Generation of a New Form of Human PrP^{Sc} *in Vitro* by Interspecies Transmission from Cervid Prions*□S

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Prion diseases are infectious neurodegenerative disorders that affect humans and animals and that result from the conversion of normal prion protein (PrP^C) into the misfolded prion protein (PrP^{Sc}). Chronic wasting disease (CWD) is a prion disorder of increasing prevalence within the United States that affects a large population of wild and captive deer and elk. Determining the risk of transmission of CWD to humans is of utmost importance, considering that people can be infected by animal prions, resulting in new fatal diseases. To study the possibility that human PrP^C can be converted into the misfolded form by CWD PrP^{Sc}, we performed experiments using the protein misfolding cyclic amplification technique, which mimics *in vitro* the process of prion replication. Our results show that cervid PrP^{Sc} can induce the conversion of human PrP^C but only after the CWD prion strain has been stabilized by successive passages *in vitro* or *in vivo*. Interestingly, the newly generated human PrP^{Sc} exhibits a distinct biochemical pattern that differs from that of any of the currently known forms of human PrP^{Sc}. Our results also have profound implications for understanding the mechanisms of the prion species barrier and indicate that the transmission barrier is a dynamic process that depends on the strain and moreover the degree of adaptation of the strain. If our findings are corroborated by infectivity assays, they will imply that CWD prions have the potential to infect humans and that this ability progressively increases with CWD spreading.

Chronic wasting disease (CWD)[2] is a disorder associated with infectious prions that affects deer, elk, and moose (1, 2). CWD is so far the only prion disease of wild animals; it is highly contagious, and the exact prevalence is currently unknown. Its origin and mechanism of transmission are also unclear. CWD-affected animals show loss of body condition, changes in behavior, ataxia, head tremors, salivation, and somnolence (1). CWD can reduce the growth and size of wild deer and elk populations in areas where the prevalence is high and is of increasing concern in North America. The disease was thought to be limited in the wild to a relatively small endemic area in northeastern Colorado and southeastern Wyoming. However, in the last decade, it has spread east and west of those areas, even across natural borders, and has been reported in 14 states, two Canadian provinces, and South Korea (1, 2). The risk of transmission of CWD to other animal species and especially to humans is unknown. Defining that risk is of utmost importance, considering that transmission of bovine prions to humans resulted in a new disease (variant Creutzfeldt-Jakob disease (CJD)) and that the number of cervids affected by CWD in the United States is increasing (3).

Transmission of prions between different species of mammals is often limited by the species barrier phenomenon (4). Currently, the strength of the barrier between different species is unpredictable and requires bioassay studies to be assessed. These experiments are costly and time-consuming and, in the case of humans, cannot be measured directly but rely on the use of animal models, such as transgenic mice and primates. It is presumed that a large number of hunters in the United States have been in contact with or consumed CWD-infected meat (5). Fortunately, no clinical evidence linking CWD-exposed humans and CJD patients has yet been found (5, 6). Experimental inoculation of CWD prions into squirrel monkeys produced the disease (7, 8); however, transgenic mice expressing human PrP did not (9–11), suggesting that the species barrier between humans and cervids is greater than that between cattle and humans.

To test the possibility that human PrP^C can be converted into the infectious form by CWD PrP^{Sc}, we performed experiments using the protein misfolding cyclic amplification (PMCA) technique. PMCA mimics prion replication *in vitro* at an accelerated speed (12); PrP^{Sc} generated *in vitro* by PMCA has been shown to be infectious to wild-type animals and to maintain the strain properties (13–16). Interestingly, new prion strains can be generated, adapted, and stabilized upon crossing species barriers *in vitro* by PMCA (16, 17). These findings demonstrate that PMCA is a valuable tool to investigate the strength of the barrier between diverse species, its molecular determinants,

and the expected features of the new infectious material produced.

EXPERIMENTAL PROCEDURES

Preparation of Brain Homogenates—Brains from healthy transgenic mice overexpressing either human PrP (129M genotype) (18) or cervid PrP (Tg1536) (19) were extracted after animals were perfused with PBS plus 5 mM EDTA. As inoculum for the PMCA reaction, we used brains from different naturally affected CWD mule deer, cattle with bovine spongiform encephalopathy (BSE), sheep with scrapie, humans affected with diverse forms of CJD, or Tg1536 mice infected with CWD prions. 10% (w/v) brain homogenates were prepared in conversion buffer (PBS containing 150 mM NaCl, 1.0% Triton X-100, and Complete^{TM} protease inhibitor mixture (Roche Applied Science)).

