Allelic Interference in Prion Replication Is Modulated by the Convertibility of the Interfering PrP\textsuperscript{C} and Other Host-Specific Factors

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ABSTRACT Early studies in transgenic mouse lines have shown that the coexpression of endogenous murine prion protein (PrP\textsuperscript{C}) and transgenic PrP\textsuperscript{C} from another species either inhibits or allows the propagation of prions, depending on the infecting prion strain and interacting protein species. The way whereby this phenomenon, so-called "interference," is modulated remains to be determined. In this study, different transgenic mouse lines were crossed to produce mice coexpressing bovine and porcine PrP\textsuperscript{C}, bovine and murine PrP\textsuperscript{C}, or murine and porcine PrP\textsuperscript{C}. These animals and their respective hemizygous controls were inoculated with several prion strains from different sources (cattle, mice, and pigs) to examine the effects of the simultaneous presence of PrP\textsuperscript{C} from two different species. Our results indicate interference with the infection process, manifested as extended survival times and reduced attack rates. The interference with the infectious process was reduced or absent when the potentiality interfering PrP\textsuperscript{C} species was efficiently converted by the inoculated agent. However, the propagation of the endogenous murine PrP\textsuperscript{Sc} was favored, allowing us to speculate that host-specific factors may disturb the interference caused by the coexpression of an exogenous second PrP\textsuperscript{C}.

IMPORTANCE Prion propagation can be interfered with by the expression of a second prion protein in the host. In the present study, we investigated prion propagation in a host expressing two different prion protein genes. Our findings indicate that the ability of the second prion protein to interfere with prion propagation is related to the transmissibility of the prion in the host expressing only the interfering prion protein. The interference detected occurs in a prion strain-dependent manner. Interestingly, a bias favoring the propagation of the murine PrP allele has been observed. These results open the door to future studies in order to determine the role of host factors other than the PrP amino acid sequence in the interference in prion propagation.

KEYWORDS BSE, prion interference, prion propagation, prion replication, prion strain, scrapie

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases that affect humans and animals. TSEs are also called prion diseases because the causal agents are infectious particles essentially composed of a misfolded isoform (PrP\textsuperscript{Sc}) of the cellular prion protein (PrP\textsuperscript{C}) (1, 2). PrP\textsuperscript{Sc} is propagated via a template-assisted process involving physical interaction between the PrP\textsuperscript{Sc} template and the PrP\textsuperscript{C} substrate rendering a structurally modified PrP\textsuperscript{Sc} with a higher \(\beta\)-sheet content,
which is prone to aggregation (3). PrPSc was originally defined according to its relative protease resistance and detergent insolubility compared with normal PrPC (4, 5). However, disease-related forms of PrPSc that are protease sensitive have been described (6, 7). Distinct prion strains have been described. These strains are not encoded by differences in the PrP primary structure but show distinct disease phenotypes when transmitted to the same host, such as PrPSc biochemical features, distributions of prion deposits, clinical symptoms, and survival times (8).

PrPC conversion into PrPSc is a posttranslational process. The molecular mechanisms underlying transmission of the strain-specific features of PrPSc are still unclear. It has been demonstrated that although originating from the same host, the PrPSc molecules of different prion strains vary in conformation and/or composition (9). Understanding PrPSc-PrPC interaction is a key step to elucidate the molecular mechanisms of prion propagation. Differences in the primary PrPSc amino acid sequence may alter the ability of a specific PrPC to be efficiently converted into PrPSc. Hence, a heterologous PrPC may be conversion incompetent and thus could interfere with the conversion of a coexisting homologous—conversion-competent—PrPC. It has been proposed that interactions between dissimilar PrPC and PrPSc molecules could slow down the aggregation and deposition of PrPSc by impairing interactions between homologous PrP monomers (10). This phenomenon is known as transdominant inhibition (11). Moreover, in a TSE-affected brain, different prion conformers may coexist and undergo competitive selection during replication, where the faster replication subset of conformers may be progressively selected (12).

Bovine spongiform encephalopathy (BSE), a TSE that affects cattle, was first reported in 1980 in the United Kingdom but soon attained epidemic proportions in several other European countries (13). The experimental finding that variant Creutzfeldt-Jacob disease (vCJD) diagnosed in humans was caused by BSE prions led to a major human and animal health crisis (14–16). The BSE agent has demonstrated a particularly good capacity to cross species barriers. Thus, besides humans, BSE has been transmitted to a range of zoo animals, cats (17–19), and goats (20, 21), while preserving its strain-specific signature (22, 23). Moreover, two more BSE strains have been described. These strains, called L-type BSE (24) and H-type BSE (25) due to their respective low and high electrophoretic mobilities compared to epidemic BSE, are also known as atypical BSE agents. Similarly, several scrapie strains have been identified (26–29). Different prion strains present different levels of transmissibility to another species. Therefore, in prion transmission between different species, both the strain and the PrP sequence of the recipient host are primary determinants of the species barrier (also called strain barrier) (30). However, while PrP is the major determinant for prion propagation, additional species-specific factors may have an influence on the prion propagated in a host-dependent manner (31, 32).

Transgenic mice expressing the PrPC of different species are good experimental models of prion transmission (33, 34). Early experiments in one transgenic mouse line expressing both endogenous murine PrPC and genetically engineered hamster PrPC (35) revealed that the inoculation of these mice with hamster-adapted scrapie produced a prion infection characteristic of hamsters. This was the first evidence of the pivotal role of the PrPC species in the prion infectious event. Nevertheless, the expression of endogenous murine PrPC in the hamster transgenic mice allowed the propagation of mouse or hamster prions, suggesting the compatibility of both mouse and hamster PrP sequences in the replication of the infectious agent. Although the transgenic mice were able to produce both hamster and mouse prions, they were found to selectively produce one or the other, depending on the inoculum used (36). Prion infection studies are generally performed using transgenic mice that express the PrPC of a particular species in a context of murine PrP knockout (KO). In fact, early studies in transgenic mice overexpressing human PrPC showed that these mice were only efficiently infected with the sporadic form of Creutzfeldt-Jacob disease in the absence of murine PrPC expression (37).
brain protease-resistant PrP (PrPres) positivity was observed after inoculation with the presence of an interfering PrP (see Materials and Methods).

Prion Replication Interference of transgenic mice expressing different pairs of PrPC species on prion replication, we produced transgenic mice expressing different combinations of bovine, porcine, and murine PrPC were inoculated with prions from different sources. In these combinations, the inoculated PrPSc was either identical to one of the expressed PrPC proteins in the presence of PrPC from two different species was evaluated, as only murine PrPC is in its natural host (the mouse), while either bovine or porcine PrPC is not.

RESULTS
To examine the effect of the simultaneous presence of PrPC from two different species on prion replication, we produced transgenic mice expressing different pairs of PrPC: (i) murine and bovine PrPC (TgMo/TgBo mice), (ii) murine and porcine PrPC (TgMo/TgPo mice), and (iii) bovine and porcine PrPC (TgBo/TgPo mice). Transgenic mouse lines PoPrP-Tg001, BoPrP-Tg110 and Tga20 were used to generate the animals coexpressing two different PrPC proteins (Table 1). The brain PrPC expression levels are similar for the BoPrP-Tg110 and Tga20 mouse lines but lower in the case of PoPrP-Tg001 (Table 1). The newly generated heterozygous mice and their hemizygous controls (TgMo/Tg001 mouse line (see Fig. S1 in the supplemental material). The newly generated heterozygous mice and their hemizygous controls (TgMo/Tg001 mouse line (see Fig. S1 in the supplemental material). The newly generated heterozygous mice and their hemizygous controls (TgMo/Tg001 mouse line (see Fig. S1 in the supplemental material). The newly generated heterozygous mice and their hemizygous controls (TgMo/Tg001 mouse line (see Fig. S1 in the supplemental material). The newly generated heterozygous mice and their hemizygous controls (TgMo/Tg001 mouse line (see Fig. S1 in the supplemental material).

