

Highly Bovine Spongiform Encephalopathy–Sensitive Transgenic Mice Confirm the Essential Restriction of Infectivity to the Nervous System in Clinically Diseased Cattle

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Transgenic mice expressing bovine prion protein (PrP)^C (Tgbov XV mice) display remarkably shorter incubation times for cattle-derived bovine spongiform encephalopathy (BSE) infectivity than do nontransgenic mice. To verify that this phenomenon reflects increased sensitivity, we challenged Tgbov XV mice and conventional RIII mice with a BSE brain-stem homogenate of known infectivity titer in cattle. An end-point titration experiment in Tgbov XV mice revealed their superior sensitivity, which exceeded that of RIII mice by at least 10,000-fold and even that of cattle by ~10-fold. Moreover, Tgbov XV mice were challenged with various tissues from cattle with end-stage clinical BSE, and infectivity was found only in the central and peripheral nervous system and not in lymphatic tissues; the only exception was the Peyer's patches of the distal ileum, which most likely are the site of entry for BSE infectivity. These results provide further indication that the pathogenesis of BSE in cattle is fundamentally different from that in sheep and mice, due to an exclusive intraneuronal spread of infectivity from the gut to the central nervous system.

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases that, under experimental conditions, can be transmitted to a wide range of mammalian species. During the pathogenesis of these diseases, the host-encoded neuronal surface protein, prion protein (PrP)^C, is converted into its fibrillogenic and partially proteinase K (PK)–resistant isoform, PrP^{Sc}. Transmission experiments in transgenic mice expressing the PrP of another species have revealed that the degree of sequence homology between the 2 PrPs involved influence the efficiency of the conversion process [1]. However, for transgenic mice expressing human PrP^C, this applies only

when the mice are bred on a wild-type murine PrP–ablated background [2] or when transgenic mice express a chimeric protein of human and murine PrP (with human sequences from codon 97 to 168) [3]. The species barrier may, therefore, be modulated by (1) polymorphisms in PrP regions that influence the conversion efficiency of the protein and (2) polymorphisms in domains that act as a chaperone binding site for the postulated protein X that assists in PrP^{Sc} formation [4]. Transgenic mice expressing bovine PrP^C on a PrP–ablated background have been reported to develop symptoms between 230 and 340 days after a bovine spongiform encephalopathy (BSE) infection, depending on the inoculum and on the infected transgenic mouse line, whereas transgenic mice expressing a chimeric protein of murine and bovine PrP with bovine sequences from codons 97 to 186 have been reported to be resistant [5].

In the past, numerous tissues from BSE-diseased cattle were tested in infectivity experiments that used a mouse inoculation bioassay with conventional RIII mice. In these experiments, the agent was detected only in the central nervous system (CNS), retina, spinal cord [6], and Peyer's patches of the distal ileum in

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Table 1. Titration of bovine spongiform encephalopathy with the British isolate BBP12/92 in Tgbov XV mice, RIII mice, and Tga 20 mice.

Category	Tgbov XV mice		RIII mice		Tga 20 mice	
	Infectivity titer per gram ^a		Infectivity titer per gram ^a		Infectivity titer per gram ^a	
Experimental result	7.67		3.27		>3.97 ^b	
	No. diseased/ no. inoculated	Mean (SE) or minimum incubation time, days	No. diseased/ no. inoculated	Mean (SE) or minimum incubation time, days	No. diseased/ no. inoculated	Mean (SE) or minimum incubation time, days
Inoculated mice, log ₁₀ dilution of the inoculum						
-1	7/7	230 (7.48)	6/6	434 (27.40)	5/5	397 (12.77)
-2	9/9	283 (12.89)	5/6	526 (27.73)	8/8	410 (12.48)
-2.7	9/9	280 (10.84)	3/5	659 (68.83)	9/9	490 (21.21)
-3.4	8/8	283 (15.19)	0/6	>770
-4.1	7/7	336 (47.04)	0/5	>770
-4.8	8/8	356 (33.33)	0/6	>770
-5.5	7/8	509 (35.23)	0/6	>770
-6.2	2/7	537 (45.01)	0/6	>770
-6.9	6/8	497 (33.95)	0/6	>770
-7.6	3/8	583 (0)	0/6	>770
Uninfected control	0/2	>742	0/7	>789	0/3	>510

NOTE. All mice were tested for prion protein (PrP)^{Sc} accumulation in the brain by Western blot and/or immunohistochemistry, and mice that were positive were considered to be diseased.

