Vertical Transmission of Bovine Spongiform Encephalopathy Prions Evaluated in a Transgenic Mouse Model

J. Castilla,¹ A. Brun,¹ F. Díaz-San Segundo,¹ F. J. Salguero,¹ A. Gutiérrez-Adán,² B. Pintado,² M. A. Ramírez,² L. del Riego,¹ and J. M. Torres^{1*}

Centro de Investigación en Sanidad Animal (CISA-INIA), Ctra. de Valdeolmos a El Casar, Valdeolmos, 28130 Madrid, Spain,¹ and Departamento de Reproducción Animal y Conservación de Recursos Zootécnicos (INIA), Avda. Puerta de Hierro s/n, Madrid 28040, Spain²

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In this work we show evidence of mother-to-offspring transmission in a transgenic mouse line expressing bovine PrP (boTg) experimentally infected by intracerebral administration of bovine spongiform encephalopathy (BSE) prions. PrP^{res} was detected in brains of newborns from infected mothers only when mating was allowed near to the clinical stage of disease, when brain PrP^{res} deposition could be detected by Western blot analysis. Attempts to detect infectivity in milk after intracerebral inoculation in boTg mice were unsuccessful, suggesting the involvement of other tissues as carriers of prion dissemination. The results shown here prove the ability of BSE prions to spread centrifugally from the central nervous system to peripheral tissues and to offspring in a mouse model. Also, these results may complement previous epidemiological data supporting the occurrence of vertical BSE transmission in cattle.

Prion diseases or transmissible spongiform encephalopathies (TSEs) belong to a class of infectious diseases characterized by the presence of an abnormally folded protein (PrP^{Sc}) that accumulates in the brains of affected individuals (24). TSEs may be of spontaneous, familial, or infectious origin. While spontaneous and familial etiologies have been described for the disease in humans (22, 23), infectious TSEs have been clustered mainly in domestic animals, from which sheep scrapie was the prototype of disease (17). The epidemic dimension of bovine spongiform encephalopathy (BSE) in the mid-1980s contributed to the spread of the disease to humans in the form of variant Creutzfeldt-Jakob disease (vCJD) (7, 8). It is now generally accepted that the consumption of contaminated meat and/or meat-derived products has been the most probable route of transmission of BSE prions to humans. Natural routes of transmission have been described for scrapie prions (16, 19, 20), although scant information is available regarding BSE natural routes of infection. The ability of scrapie prions to accumulate in placental tissues from genetically susceptible ewes (1, 25, 27) might be a contributing factor in scrapie epidemiology (16). However, this picture still remains diffuse for BSE. No PrP^{Sc} accumulation is detected in placentas from BSE-infected cattle (31), and neither blood nor milk from BSE-infected animals have yet been shown to be infectious, consistent with the apparent absence of the prion agent in peripheral tissues (3). Experiments to test maternal transmission in cattle showed that approximately 10% of calves born to cows with confirmed BSE developed disease (2). This transmission rate, however, was obtained in a scenario of disease prevalence, since some of the calves were born after the feed ban was fully effective.

The ability of prions to move from the central nervous sys-

tem (CNS) through afferent nerve fibers has been described for several TSEs, including genetic and sporadic human prion diseases (14, 15) and scrapie (28), and was suggested for chronic wasting disease (CWD) (26). Recently, it has been shown how vCJD and Gerstmann-Sträussler-Scheinker syndrome (strain Fukuoka-1) prions retaining full infectivity can be detected in the blood of mice after intracerebral inoculation (6). To test the ability of BSE prions to spread from CNS to peripheral tissues, we studied the efficiency of BSE transmission from intracerebrally BSE-inoculated mothers to their offspring in a transgenic mouse line (boTg110) expressing bovine PrP (4). boTg110 mice express boPrP controlled by the mouse PrP promoter at a level eight times that of the level of bovine PrP in cattle brain as previously described (4). Groups of boTg110 females were intracerebrally infected with a BSE inoculum named BSE₁ consisting of a pool from 49 BSE-infected cattle brains (TSE/08/59) supplied by the Veterinary Laboratories Agency (New Haw, Addlestone, Surrey, United Kingdom). The titer of this inoculum was $\sim 10^8$ 50% infective dose units per gram of bovine brainstem when measured in the boTg110 mouse line (data not shown). At different times postinoculation, infected female mice were mated with healthy homologous males (Table 1). Group I female mice (mated at 195 and 223 days postinoculation [d.p.i.]) showed a strong PrP^{res} signal as judged by Western blot analysis of brain extracts (data not shown). In contrast, only mouse 09 from group II (mated at 160 d.p.i.) showed detectable brain PrP^{res} accumulation, in good agreement with the kinetics of PrP^{res} deposition in this mouse model (4).

