

# PrP Expression Level and Sensitivity to Prion Infection

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**Mice overexpressing the prion protein (PrP) sequence from various host species are widely used for measuring infectious titers in prion disease. However, the impact that the transgene expression level might have on the susceptibility to infection raises some concerns about the final biological relevance of these models. Here we report that endpoint titration of a sheep scrapie isolate in sheep and in mice overexpressing the ovine PrP results in similar estimates of the infectious titer.**

Bioassays play a pivotal role in transmissible spongiform encephalopathy (TSE) research. They are used to characterize the nature of the agent (strain typing) and/or to measure the infectious titer, which is a critical parameter for assessing the risk of disease transmission (1).

Historically, laboratory rodents have been used for titrating infectivity. However, the difficulties of transmitting certain TSE isolates, such as, for instance, sporadic Creutzfeldt-Jakob disease (CJD), in conventional rodents remained a major limitation to their use. This “transmission barrier” phenomenon is mainly attributed to the amino acid divergences in the prion protein (PrP) sequences between the donor and the recipient (2).

The apparent abrogation of the transmission barrier in transgenic hosts that express an homologous PrP sequence that results in transmission to that of the donor species has led to the development of a variety of transgenic mouse models expressing the sheep, bovine, porcine, and human PrP. An inverse correlation between the survival time and expression level of the transgene in the brain has been noticed in mice transgenic for mouse, hamster, sheep, and bovine PrP (1). Whether the transgene expression level has an impact on the final susceptibility of the model to infection remains a subject of debate, and the final pertinence of infectious titers as measured in mice overexpressing PrP to the risk of transmitting the disease in the natural host species is uncertain (3).

In this study, we first produced a large batch of stock inoculum, using the brain stems from 70 ARQ/VRQ sheep clinically affected with scrapie. All these animals were born and raised in a flock naturally affected by scrapie (Langlade flock) and belonged to the 2005/2006 birth cohort (4). A 1/10 dilution series of the inoculum (starting from a 4% [wt/vol] tissue homogenate) was prepared in negative sheep brain homogenate, and aliquots were stored at  $-80^{\circ}\text{C}$ .

Groups of (i) tg338 mice that are homozygotes for the sheep PrP VRQ variant and knock down for the mouse PrP ( $\text{PrP}^{\text{K}^0}$ ) (5), (ii) tg338 mice cross bred with  $\text{PrP}^{\text{K}^0}$  mice, and (iii) tg338 mice cross bred with tgShpXI (that are homozygotes for the sheep PrP ARQ variant and  $\text{PrP}^{\text{K}^0}$ ) were produced (1).

The expression levels in the brain of the tg338, tg338  $\times$   $\text{PrP}^{\text{K}^0}$ , and tg338  $\times$  tgShpXI mice, as estimated by Western blot analysis, were approximately 8-fold, 4-fold, and 8-fold higher than in the brain of ARQ/VRQ TSE-free sheep (Fig. 1).

Groups of six mice belonging to these three different mice lines were intracerebrally (IC) inoculated with the prepared dilution series. Similarly, groups of six ARQ/VRQ sheep were IC chal-

lenged using the same dilution series (Table 1). For each tested dilution, each of the mice and sheep received the same amount of brain material. Both mice and sheep were then monitored for clinical TSE occurrence. In clinically suspect animals, TSE transmission was confirmed by abnormal PrP ( $\text{PrP}^{\text{Sc}}$ ) detection in the animals' tissues using Western blot analysis (Sha31 anti-PrP monoclonal antibody; epitope YEDRYRE) (6).

On the basis of these results (Table 1), the infectious titer in sheep was estimated to be  $10^{7.1}$  50% infective doses ( $\text{ID}_{50}$ ) per gram (95% confidence interval [CI 95%],  $10^{6.69}$  to  $10^{7.51}$ ) by the Spearman-Kärber method (7). In both tg338 and tg338  $\times$   $\text{PrP}^{\text{K}^0}$  mice, the infectious titer was  $10^{7.26}$   $\text{ID}_{50}$  per gram (CI 95%,  $10^{6.88}$  to  $10^{7.65}$ ). In tg338  $\times$  tgShpXI mice, the inoculum displayed an infectious titer of  $10^{6.93}$   $\text{ID}_{50}$  per gram (CI 95%,  $10^{6.55}$  to  $10^{7.32}$ ). According to these results, the infectious titers measured in the different animal models were not statistically different.