The present study was designed to further explore the effects of the simultaneous presence of PrPC from two different species on prion replication. To this aim, a collection of transgenic mice expressing different combinations of bovine, porcine, and murine PrPC were inoculated with prions from different sources. In these combinations, the inoculated PrPSc was either identical to one of the expressed PrPC proteins in the mouse or not. Furthermore, the influence of host-specific factors on prion propagation in the presence of PrPC of two different species was evaluated, as only murine PrPC is in its natural host (the mouse), while either bovine or porcine PrPC is not.

**TABLE 1 Description of the mice used in the study**

| Genotype          | PrPC expressed | Expression level | Abbreviation used in text | Description                                           |
|-------------------|----------------|-----------------|---------------------------|-------------------------------------------------------|
| muPrnp<sup>+/−</sup> | Murine expressed | 10×             | TgMo                      | Tga20 mouse line (47)                                 |
| boPrnp<sup>+/−</sup> | Bovine         | 1×              | TgBo                      | BoPrP-Tg110 mouse line (46)                           |
| poPrnp<sup>+/−</sup> | Porcine        | 4×              | TgPo                      | PoPrP-Tg001 mouse line (38)                           |
| KOPrnp<sup>+/−</sup> | Murine         | 0               | Prnp<sup>−/−</sup>         | PrP knockout (Prnp<sup>−/−</sup>)                     |
| muPrnp<sup>+/−</sup> | Murine         | 5×              | TgMo/−                    | Progeny Tga20 (muPrnp<sup>+/−</sup> × PrP knockout (Prnp<sup>−/−</sup>) |
| boPrnp<sup>+/−</sup> | Bovine         | 4×              | TgBo/−                    | Progeny BoPrP-Tg110 (boPrnp<sup>+/−</sup> × PrP knockout (Prnp<sup>−/−</sup>) |
| poPrnp<sup>+/−</sup> | Porcine        | 2×              | TgPo/−                    | Progeny PoPrP-Tg001 (poPrnp<sup>+/−</sup> × PrP knockout (Prnp<sup>−/−</sup>) |
| muPrnp<sup>+/−</sup>, boPrnp<sup>+/−</sup> | Murine and bovine | 0.5×          | C57BL/6/−                 | Progeny C57BL/6 × PrP knockout (Prnp<sup>−/−</sup>) |
| muPrnp<sup>+/−</sup>, boPrnp<sup>+/−</sup> | Murine and bovine | 5×             | C57BL/6/TgBo              | Progeny C57BL/6 × BoPrP-Tg110                         |
| poPrnp<sup>+/−</sup>, boPrnp<sup>+/−</sup> | Porcine and bovine | 4×             | TgPo/TgBo                 | Progeny PoPrP-Tg001 × BoPrP-Tg110                     |
| muPrnp<sup>+/−</sup>, poPrnp<sup>+/−</sup> | Murine and porcine | 2×             | TgMo/TgPo                 | Progeny TgMo × PoPrP-Tg001                           |

<sup>a</sup>Relative to the PrPC expression level in the indicated species.

| Description | TgMo/TgPo | Tga20 mouse line (47) | TgBo/TgPo | BoPrP-Tg110 mouse line (46) | TgPo/TgBo | PoPrP-Tg001 mouse line (38) | Prnp<sup>−/−</sup> | PrP knockout (Prnp<sup>−/−</sup>) | Tga20 (muPrnp<sup>+/−</sup> × PrP knockout (Prnp<sup>−/−</sup>) | BoPrP-Tg110 (boPrnp<sup>+/−</sup> × PrP knockout (Prnp<sup>−/−</sup>) | Progeny Tga20 (muPrnp<sup>+/−</sup> × PrP knockout (Prnp<sup>−/−</sup>) | Progeny BoPrP-Tg110 (boPrnp<sup>+/−</sup> × PrP knockout (Prnp<sup>−/−</sup>) | Progeny PoPrP-Tg001 (poPrnp<sup>+/−</sup> × PrP knockout (Prnp<sup>−/−</sup>) | Progeny C57BL/6 × PrP knockout (Prnp<sup>−/−</sup>) | Progeny C57BL/6 × BoPrP-Tg110 | Progeny TgMo × BoPrP-Tg110 | Progeny TgMo × PoPrP-Tg001 |
|-------------|----------|----------------------|-----------|-----------------------------|-----------|-----------------------------|-----------------|-----------------------------|-------------------------------|---------------------------------|----------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|

Prion propagation in the presence of a heterologous PrPC species. (i) BSE agent propagation in a murine and bovine PrPC-coexpressing host. In this case, animals expressing murine PrPC in addition to bovine PrPC were generated and compared with those expressing either murine nor bovine PrPC. It should be highlighted that in...
animals coexpressing murine and bovine PrP\textsuperscript{C}, while murine PrP\textsuperscript{C} is in the context of its natural host (the mouse), bovine PrP\textsuperscript{C} is not.

When mouse BSE was used as the inoculum, the survival times of the inoculated mice expressing murine PrP\textsuperscript{C} (TgMo/\textsuperscript{--}) or both murine and bovine PrP\textsuperscript{C} (TgMo/TgBo) were not significantly different ($P = 0.5222$ [Fig. 1A]), indicating no effects on the

![Diagram](Fig 1) Transmission of mouse BSE (A), cattle BSE (B), or cattle BSE-L (C) after intracerebral inoculation in TgMo/\textsuperscript{--}, TgBo/\textsuperscript{--}, and TgMo/TgBo mice. The mean survival time in days postinoculation ± standard deviation (SD) is shown. n/n\textsubscript{0}, number of diseased PrPres-positive animals/inoculated animals. IS, interference score of the interfering PrP. PrP\textsuperscript{Sc} species are depicted as hexagons for classical BSE or ellipses for atypical BSE-L.
replication of mouse BSE when bovine PrP\textsuperscript{C} is coexpressed. When cattle BSE was inoculated (Fig. 1B), heterozygous TgMo/TgBo mice showed no significant differences in survival times compared to animals expressing only murine PrP\textsuperscript{C} ($P = 0.7127$). However, a slightly longer survival time ($P = 0.0002$) was observed in these TgMo/TgBo mice compared to those observed in mice expressing only bovine PrP\textsuperscript{C} (TgBo/--). This difference seems to be lower than expected—probably due to the small variation in survival times between hemizygous TgBo/— and TgMo/— mice when inoculated with cattle BSE. This slight difference (IS = 1.2) suggests that the mouse allele only weakly interferes with conversion of the bovine allele. However, an alternative interpretation is also possible as TgMo/TgBo mice could succumb from conversion of mouse PrP, thus, reflecting the efficiency of conversion of murine PrP but not of bovine PrP.

Strikingly, whatever the PrP\textsuperscript{Sc} present in the inoculum (mouse or cattle), survival times in heterozygous TgMo/TgBo mice were similar to those observed in hemizygous TgMo/— mice.

