difference in CerPrPSc signal in the un inoculated Tg(CerPrP-L132)1973+/− mouse brain extract may be the result of partial PrPSc degradation. Tg(CerPrP)1536+/− and Tg(CerPrP-L132)1973+/− mice are referred to as Tg1536 and Tg1973, respectively. The positions of protein molecular mass markers at 37, 25 and 20 kDa (from top to bottom) are shown.

Table 2. Transmission of SSBP/1 scrapie prions

<table>
<thead>
<tr>
<th>Inocula</th>
<th>Inoculum genotype</th>
<th>Mean ± SEM incubation time (days)*</th>
</tr>
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<tbody>
<tr>
<td>SSBP/1</td>
<td>M/M</td>
<td>241 ± 16 (8/8) 290 ± 6 (7/7)</td>
</tr>
<tr>
<td>SSBP/1-Cer</td>
<td>L132</td>
<td>165 ± 5 (7/7) 147 ± 7 (8/8)</td>
</tr>
<tr>
<td>SSBP/1-Cer</td>
<td>M132</td>
<td>163 ± 1 (8/8) 189 ± 2 (7/7)</td>
</tr>
<tr>
<td>SSBP/1-Cer</td>
<td>L132</td>
<td>136 ± 3 (8/8) 168 ± 1 (8/8)</td>
</tr>
</tbody>
</table>

*The number of mice developing prion disease divided by the original number of inoculated mice is shown in parentheses.
were significant differences in the glycosylation of CerPrPSC-M132 in the brains of SSBP/1-infected Tg(CerPrP)1536+/− mice (P<0.05 for the di- and unglycosylated bands; unpaired t-test), but no difference in the glycosylation of CerPrPSC-L132 in the brains of SSBP/1-infected Tg(CerPrP-L132)1973+/− mice. We also found statistically significant differences in the proportions of di- and unglycosylated CerPrPSC glycoforms in the brains of Tg(CerPrP)1536+/− mice inoculated with CWD prions from the 132 L/L elk, which disappeared following second passage in Tg(CerPrP)1536+/− mice. Finally, there were also statistically significant differences in the extent of CerPrPSc glycosylation in the brains of asymptomatic Tg(CerPrP-L132)1973+/− inoculated with CWD prions from the 132 M/M 99W12389 elk isolate. Collectively, these findings indicate that the M/L polymorphism at 132 influences CerPrPSc glycosylation but that these differences are primarily the result of the host rather than the agent strain effects.

Histoblot analysis (Taraboulos et al., 1992) is a useful tool for assessing strain-related differences in the global distribution of PrPSc (DeArmond et al., 1993; Hecker et al., 1992; Masriani et al., 1999; Telling et al., 1996). Histoblot patterns of PrP in the brains of SSBP/1-infected Tg(CerPrP)1536+/− and Tg(CerPrP-L132)1973+/− mice showed widespread and diffuse distribution of CerPrPSc (Fig. 5). The most intense PrPSc signals occurred primarily in sites largely devoid of PrPC, a phenomenon detailed in the seminal studies of Taraboulos et al. (1992) and subsequently confirmed in studies of scrapie-infected mice (Yokoyama et al., 2001), and in the brains of BSE- or scrapie-affected cattle and sheep (Kimura et al., 2002). While patterns of immunostaining were not identical among histoblots of Tg(CerPrP)1536+/− mice infected with CWD prions from the 132 M/M, 132 M/L and 132 L/L elk isolates, or compared to previously described Tg(CerPrP)1536+/− mice infected with elk or mule deer CWD prions (Browning et al., 2004), widespread punctate deposition of CerPrPSc-M132 was a consistent feature, which contrasted sharply with the diffuse distribution of CerPrPSc in Tg mice infected with SSBP/1 prions (Fig. 5). Punctate staining, which was not present in uninfected Tg mice [Supplementary Fig. S1(b–e) available in JGVM Online] or in Tg mice infected with SSBP/1 prions (Fig. 5), was seen to greatest effect in the absence of PK treatment, suggesting that a subset of CerPrPSc in these deposits was protease sensitive.

We performed immunohistochemistry to precisely characterize the PrP immunostaining in the brains of CWD-infected Tg(CerPrP)1536+/− mice. While plaque deposition, particularly in the hippocampus, was a neuropathologic feature of diseased Tg(CerPrP)1536+/− mice infected with CWD prions from 132 M/M, 132 M/L and 132 L/L elk isolates (Fig. 6), there were differences in the patterns of PrP immunostaining in all three cases. In Tg mice infected with the 132 M/M and 132 M/L isolates (Fig. 6a and b), plaques were compact and densely stained. Consistent with the histoblot pattern of immunostaining, the corpus callosum, was particularly affected, especially
in the case of Tg mice infected with the 132 M/M isolate (Fig. 6a). In contrast, PrP immunostaining in Tg(CerPrP)1536+/2 mice infected with the 132 L/L isolate was more diffuse, plaques displayed a less regular shape, and were devoid of a central dense core (Fig. 6c).