^a Calculated by the method of Spearman and Kaerber.

^b The infectivity titer was estimated, because the end point was not reached.

Table 2. Primary transmission and subpassages of bovine spongiform encephalopathy spleen pool SE1809/2 in Tgbov, RIII, and Tga 20 mice.

Passage, mouse line	Inoculum	Minimum incubation time (no. diseased/no. inoculated), days
Primary transmission		
RIII	SE1809/2	>785 (0/7)
	Tgbov XV (uninfected)	>700 (0/3)
Tga 20	SE1809/2	>611 (0/7)
	Tgbov XV	SE1809/2
Tgbov XV	Tgbov XV (uninfected)	>728 (0/5)
	SE1809/2	>790 (0/10)
Tgbov XII	SE1809/2	>847 (0/3)
Subpassage		
Tga 20	Tga 20/SE1809/2, mouse 1	>513 (0/4)
RIII	Tga 20/SE1809/2, mouse 1	>716 (0/5)
Tga 20	Tga 20/SE1809/2, mouse 2	>499 (0/5)
RIII	Tga 20/SE1809/2, mouse 2	>437 (0/4)
Tgbov XV	Tgbov XV/SE1809/2, mouse 1	>709 (0/4) ^a
Tgbov XV	Tgbov XV/SE1809/2, mouse 7	>728 (0/4) ^a
RIII	Tgbov XV/SE1809/2, mouse 7	>628 (0/4)
Tgbov XV	Tgbov XV/SE1809/2, mouse 9	>785 (0/5) ^a
RIII	Tgbov XV/SE1809/2, mouse 9	>643 (0/4)
Tgbov XV	Tgbov XV/SE1809/2, mouse 20	>643 (0/6)

NOTE. All mice were tested for prion protein (PrP)^{Sc} accumulation in the brain by Western blot and/or immunohistochemistry, and mice that were positive were considered to be diseased.

^a A second subpassage experiment from these groups had been ongoing in Tgbov XV mice without any clinical signs of transmission for >600 days at the time of manuscript preparation.

experimentally infected calves [7]. All other investigated tissues—including the spleen, other lymphatic tissues, skeletal muscle, et cetera—were reported to be free of detectable infectivity [6, 8]. This is in sharp contrast to what is observed during scrapie in sheep, hamsters, and mice, in which the agent is easily detectable in peripheral nervous system (PNS) tissue [9–11], spleen, tonsils, other lymphatic tissues [12, 13], and skeletal muscle [14, 15], even during the preclinical state. However, as in the above-mentioned experiments, the BSE agent had to cross the species barrier between cattle and mouse, and negative results of challenge experiments with tissues from BSE-diseased cattle in conventional RIII mice could not reliably exclude low levels of infectivity therein.

To obtain more-sensitive mouse models for the detection of BSE infectivity—ones that might even lack species-barrier effects—we generated and characterized transgenic mice overexpressing bovine PrP^C (Tgbov XV mice) and murine/bovine chimeric PrP^C (Tgmubo XIII mice). Tgbov XV mice turned out to be at least 10,000-fold more sensitive to BSE infection than conventional RIII mice and ~10-fold more sensitive than cattle. Therefore, Tgbov XV mice are extremely valuable for infectivity bioassays of CNS and PNS tissue from BSE-diseased cattle.

MATERIALS AND METHODS

Mouse lines. The generation of Tgbov XV and Tgmubo XIII mice on the basis of the half-genomic vector pPrPHG has been

described elsewhere [16]. Tga 20 mice were provided by C. Weissmann (Scripps Institute; Miami, FL). RIII mice were a gift from M. Bruce (Neuropathogenesis Unit, Institute for Animal Health; Edinburgh).