The incubation periods observed in tg338 mice were significantly (Student's test;  $P < 0.05$ ) shorter than in tg338  $\times$   $\text{PrP}^{\text{K}^0}$  and tg338  $\times$  tgShpXI mice ( $10^{-3}$  dose; see Table 1). However, no statistical difference was observed between incubation periods in tg338  $\times$   $\text{PrP}^{\text{K}^0}$  and tg338  $\times$  tgShpXI mice. These results were unexpected, since the TSE incubation period is supposed to be shorter in hosts expressing a higher level of  $\text{PrP}^{\text{C}}$ . The results suggest that the coexpression of ARQ and VRQ  $\text{PrP}^{\text{C}}$  in the mice somehow interfered with the dynamics of the TSE agent propagation. Further experiments are ongoing to clarify the mechanism underlying this phenomenon.

In parallel to the IC endpoint titration experiment, newborn ARQ/VRQ lambs were orally challenged (natural suckling) with decreasing amounts of infectious material. Lambs ( $n = 50$ ) were separated from their mothers within the first 6 h following birth, and each received a single dose of inoculum corresponding to  $10^{2.7}$ ,  $10^{3.7}$ ,  $10^{4.7}$ , or  $10^{5.7}$  IC  $\text{ID}_{50}$  in sheep (natural suckling). The experimental challenge was performed

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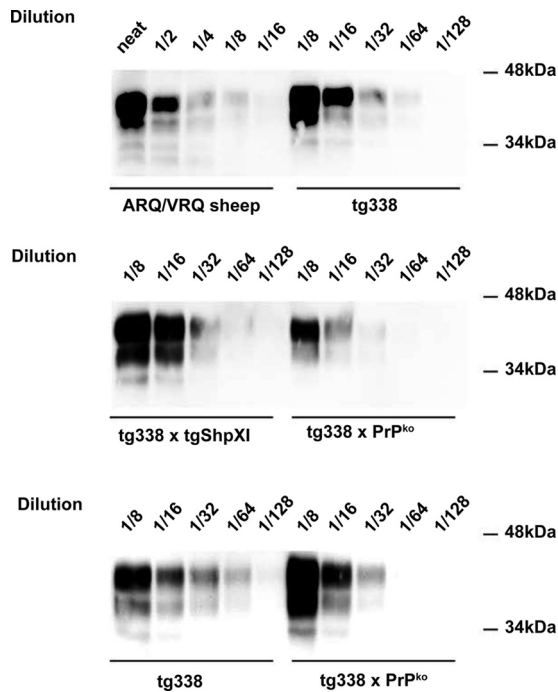
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**FIG 1** Cellular PrP expression level in the brain of ARQ/VRQ sheep and tg338, tg338 × PrP<sup>ko</sup>, and tg338 × tgShpXI mice. tg338 and tgShpXI mice express the ovine VRQ and ARQ variants of PrP, respectively. For each of the three mouse lines and a control sheep brain (cerebral cortex), a 10% brain homogenate was prepared. After denaturation in Laemmli's buffer, samples (either neat or diluted in Laemmli's buffer) were run on a 12% acrylamide gel and transferred onto a polyvinylidene difluoride (PVDF) membrane. PrP<sup>C</sup> was then detected using 8G8 antibody (epitope, <sub>97</sub>SQWNK<sub>102</sub>).

within the first 48 h of the life of the lamb. A group of animals was kept unchallenged (Table 2).