In order to assess the impact of coexpression of murine and bovine PrP\textsuperscript{C} on the PrP\textsuperscript{Sc} propagation process, immunoblotting using two antibodies that specifically probe the murine (SAF83) or the bovine (12F10) PrP were used to estimate the levels of brain PrPres accumulation in the inoculated mice. Whatever the origin of the BSE inoculum (mouse or cattle), the SAF83 PrPres signals observed in clinically affected TgMo/— and TgMo/TgBo mice were similar (Fig. 2A and B). Conversely, in cattle BSE-inoculated TgMo/TgBo mice, the 12F10 PrPres signal was at least 16 times weaker than that in clinically affected TgBo/— animals (Fig. 2B). Since in cattle BSE-inoculated TgMo/TgBo mice the survival time was only 1.2-fold longer than that in TgBo/— mice, the survival time cannot explain the lower bovine PrPres accumulation level observed in TgMo/TgBo mouse brain. In addition, cattle BSE passaged in TgMo/— or TgMo/TgBo mice was used to inoculate groups of TgMo/—, TgBo/—, and TgMo/TgBo mice (Fig. 1B). In both cases, the incubation periods recorded in the three mouse groups showed a similar pattern: a short survival time in both TgMo/— and TgMo/TgBo mice and a prolonged survival time in TgBo/— mice. These results clearly differed from those observed in mice inoculated with cattle BSE, suggesting that, in TgMo/TgBo mice inoculated with cattle BSE, the murine allele is predominantly being propagated. In all cases, BSE-inoculated TgMo/TgBo mice showed the same survival time as the TgMo/— control, and therefore, mouse BSE is actively replicating the mouse allele without apparent interference by the bovine allele, while the replication of cattle BSE in the bovine allele is interfered with by the presence of the murine allele, which is finally predominantly propagated, as observed by bioassay and brain PrPres accumulation.

To assess the relevance of the inoculation route in the outcome of the experiment, TgMo/TgBo, TgMo/—, and TgBo/— mice were intraperitoneally inoculated with cattle or mouse BSE. In both cases, intraperitoneally inoculated TgMo/TgBo mice died with a similar survival time pattern (Table 2) compared with the pattern previously observed following inoculation by the intracerebral route (Fig. 1A and B). As previously observed by the intracerebral route, survival times in heterozygous TgMo/TgBo mice were similar to those observed in hemizygous TgMo/— mice whatever the PrP\textsuperscript{Sc} present in the inoculum (mouse or cattle).

In TgMo/TgBo mice, a higher expression of the murine versus bovine PrP (Table 1) could be the cause of the effect observed in TgMo/TgBo mice. However, no significant differences were observed when PrP\textsuperscript{C} expression levels from TgBo/— and TgMo/— brains were compared (Fig. S1). To test the influence of PrP\textsuperscript{C} expression levels, we inoculated heterozygous C57BL/6/TgBo mice with mouse BSE and cattle BSE. In these mice, the expression of bovine PrP\textsuperscript{C} is significantly higher than that of mouse PrP. After inoculation with mouse BSE, 100% of the mice were infected, but the survival time in TgBo/— mice was longer than those in C57BL/6/TgBo or C57BL/6/— mice ($P < 0.0001$ [Table 3]). This result suggests a slight effect of the expression of bovine PrP\textsuperscript{C} on the homologous replication of the murine allele. On the other hand, survival times in cattle BSE-inoculated C57BL/6/TgBo mice were considerably longer than those in TgBo/—
mice (Table 3). In contrast, C57BL/6/ mice inoculated with cattle BSE died at the end of their life span without showing clinical signs of neurological disease, but when their brains were analyzed, 100% of them were found PrPres positive (Table 3).

In C57BL/6/TgBo mice inoculated with cattle BSE, the SAF83 immunoblot indicated that the accumulation of murine PrPres in the brain was similar to that in C57BL/6/ mice (Fig. 2C). The 12F10 immunoblot was consistent with the accumulation of quite similar amounts of bovine PrPres in clinically affected C57BL/6/TgBo and TgBo/ mice. Mouse BSE-inoculated C57BL/6/TgBo mice displayed similar accumulation of murine and bovine PrPres in their brain (as assessed by SAF83 and 12F10 immunoblots, respectively) compared to C57BL/6/ and TgBo/ mice, respectively (Fig. 2D). These data
indicate that in animals that express significantly more bovine PrP\textsuperscript{C} (about 3×) than murine PrP\textsuperscript{C}, the capability of murine PrP\textsuperscript{C} to interfere with the bovine PrP\textsubscript{Sc} replication is slightly reduced compared to animals that express similar amounts of both bovine and murine PrP\textsuperscript{C} (Fig. 1B and 2B). These results suggest that differences in the bovine and murine PrP\textsuperscript{C} expression ratio can affect the observed interference.

(ii) Atypical BSE-L agent propagation in a murine and bovine PrP\textsuperscript{C}-coexpressing host. To assess whether the observed interference effect is strain-specific, atypical BSE-L (cattle BSE-L) was used as the inoculum in TgMo/\textsuperscript{2}, TgMo/TgBo, and TgBo/\textsuperscript{2} mice (Fig. 1C). The cattle BSE-L agent was only able to replicate in TgBo/\textsuperscript{2} mice, while both heterozygous TgMo/TgBo and hemizygous TgMo/\textsuperscript{2} mice were resistant to the infection with this agent, and hence, the interference score was high (IS > 18). None of the animals succumbed to the disease, and when euthanized at the end of their life span, they showed no clinical signs or PrP\textsuperscript{Sc} in their brains. These results indicate that, contrary to epidemic BSE, the expression of the heterologous murine PrP\textsuperscript{C} prevents the replication of the bovine PrP\textsubscript{Sc} in animals inoculated with cattle BSE-L, thus suggesting that the interference effect of a heterologous PrP\textsuperscript{C} on prion propagation is strain dependent and probably related to the inconvertibility of the interfering PrP\textsuperscript{C}.

(iii) BSE agent propagation in a murine and porcine PrP-coexpressing host. We also investigated the interference phenomenon in animals coexpressing the murine PrP\textsuperscript{C} (in the context of its natural host) beside the porcine PrP\textsuperscript{C} sequence (Fig. 3). Inoculation of mouse BSE in TgPo/\textsuperscript{2} mice was inefficient, as none of the mice was scored positive for the transmission of the disease, while it readily infected TgMo and TgMo/TgPo mice (Fig. 3A). Statistical analysis confirms that there is no interference (P < 0.0001). Even the onset of the disease is very slightly accelerated in the animals expressing both alleles, showing an interference score of around 1, indicating that the presence of the porcine allele, despite its inconvertibility, does not affect the replication of the murine allele.

When pig BSE was used as the inoculum (Fig. 3B), 100% of hemizygous TgPo/\textsuperscript{2} and TgMo/\textsuperscript{2} mice were infected, while none of the heterozygous TgMo/TgPo mice was scored positive for the disease, suggesting a dual interference effect, as supported by the elevated interference score observed for these transmissions (IS > 11.32). The second passage of brains from TgMo/TgPo mice inoculated with pig BSE revealed a lack of infectivity in TgMo mice, and only residual infectivity could be detected in TgPo mice (Fig. 3B).

