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Chronic wasting disease of deer and elk in transgenic mice: Oral transmission and pathobiology

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Abstract

To study the pathogenesis of chronic wasting disease (CWD) in deer and elk, transgenic (tg) mice were generated that expressed the prion protein (PrP) of deer containing a glycine at amino acid (aa) 96 and a serine at aa 225 under transcriptional control of the murine PrP promoter. This construct was introduced into murine PrP-deficient mice. As anticipated, neither non-tg mice nor PrP ko mice were susceptible when inoculated intracerebrally (i.c.) or orally with CWD brain material (scrapie pool from six mule deer) and followed for 600+ days (dpi). Deer PrP tg mice were not susceptible to i.c. inoculation with murine scrapie. In contrast, a fatal neurologic disease occurred accompanied by conversion of deer PrPsen to PrPres by western blot and immunohistochemistry after either i.c. inoculation with CWD brain into two lines of tg mice studied (312+32 dpi [mean+2 standard errors] for the heterozygous tg line 33, 275+46 dpi for the heterozygous tg line 39 and 210 dpi for the homozygous tg line 33) or after oral inoculation (381+55 dpi for the homozygous tg line 33 and 370+26 dpi for the homozygous tg line 39). Kinetically, following oral inoculation of CWD brain, PrPres was observed by day 200 when mice were clinically healthy in the posterior surface of the dorsum of the tongue primarily in serous and mucous glands, in the intestines, in large cells at the splenic marginal zone that anatomically resembled follicular dendritic cells and macrophages and in the olfactory bulb and brain stem but did not occur in the cerebellum, cerebral cortex or hippocampus or in hearts, lungs and livers of infected mice. After 350 days when mice become clinically ill the cerebellum, cerebral cortex and hippocampus became positive for PrPres and displayed massive spongiosis, neuronal drop out, gliosis and florid plaques.

Keywords: Chronic wasting disease; Scrapie; Oral transmission; Pathobiology

Introduction

Transmissible spongiform encephalopathies (TSE, scrapie or prion) are a group of rare uniformly fatal neurodegenerative diseases of humans and animals (Chesebro, 1999; Prusiner, 2001). Prion diseases occur in various mammalian species, including humans with Kuru and Creutzfeldt-Jakob disease (CJD), cattle with bovine spongiform encephalopathy (BSE) or "mad cow disease", sheep with scrapie and deer and elk with chronic wasting disease (CWD). Disease is associated with the conversion of the normal host PrP (PrPsen) found in most cells to abnormally folded beta sheath enriched protease resistant isoform PrPres (PrPsc). Although prion diseases dramatically damage the central nervous system (CNS), infection as well as the protein PrPres can be detected within peripheral tissues, including lymphoid organs (Bosque et al., 2002; Chesebro, 1999; Peden et al., 2006; Prusiner, 2001), skeletal muscle (Angers et al., 2006; Bosque et al., 2002; Peden et al., 2006), tongue, oral and nasal mucosa (DeJoia et al., 2006; Mulcahy et al., 2004), kidney,[1] pancreas[1] (Heikenwalder et al., 2005), intestine[1], blood and heart (Aguzzi and Glatzel, 2006; Hewitt et al., 2006; Houston et al., 2000; Jewell et al., 2006; Trifilo et al., 2006).

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CWD represents both a major economic and a public health concern (Medicine, 2004). Deer raised on farms and in the wild have CWD. Spread of CWD from one infected deer to others in holding pens strongly suggests horizontal transmission of the disease. This is supported by the recent detection of infectious transmissible material in deer saliva (Mathiason et al., 2006). Although there is a species barrier that usually prevents passage of scrapie from one species to another, it is currently unknown whether CWD in deer and elk is transmissible to humans. Concern about this possibility stems from three observations. First, experimental results indicate that human PrPsen converts to human PrPres *in vitro* in the presence of CWD PrPres (Caughey, 2001; Caughey and Chesebro, 2001). Although conversion is of low efficiency, it is in the range observed when human PrPsen is changed to human PrPres in the presence of BSE PrPres, and BSE has been transmitted to humans (reviewed, Aguzzi and Glatzel, 2006; Chesebro, 1999; Prusiner, 1999). Second, CWD has been transmitted to non-human primates (Marsh et al., 2005). Third, saliva and blood from deer with CWD produced disease after inoculation into uninfected deer hosts (Mathiason et al., 2006), demonstrating that materials which come in contact with humans harbor the infection. However, there is as yet no evidence that CWD can transmit disease to humans as tg mice expressing human PrP fail to develop disease when given deer/elk scrapie (Kong et al., 2005) and public health surveillance of humans in contact with deer has not revealed transmission of disease (Medicine, 2004).