DISCUSSION

Since our seminal reports of accelerated CWD prion transmission from deer and elk to Tg(CerPrP) mice (Browning et al., 2004), several other groups have reported similar results using comparable Tg mouse models (Kong et al., 2005; LaFauci et al., 2006; Meade-White et al., 2007; Tamguney et al., 2006; Trifilo et al., 2007). In the current study, we used Tg(CerPrP) mice and created Tg(CerPrP-L132) mice to investigate the effects of the elk codon 132 M/L polymorphism on CWD pathogenesis. While Tg(CerPrP)1536+/2 and Tg(CerPrP)1534+/+ mice expressing CerPrP155–M132 are susceptible to CWD prions (Angers et al., 2006; Browning et al., 2004) (Table 1), we show here that Tg(CerPrP-L132)1970+/+ and Tg(CerPrP-L132)1973+/+ mice fail to develop disease following challenge with CWD prions. While two Tg(CerPrP-L132) lines were resistant to CWD prions from elk of all three codon 132 genotypes, the susceptibility of Tg(CerPrP-L132)1973+/+ mice to SSBP/1 prions showed that their resistance to CWD prions was not due to a non-specific deficit in prion replication. We conclude that the differences in susceptibility between Tg(CerPrP) and Tg(CerPrP-L132) mice are most likely the effect of the 132 polymorphism. While our results are consistent with oral transmission experiments of CWD prions in elk of various codon 132 genotypes, which suggested that the L132 allele may protect against CWD (Hamir et al., 2006), our studies provide some insight into the mechanistic basis of this protection.

Since disease transmission of CWD only occurred in Tg(CerPrP) mice expressing CerPrP155–M132, we reasoned that the resistance of Tg(CerPrP-L132) mice might be the direct result of amino acid mismatches at residue 132.

Fig. 3. Conformational stability of PrPSc in the brains of diseased Tg mice provides quantitative assessment of strain differences between CWD and SSBP/1 prions. (a) Densitometric analysis of immunoblots showing the percentage of protease-resistant PrPSc as a function of GdnHCl concentration. The sigmoidal dose–response was plotted using a four-parameter algorithm and non-linear least-square fit. Each point shown is the mean value from three different animals from each study group. Error bars, SEM. Tg(CerPrP)1536+/2 mice inoculated with CWD prions from M/M elk (filled squares), M/L elk (open squares), L/L elk (filled circles) and Tg(CerPrP-L132)1973+/+ mice inoculated with M/M elk (open circles). (b) Conformational stability of PrPSc in Tg(CerPrP)1536+/+ mice (filled triangles) and Tg(CerPrP-L132)1973+/+ mice (open triangles) inoculated with sheep scrapie SSBP/1 prions. Representative examples of Western immunoblots from each assay are shown to the right of the figure. The positions of protein molecular mass markers at 37, 25 and 20 kDa (from top to bottom) are shown.
Since CWD prions from homozygous 132 L/L elk transmit genotypes allowed us to address this hypothesis directly. Characteristics of CWD prions from elk of defined genotypes have been investigated extensively (Collinge et al., 1995). Telling et al. demonstrated the influence of this residue on CJD prion propagation, with incubation times being substantially shortened when the 129 residue was the same in PrPSc of the inoculum and PrPC expressed in the host (Asante et al., 2002; Collinge et al., 1995; Hill et al., 1997; Korth et al., 2003; Telling et al., 1995). The ability to assess the transmission characteristics of CWD prions from elk of defined PRNP genotypes allowed us to address this hypothesis directly. Since CWD prions from homozygous 132 L/L elk transmit to Tg(CerPrP)L132(1973 +/− ) mice but fail to induce disease in Tg(CerPrP-L132)1973 +/− mice, our studies demonstrate that amino acid mismatches at residue 132 between PrPSc in the inoculum and PrPC expressed in the host are not the direct cause of resistance to CWD prions.

Fig. 4. Ratio of three protease-resistant PrP glycoforms produced in the brains of diseased Tg(CerPrP)1536+/− or Tg(CerPrP-L132)1973+/− mice infected with CWD or scrapie prions. Data points represent the mean relative proportions of di-, mono- and unglycosylated PrP as a percentage. Error bars, SEM, which, in some cases, was smaller than the symbols used. Three or four individual diseased mice were analysed in each case. M/M elk, M/L elk and L/L elk refer to the 99W12389, 03W3297 and 03W3355 isolates, respectively. Statistically significant differences in the proportions of di- and unglycosylated PrPSc glycoforms are indicated by asterisks.