No disease transmission or PrP<sup>Sc</sup> accumulation was observed in lymphoid tissues (spleen, tonsil, mesenteric lymph node) or the central nervous system of the unchallenged control sheep. A 100% attack rate of disease was observed in sheep challenged with inocula containing  $10^{7.4}$  to  $10^{5.4}$  IC ID<sub>50</sub> units in sheep. In the animals

that were challenged with  $10^{4.4}$  IC ID<sub>50</sub> units in sheep, transmission was observed in 6 of the 9 animals. Clinical suspicions were confirmed by the presence of PrP<sup>Sc</sup> in the animals' tissues using Western blot analysis.

Together, these results indicate that in the investigated scrapie isolate, the infectious titers as measured by intracerebral endpoint titration in the different ovine PrP-expressing mice and in the ARQ/VRQ sheep were not statistically different. These findings are in agreement with data reported by Peretz et al. in hamster and transgenic mice overexpressing the hamster PrP and by Thackray et al. in conventional mice and transgenic mice overexpressing the mouse PrP (8, 9). They contradict the view that animal models that overexpress PrP have an intrinsic higher susceptibility to TSE agent infection than the natural host. They also strongly support the contention that the infectious titers measured by intracerebral endpoint titration in the transgenic mouse model and in the natural host are equally relevant for elaboration of TSE transmission risks.

Data reported in some mouse models (10) and bovine spongiform encephalopathy (BSE) in cattle (11) indicated that 1 ID<sub>50</sub> administered by the oral route is approximately equivalent to  $10^{5.5}$  to  $10^{5.6}$  IC ID<sub>50</sub> units. This range of values was the one we used to design our experiment. The results we obtained, using a sheep scrapie isolate and ARQ/VRQ sheep, indicated that 1 ID<sub>50</sub> oral unit in our paradigm is equivalent to less than  $10^{4.4}$  IC ID<sub>50</sub> units.

This discrepancy is a likely consequence of the complexity of the phenomena determining the capacity of TSE agents to transmit following peripheral exposure. Experimental evidence supports the view that both the nature of the TSE agent and the host species/genetic background have a direct impact on such transmission efficacy; whereas administration of 40,000 IC 50% lethal dose (LD<sub>50</sub>) units of 263K was shown to give 1 LD<sub>50</sub> by the intraperitoneal (IP) route in hamsters (12), in CW mice, 1 IP LD<sub>50</sub> of the 139A strain was equivalent on average to 430 IC LD<sub>50</sub> units (13).

In any case, the data we report indicate that the minimal oral infectious dose enabling the oral transmission of certain TSE agents might be significantly lower than it is usually considered to be.

**TABLE 1** Endpoint titration by the intracerebral route of a reference scrapie isolate in transgenic mice that express the ovine PrP and in ARQ/VRQ sheep<sup>a</sup>

|  |                               | Transmission in <sup>b</sup> :                   |                                      |  |                                      |  |                                      |  |                                      |
|--|-------------------------------|--|--------------------------------------|--|--------------------------------------|--|--------------------------------------|--|--------------------------------------|
|  |                               | tg338 mice                                       |                                      | tg338 × PrP <sup>ko</sup> mice                   |                                      | tg338 × tgShpXI mice                             |                                      | ARQ/VRQ sheep                                      |                                      |
| Dilution of the 4% (wt/vol) brain homogenate | Brain material per animal (g) | No. of infection-positive mice/total no. of mice | Incubation period (days) (mean ± SD) | No. of infection-positive mice/total no. of mice | Incubation period (days) (mean ± SD) | No. of infection-positive mice/total no. of mice | Incubation period (days) (mean ± SD) | No. of infection-positive sheep/total no. of sheep | Incubation period (days) (mean ± SD) |
| $10^{-3}$                                    | $8 \times 10^{-7}$            | 6/6  | 94 ± 6                               | 6/6  | 103 ± 4                              | 6/6  | 111 ± 9                              | 6/6  | 212 ± 16                             |
| $10^{-4}$                                    | $8 \times 10^{-8}$            | 4/6  | 117 ± 12                             | 4/6  | 128 ± 12                             | 2/6  | 124, 154                             | 3/6  | 242, 263, 275                        |
| $10^{-5}$                                    | $8 \times 10^{-9}$            | 0/6  | >250                                 | 0/6  | >250                                 | 0/6  | >250                                 | 0/6  | >650                                 |
| $10^{-6}$                                    | $8 \times 10^{-10}$           | 0/6  | >250                                 | 0/6  | >250                                 | 0/6  | >250                                 | 0/6  | >650                                 |
| $10^{-7}$                                    | $8 \times 10^{-11}$           | 0/6  | >250                                 | 0/6  | >250                                 | 0/6  | >250                                 | 0/6  | >650                                 |