PMCA Procedure—Aliquots (100 μl) of 10% healthy brain homogenate were loaded onto 0.2-ml PCR tubes. Tubes were positioned on an adaptor placed on the plate holder of a microsonicator (Misonix Model 4000), programmed to perform 144 cycles of 30 min of incubation at 37 °C, followed by a 20-s pulse of sonication set at an amplitude of 70. For serial PMCA, after each round of cycles, a 10-μl aliquot of the amplified material was diluted into 90 μl of normal brain homogenate, and a new round of PMCA cycles was performed. In some experiments, 0.05% digitonin and 4 mM EDTA were added to the conversion buffer. The detailed protocol for PMCA, including reagents, solutions and troubleshooting, has been published previously (20, 21).

PrP^{Sc} Detection by Western Blotting after Proteinase K Digestion—The standard procedure to digest PrP^{Sc} consists of subjecting samples to incubation in the presence of proteinase K (PK; 50 μg/ml) for 60 min with shaking at 37 °C. The digestion was stopped by the addition of electrophoresis sample buffer, and protease-resistant PrP was revealed by Western blotting. Proteins were fractionated by SDS-PAGE, electroblotted onto nitrocellulose membrane, and probed with antibody 6D11 (1:5000 dilution) to recognize cervid, cattle, or sheep PrP proteins or with antibody 3F4 (1:10,000 dilution) to detect exclusively human PrP. The immunoreactive bands were visualized by enhanced chemiluminescence assay (Amersham Biosciences) using a UVP image analysis system.

RESULTS

Having validated the PMCA technology to assess the species barrier *in vitro* (16, 17), we investigated the possibility that CWD PrP^{Sc} can convert human PrP^C to PrP^{Sc}. Standard PMCA using samples from three different CWD brains failed to produce a signal for human PrP^{Sc} (Fig. 1*A*). For these studies, we took advantage of the fact that human (but not deer) PrP is detectable with antibody 3F4 (22), so any PK-resistant signal detectable with this antibody corresponds to human PrP^{Sc} material. To further attempt the generation of human PrP^{Sc} from deer, we performed several rounds of PMCA, a process that has been shown to dramatically increase the amplification efficiency (23, 24). As shown in Fig. 1*B*, no human PrP^{Sc} signal was detectable even after three rounds of PMCA. Importantly, the same CWD inoculum efficiently induced the conversion of

deer PrP^C into PrP^{Sc} (Fig. 1*C*). We used brains of transgenic mice expressing human PrP^C as the substrate under conditions that were previously shown to efficiently propagate human PrP^{Sc} from various forms of sporadic and variant CJD (see also supplemental Fig. 1) (14). To further attempt conversion of human PrP^C by deer PrP^{Sc}, we added 0.05% digitonin and 4 mM EDTA to the conversion buffer, which we have found to increase the efficiency of PMCA amplification in human samples (supplemental Fig. 1). However, these conditions were unsuccessful in generating PK-resistant human PrP^{Sc} (Fig. 1*D*). In our previous experiments to cross the mouse-hamster species barrier by PMCA, we found that adding a more concentrated solution of the PrP^{Sc} inoculum was successful in producing interspecies conversion (17). To evaluate this possibility, we used more concentrated CWD material (from 2- to 0.2-fold) as inoculum. Again, no human PrP^{Sc} product was observed under these conditions (Fig. 1*E*). The conclusion from these experiments is that either the deer-human barrier is absolute or the PMCA conditions were not optimal to cross it. In a previous study, Hoover and co-workers (25) were also unable to replicate CWD PrP^{Sc} at the expense of human PrP^C in serial PMCA experiments.

On the basis of our previous studies, we noted that PMCA not only enables interspecies conversion but also allows adaptation of the new strain generated (17). Indeed, a recent study showed that serial rounds of PMCA produce the same adaptation of a natural CWD prion strain as do serial passages *in vivo* (26). Because the CWD prions used in our experiments are derived from naturally affected mule deer, we hypothesized that the CWD strain most likely corresponds to an unstable prion strain. To assess whether strain adaptation by serial PMCA rounds might influence the ability of CWD prions to cross the human species barrier, we performed several passages of CWD PrP^{Sc} into deer PrP^C by PMCA (Fig. 2*A*). For this experiment, six replicate experiments were performed in parallel. Interestingly, after one PMCA round, the deer PrP^{Sc} produced was able to convert human PrP^C with low efficiency (Fig. 2*B*, *upper panel*). One of the six replicates showed a clear protease-resistant band with a molecular weight reminiscent of PrP27–30. Two or three other replicates showed faint immunoreactive bands (Fig. 2*B*, *upper panel*). Moreover, after a second and third passage of CWD PrP^{Sc} into deer PrP^C, a much more clear and efficient conversion of human PrP^C to PrP^{Sc} was observed (Fig. 2*B*, *middle* and *lower panels*). These results suggest that CWD prions might be able to affect human beings but only after the CWD strain has been stabilized by successive passaging. To further test this hypothesis and to determine that the ability to convert human PrP was not an artifact produced by *in vitro* PMCA amplification, we tested samples of CWD that were serially passaged in transgenic mice expressing cervid PrP^C. Samples from the first and second passages *in vivo* in mice from two different inocula were used to trigger conversion of human PrP^C. As shown in Fig. 3, cervid PrP^{Sc} obtained from these two inocula, after either one or two passages in transgenic mice, was able to convert human PrP^C into PK-resistant human PrP^{Sc}.

