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Trans-species amplification of PrP^{CWD} and correlation with rigid loop 170N

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ABSTRACT

Chronic wasting disease (CWD) is an efficiently transmitted spongiform encephalopathy of cervids. Whether CWD could represent a threat to non-cervid species remains speculative. Here we show that brain homogenates from several CWD-susceptible non-cervid species, such as ferrets and hamsters, support amplification of PrP^{CWD} by sPMCA, whereas brain homogenates from CWD-resistant species, such as laboratory mice and transgenic mice expressing human PrP^{C} [Tg(HuPrP) mice], do not. We also investigated whether several North American species that share the environment with cervids would support amplification of PrP^{CWD} by sPMCA. Three native rodent species, including voles and field mice, supported PrP^{CWD} amplification, whereas other species (e.g. prairie dog, coyote) did not. Analysis of PrP sequences suggests that an ability to support amplification of PrP^{CWD} in trans-species sPMCA is correlated with the presence of asparagine at position 170 of the substrate species PrP. Serial PMCA may offer insights into species barriers to transmission of CWD.

Introduction

Chronic wasting disease (CWD) of deer, elk and moose is a prion disease first identified in the Rocky Mountain region and now recognized in 15 states, Canada, and one Asian country (Williams, 2005; Williams and Young, 1980). Like other transmissible spongiform encephalopathies (TSEs) such as ovine scrapie, bovine spongiform encephalopathy (BSE) and human Creutzfeldt–Jakob Disease (CJD), CWD is caused by the conversion of normal, protease-sensitive PrP^{C} protein to a misfolded, protease-resistant conformation (PrP^{RES}) which accumulates in the central nervous and lymphoid systems and leads to wasting and spongiform encephalopathy (Sigurdson et al., 2002, 1999; Spraker et al., 2002).

The facile spread of CWD is different from most TSEs and may reflect the transmission of infectious prions from the saliva and excreta of infected cervids (Mathiason et al., 2006; Safar et al., 2008). While the known natural host range for CWD is limited to cervids, some non-cervid species, e.g. ferrets and hamsters, can be infected experimentally (Bartz et al., 1998; Harrington et al., 2008; Raymond et al., 2007; Sigurdson et al., 2008a). Trans-species transmission of prion diseases is infrequent due to the species barrier phenomenon, which may be mediated by differences in PrP^{C} sequence, prion strain, and other still unknown factors (Bartz et al., 1994; Harrington et al., 2008; Kong et al., 2005; Piening et al., 2006; Raymond et al., 2000). Because *in vivo* susceptibility studies in candidate outbred species are protracted and costly, a comprehensive analysis of CWD species barriers by direct *in vivo* exposure has yet to emerge. Thus, the existence of non-cervid reservoirs for CWD in the wild remains conceivable.

The advent of protein misfolding cyclic amplification (PMCA) for *in vitro* prion amplification (Saborio et al., 2001) offers the potential to assess the CWD species barrier by evaluating the permissiveness of a given species brain substrate to support PrP^{C}-to-PrP^{RES} conversion (Jones et al., 2007; Saa et al., 2006; Soto et al., 2005). When PrP^{C} and PrP^{RES} from the same species are used, *in vitro* amplification preserves the biochemical characteristics, infectivity and species barriers of the seed PrP^{RES} (Bossers et al., 1997; Castilla et al., 2008, 2005; Kocisko et al., 1995; Lucassen et al., 2003). We have demonstrated efficient amplification of CWD PrP^{RES} (PrP^{CWD}) by serial protein misfolding cyclic amplification (sPMCA) using transgenic mice [Tg(CerPrP) mice] over-expressing cervid PrP^{C} as brain substrate (Green et al., 2008; Kurt et al., 2007; Meyerett et al., 2008).