While CWD prions from elk or deer of any PRNP genotype were unable to induce disease in Tg(CerPrP-L132) mice, SSBP/1 prions induced the conversion of CerPrPC-L132 and caused disease in Tg(CerPrP-L132)1973+/− mice with a 100% attack rate and rapid incubation times. Notably, prions consisting of ovine PrPSc were able to overcome species-specific PrP primary structural differences in Tg(CerPrP-L132)1973+/− mice and propagate more efficiently than CWD-cervid prions consisting of homologous CerPrpSc. Moreover, the efficiency of SSBP/1 scrapie prion transmission on primary passage in Tg(CerPrP)L1536+/− mice was comparable to that of naturally occurring CWD prions from M/M deer and elk (Tables 1 and 2). Transmission of SSBP/1 prions allowed us to isolate and characterize novel ‘cervid’ prions with strain properties that were distinct from naturally occurring CWD isolates, and to further investigate the effects of the codon 132 polymorphism on the efficiency of replication of this strain. While the shorter incubation times on serial passage of SSBP/1-derived prions in Tg(CerPrP)L1536+/− and Tg(CerPrP-L132)1973+/− mice indicated that transmission was more efficient during isologous conversion of CerPrpC by CerPpSc, the ready transmission of SSBP/1-derived prions comprised of either CerPrpSc-M132 or CerPrpSc-L132 to either line of Tg mice confirmed that the effects of the codon 132 polymorphism on SSBP/1-derived prion transmission were minimal.

The strain properties of SSBP/1-derived prions in Tg(CerPrP)L1536+/− mice, assessed by a number of criteria, including the neuroanatomical distribution and conformational stability profiles of CerPpSc, were different from those of naturally occurring CWD prion isolates. Our findings therefore suggest that the elk 132 polymorphism controls prion susceptibility at the level of prion strain selection. The contrasting ability of CWD and SSBP/1 prions to overcome the inhibitory effects of the CerPrpC-L132 allele is reminiscent of the results of studies detailing the effects of the human codon 129 M/V polymorphism on vCJD/BSE prions in Tg mice, which concluded that human PrP V129 severely restricts propagation of the BSE prion strain (Wadsworth et al., 2004). Our finding that the L132 polymorphism severely restricts propagation of CWD prions is consistent with this interpretation and we speculate that the L132 polymorphism results in less efficient conversion of CerPrpC-L132 by CWD prions, an effect that is overcome by the SSBP/1 strain. Consistent with this interpretation differences in the efficiencies of transmission of CWD prions from 132 M/M, 132 M/L and 132 L/L elk to susceptible Tg(CerPrP)L1536+/− mice, as well as the accumulation of low levels of CerPpSc-L132 in the brains of asymptomatic Tg(CerPrP-L132)1973+/− mice inoculated with CWD prions from M/M elk, appear to be a reflection of differences in CWD prion titres in these various genetic backgrounds. Since the protective effects of the L132 allele against CWD prions are not absolute, our findings suggest that breeding strategies designed to eliminate the more susceptible elk polymorph-
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**Fig. 5.** Regional distribution of PrPSc in the CNS of diseased Tg mice infected with CWD and scrapie prions. PK-treated (+) or untreated (−) histoblotted coronal sections at the level of the hippocampus or midbrain of terminally sick Tg(CerPrP)1536+/− or Tg(CerPrP-L132)1973+/− mice inoculated with CWD prions from elk of different genotypes or SSBP/1 prions.

Isolation might inadvertently establish a carrier state for CWD prions. Whether strain differences exist between CWD prions in the brains of elk expressing M and L at codon 132 awaits the outcome of extensive serial transmission experiments; however, this polymorphism does appear to affect the properties of prions as assessed by PrPSc deposition (Figs 5 and 6) as well as PrPSc conformational stability and glycosylation (Figs 3 and 4).

**Fig. 6.** Immunohistochemical detection of PrPSc in the brains of diseased Tg(CerPrP)1536+/− mice. Sections through the hippocampus of diseased mice inoculated with CWD prions from (a) the M/M 99W12389 elk isolate, (b) the M/L 03W3297 elk isolate and (c) the L/L 03W3355 elk isolate are shown. Haematoxylin was used as counterstain. Bar, 100 μm in all cases.
Recently, Meade-White et al., (2007) demonstrated resistance to CWD in Tg mice expressing serine (S) at residue 96, a naturally occurring allelic variant of white-tailed deer. In contrast to our findings, which indicate that a subclinical carrier state for CWD prion infection may be established in animals expressing CerPrP<sup>PC-L132</sup>, the resistance of Tg mice expressing CerPrP-S96 appeared to be absolute (Meade-White et al., 2007). Interestingly, however, CWD-affected deer homozygous for the S96 allele have been identified (Johnson et al., 2006; O’Rourke et al., 2004), demonstrating that the S96 allele is not absolutely protective against CWD in deer. Unfortunately, only pools of CWD brain materials were available for studies in Tg mice expressing CerPrP-S96 and it was not possible to genotype the brains of the white-tailed deer used in that inculmum pool. While the results of both studies demonstrate the importance of PrP polymorphisms in susceptibility to CWD infection, the ability to assess the strain properties of scrapie prions and CWD isolates from elk of defined genotypes provides important information about the influence of the 132 polymorphism on prion strain selection and demonstrates that the protective effects of cervid PRNP polymorphisms are highly strain dependent.

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