Interestingly, when the Western blot profile of this newly generated form of human PrP^{Sc} (termed CWD-huPrP^{Sc}) was compared with those of known strains of human prions, it was

clear that CWD-huPrP^{Sc} exhibited a different pattern (Fig. 4*A*). The electrophoretic migration of this protein after PK digestion is similar to that of the type 1 strain of sporadic CJD, but its glycosylation profile is clearly different, showing a highly predominant diglycosylated form (Fig. 4, *A* and *B*). This result suggests that CWD-huPrP^{Sc} corresponds to a new human prion strain. Interestingly, a detailed previous study by Chen and co-workers (6) comparing the biochemical characteristics of PrP^{Sc} from cervids and humans showed that CWD PrP^{Sc} is similar to sporadic CJD MM1 in terms of electrophoretic mobility. How-
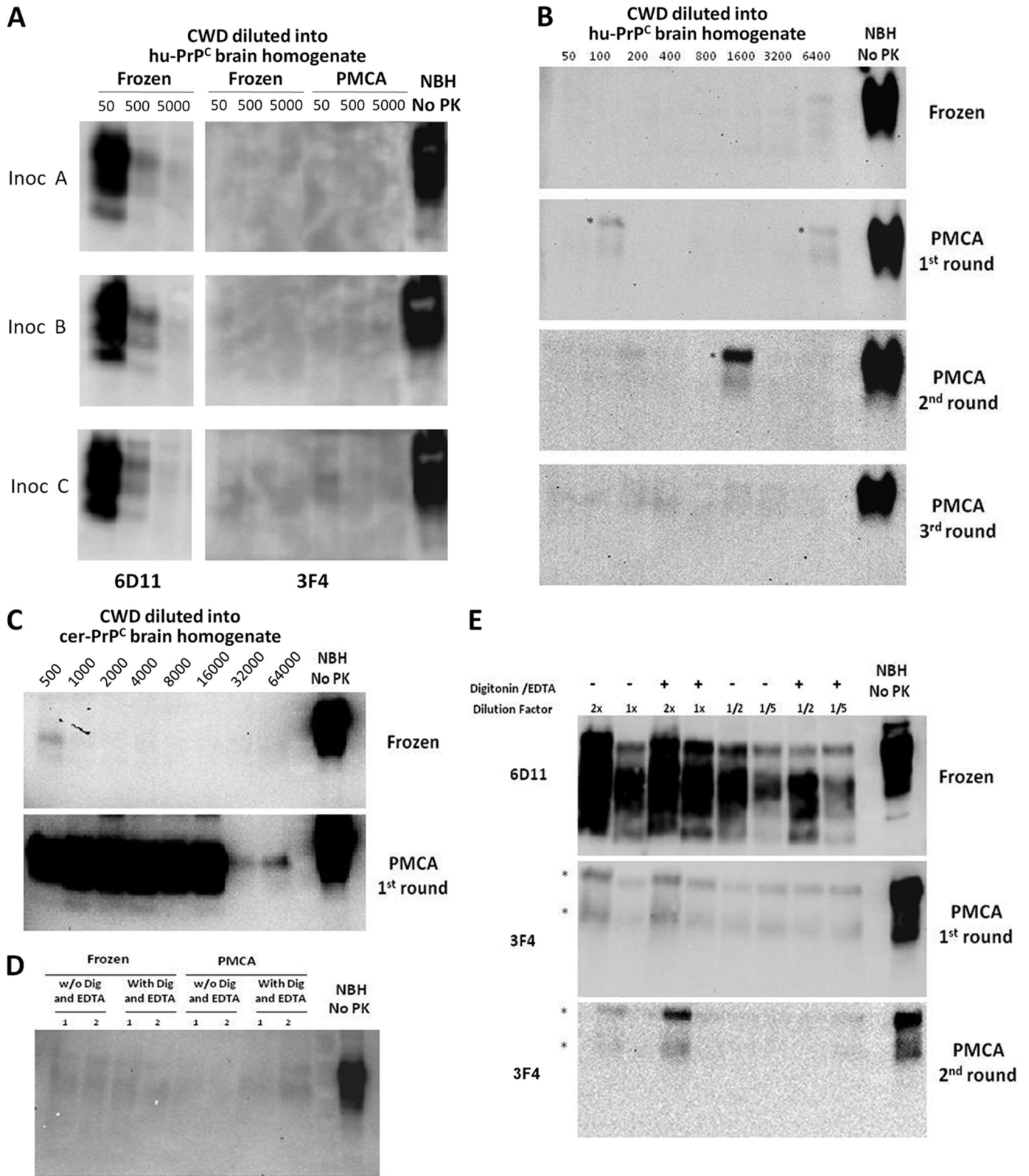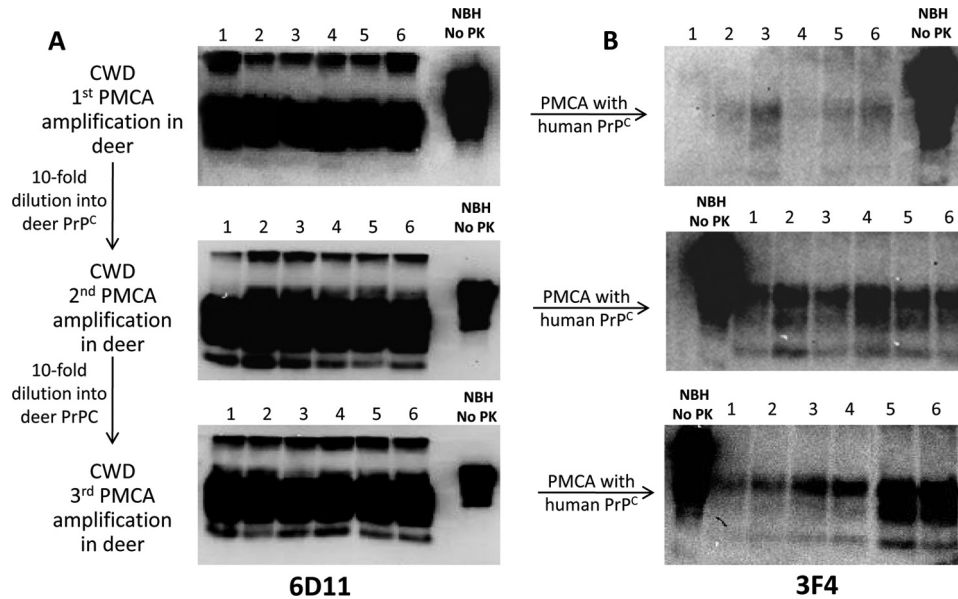
FIGURE 2. Conversion of human PrP^C induced by CWD PrP^Sc after PMCA strain adaptation. *A*, six separate samples of a 1000-fold dilution of CWD brain homogenate (inoculum A) were used to convert cervid PrP^C in three successive rounds of 144 PMCA cycles. Protease-resistant deer PrP^Sc was measured by Western blotting using antibody 6D11. *B*, samples from the amplified material were used to attempt conversion of human PrP^C by PMCA, and the protease-resistant signal was checked using the human-specific antibody 3F4. *NBH No PK*, normal brain homogenate used as a control.
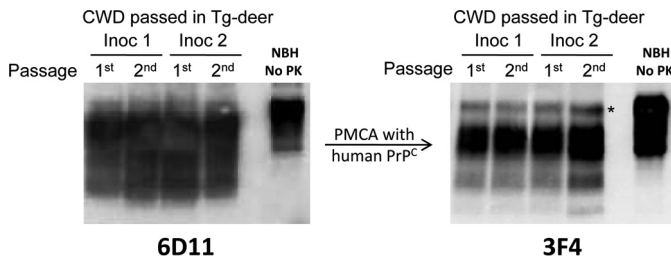