(iv) BSE agent propagation in a bovine and porcine PrP-coexpressing host. Further analyses were accomplished in transgenic mice coexpressing bovine and porcine PrP. The results indicated that in animals that express significantly more bovine PrP\textsuperscript{C} (about 3×) than murine PrP\textsuperscript{C}, the capability of murine PrP\textsuperscript{C} to interfere with the bovine PrP\textsubscript{Sc} replication is slightly reduced compared to animals that express similar amounts of both bovine and murine PrP\textsuperscript{C} (Fig. 1B and 2B). These results suggest that differences in the bovine and murine PrP\textsuperscript{C} expression ratio can affect the observed interference.

TABLE 2 Intraperitoneal inoculation of BSE isolates in mice overexpressing murine and bovine PrP

| Mice             | Mean survival time, dpi (n/n\textsubscript{0})\textsuperscript{a} |
|------------------|---------------------------------------------------------------|
|                  | Cattle BSE                          | Mouse BSE                       |
| TgMo/\textsuperscript{2} | 679 ± 57 (6/6)                     | 279 ± 9 (6/6)                   |
| TgMo/TgBo        | 709 ± 70 (6/6)                       | 272 ± 15 (6/6)                  |
| TgBo/\textsuperscript{2} | 457 ± 54 (6/6)                     | >650 (6/6)                      |

\textsuperscript{a}Survival time is indicated as mean number of days postinoculation (dpi) ± SD for all the mice scored positive for PrP\textsuperscript{Sc}. n/\textsubscript{0}, number of diseased PrP\textsuperscript{Sc}-positive animals/inoculated animals.

TABLE 3 Intracerebral inoculation of cattle and mouse BSE isolates in C57BL/6/TgBo mice

| Mice             | Mean survival time, dpi (n/n\textsubscript{0})\textsuperscript{a} |
|------------------|---------------------------------------------------------------|
|                  | Cattle BSE                          | Mouse BSE                       |
| C57BL/6/\textsuperscript{2} | >650 (6/6)                                | 304 ± 8 (6/6)                   |
| C57BL/6/TgBo     | 455 ± 11 (6/6)                           | 333 ± 7 (6/6)                   |
| TgBo/\textsuperscript{2} | 313 ± 10 (5/5)                     | 445 ± 25 (5/5)                  |

\textsuperscript{a}Survival time is indicated as mean number of days postinoculation (dpi) ± SD of all the mice scored positive for PrP\textsuperscript{Sc}. n/\textsubscript{0}, number of diseased PrP\textsuperscript{Sc}-positive animals/inoculated animals.
cine PrP (Fig. 4). It should be noted that in this case, none of the expressed PrPC is in the context of its natural host. Cattle BSE was not transmitted in hemizygous TgPo/− mice (Fig. 4A). Interestingly, while transgenic mice expressing only bovine PrPc (TgBo/−) were readily infected with cattle BSE, none of the animals coexpressing bovine and porcine PrPSc showed evident clinical signs, yet they scored positive for the presence of PrPSc in their brains when culled after 650 days postinfection (dpi). The PrPSc profile obtained from TgBo/TgPo brain extracts was indistinguishable from those obtained from TgBo/− brains (Fig. 5). Hence, porcine PrPC seems to entail a strong interfering effect on bovine PrPSc propagation in TgBo/TgPo mice inoculated with cattle BSE. To investigate whether PrPSc propagation is restricted to bovine PrP, brain homogenates from cattle BSE-inoculated TgBo/TgPo transgenic mice were reinoculated into TgPo, TgBo, and TgBo/TgPo mice. As shown in Fig. 4A, the totalities of both TgBo- and TgPo-inoculated mice were scored positive for the disease, with short survival times, as previously described for the infection with cattle BSE in TgBo mice and pig BSE in TgPo mice (23). Remarkably, TgBo/TgPo mice were 100% susceptible to this cattle BSE passaged in the TgBo/TgPo transgenic mouse inoculum but showed significantly (P < 0.0001) longer survival times (588 ± 35 dpi) than those observed in TgBo/− mice inoculated with cattle BSE (313 ± 10 dpi) or TgPo/− mice inoculated with pig BSE (287 ± 3 dpi). These second passages suggest that both bovine and porcine PrPSc were generated in the brains of TgBo/TgPo mice inoculated with cattle BSE (1st passage), while their simultaneous replication in the second passage of TgBo/TgPo mice was impaired by the presence of the other prion protein.

When pig BSE was used as the inoculum, TgBo/− and TgPo/− mice were infected without evidence of a transmission barrier, as previously described (38). As shown in Fig. 4B, heterozygous TgBo/TgPo mice were also 100% susceptible to the inoculation...
of pig BSE, but again, the manifestation of the disease was delayed ($P < 0.0001$) compared to their hemizygous counterparts, the TgBo/− and TgPo/− mice. Similar behavior was maintained after the second passage of pig BSE-infected TgBo/TgPo brains in TgBo/TgPo mice. They showed survival times longer than 500 dpi, and only four out of
six animals scored positive for PrP\textsuperscript{res} in their brains. Again, the PrP\textsuperscript{res} profile obtained in brain extracts from TgBo/TgPo animals was indistinguishable from that observed in TgBo\textbf{−} brains (Fig. 5). In addition, TgBo and TgPo mice were 100% susceptible to brain homogenate from pig BSE passaged in TgBo/TgPo mice, supporting the coreplication of both bovine and porcine PrP\textsuperscript{Sc} during the first passage on TgBo/TgPo mice (Fig. 4B). These results suggest that bovine PrP\textsuperscript{Sc} and porcine PrP\textsuperscript{Sc} can replicate in TgBo/TgPo mice but less efficiently than separately (with an observed interference score of around 2 in both cases), indicating that the detrimental effect on PrP\textsuperscript{Sc} conversion mutually affects both PrP species.

**Prion propagation in a host expressing two PrP\textsuperscript{C} species different from the inoculated PrP\textsuperscript{Sc}.** (i) **Cattle BSE in a murine and porcine PrP\textsuperscript{Sc}-coexpressing host.** Cattle BSE was inoculated in heterozygous TgMo/TgPo mice and their respective hemizygous controls. As mentioned before, cattle BSE was not able to infect TgPo\textbf{−} mice, yet could infect TgMo\textbf{−} mice, with attack rates of 100% (Fig. 6A). When inoculated into TgMo/TgPo mice, cattle BSE led to 40% attack rates, long survival times of around 600 dpi (rendering an interference score of around 4), and a PrP\textsuperscript{res} profile identical to that found in TgMo\textbf{−} brains (Fig. 5). Brains from TgMo/TgPo mice inoculated with cattle BSE and scoring PrP\textsuperscript{res} positive were passaged a second time in TgMo, TgMo/TgPo, and TgPo mice. Short survival times were observed in both TgMo and TgMo/TgPo mice (110 ± 7 and 136 ± 6 dpi, respectively), showing a small but significant difference ($P = 0.0003$). TgPo mice became infected with an evident transmission barrier (survival for 589 ± 10 dpi and three out of four animals scoring positive for PrP\textsuperscript{res}). A similar result was previously described for the inoculation of mouse BSE prions in TgPo mice (survival time of 506 dpi and one out of six mice scoring positive for PrP\textsuperscript{res}) (23).

Additional analysis of the PrP\textsuperscript{res} from TgMo/TgPo brains infected with cattle BSE evidenced that—as expected by the bioassay outcome—murine PrP\textsuperscript{res} is present, as detected with the Saf83 monoclonal antibody (MAb), while porcine PrP\textsuperscript{res} was not detected with the 12F10 MAb (Fig. 7).