BSE end-point titration experiment. In previously described BSE challenge experiments, Tgbov XV mice died of the disease after markedly reduced incubation times, whereas incubation times in Tgmubo XIII mice were extremely prolonged, even after subpassage [16]. A BSE titration experiment was conducted with the British isolate BBP12/92 in Tgbov XV mice, conventional RIII mice, and Tga 20 mice overexpressing murine PrP^C. This isolate has been used previously in the United Kingdom for titration experiments in RIII mice and cattle, and titers of 10^{3.3} and 10^{6.0} ID₅₀/g were determined [17]. Starting from a 10% homogenate of this sample, serial dilutions in 10 log₃ steps, ending at 10^{7.6}, were prepared and inoculated into 6–8 mice/group (20 μL intracerebrally [ic] plus 100 μL intraperitoneally [ip]). The mice were supervised for the development of clinical symptoms as described below, and all were tested for the accumulation of PrP^{Sc} in their brains at the end of the experiment. On the basis of these data, the number of infectious units per gram of tissue sample that led to an infection in 50% of the mice (i.e., the ID₅₀/g) was calculated by the method of Spearman [18] and Kaerber [19].

Challenge with a pool of spleens from BSE-diseased cattle. A pool of spleens from 3 clinically diseased British cattle (des-

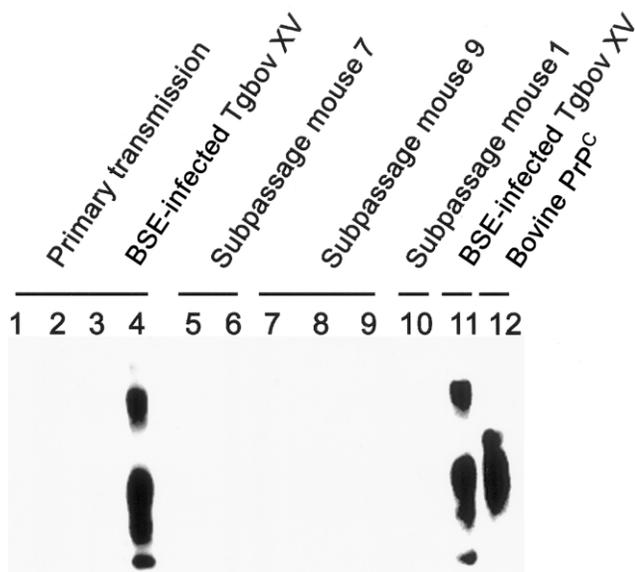


Figure 1. Scrapie-associated fibril preparation and Western blot of Tgbov XV mice challenged with spleen pool SE1809/2. Shown are results for primary transmission of the spleen pool (lanes 1–3), a bovine spongiform encephalopathy (BSE)–infected Tgbov XV mouse (lanes 4 and 11), subpassages into Tgbov XV mice using homogenates from Tgbov XV mice inoculated with the spleen pool (lanes 5–10), and bovine prion protein (PrP^C) (lane 12).

ignated SE1809/2) that has also been used for transmission experiments in cattle (S. Hawkins, personal communication) was inoculated into Tgbov XV, RIII, and Tga 20 mice. Eight mice per group were challenged (20 μ L ic plus 100 μ L ip), and subpassages were performed in Tgbov XV and Tga 20 mice. All groups were observed for at least 700 days, and mouse brains were tested for the accumulation of PrP^{Sc} by both Western blot and immunohistochemistry (IHC). To completely exclude the possibility of any accidental transmission to these inoculated mice, they were kept in a room where no BSE infection studies had ever been performed. All mouse cages and other equipment were new, and all staff changed all of their clothing before entering the room.