FIGURE 3. Conversion of human PrP^C induced by CWD PrP^Sc after *in vivo* strain adaptation. Transgenic (*Tg*) mice expressing the cervid PrP gene were used to serially passage CWD prions from two different natural inocula (*Inoc*). Brain homogenate from sick transgenic mice after one or two *in vivo* passages was used to convert human PrP^C by subjecting the sample to 144 PMCA cycles. The generation of human PrP^Sc after PMCA was assessed by Western blotting employing antibody 3F4 (*right panel*). The PrP^Sc reactivity of the CWD inoculum from cervid transgenic mice was evaluated by Western blotting using antibody 6D11 (*left panel*). All samples were digested with PK (50 μg/ml), except for the normal brain homogenate (*NBH No PK*), which was used as a marker of full-length PrP^C electrophoretic migration. The *asterisk* represents incomplete digestion of PrP^C. As shown before, amplification of the original CWD inoculum at the expense of human PrP^C failed to show any PK-resistant PrP^Sc band.

ever, the misfolded protein associated with CWD is predominantly diglycosylated, whereas PrP^Sc from type 1 sporadic CJD is mostly monoglycosylated (6). On the basis of the fact that

transmission of BSE prions to humans resulted in a new form of PrP^Sc very similar to the one in cattle (6, 27), these authors predicted that if humans were infected by CWD, it is likely that PrP^Sc would be type 1, with a predominance of the diglycosylated isoform (6). Our results agree with that prediction and suggest that the newly generated CWD-huPrP^Sc acquires the biochemical properties of the cervid infectious material (Fig. 4, *A* and *B*). We and others have shown that PMCA replication of PrP^Sc obtained from experimental rodents, sheep, cervid, and human samples faithfully maintains the prion strain characteristics (14, 16, 26, 28–30). To further support the relevance of our results, we performed experiments in which we attempted to convert human PrP^C by either cattle BSE PrP^Sc or sheep scrapie PrP^Sc. Whereas the typical variant CJD type of PrP^Sc was generated when human PrP^C was converted by BSE PrP^Sc, no human PrP^Sc was generated under any condition when sheep scrapie PrP^Sc was used as inoculum (Fig. 4*B*). These results further validate our PMCA assay.

DISCUSSION

CWD is possibly the most worrisome prion zoonosis because it affects free-ranging animals, making it very difficult to con-

FIGURE 1. Unsuccessful conversion of human PrP^C by CWD PrP^Sc. *A*, three different samples of brain homogenates from mule deer naturally affected with CWD were used to attempt conversion of human PrP^C (*hu-PrP^C*). 10% CWD brain homogenates were diluted 50-, 500-, or 5000-fold into 10% brain homogenates from transgenic mice expressing the human (129M) PrP gene-coding segment. Samples were either frozen or subjected to 144 cycles of PMCA. Formation of PrP^Sc was assessed by PK digestion, followed by Western blotting using antibody 3F4, which recognizes human, but not cervid, PrP (22). The amount of cervid PrP^Sc in the inoculum (*Inoc*) was checked by Western blotting using antibody 6D11. *B*, to further attempt conversion of human PrP^C with cervid prions, several serial rounds of PMCA were done using standard conditions. As reported previously, three serial rounds of PMCA should lead to ~100,000-fold amplification of any PrP^Sc signal present in the sample (24). *C*, the CWD PrP^Sc conversion of deer PrP^C by PMCA was studied using samples of 10% CWD brain homogenate (inoculum A) that were diluted into 10% brain homogenate prepared from healthy transgenic mice expressing cervid PrP^C (*cer-PrP^C*). The formation of cervid PrP^Sc was assessed by Western blotting using antibody 6D11. *D*, crossing the cervid-human species barrier was further attempted by adding detergents and EDTA to the conversion buffer, which has been shown to increase PMCA efficiency (37), particularly for human samples (supplemental Fig. 1). *E*, the CWD inoculum was concentrated by the Sarkosyl procedure described under "Experimental Procedures" to add larger amounts of PrP^Sc. 2-, 1-, 0.5, or 0.2-fold PrP^Sc relative to the 10% CWD brain homogenate was added to the human transgenic mouse brain homogenate and subjected to PMCA in either the presence or absence of digitonin (*Dig*) and EDTA. In the experiments shown in all panels, samples were treated with PK (50 μg/ml), except for the normal brain homogenate (*NBH No PK*) used as control. The *asterisks* in *B* and *E* represent incomplete digestion of PrP^C, which is clearly appreciated because the electrophoretic mobility is identical to that of full-length PrP^C.
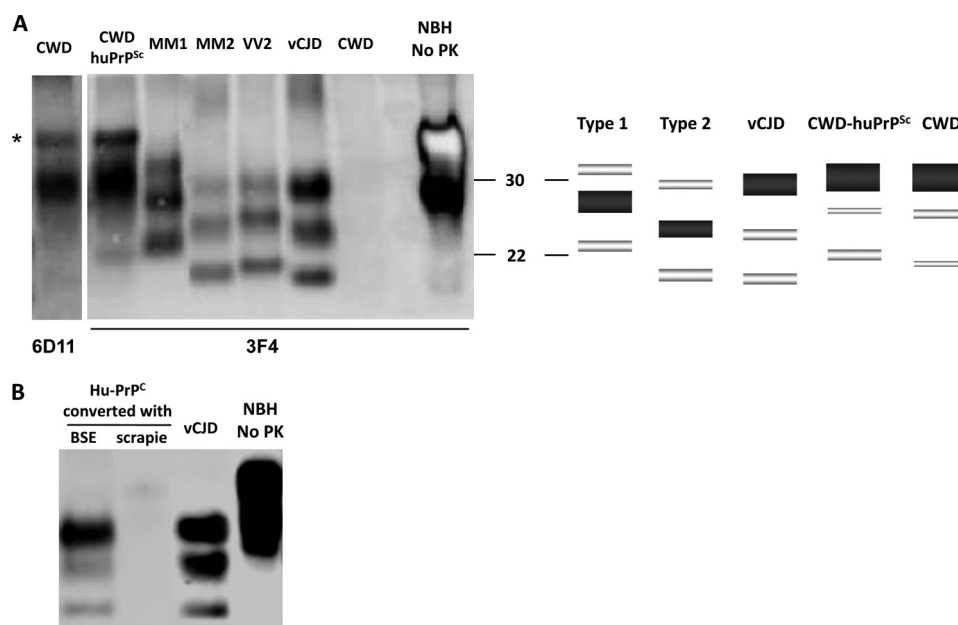
FIGURE 4. **Western blot profile of human PrP^{Sc} generated *in vitro* from CWD.** *A*, aliquots of CWD-huPrP^{Sc} obtained by *in vitro* conversion of human PrP^C with CWD after two rounds of PMCA in deer (see Fig. 2) were loaded together with huPrP^{Sc} associated with variant CJD (*vCJD*) and sporadic CJD types MM1 and MM2. Samples were treated with PK and developed using antibody 3F4. A schematic representation of the mobility and intensity of the different PrP glycoforms in each sample is shown to the right. *B*, samples of PrP^{Sc} from either cattle affected with BSE or sheep with typical scrapie were used to induce conversion of human PrP^C (*Hu-PrP^C*) by 144 PMCA cycles. Newly generated human PrP^{Sc} was detected by Western blotting using antibody 3F4, which recognizes human, but not cattle or sheep, PrP. As a control of the electrophoretic pattern of human PrP^{Sc} associated with variant CJD, a brain sample from a patient affected with this disease was loaded. The *asterisk* represents a signal coming from incomplete digestion of PrP^C. *NBH No PK*, normal brain homogenate used as a control.