The plausibility of trans-species sPMCA is supported by cell-free conversion studies which have shown that some conversion may

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occur when combining PrP^{C} and PrP^{RES} from different species (Kocisko et al., 1994; Piening et al., 2006; Priola et al., 2001; Raymond et al., 2000). Here we apply sPMCA to demonstrate that brain homogenates from species shown to be susceptible to CWD infection *in vivo* also support amplification of CWD prions *in vitro* (e.g. ferrets and hamsters) whereas relatively resistant species [e.g. laboratory mice (*Mus* spp.)] (Browning et al., 2004; Raymond et al., 2007; Sigurdson et al., 2006; Williams and Young, 1980) do not. We extended this approach to include species abundant in North America likely to be exposed to CWD in the wild and that therefore have potential to serve as reservoirs or laboratory models for CWD. Interestingly, we found that all tested species that expressed asparagine at PrP position 170 supported trans-species amplification of PrP^{CWD}. All but one species that expressed serine at PrP position 170 failed to support trans-species amplification of PrP^{CWD}.

Results

Species susceptible to CWD infection in vivo

Deer, Tg(CerPrP) mouse, and ferret brain homogenates support PrP^{CWD} amplification

To determine whether *in vitro* PrP^{CWD} amplification is demonstrable in a susceptible species, we first performed sPMCA using normal-brain homogenates (NBH) from white-tailed deer (*Odocoileus virginianus*), a natural host for CWD. For these experiments, CWD-positive deer brain 104 was diluted 1:10 into NBH from white-tailed deer and subjected to sPMCA with 1:2 dilutions into fresh NBH at each successive round for a total of 4 rounds. We previously reported (Kurt et al., 2007), and here confirm, that deer brain homogenates support ~5 fold increases in PrP^{RES} in sPMCA (Fig. 1A, left panel), thus NBH from the native CWD-susceptible species will support PrP^{C}-to-PrP^{RES} conversion *in vitro*. We extended this work using NBH from Tg(CerPrP)$1536^{+/-}$ mice, which express cervid PrP^{C} at ~4-fold the concentration of that in deer brain. Using Tg(CerPrP)$1536^{+/-}$ NBH and the CWD-positive deer brain D10, PrP^{RES} amplification was at least 100 to 250-fold per round of PMCA (Kurt et al., 2007) and amplification was consistently achieved with starting dilutions up to 1:16,000, whereas the equivalent un-amplified dilutions (−PMCA samples) were not detectable by Western blot (Fig. 1A, right panel).

To initiate trans-species sPMCA studies, we first used NBH from ferrets (*Mustela putorius futo*), a species that is susceptible to CWD (Bartz et al., 1998; Sigurdson et al., 2008a), as a PrP^{C} conversion substrate. Ferret NBH supported amplification at starting dilutions of up to 1:16,000 of D10 (Fig. 1B).

In control experiments, D10 added to PrP-null mouse ($PrP^{0/0}$) brain homogenate did not amplify, indicating that the majority of PrP^{C} which is converted in sPMCA comes from the NBH vs. the PrP^{RES} seed material (not shown).

Species relatively less-susceptible to CWD infection in vivo

Hamster brain homogenates have varying ability to support PrP^{CWD} amplification

Raymond et al. (2007) have demonstrated that Syrian golden (*Mesocricetus auratus*), Chinese (*Cricetulus griseus*) and Armenian (*Cricetulus migratorius*) hamsters are variably susceptible to intracerebral inoculation of CWD. We have recently confirmed the *in vivo* susceptibility of Syrian golden hamsters to CWD (100% infected after inoculation with D10, Hoover lab, unpublished). To investigate differences in the ability of hamster species to support CWD amplification *in vitro*, we harvested NBH from Armenian, Chinese and Syrian golden hamsters for sPMCA. We found that in three experiments, Syrian golden hamster NBH supported amplification of 1:8000–1:16,000 dilutions of PrP^{CWD} (Fig. 2A). Chinese hamster NBH consistently supported amplification of up to 1:2000 dilutions of mule deer PrP^{CWD} (Fig. 2B) and Armenian hamsters supported amplification of up to ~1:1000 dilutions of D10 (Fig. 2C).