CWD was identified in the late 1960s among captive mule deer at a Colorado research facility and designated as a TSE disease that affects white tailed and mule deer as well as elk (Williams and Young, 1980, 1982). CWD bears many similarities to other TSE diseases, e.g., unremitting neurologic disease with PrPres accumulation, neuronal loss, widespread spongiform formation and gliosis within the CNS, and presence of PrPres in follicular dendritic cells in the spleen. CWD, scrapie in sheep and human vCJD, initially transmitted by BSE, display PrPres prominently in lymphoid tissues.

To understand the spread of infectious material, host genetic susceptibility and the pathogenesis of CWD, we generated a tg mouse model (Meade-White et al., 2007). Here we report the utilization of such tg mice to study infectivity, spread and disease following oral inoculation with CWD brain. After infection, mice homologous for the deer PrP gene remain healthy up to 300 days but show expression of PrPres by both western blot and immunohistochemistry in the tongue, gut, spleen, olfactory bulb and brain stem, but not in the cerebellum, cerebral cortex or hippocampus. After 350 days clinical disease occurs and is associated with both PrPres deposits and spongiform patterns classic of scrapie disease throughout the cerebellum, cerebral cortex, entorhinal cortex and hippocampus. Progression of clinical signs of ataxia, tremor, lethargy and weakness leads to marked disease usually by 380 to 410 days post-infection. At this time the brain displays histopathologic evidence of advanced spongiosis, pyknotic neurons, neuronal drop out, florid plaques and astrogliosis.

Results

Generation and phenotyping of deer PrP tg mice

Reports that deer PrP allelic variation correlated with susceptibility of deer to natural disease suggested a greater susceptibility with glycine instead of a serine at residue 96 of deer PrP and a serine instead of a phenylalanine at position 225 (Jewell et al., 2005; Johnson et al., 2006; O'Rourke et al., 2004) lead to the construction of a plasmid containing deer PrP 96 gly/225 ser under transcriptional control of murine PrP (Meade-White et al., 2007). The resultant DNA fragment was inoculated into fertilized eggs from PrP ko mice (129/Ola back-crossed to C57Bl/6 mice for over six generations) at The Scripps Research Institute transgenic facility. Eggs were then transplanted into pseudo-ovulating foster mothers using standard techniques (Race et al., 1995; Trifilo et al., 2006). Due to the low efficiency employing eggs from PrP ko donors, 642 eggs were transplanted which yielded 14 founder mice, an efficiency slightly over 2% as compared to the usual efficiency of 20 to 30% processing eggs from C57Bl/6 mouse donors. Of the 14 founder mice, two were selected, numbers 33 and 39, to generate lines for subsequent studies because first, they passed the transgene with an expression level roughly equivalent to that of murine PrP in C57Bl/6 mice and, second, bred robustly. Initial we bred heterologous mice (±) by crossing line 33 or line 39 to PrP ko mice. Thereafter homologous (+/+) breeding was done.

Phenotyping to judge the utility of the two deer PrP tg lines was accomplished by inoculating heterologous tg mice from both lines 33 and 39 intracerebrally (i.c.) with a 2% cleared brain homogenate from a pool of CWD (Meade-White et al., 2007) (pool 2). As shown in Table 1, both lines 33 and 39 developed disease with a mean incubation time for line 33 of 312+32 days and for line 39 of 275+46 days. Clinically ill mice displayed ataxia, tremors, reduced movement, kyphosis and leg weakness which was first detected at day 225 in some mice with continuous progression involving all mice until severity of disease required sacrifice. When homologous mice were used these signs appeared approximately 70 days earlier. Histologic examination of brains of severely ill mice displayed classic spongiosis, neuronal drop out and gliosis, similar to but in several mice more intense than the picture usually observed with other TSE diseases. Immunohistochemistry revealed deposition of PrPres but staining with either Thioflavin-S or Congo red failed to document amyloid positive plaques. Western blots, as shown in Fig. 1, documented the conversion of deer PrPsen to deer PrPres. PrPres was glycosylated with diglycosylated PrPres as the predominant species, although the nonglycosylated form was also present. The glycosylated pattern varied among individual mice studied similar to observations for CWD infected deer and elk (Race et al., 2002). By contrast, neither heterologous nor homologous line 33 or 39 deer PrP tg mice when inoculated with diluent of sterile phosphate buffered saline free of CWD brain or either non-tg C57Bl/6 mice or PrP ko mice inoculated with pooled CWD brain developed disease or showed conversion of deer PrPsen to