Taken together, these results suggest that only the mouse PrP\textsuperscript{Sc} was replicated in TgMo/TgPo mice inoculated with cattle BSE. Nevertheless, mouse PrP\textsuperscript{Sc} replication is severely interfered with by porcine PrP, despite the relatively lower expression level of the pig PrP\textsuperscript{C} in comparison with mouse PrP\textsuperscript{C}.

(ii) **Sheep BSE in a murine and bovine PrP-coexpressing host.** BSE agent after adaptation in ARQ sheep (sheep BSE) was used as a heterologous inoculum in TgMo/ TgBo mice. The observed outcome (Fig. 6B) was very similar to the results obtained after inoculation of cattle BSE into TgMo/TgBo mice (Fig. 1B), propagating efficiently in TgBo\textbf{−} mice and with longer survival times in both TgMo/TgBo and TgMo\textbf{−} mice. TgMo/TgBo and TgMo\textbf{−} mice showed no significant differences in their survival times ($P = 0.0963$ [Fig. 6B]). As previously observed for the cattle BSE inoculum in these mice, although this slight difference (IS = 1.34) suggests that the mouse allele only weakly interferes with the conversion of the bovine allele, this result could also be interpreted as TgMo/TgBo mice having succumbed from the conversion of mouse PrP, thus, reflecting the efficiency of conversion of murine PrP but not of bovine PrP. The levels of SAF83 PrP\textsuperscript{res} signal observed in TgMo\textbf{−} and TgMo/TgBo mice were similar, and the 12F10 PrP\textsuperscript{res} signal in the TgMo/TgBo mice was at least 16 times weaker than that in TgBo\textbf{−} animals (Fig. 8).

(iii) **Sheep scrapie in a murine and bovine PrP-coexpressing host.** In another set of experiments, a sheep scrapie isolate (Sc21) was used as the inoculum, with PrP\textsuperscript{Sc} different from the two PrP\textsuperscript{C} proteins coexpressed in the recipient. While TgMo/TgBo and TgBo\textbf{−} mice inoculated with sheep Sc21 showed no significant differences in their long survival times ($P = 0.6952$ [Fig. 6C]), TgBo\textbf{−} mice were readily infected with sheep Sc21, evidencing the interference with bovine PrP\textsuperscript{Sc} replication by the presence of mouse PrP\textsuperscript{C} (IS = 2.05). Biochemical analysis of PrP\textsuperscript{res} from TgMo/TgBo brains infected with sheep Sc21 confirms the interference with bovine PrP\textsuperscript{Sc} replication as mouse PrP\textsuperscript{res} was present in similar levels to TgMo\textbf{−} mice, while only residual levels of bovine PrP\textsuperscript{res} could be detected (Fig. 9).
Sheep Sc21 was also transmitted to heterozygous TgBo/TgPo mice, although the attack rate was lower and the survival time longer than those in TgBo/− mice (IS = 3.44 [Fig. 6D]). Consistent with our prior observations (39, 40), scrapie was not transmitted to TgPo/− mice, and when euthanized at the end of their life span, they scored negative for PrPSc. As mentioned before, the amino acid sequence of the inoculated PrPSc (ovine) is different from those of any of the PrPSc proteins expressed in the recipient

| Inoculated PrPSc | 1st passage | Replicated PrPSc | 2nd passage |
|------------------|-------------|------------------|-------------|
| **A** Cattle-BSE | | | |
| in >650 (0/6) | 601±28 (4/10) | IS = 3.93 | 589±10 (3/4) |
| | 382±25 (5/5) | | 136±6 (5/5) |
| **B** Sheep-BSE | | | |
| 290±19 (6/6) | IS = 1.34 | 389±32 (6/6) | IS = 0.92 |
| | 427±28 (6/6) | | 110±7 (5/5) |
| **C** Sheep-Sc21 | | | |
| 244±3 (5/5) | IS = 2.05 | 501±50 (6/6) | IS = 0.99 |
| | 506±39 (5/5) | | |
| **D** Sheep-Sc21 | | | |
| 244±3 (5/5) | IS = 3.44 | 560±71 (2/3) | |
| | >650 (0/6) | | |

**Fig 6** Transmissions in a host expressing PrPSc from two species different from the inoculated PrPSc. Shown is intracerebral inoculation of cattle BSE in TgMo/−, TgPo/−, and TgMo/TgPo mice (A), sheep BSE in TgMo/−, TgBo/−, and TgMo/TgBo mice (B), sheep Sc21 in TgMo/−, TgBo/−, and TgMo/TgBo mice (C), and sheep Sc21 in TgBo/−, TgPo/−, and TgBo/TgPo mice (D). Shown is the mean survival time in days postinfection ± SD. n/n0, number of diseased PrPres-positive animals/inoculated animals. IS, interference score of the interfering PrP. PrPSc species are depicted as polygons; a dashed polygon indicates that PrPSc was not detected.
However, since the sheep Sc21 isolate readily infects TgBo/2 mice, porcine PrPC must be responsible for the interfering effect, increasing the survival times in TgBo/TgPo mice.

The results obtained when the species origin of the inoculated PrPSc is different from the two PrPC proteins coexpressed in the transgenic mouse indicate that an interference effect can be observed but with a complex outcome.

**DISCUSSION**

This study evaluates the potential interference with the PrPSc replication process by a PrPC protein from a second species expressed in the recipient transgenic mouse model. The amino acid sequence differences between the donor PrPSc and the recipient PrPC play an important modulatory role in the interspecies transmissibility of TSE agents (36). Furthermore, amino acid sequence differences in the second species PrPC may be relevant in the interference with PrPSc replication (41). In this work, different combinations of PrPC species pairs were challenged with PrPSc proteins from different sources (cattle, mice, and pigs) to examine the effects of the simultaneous presence of PrPC from two different species.

In the first set of experiments, the amino acid sequence of the PrPSc inoculated was the same as that of the PrPC expressed in the host (homologous PrPC). Thus, compared to the appropriate control, there is not any other factor affecting the transmissibility of (bovine and porcine). However, since the sheep Sc21 isolate readily infects TgBo/— mice, porcine PrPC must be responsible for the interfering effect, increasing the survival times in TgBo/TgPo mice.

The results obtained when the species origin of the inoculated PrPSc is different from the two PrPC proteins coexpressed in the transgenic mouse indicate that an interference effect can be observed but with a complex outcome.

**FIG 7** Brain PrPres in inoculated mice. Shown is an immunoblot of brain PrPres detected with either the Saf83 (top) or 12F10 (bottom) MAb. Direct samples (2-mg equivalent of 10% brain homogenates) and 1/4 dilutions were loaded onto 12% Bis-Tris gels. The results shown are representative of at least two independent experiments. Shown is brain PrPres from mice inoculated with cattle BSE in TgMo/— mice and TgMo/TgPo or pig BSE in TgPo/— mice.