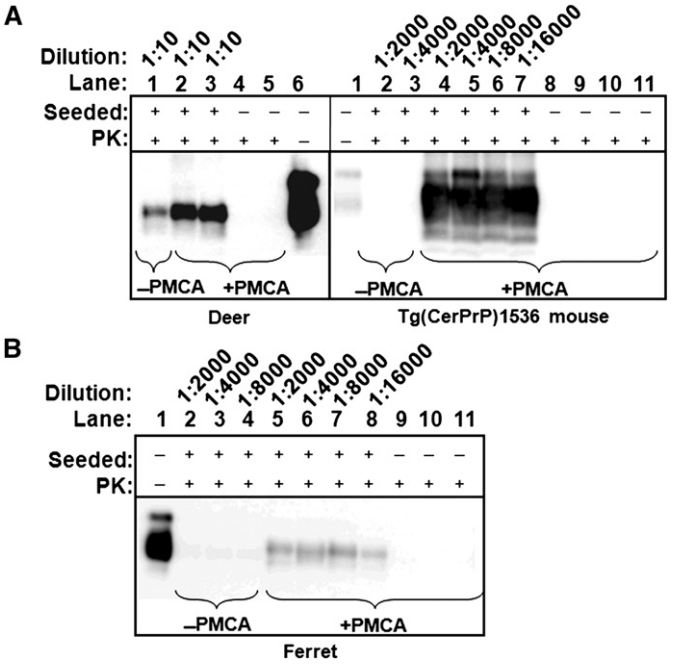

Fig. 1. NBH from deer, Tg(CerPrP)1536 mouse and ferret support amplification of PrP^{CWD}. (A) Left panel: Amplification of PrP^{CWD} in white-tailed deer NBH initiated by diluting CWD-positive brain deer brain 104 1:10 into the NBH. Serial PMCA was performed with 1:2 dilutions into fresh NBH at each subsequent round for a total of 4 rounds. Two replicates each of PrP^{CWD}-seeded (lanes 2–3) and unseeded (lanes 4–5) samples are shown. Lane 1: A dilution (labeled −PMCA) frozen at −70 °C for the duration of the experiment and equivalent to the amplified samples (labeled +PMCA) after sPMCA. Lane 6: Deer NBH only, showing PrP^{C} not digested with PK. (A) Right panel: Amplification in Tg1536 NBH was initiated by 1:2000–1:16,000 dilutions of CWD-positive brain D10, followed by 1:2 dilutions into fresh NBH at each subsequent round for a total of 4 rounds. Lane 1: Tg1536 NBH only, showing PrP^{C} not digested with PK. Lanes 2–3: Dilutions (labeled −PMCA) frozen at −70 °C for the duration of the experiment and equivalent to the amplified samples (labeled +PMCA) after sPMCA. (B) Amplification in ferret NBH was accomplished by 1:2000–1:16,000 dilutions of D10 and 4 rounds of sPMCA. Lane 1: Ferret NBH only, showing PrP^{C} not digested with PK. Lanes 2–3: Dilutions (labeled −PMCA) frozen at −70 °C for the duration of the experiment and equivalent to the amplified samples (labeled +PMCA) after sPMCA. "Seeded": samples seeded (+) or not seeded (−) with CWD-positive brain homogenate, "PK": samples digested (+) or not (−) with proteinase K, "PMCA": samples subjected (+) or not (−) to the sPMCA protocol described.

Mink brain homogenates did not support PrP^{CWD} amplification

American mink (*Mustela vison*) are closely related to ferrets and differ from the latter by very few residues in PrP amino acid sequence (Bartz et al., 1994), however, recent studies suggest that a relatively strong species barrier exists restricting CWD transmission to mink by even the intracranial route (Harrington et al., 2008). In our experiments mink NBH did not support amplification of PrP^{CWD} even when a high concentration (a 1:10 dilution) of D10 seed was used in order to provide as much seed material as possible (Fig. 3). Higher concentrations of D10 were not feasible due to the difficulty in distinguishing potentially new PrP^{RES} from input seed. In these experiments Western blot PrP^{CWD} signals degraded with successive rounds of sPMCA (Fig. 3).

Species relatively resistant to CWD infection in vivo

BALB/c mouse brain failed to support PrP^{CWD} amplification

Common laboratory mouse (*Mus*) strains are considered to be resistant to CWD (Browning et al., 2004; Raymond et al., 2007; Sigurdson et al., 2006). Therefore we evaluated NBH from BALB/c mice (selected as a common laboratory mouse strain expressing wild-type