<sup>a</sup> Successive 1/10 dilutions of a 4% tissue homogenate prepared using the brainstem from 70 ARQ/VRQ scrapie affected sheep (Langlade flock) were inoculated into groups of tg338 mice ( $n = 6$ ), tg338 × PrP<sup>ko</sup>, tg338 × tgShpXI and ARQ/VRQ sheep ( $n = 6$ ). In comparison with ARQ/VRQ sheep, (i) tg338 mice express about 8-fold, (ii) Tg338 × PrP<sup>ko</sup> mice express about 4-fold, and (iii) tg338 × tgShpXI mice express about 8-fold the ovine PrP<sup>C</sup> in their brain (see Fig. 1). Animals were euthanized when they showed clinical signs of infection or after 250 days postinoculation (mice) or 650 days postinoculation (sheep). Animals were considered infected when PrP<sup>Sc</sup> deposition was detected in the brain by Western blot analysis using the Sha31 monoclonal antibody which recognizes amino acids 145 to 152 (YEDRYRE) of the sheep PrP. Infectious titers were estimated by the Spearman-Kärber method (7), and they are reported as the number of ID<sub>50</sub> per gram of brain tissue.

<sup>b</sup> The most likely value and the lower and upper values of the 95% confidence intervals (in parentheses) of the infectious titers (ID<sub>50</sub> per gram of tissue) are as follows: for tg338 mice,  $10^{7.26}$  ( $10^{6.88}$  to  $10^{7.65}$ ); for tg338 × PrP<sup>ko</sup> mice,  $10^{7.25}$  ( $10^{6.88}$  to  $10^{7.65}$ ); for tg338 × tgShpXI mice,  $10^{6.93}$  ( $10^{6.55}$  to  $10^{7.32}$ ); and for ARQ/VRQ sheep,  $10^{7.1}$  ( $10^{6.69}$  to  $10^{7.51}$ ).

TABLE 2 Oral inoculation of ARQ/VRQ lambs with decreasing amounts of a reference endpoint titrated (IC route in ARQ/VRQ sheep) scrapie isolate<sup>a</sup>

| Equivalent brain material amount per lamb | No. of ID <sub>50</sub> IC units in ARQ/VRQ sheep | Transmission in orally challenged ARQ/VRQ sheep |                                      |
|---|---|---|--------------------------------------|
|   |   | No. of affected sheep/total no. of sheep        | Incubation period (days) (mean ± SD) |
| 2 g                                       | 10 <sup>7.4</sup>                                 | 10/10   | 257 ± 18                             |
| 200 mg                                    | 10 <sup>6.4</sup>                                 | 10/10   | 279 ± 37                             |
| 20 mg                                     | 10 <sup>5.4</sup>                                 | 8/8 <sup>b</sup>                                | 305 ± 44                             |
| 2 mg                                      | 10 <sup>4.4</sup>                                 | 6/9 <sup>b</sup>                                | 545 ± 61                             |
| Noninoculated control                     |   | 0/10  | >1,200                               |

<sup>a</sup> Groups of 10 ARQ/VRQ lambs were orally challenged with decreasing amounts of a reference scrapie isolate. This isolate had previously been endpoint titrated by the intracerebral route in ARQ/VRQ (Table 1). Lambs were challenged within their first 48 h of life by natural suckling.

<sup>b</sup> Some of the challenged animals died from intercurrent disease within the first months of life. At death, to confirm the scrapie infection status, PrP<sup>Sc</sup> deposition in lymphoid tissues and central nervous system was assessed in each animal by Western blot analysis.

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