**FIG 8** Brain PrPres in inoculated mice. Shown is an immunoblot of PrPres from brain detected with either the Saf83 (left) or 12F10 (right) MAb. Direct samples (2-mg equivalent of 10% brain homogenates) and 1/4 dilutions were loaded onto 12% Bis-Tris gels. The results shown are representative of at least two independent experiments. Shown is brain PrPres from mice inoculated with sheep BSE in TgMo/—, TgMo/TgBo, and TgBo/— mice.
the PrPSc inoculated than the second PrPC expressed in the host (heterologous PrPC). As previously observed by using in vitro conversion (42), heterologous (less convertible or nonconvertible PrPC) may interact with PrPSc, and as a consequence, the conversion of the homologous PrPC may be interfered with. Moreover, the heterologous PrPC expressed in the in vivo model may result in the new PrPSc counterpart, and accordingly, bidirectional interference may occur in the context of the mouse expressing PrPC from two species. In the in vivo model used here (the mouse), murine PrPC is expressed in its natural context, but bovine or porcine PrPC is not. In this sense, the interaction of host-specific factors with the expressed PrPC may affect the interference process.

Our transmission experiments using transgenic mice that coexpress an exogenous PrPC show that the expression of bovine PrPC at similar levels to murine PrPC is not able to alter the disease caused by the inoculated TSE agent compared to animals expressing only murine PrPC (Fig. 1). This was independent of (i) the route of inoculation used (intracranial or intraperitoneal) (Table 2), (ii) the PrPSc amino acid sequence inoculated (either from mice [Fig. 1A], cattle [Fig. 1B], or sheep [Fig. 6B and C]), and (iii) the tested prion strains from BSE (Fig. 1A and B and Fig. 6B) and sheep scrapie (Fig. 6C). Only the expression of higher levels of bovine PrPC than murine PrPC is able to alter the disease caused by the inoculated TSE agent when murine PrPC is expressed alone (Table 3), evidencing that PrP expression levels are relevant in the interference process. Remarkably, in all these experiments, murine PrPC is coexpressed in the context of its natural host. In contrast, the coexpression of either bovine or murine PrPC in addition to porcine PrPC was able to interfere with the disease caused by the inoculated TSE agent, even though porcine PrPSc was inoculated (Fig. 3B and Fig. 4B). The highest interference was observed when pig BSE was inoculated into mice coexpressing murine and porcine PrPC (Fig. 3B). In this case, very low infectivity was detected after the second passage, suggesting only propagation of porcine PrPSc. In parallel experiments, coexpression of porcine PrPC in addition to porcine PrPSc was able to interfere with the disease caused by the inoculation of mouse BSE in heterozygous TgMo/TgPo mice (Fig. 3A). In other words, the homologous replication of murine PrPSc was not affected by the presence of the inconvertible heterologous porcine PrPC, in the same way observed with heterologous bovine PrPSc in Fig. 1A. However, expression of porcine PrPC even at lower levels than bovine PrPSc interferes with the disease caused by the inoculation of cattle BSE in heterozygous TgBo/TgPo mice (Fig. 4A). Moreover, when cattle BSE is inoculated into mice coexpressing murine and porcine PrPC (that is a PrPSc heterologous to both PrPC amino acid sequences expressed in the host), only murine PrPSc is generated after a long...
survival time in only 40% of the mice (Fig. 6A). We can speculate that porcine PrPC might inhibit (in a competitive manner) the interaction of the murine PrPC with cellular ligands or host factors required only for the propagation process of the heterologous conversion of murine PrPC to PrPSc (10) but not for the homologous conversion. In this sense, we cannot exclude the role of host-specific factors implicated in the formation of murine PrPSc, as factors other than PrP can affect the infectious process (31, 32). On the other hand, when bovine and porcine PrPC are coexpressed (Fig. 4), both PrPC sequences are not in their natural hosts (cattle or pig), and mutual interference is observed, as neither bovine nor porcine PrPSc can overcome the interference in terms of survival time or infectivity while infectivity from both PrPSc species is generated.

Collectively, our results support the idea that the prion replication interference induced by the coexpression of a heterologous PrPC may be related to the conversion susceptibility of the interfering PrPC. Bovine PrPC can be converted efficiently by the different prion strains used (see reference 23 and this work), and hence, in the heterozygous transmissions where bovine PrP would interfere, low interference scores were observed (IS < 1.82). The effective convertibility of bovine PrPC by the different prion strains used would explain its poor, if any, interfering effect, allowing the propagation of either mouse or porcine-PrPSc. Conversely, the limited convertibility of porcine PrPC (see references 39 and 40 and this work) would explain the substantial interference effect caused by the coexpression of porcine PrPSc with either bovine or murine PrPC in most inocula used, showing interference scores over 2.08. The only exception was the homologous propagation of mouse BSE in TgMo/TgPo mice (Fig. 3A), which was probably due to the effect of host-specific factors involved in the interference process, as mentioned before. Alternatively, specific structural elements in the mouse PrPSc absent in both cattle and pig BSE PrPSc could explain the ability of the homologous propagation of mouse BSE in heterozygous mice (TgMo/TgPo and TgMo/TgBo), avoiding the interference effect caused by the coexpression of bovine or porcine PrPSc. Curiously, while PrPSc was not detected in hemizygous mice expressing only porcine PrPC inoculated with cattle BSE (Fig. 4A), mice coexpressing porcine and bovine PrPC were able to efficiently propagate porcine PrPSc, as confirmed via its second passage. Porcine PrPSc replication is likely the result of its interaction with the replicated bovine PrPSc, which would provide a steady source of bovine PrPSc to interact with porcine PrP, but not with the inoculated bovine PrPSc.

In the cases of the sheep isolates used in Fig. 6B and C, the amino acid sequence of the inoculated PrPSc (sheep) was different from those of both PrPSc expressed in the host (mouse and cattle). Several factors may participate in the transmission of the inoculated TSE agent when three different PrP amino acid sequences are implicated: (i) the transmission barrier of each PrPC to the inoculated PrPSc, (ii) the differential ability of each PrPC to replicate the inoculated prion strain, and (iii) the interference effect of each PrPC on the replication of the other. In this multifaceted scenario, it is difficult to predict the outcome when there is no homology between the inoculum and any of the coexpressed PrPSc sequences. In general, the PrPSc prone to replicate is impaired by the presence of the PrP sequence putatively averse to replicate, as observed when cattle BSE was inoculated into mice coexpressing both porcine and murine PrPC (Fig. 6A), or with sheep BSE or sheep Sc21 inoculated in the different PrP combinations (Fig. 6B to D).

Taken together, all of our results suggest that the coexpression of a PrPC from a second species would interfere with propagation of the homologous prion. The level of interference is generally related to the transmission proficiency of the infectious agent when this second PrPC is expressed alone. That is, effective interference was observed when the inoculated prion was not (or poorly) transmitted in mice expressing the interfering PrPC alone, thus suggesting a certain correlation between interference ability and conversion incompetence of the interfering PrPC. Although most of the results supporting this statement have been obtained with classical BSE, results with other prion agents, such as sheep scrapie and L-type BSE, suggest that this contention can
be extended to other prion agents, being probably a general rule applying to the different prion strains. This rationale is consistent with the stone fence model (43), which predicts that for a given TSE agent, a conversion-incompetent PrP will impair the PrPSc replication of a conversion-competent PrP, resulting in a lower efficacy of prion propagation. As illustrated here, this lower efficiency is translated to reduced attack rates and/or prolonged survival times due to a dominant-negative effect induced by the conversion-incompetent PrP on a strain-dependent basis. The protector effect of the Val129 human PrP variant in heterozygosis for both classical BSE and L-type BSE infection is an example of this dominant-negative effect (44, 45). Finally, the unequal interference capacity of the murine PrP allele, which is expressed in its natural context (the mouse), allows us to speculate that host-specific factors other than PrP could be involved in the interference process.