trol its spread, and because it is highly efficiently transmitted (1, 2). Indeed, in dense free-ranging cervid populations, CWD prevalence can reach as high as 30%, and among captive herds, the prevalence can climb to nearly 100%. The mechanisms and routes of transmission are currently unknown but likely involve horizontal spread through exposure to prion-infected secretions, excretions, or decomposed carcasses (1, 2). Moreover, it is likely that CWD prions are progressively accumulating in the environment because PrP^{Sc} binds tightly to soil and can maintain infectivity for a long time (31–33). Currently, it is unknown what proportion of natural CWD cases arises sporadically or comes from horizontal transmission among animals. Based on the available knowledge of the emergence, adaptation, and stabilization of prion strains, it is likely that prions appear either spontaneously, through interspecies transmission, or by genetic mutations. These "first generation" prions are unstable strains that begin a progressive and gradual process of adaptation that may take several passages and years or decades to complete. In addition, natural strain stabilization may take considerably more time than the controlled adaptation done by intracerebral inoculation of brain homogenates in experimental animals. In natural cases, animals usually get infected by peripheral (most likely oral) exposure to small quantities of prions present in peripheral tissues or secretion fluids. Recent data indicate that, in some cases, the strain characteristics of natural prions in peripheral organs are different from those in the brain even in the same individuals.[3,4] In cervids, there are at least two different strains that can be differentiated by the incubation time and neuropathological characteristics produced when

inoculated into transgenic mice expressing deer PrP (34). The susceptibility of these two strains to human transmission is currently unknown.

Our findings demonstrate that upon strain adaptation by serial passages *in vitro* or in cervid transgenic mice, cervid PrP^{Sc} is capable of converting human PrP^C to produce PrP^{Sc} with unique biochemical properties, likely representing a new human prion strain. The newly generated CWD-huPrP^{Sc} material has been inoculated into transgenic mice expressing human PrP to study infectivity and disease phenotype, and these data will be published elsewhere. It is highly unlikely that the human PrP^{Sc} generated in these studies is coming from spontaneous "*de novo* generation" because, under the conditions used, no spontaneous PK-resistant band was detected in brain homogenates of humans or transgenic mice expressing human PrP^C, even after >20 serial rounds of PMCA (35). Furthermore, none of the many controls included in our experiments in which no PrP^{Sc} was added to the reaction showed any PK-resistant PrP band.