Challenge with tissues from a BSE-diseased cow. Numerous samples, including samples of CNS and PNS tissues as well as lymphatic tissues, had been collected from a clinically diseased cow. This cow had been delivered live to the German National Reference Laboratory while showing severe symptoms of late-stage clinical BSE, such as extreme nervousness, ataxia, and loss of balance. The cow was in late-stage pregnancy (2–3 weeks before calving), but it had to be killed for the welfare of the animal. After it was killed, a wide panel of neural and nonneural tissues were collected under TSE-sterile conditions, to prevent any infectivity from being carried over from one sample to the next. This was ensured by using fresh, single-use instruments for each sample biopsy and by taking a central

piece from each sample with fresh, single-use instruments for the challenge inoculate. Ten percent homogenates were prepared in 0.9 % NaCl solution by aspiration with syringes and needles (18G–23G). These homogenates were then inoculated (20 μ L ic plus 100 μ L ip) into Tgbov XV and RIII mice. Body fluids (colostrum, amniotic fluid, and cerebrospinal fluid) were inoculated undiluted under the same conditions as were tissue homogenates. Subpassage experiments with selected groups of mice were performed by pooling 3 mice brains from challenged Tgbov XV mice and inoculating ic 30 μ L of a 10% homogenate thereof into 10 Tgbov XV mice.

Groups of mice that were challenged with tissue homogenates in which the presence of BSE infectivity was uncertain were also housed in a room where no BSE infection studies had ever been performed. The equipment employed had not been used in other TSE experiments.

Supervision of infected mice. Mice were checked for their health status every other day. Those showing at least 2 clinical symptoms of disease—such as hind limb paresis, abnormal tail tonus, behavioral changes, and weight loss—over several consecutive days were killed, and samples were collected for diagnostic evaluation. Incubation times were calculated as the time between inoculation and death. All mice in an experiment were tested for PrP^{Sc} accumulation in their brains, and only those with positive results were included in the calculation of incubation times.

Histological examination. Mouse brains were fixed in 3.5% formol and embedded in paraffin by routine methods [20]. Tissue sections were examined by hematoxylin-eosin staining and IHC (section thickness, 5 and 3 μ m, respectively). For the detection of murine PrP^{Sc} in RIII and Tga 20 mice, rabbit serum Ra10/7 diluted 1:7000 was applied [21], after a pretreatment step that included incubation of slides in formic acid for 15 min, incubation of slides with 4 μ g/mL PK for 15 min at 37 °C, and hydrated autoclaving for 15 min, to improve the accessibility of the antibody epitopes. For detection of bovine PrP^{Sc}, slides were incubated with monoclonal antibody (MAb) L42 diluted 1:100 [22], after the same pretreatment step. To prove the specificity of this IHC staining, selected sections from Tgbov XV mice were also stained with MAb SAF70 diluted 1:2000 [23]. A biotinylated goat anti–mouse or goat anti–rabbit conjugate (Vector) was used to visualize the binding of the primary antibody. For signal detection, an avidin-biotin–complex system (Vectastain ABC Elite kit), in combination with VIP substrate (Vector), was used.

Scrapie-associated fibril preparation and Western blot. Two hundred microliters of a 5% brain homogenate was incubated with 50 μ g/mL PK (Boehringer Mannheim) for 60 min at 55°C, to completely digest all PrP^C. The reaction was stopped by the addition of 10 mmol/L phenylmethylsulfonyl fluoride. After addition of sarcosine (to a final concentration of 10%) and Tris-HCl (to a final concentration of 10 mmol/L), the

Table 3. Challenge of Tgbov XV mice and RIII mice with 10% tissue homogenates from a terminally bovine spongiform encephalopathy-diseased cow.