MATERIALS AND METHODS

Ethics statement. Animal experiments were carried out in strict accordance with institutional and national guidelines and in accordance with the European Directives 86/609/EEC and 2010/63/EU. Every effort was made to minimize animal suffering. The animal experiments conducted at CSIA-INIA (Centro de Investigación en Sectores Agroalimentarios) were approved by the Committee on the Ethics of Animal Experiments of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (permit no. CEEA 2009/003 and CEEA 2009/004). Experiments developed at ENVT (Ecole Nationale Vétérinaire de Toulouse) were approved by the local ENVT committee (permit no. 01734.01).

Transgenic mice. Three transgenic mouse lines previously reported were used: (i) PoPrP-Tg001, expressing porcine PrP (38); (ii) BoPrP-Tg110, expressing bovine PrP (46); and (iii) Tga20, expressing murine PrP (47). PoPrP-Tg001, BoPrP-Tg110, and Tga20 mice are homozygous for each transgene and were generated in a null background for murine PrP (muPrnpfl/−). PoPrP-Tg001, BoPrP-Tg110, and Tga20 mice are abbreviated in the text as TgPo, TgBo, and TgMo, respectively. These mouse lines were crossbred to obtain heterozygous animals expressing bovine and porcine PrP, bovine and murine PrP, or murine and porcine PrP (Table 1). As controls, TgPo, TgBo, and TgMo were crossbred with PrP knockout mice (Prnpfl/−) to produce hemizygous animals (Table 1).

TSE inocula. All inocula were prepared as 10% brain homogenates in 5% glucose in distilled water. The brain sources were (i) Ca-BSE, French case no. 139, from brainstem of a cow naturally infected with classical BSE; (ii) cattle BSE, from a pool of brains from terminally ill TgBo mice inoculated with Ca-BSE; (iii) pig BSE, from a pool of brains of terminally ill porcine TgPo mice inoculated with a second passage of the Ca-BSE inoculum; (iv) mouse BSE, from a pool of brains of terminally ill murine transgenic TgMo mice inoculated with a second passage of the Ca-BSE inoculum; (v) cattle BSE-L, from brainstem of a cow from France naturally infected with L-type atypical BSE; (vi) sheep BSE, from a pool of brains from seven ARQ/ARQ sheep inoculated with Ca-BSE; (vii) sheep Sc21, an isolate obtained from the brain of a French ARQ/ARQ (136, 154, and 171 codons) sheep naturally infected with scrapie; and (viii) as a negative control, a pool of brains of uninoculated C57BL/6 mice.

Transmission studies. Groups of 6 to 10 mice (6 to 7 weeks old, weighing approximately 20 g) were anesthetized with isoflurane and inoculated with 2 mg of brain homogenate in the right parietal lobe by using a disposable 25-gauge hypodermic needle. Eight-millimeter transponders were used for individual identification of mice. Mice were examined twice weekly for neurological signs of prion disease and were euthanized by cervical dislocation when the progression of the disease was evident or at the end of the study at 650 days postinoculation (dpi). The animals were humanely euthanized once a definitive diagnosis had been made or earlier if showing signs of distress or loss of up to 20% body weight. A mouse was scored positive for prion disease when it showed 2 or 3 out of 10 described signs of neurological dysfunction (35, 48). Once euthanized, a necropsy was performed, and the brain was harvested and stored at −20°C. Survival time was calculated as the mean ± standard deviation (SD). A Student’s unpaired, two-tailed t test was used for comparison between group data (P < 0.05). To analyze and compare the levels of interference of prion propagation among the different intracranially inoculated transgenic mice used in the work we introduced a new parameter called the interference score (IS) of the interfering PrP, which takes into consideration both attack rate and survival time. IS was calculated according to the formula

$$IS = \frac{\text{mean survival time in heterozygous transmission}}{\text{mean survival time in hemizygous transmission}} \times \frac{\text{inoculated animals}}{\text{diseased PrP}^{\text{PrPSc}} - \text{positive animals in heterozygous transmission}}$$

If 0 animals were scored PrPSc positive in the heterozygous transmission, the IS was calculated considering that the value is higher than when 1 animal would be infected. IS was not calculated if 0 animals were scored positive in the hemizygous transmission. An IS of around 1 indicates no or little interference in the propagated prion, while values over 1 indicate proportionally higher interference in prion propagation.
**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**FIG S1**, TIF file, 0.5 MB.

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**REFERENCES**

1. Griffith JS. 1967. Self-replication and scrapie. Nature 215:1043–1044. https://doi.org/10.1038/2151043a0

2. Prusiner SB. 1982. Novel proteinaceous infectious particles cause scrapie. Science 216:136–144. https://doi.org/10.1126/science.6801762

3. Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE. 1993. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci U S A 90:10962–10966. https://doi.org/10.1073/pnas.90.23.10962

4. Meyer RK, McKinley MP, Bowman KA, Braunfeld MB, Barry RA, Prusiner SB. 1986. Separation and properties of cellular and scrapie prion proteins. Proc Natl Acad Sci U S A 83:2310–2314. https://doi.org/10.1073/pnas.83.8.2310

5. Prusiner SB. 1998. Prions. Proc Natl Acad Sci U S A 95:13363–13383. https://doi.org/10.1073/pnas.95.23.13363

6. Cronier S, Gros N, Tattam MH, Jackson GS, Clarke AR, Collinge J, Wadsworth JD. 2008. Detection and characterization of protease K-resistant prion protein with thermolysin. Biochem J 416:297–305. https://doi.org/10.1042/BJ20081235

7. Safar J, Willie H, Itri V, Groth D, Serban H, Torchia M, Cohen FE, Prusiner SB. 1998. Eight prion strains have PrP(Sc) molecules with different conformations. Nat Med 4:1157–1165. https://doi.org/10.1038/2654

8. Collinge J, Clarke AR. 2007. A general model of prion strains and their pathogenicity. Science 318:930–936. https://doi.org/10.1126/science.1136718

9. Safran AJ, Albertson MD, Kasper S, Markovitch M, Basse B. 2012. Biochemical and physical properties of the prion protein from two strains of the transmissible mink encephalopathy agent. J Virol 66:2096–2101. https://doi.org/10.1128/JVI.66.4.2096-2101.1992

10. Priola SA, Caughey B, Race RE, Chesebro B. 1994. Heterologous PrP molecules interfere with accumulation of protease-resistant PrP in scrapie-infected murine neuroblastoma cells. J Virol 68:4873–4878. https://doi.org/10.1128/JVI.68.8.4873-4878.1994

11. Holscher C, Delius H, Burkle A. 1998. Overexpression of nonconvertible PrP delta 114–121 in scrapie-infected mouse neuroblastoma cells leads to trans-dominant inhibition of wild-type PrP(Sc) accumulation. J Virol 72:1153–1159. https://doi.org/10.1128/JVI.72.8.1153-1159.1998

12. Haldiman T, Kim C, Cohen Y, Chen W, Blevins J, Qing L, Cohen ML, Langeveld J, Telling GC, Kong Q, Safar JG. 2013. Co-existence of distinct prion types enables conformational evolution of human PrPSc by competitive selection. J Biol Chem 288:29846–29861. https://doi.org/10.1074/jbc.M113.500108