PrP^{res} was clearly detected by Western blotting in 2 out of 10 mice born from group I females (mated at 195 and 223 d.p.i.) but in only 1 out of 40 in group II (mated at 160 d.p.i.). The PrP^{res} banding pattern observed for group I positive brains was similar to that for brains from Tg110 mice intracerebrally challenged with the BSE₁ inoculum, and no differences could be observed in their relative molecular weight mobilities (Fig. 1A)

* Corresponding author. Mailing address: Centro de Investigación en Sanidad Animal INIA, Valdeolmos, 28130 Madrid, Spain. Phone: 34 91 620 23 00. Fax: 34 91 620 22 47. E-mail: jmtorres@inia.es.

TABLE 1. Vertical transmission of BSE in bo-PrP-Tg110 mice after intracerebral inoculation

Group	Inoculum	Mouse	d.p.i. to:				Clinical signs	PrP ^{res} in mothers ^a	No. of offspring with PrP ^{res} /total no. of offspring (d.p.i.)
			Mating	Offspring	Milking	Culling			
I	BSE ₁	01	195	246	256	274	Yes	+++	1/5 (622)
I	BSE ₁	02	223	250	258	274	Yes	+++	1/5 (613)
II	BSE ₁	09	160	182	190	237	Yes	++	1/13 (536)
II	BSE ₁	12	160	182	190	210	No	—	0/14
II	BSE ₁	14	160	182	190	210	No	—	0/13
III	None (control)	03	220	246	256	276	No	—	0/12
III	None (control)	05	220	246	256	276	No	—	0/10

^a +++ , strong PrP^{res} signal; ++ , PrP^{res} accumulation detectable in brain; — , no detectable PrP^{res} in brain.

Deglycosylation experiments with *N*-glycosidase F (PNGase F) confirmed this observation (Fig. 1B). However, differences in the amounts of immunoreactive PrP^{res} were found between group I and II: PrP^{res} levels in mouse 09/02 from group II were found to be clearly lower than those in mice from group I. This fact might be explained by the shorter survival time of this mouse (time to death, 536 d.p.i.) relative to those of mice from group I, which died at 622 and 613 days postinfection. Differences in the percentages of PrP^{res}-positive offspring among groups I and II (20% versus 2.5%; $P_{\text{test}} = 0.098$) might be related to the time after intracerebral BSE prion inoculation after which mating was allowed. Thus, higher transmission rates, defined by the presence of detectable PrP^{res}, are obtained if the accumulation of pathogenic PrP in brain is allowed to reach certain nonpathological levels without disturbing the reproductive competence of female mice. The high percentage of PrP^{res}-negative littermates could be attributed to the limited sensitivity of the Western blot technique (5). In addition, exploring the presence of PrP^{res} depositions by im-

munochemistry in brains from mice negative for PrP^{res} by Western blotting was consistently unsuccessful (data not shown). The lack of PrP^{res} detection, however, cannot exclude completely the existence of subclinical infections in the PrP^{res}-negative offspring. This assumption can be supported by the statistically significant differences ($P = 0.020$) observed in the survival times between offspring from infected (585 ± 60 , 589 ± 71 , 583 ± 36 , 566 ± 63 and 608 ± 20 d.p.i.) and control (637 ± 57 d.p.i.) mothers (Fig. 2). Moreover, there was no difference between the survival times of PrP^{res}-positive and PrP^{res}-negative offspring mice. To confirm the fact of subclinical infection, works on second-passage experiments are in progress.

The fact that BSE prions delivered into mice brains can be transmitted to a next generation is indicative of their intrinsic ability to centrifugally spread from the CNS to other peripheral tissues. In fact, the ability of prions to move from CNS through afferent nerve fibers has been also described for other TSEs, including genetic and sporadic human prion diseases (14, 15) and scrapie (28), and was suggested for chronic wast-

FIG. 1. (A) Comparison of Western blot profiles in brain detergent-insoluble fractions from PrP^{res}-positive offspring. Tg05/01, mouse born from the Tg05-uninfected female; Tg01/02 and Tg02/02, mice born from the Tg01 and Tg02 BSE₁ inoculum-infected females, respectively; Tg09/02, mouse born from the Tg09-infected female; C+, brain extract from a Tg110 mouse intracerebrally inoculated with BSE₁ inoculum; Mr, Relative molecular mass expressed in kilodaltons; PK, proteinase K treatment. Protein loads per lane are equivalent in progeny mice. In the Tg05/01 mouse the PK — lane shows soluble brain fraction. (B) Deglycosylation studies of PrP^{res} from control (C+) and progeny Tg02/02 brain extracts.