Tissue sample	RIII mice		Tgbov XV mice		Second subpassage, ^a no. negative ^b / no. inoculated (no. of days)
	No. diseased/ no. inoculated	Mean (SE) or minimum incubation time, days	No. diseased/ no. inoculated	Mean (SE) or minimum incubation time, days	
Brain stem	5/16	394 (10.04)	14/14	208 (2.33)	...
Spinal cord T11	7/15	441 (35.11)	12/12	262 (9.56)	...
Spinal cord L4	5/10	492 (5.85)	15/15	236 (5.33)	...
Retina	6/12	538 (16.57)	10/13	331 (5.90)	...
Optical nerve	0/5	>536	13/14	407 (18.19)	...
Facial nerve	0/12	>719	11/14	526 (31.76)	...
Sciatic nerve	0/15	>719	9/13	438 (27.33)	...
Radial nerve	0/12	>644	0/13	>714	5/7 (>540)
Distal ileum	0/12	>727	3/13	574 (13.00)	6/8 (>540)
Cerebrospinal fluid	0/12	>727	0/12	>727	4/6 (>540)
Spleen	0/12	>720	0/14	>727	5/7 (>540)
Tonsil	0/14	>735	0/11	>706	5/6 (>540)
Mesenteric lymph node	0/11	>726	0/12	>715	...
Musculus semitendinosus	0/10	>686	1/10	520	8/10 (>600)
Musculus longissimus dorsi	0/10	>721	0/13	>715	7/10 (>600)
Heart	0/7	>727	0/11	>727	...
Caruncle	0/14	>715	0/12	>727	3/5 (>540)
Amniotic fluid	0/10	>726	0/13	>726	...
Colostrum	0/11	>721	0/13	>715	8/10 (>600)
Negative cattle brain (brain stem)	0/14	>735	0/12	>706	...

NOTE. All mice were tested for prion protein (PrP)^{Sc} accumulation in the brain by Western blot and immunohistochemistry, and mice that were positive were considered to be diseased.

^a A second subpassage experiment from the indicated groups had been ongoing in Tgbov XV mice for the indicated no. of days at the time of manuscript preparation without any clinical signs or detection of PrP^{Sc} accumulation in the brains of these mice.

^b By Western blot.

samples were incubated for 15 min at room temperature. The samples were then carefully loaded in 250 μ L of a 10 mmol/L Tris-HCL solution containing 10% sucrose and were centrifuged at 540,000 g for 45 min. The pellets were resuspended in 40 μ L of gel loading buffer containing 2% SDS and were incubated for 5 min at 95°C. Samples were separated in a 16% SDS-PAGE gel and were investigated by Western blot, with MAb L42 cell-culture supernatant diluted 1:10 used as detection antibody for ruminant PrP^C and with rabbit serum Ra10/7 diluted 1:5000 used for detection of murine PrP^C. To verify the specificity of the applied antibody, selected samples were also investigated by use of MAb 6H4 diluted 1:10,000 [24].

RESULTS

High BSE susceptibility of Tgbov XV mice. A parallel end-point titration experiment with the inoculum BBP12/92 was performed in Tgbov XV, RIII, and Tga 20 mice. Although an infectivity titer of $10^{3.27}$ ID₅₀/g was determined for RIII mice (which corresponded well to the $10^{3.3}$ ID₅₀/g that had previously been determined for this isolate [17]), the infectivity titer determined for Tgbov XV mice was $10^{7.67}$ ID₅₀/g (table 1). All

mice were individually tested for PrP^{Sc} accumulation in the brain by Western blot and/or IHC. An exact infectivity titer could not be calculated for Tga 20 mice, because all mice from all infected groups (lowest dilution, $10^{-2.7}$ ID₅₀/g) died of the disease, but it was determined that the infectivity titer for these mice must be $>10^{3.97}$ ID₅₀/g. A subpassage experiment using Tgbov XV mice that received the 10^{-1} dilution in the above-described titration experiment revealed no significant reduction in incubation time: 235 days after primary transmission and 240, 225, 211, and 238 days in 4 independent subpassage experiments. In contrast to these results, BSE susceptibility of Tgmubo XIII mice turned out to be extremely low, irrespective of their genetic PrP background (authors' unpublished data).