Analyses of the transmission of CWD to transgenic mice expressing human PrP have consistently given negative results (9–11), indicating a strong species barrier. This conclusion is consistent with our many failed attempts to convert human PrP^C with natural CWD, even after pushing the PMCA conditions (see Fig. 1). We found successful conversion only after adaptation of the CWD prion strain by successive passages *in vitro* or in cervid transgenic mice. We are not aware that the inoculum used in any of the transgenic mice studies was a previously stabilized CWD strain. Although it has been shown that strain stabilization *in vitro* by PMCA (17, 26) and *in vivo* using experimental rodents (36) has similarities to the strain adapta-

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[3] G. C. Telling, unpublished data.
[4] M. A. Barria and C. Soto, unpublished data.

tion process occurring in natural hosts, we cannot rule out that the type of CWD strain adaptation that is required to produce strains transmissible to humans may take much longer time in cervids or not occur at all. An important experiment will be to study transmissibility to humanized transgenic mice of CWD passaged experimentally in deer several times.

Besides the importance of our results for public health in relation to the putative transmissibility of CWD to humans, our data also illustrate a very important and novel scientific concept related to the mechanism of prion transmission across species barriers. Today, the view is that the species barrier is controlled mostly by the degree of similarity of the sequence of the prion protein between the host and the infectious material (4). In our study, we have shown that the strain and, moreover, the stabilization of the strain play a major role in the interspecies transmission. In our system, there is no change in the protein sequence, yet strain adaptation results in a complete change in prion transmissibility with potentially dramatic consequences. Therefore, our findings lead to a new view of the species barrier that should not be seen as a static process but rather a dynamic biological phenomenon that can change over time when prion strains mature and evolve. It remains to be investigated whether other species barriers also change upon progressive strain adaptation of other prion forms (*e.g.* the sheep-human barrier).

Our results have far-reaching implications for human health because they indicate that cervid PrP^Sc can trigger the conversion of human PrP^C into PrP^Sc, suggesting that CWD might be infectious to humans. Interestingly, our findings suggest that unstable strains from CWD-affected animals might not be a problem for humans, but upon strain stabilization by successive passages in the wild, this disease might become progressively more transmissible to man.