FIG. 2. Mean survival times of mice born from infected mothers. (A) Histograms showing survival times of the offspring of each female and of all offspring (boTg-Tot). BoTg-C-, offspring from uninoculated group III mothers. The values within the bars represent the days after inoculation $\pm$ standard deviations. The numbers of mice of each type inoculated are in parentheses. (B) Kaplan-Meier curves correspond to the overall groupings of the offspring (groups I and II).

ing disease (CWD) (26). Recently it has been shown how vCJD and Gerstmann-Sträussler-Scheinker syndrome (strain Fukuoka-1) prions retaining full infectivity can be detected in the blood of mice after intracerebral inoculation (6). The role of blood in BSE prion dissemination became more evident after the demonstration of BSE transmission to sheep via blood transfusion even during the preclinical phase of an experimental oral BSE inoculation in sheep (18). Our results indicated that BSE prions could be transmitted to the offspring after intracerebral inoculation in a process that seems to be more efficient when detectable amounts of PrP^{res} are present in the brain. The way by which prion infectivity is transmitted through a next generation could be then, based on previous work, be identified as blood dissemination. Other investigated tissues (placenta, lymphoid tissues, and gastrointestinal tract) were negative for PrP^{res} either by Western blotting or by analysis with immunohistochemistry (data not shown). However, these negative results do not allow one to conclude that there is a lack of infectivity in these tissues. In our experimental model, other fluids cannot be disregarded as vehicles for prion spread. To assess whether the route of infection through milk feeding was involved, we carried out experimental inoculations of milk extracted from mothers. For this purpose, 0.5 ml of pooled milk extracted from both infected and uninfected mothers was delipidated and intracerebrally injected into boTg110 mice after a concentration step (centrifugation at $25,000 \times g$ for 30 min). We estimate that the amount of milk used for the inoculations represents 25% of the milk intake during lactancy. Analysis of the survival times of mice inoculated or mock inoculated did not show any significant difference (Fig. 3). Brains from these mice were then analyzed with both histopathology and immunohistochemistry for the pres-

ence of PrP^{res}. Similarly, no PrP^{res} was detected (data not shown). This negative result does not exclude the potential of milk to transmit prions but suggests that the relevance of this fluid in infectivity might be very low if it exists at all. Thus, the centrifugal dispersion of prions together with the ability of blood to retain prion infectivity might account for the transmission of BSE prions to the offspring without excluding other possible ways.

With regard to BSE in cattle, previous fieldwork studies

FIG. 3. Survival times of boTg110 mice inoculated with donor milk samples. Survival times for mice inoculated with milk from healthy female boTg110 mice (MILK/C) or milk (MILK/Tg) and brain (ENC/Tg) pools from BSE₁-infected female boTg110 mice are shown. The values within the bars indicate the days postinoculation $\pm$ standard deviations. The numbers of mice inoculated with each type of sample are indicated in parentheses.

suggested that the disease may be passed from cow to calf (29, 30). However, there has been controversy and uncertainties regarding whether or not maternal transmission has implications in the prevalence of this disease similar to those that it has for sheep scrapie (9, 10). Our results reveal an enhanced risk of disease in mice born from BSE-infected mothers at the end stage of the incubation time. The same type of risk may apply to the offspring from BSE-infected cattle, as has been suggested from the epidemiological data (9). However, it is necessary to point out here some differences between our transgenic mouse model and bovine species. Firstly, boTg110 mice express boPrP at a level eight times that of bovine PrP in cattle brain; therefore, there is more PrP^C substrate available for conversion to PrP^{Sc}. Secondly, there are some evident differences with respect to the architectural anatomies of mouse and cattle placentations. In cattle, the placenta is bridged to the uterus by a cotyledonary form of attachment, and the structure is of the syndesmochorial type, in which the embryo trophoblastic layer and the maternal uterine epithelium are not fused. In contrast, mouse embryonic and uterine epithelia are completely fused (hemochorial). This type of structure allows blood from the uterine endothelium to be in close contact with the fetal placenta, therefore facilitating the chances for prion dissemination and embryonic contamination.

The BSE agent can propagate efficiently in sheep (11), and the possibility of sheep flocks becoming infected with BSE was raised (21). However, in contrast to findings for sheep scrapie, no evidence of PrP^{Sc} has been found in the reproductive tissues of sheep infected with BSE (13), nor has BSE been reported in the offspring of experimentally infected ewes (12). Since transmission of BSE prions to the offspring occurs in the mouse model, it is reasonable to assume that host-specific restrictions may compromise the ability of BSE prions to be vertically transmitted.

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