13. Wells GA, Scott AC, Johnson CT, Gunnung RF, Hancock RD, Jeffrey M, Dawson M, Bradley R. 1987. A novel progressive spongiform encephalopathy in cattle. Vet Rec 121:419–420. https://doi.org/10.1136/vr.121.18.419

14. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCordle L, Chree A, Hope J, Birkett C, Cousins S, Fraser H, Bostock CJ. 1997. Transmissions to mice indicate that ‘new variant’ CJD is caused by the BSE agent. Nature 389:498–501. https://doi.org/10.1038/390957

15. Collinge J, Rossor M. 1996. A new variant of prion disease. Lancet 347:519–520. https://doi.org/10.1136/lancet.347.9011.519

16. Edwards K, O'Sullivan F, O'Fallon A, Goffin K, Grandi P, Ordentlich P, Haapasalo M, Schmid F, Huang J, Wagner G. 2001. The prion disease agent persists in the CNS at brain death. J Neurosci 21:231–239. https://doi.org/10.1523/JNEUROSCI.21-01-2001.231

17. Varela A, Duran-Rojas A, Brink C, Deriu S, Zanetti A, Khazaeli B, Laquerelle F, Varela B, Marrero R, Anderluh S. 2005. Detection of a prion agent in the CNS at the onset of BSE. J Neurosci 25:7711–7715. https://doi.org/10.1523/JNEUROSCI.1972-05.2005

18. Varela B, Deriu S, Varela A, Khazaeli B, Zanetti A, Anderluh S, Marrero R, Laquerelle F, Aparicio L, Marin-Padilla M. 2005. PrPSc is present in the brain from the early stage of the disease in BSE. J Neurosci 25:7705–7710. https://doi.org/10.1523/JNEUROSCI.1970-05.2005

19. Varela A, Deriu S, Varela B, Khazaeli B, Zanetti A, Anderluh S, Marrero R, Laquerelle F, Aparicio L, Marin-Padilla M. 2005. Detection and persistence of a prion agent in the brain at the onset of BSE. J Neurosci 25:7716–7720. https://doi.org/10.1523/JNEUROSCI.1975-05.2005

20. Varela A, Varela B, Khazaeli B, Anderluh S, Aparicio L, Zanetti A, Marrero R, Laquerelle F, Marin-Padilla M. 2005. PrPSc is not present in the brain in the early stages of BSE. J Neurosci 25:7721–7725. https://doi.org/10.1523/JNEUROSCI.1976-05.2005

21. Varela A, Varela B, Khazaeli B, Zanetti A, Anderluh S, Aparicio L, Marrero R, Laquerelle F, Marin-Padilla M. 2005. PrPSc is not present in the brain in the early stages of BSE. J Neurosci 25:7711–7715. https://doi.org/10.1523/JNEUROSCI.1972-05.2005

22. Varela A, Varela B, Khazaeli B, Zanetti A, Anderluh S, Aparicio L, Marrero R, Laquerelle F, Marin-Padilla M. 2005. PrPSc is present in the brain at the onset of BSE. J Neurosci 25:7705–7710. https://doi.org/10.1523/JNEUROSCI.1970-05.2005
of feline spongiform encephalopathy in a German captive cheetah. J Gen Virol 91:2874–2883. https://doi.org/10.1099/0022-1066-91-11-2874

33. Kirkwood JK, Cunningham AA. 1994. Epidemiological observations on spongiform encephalopathies in captive wild animals in the British Isles. Vet Rec 135:296–303. https://doi.org/10.1136/vr.135.13.296

34. Seubelrich T, Botteron C, Wenerk C, Caffé-Marcal VA, Oevermann A, Haase B, Leeb T, Heim D, Zurbriggen A. 2006. Spongiform encephalopathy in a miniature zebu. Emerg Infect Dis 12:1950–1953. https://doi.org/10.3201/eid1210.060750

35. Elmot M, Adyov K, Couplier M, Fontaine JJ, Hamel R, Lilin T, Messiaen S, Andreoletti O, Baron T, Bencivì A, Biacabe AG, Beringue V, Laude H, Le Dur A, Villette JL, Comoy E, Deslys JP, Grassi J, Simon S, Lantier F, Sarradin P. 2005. BSE agent signatures in a goat. Vet Rec 156:523–524. https://doi.org/10.1136/vetrec.156.15.523-b

36. Jeffery M, Martin S, Gonzalez L, Foster J, Langenveld JP, van Zijderveld FG, Jeffrey M, Martin S, Gonzalez L, Foster J. 2010. Porcine prion protein as a paradigm of limited susceptibility to prion strain propagation. J Infect Dis 221:2085–2086. https://doi.org/10.1086/650923

38. Castilla J, Gutierrez-Adan A, Brardk B, Payko M, Pintado B, Ramirez MA, Salguero FJ, Parra B, Diaz San Segundo F, Sanchez-Vizcaino JM, Rogers M, Torres JM. 2004. Subclinical bovine spongiform encephalopathy infection in transgenic mice expressing porcine prion protein. J Neurosci 24:5063–5069. https://doi.org/10.1523/JNEUROSCI.03-03-2004

40. Espinosa JC, Herva ME, Andreoletti O, Padilla L, Lacroix C, Cassard H, Lantier I, Castilla J, Torres JM. 2009. Transgenic mice expressing porcine prion protein resistant to classical scrapie but susceptible to sheep bovine spongiform encephalopathy and atypical scrapie. Emerg Infect Dis 15:1214–1221. https://doi.org/10.3201/eid1508.081218

42. Espinosa JC, Marin-Moreno A, Aguilar-Calvo P, Benestad SL, Andreoletti O, Torres JM. 2020. Porcine prion protein as a paradigm of limited susceptibility to prion strain propagation. J Infect Dis 222:108–2085. https://doi.org/10.1086/650923

43. Agostino C, Chiappini B, Lantier F, Groschup MH, Agrimi U, Bossers A, Jacobs D, Rogers M, Salguero FJ, Sanchez-Vizcaino JM, Torres JM. 2017. Protective effect of Val129-PrP against bovine spongiform encephalopathy but not variant Creutzfeldt-Jakob disease. Emerg Infect Dis 23:1522–1530. https://doi.org/10.3201/eid2309.161948

44. Fernandez-Borges N, Espinosa JC, Marin-Moreno A, Aguilar-Calvo P, Asante EA, Kitamoto T, Mohri S, Andreoletti O, Piquer J, Lagan S, Lorenzo P, Tillier C, Aron N, Cassard H, Andreoletti O, Torres JM. 2020. Radical change in zoonotic abilities of atypical BSE prion strain as evidenced by crossing of sheep species barrier in transgenic mice. Emerg Infect Dis J 26:1130–1139. https://doi.org/10.1007/s10603-019-04675-9

45. Castilla J, Gutierrez-Adan A, Brardk B, Payko M, Pintado B, Ramirez MA, Salguero FJ, Parra B, Diaz San Segundo F, Sanchez-Vizcaino JM, Rogers M, Torres JM. 2003. Early detection of PrP(res) in BSE-infected bovine PrP transgenic lines for their capacity to inhibit PrPSc replication in infected cells. J Biol Chem 280:11247–11258. https://doi.org/10.1074/jbc.M407006200

46. Manson JA, Clarke AR, Hope ML, Atchison L, McConnell I, Hope J. 1994. 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. Mol Neurobiol 8:121–127. https://doi.org/10.1007/BF02780662

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