No detectable infectivity after challenge with a bovine spleen pool. Tgbov XV, RIII, and Tga 20 mice were challenged with a 10% homogenate of a pool of 3 spleens from cattle with clinical BSE by a combined ic and ip inoculation, and none of the mice developed symptoms associated with clinical BSE, even after 700 days. All mice that had died during or were killed at the end of the experiment tested negative for PrP^{Sc} accumulation in their brains, by both Western blot and IHC. Moreover,

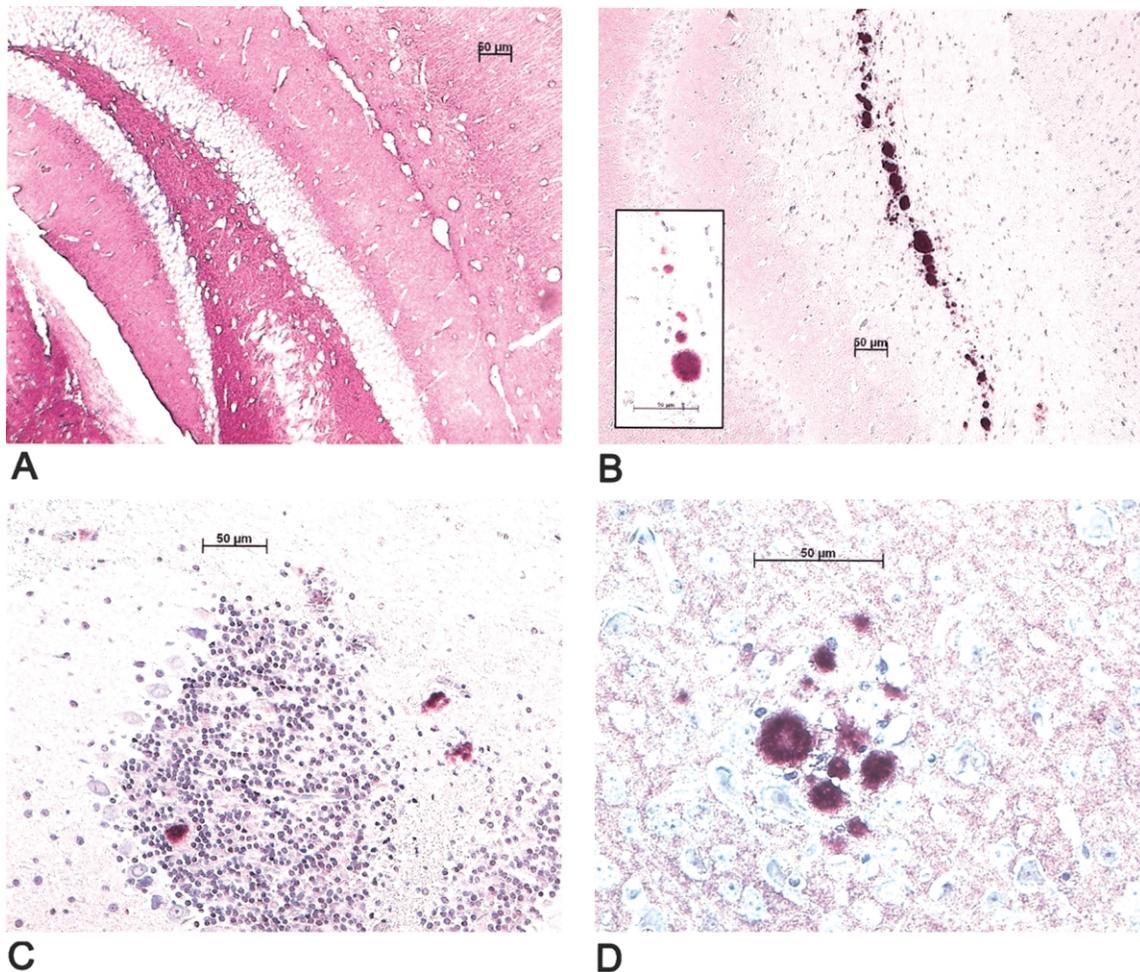


Figure 2. Immunohistochemical staining of Tgbov XV mice, to detect prion protein (PrP)^{Sc} accumulation. Shown are results for uninfected Tgbov XV mice (A) and Tgbov XV mice infected with tissue from a bovine spongiform encephalopathy–diseased cow showing severe accumulation of PrP^{Sc} plaques in the hippocampus (bovine brain homogenate) (B), cerebellum (bovine retina homogenate) (C), and thalamus (bovine distal ileum) (D). Monoclonal antibody L42 was applied for specific detection of bovine PrP^{Sc}.