REFERENCES

1. Miller, M. W., and Williams, E. S. (2004) *Curr. Top. Microbiol. Immunol.* **284,** 193–214
2. Sigurdson, C. J., and Aguzzi, A. (2007) *Biochim. Biophys. Acta* **1772,** 610–618
3. Bosque, P. J. (2002) *Curr. Neurol. Neurosci. Rep.* **2,** 488–495
4. Moore, R. A., Vorberg, I., and Priola, S. A. (2005) *Arch. Virol. Suppl.* **19,** 187–202
5. Belay, E. D., Gambetti, P., Schonberger, L. B., Parchi, P., Lyon, D. R., Capellari, S., McQuiston, J. H., Bradley, K., Dowdle, G., Crutcher, J. M., and Nichols, C. R. (2001) *Arch. Neurol.* **58,** 1673–1678
6. Xie, Z., O'Rourke, K. I., Dong, Z., Jenny, A. L., Langenberg, J. A., Belay, E. D., Schonberger, L. B., Petersen, R. B., Zou, W., Kong, Q., Gambetti, P., and Chen, S. G. (2006) *J. Biol. Chem.* **281,** 4199–4206
7. Marsh, R. F., Kincaid, A. E., Bessen, R. A., and Bartz, J. C. (2005) *J. Virol.* **79,** 13794–13796
8. Race, B., Meade-White, K. D., Miller, M. W., Barbian, K. D., Rubenstein, R., LaFauci, G., Cervenakova, L., Favara, C., Gardner, D., Long, D., Parnell, M., Striebel, J., Priola, S. A., Ward, A., Williams, E. S., Race, R., and Chesebro, B. (2009) *Emerg. Infect. Dis.* **15,** 1366–1376
9. Kong, Q., Huang, S., Zou, W., Vanegas, D., Wang, M., Wu, D., Yuan, J., Zheng, M., Bai, H., Deng, H., Chen, K., Jenny, A. L., O'Rourke, K., Belay, E. D., Schonberger, L. B., Petersen, R. B., Sy, M. S., Chen, S. G., and Gambetti, P. (2005) *J. Neurosci.* **25,** 7944–7949
10. Tamgüney, G., Giles, K., Bouzamondo-Bernstein, E., Bosque, P. J., Miller, M. W., Safar, J., DeArmond, S. J., and Prusiner, S. B. (2006) *J. Virol.* **80,** 9104–9114
11. Sandberg, M. K., Al-Doujaily, H., Sigurdson, C. J., Glatzel, M., O'Malley, C., Powell, C., Asante, E. A., Linehan, J. M., Brandner, S., Wadsworth, J. D., and Collinge, J. (2010) *J. Gen. Virol.* **91,** 2651–2657
12. Saborio, G. P., Permanne, B., and Soto, C. (2001) *Nature* **411,** 810–813
13. Castilla, J., Saá, P., Hetz, C., and Soto, C. (2005) *Cell* **121,** 195–206
14. Castilla, J., Morales, R., Saá, P., Barria, M., Gambetti, P., and Soto, C. (2008) *EMBO J.* **27,** 2557–2566
15. Deleault, N. R., Harris, B. T., Rees, J. R., and Supattapone, S. (2007) *Proc. Natl. Acad. Sci. U.S.A.* **104,** 9741–9746
16. Green, K. M., Castilla, J., Seward, T. S., Napier, D. L., Jewell, J. E., Soto, C., and Telling, G. C. (2008) *PLoS Pathog.* **4,** e1000139
17. Castilla, J., Gonzalez-Romero, D., Saá, P., Morales, R., De Castro, J., and Soto, C. (2008) *Cell* **134,** 757–768
18. Telling, G. C., Scott, M., Mastrianni, J., Gabizon, R., Torchia, M., Cohen, F. E., DeArmond, S. J., and Prusiner, S. B. (1995) *Cell* **83,** 79–90
19. Browning, S. R., Mason, G. L., Seward, T., Green, M., Eliason, G. A., Mathiason, C., Miller, M. W., Williams, E. S., Hoover, E., and Telling, G. C. (2004) *J. Virol.* **78,** 13345–13350
20. Castilla, J., Saá, P., Morales, R., Abid, K., Maundrell, K., and Soto, C. (2006) *Methods Enzymol.* **412,** 3–21
21. Saá, P., Castilla, J., and Soto, C. (2005) *Methods Mol. Biol.* **299,** 53–65
22. Kascsak, R. J., Rubenstein, R., Merz, P. A., Tonna-DeMasi, M., Fersko, R., Carp, R. I., Wisniewski, H. M., and Diringer, H. (1987) *J. Virol.* **61,** 3688–3693
23. Castilla, J., Saá, P., and Soto, C. (2005) *Nat. Med.* **11,** 982–985
24. Saá, P., Castilla, J., and Soto, C. (2006) *J. Biol. Chem.* **281,** 35245–35252
25. Kurt, T. D., Telling, G. C., Zabel, M. D., and Hoover, E. A. (2009) *Virology* **387,** 235–243
26. Meyerett, C., Michel, B., Pulford, B., Spraker, T. R., Nichols, T. A., Johnson, T., Kurt, T., Hoover, E. A., Telling, G. C., and Zabel, M. D. (2008) *Virology* **382,** 267–276
27. Collinge, J., Sidle, K. C., Meads, J., Ironside, J., and Hill, A. F. (1996) *Nature* **383,** 685–690
28. Soto, C., Anderes, L., Suardi, S., Cardone, F., Castilla, J., Frossard, M. J., Peano, S., Saa, P., Limido, L., Carbonatto, M., Ironside, J., Torres, J. M., Pocchiari, M., and Tagliavini, F. (2005) *FEBS Lett.* **579,** 638–642
29. Jones, M., Peden, A. H., Wight, D., Prowse, C., Macgregor, I., Manson, J., Turner, M., Ironside, J. W., and Head, M. W. (2008) *Neuroreport* **19,** 1783–1786
30. Thorne, L., and Terry, L. A. (2008) *J. Gen. Virol.* **89,** 3177–3184
31. Seidel, B., Thomzig, A., Buschmann, A., Groschup, M. H., Peters, R., Beekes, M., and Terytze, K. (2007) *PLoS ONE* **2,** e435
32. Brown, P., and Gajdusek, D. C. (1991) *Lancet* **337,** 269–270
33. Johnson, C. J., Pedersen, J. A., Chappell, R. J., McKenzie, D., and Aiken, J. M. (2007) *PLoS Pathog.* **3,** e93
34. Angers, R. C., Kang, H. E., Napier, D., Browning, S., Seward, T., Mathiason, C., Balachandran, A., McKenzie, D., Castilla, J., Soto, C., Jewell, J., Graham, C., Hoover, E. A., and Telling, G. C. (2010) *Science* **328,** 1154–1158
35. Barria, M. A., Mukherjee, A., Gonzalez-Romero, D., Morales, R., and Soto, C. (2009) *PLoS Pathog.* **5,** e1000421
36. Race, R., Meade-White, K., Raines, A., Raymond, G. J., Caughey, B., and Chesebro, B. (2002) *J. Infect. Dis.* **186,** S166–S170
37. Murayama, Y., Yoshioka, M., Yokoyama, T., Iwamaru, Y., Imamura, M., Masujin, K., Yoshiba, S., and Mohri, S. (2007) *Neurosci. Lett.* **413,** 270–273