a subpassage experiment that used brain homogenates from selected Tgbov XV and Tga 20 mice from the first transmission experiment and that again included mice from both transgenic lines did not reveal subclinical infections (table 2). At the end of the experiment, all mice were again negative for PrP^{Sc} accumulation in their brains, by both Western blot (figure 1) and IHC. A second subpassage experiment had been ongoing for >600 days at the time of manuscript preparation, and no mice had succumbed to the disease, reconfirming the results of the first transmission experiment.

Agent distribution in a terminally BSE-diseased cow. A variety of tissue homogenates collected from a terminally BSE-diseased German cow was inoculated into Tgbov XV and RIII mice (table 3). As expected, the samples collected from the CNS (brain stem, spinal cord, and retina) transmitted well to both mouse lines. The shortest incubation time was observed after Tgbov XV mice were challenged with a 10% homogenate

of brain stem from the cow (208 days). PNS tissue also turned out to carry BSE infectivity, albeit at lower levels. Tgbov XV mice, but not RIII mice, inoculated with either sciatic or facial nerve homogenates died of BSE after incubation times of 438 and 538 days, respectively. The optical nerve, which ontogenetically is part of the CNS, transmitted the disease only to Tgbov XV mice (mean incubation time, 407 days), whereas the retina was found to transmit the disease to both mouse lines. On the other hand, the radial nerve did not transmit the disease to Tgbov XV mice during the first or second passage (the subpassage experiment had been ongoing for 540 days at the time of manuscript preparation). A sample of cerebrospinal fluid was negative in the first transmission experiment and after 540 days of a subpassage experiment in Tgbov XV mice.

It has been previously reported that the lymphatic tissue in the Peyer's patches attached to the distal ileum of experimentally infected calves contained BSE infectivity by the RIII mouse bio-

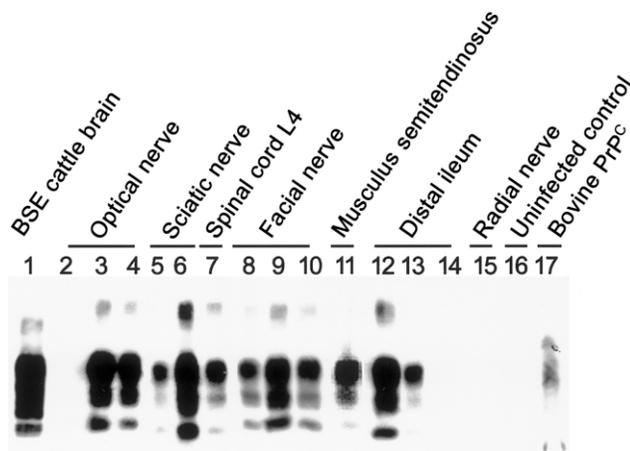


Figure 3. Scrapie-associated fibril (SAF) preparation and Western blot of selected mice challenged with homogenates of tissue from a bovine spongiform encephalopathy (BSE)-diseased cow. Shown are results for a BSE-positive cattle brain (*lane 1*), Tgbov XV mice challenged with bovine tissues (*lanes 2–15*), an uninfected Tgbov XV mouse (*lane 16*), and bovine prion protein (PrP^C) (*lane 17*). SAFs were prepared by ultracentrifugation through a sucrose cushion. Monoclonal antibody L42 was used as detection antibody in the Western blots.

assay, whereas naturally infected cattle were negative by the same bioassay. In the present study, infectivity also could be detected in the Peyer's patches of the distal ileum of the naturally infected German cow by use of the highly BSE-susceptible Tgbov XV mice (mean incubation time, 574 days), whereas the RIII mouse bioassay result was negative. A subpassage from mice that were negative after the first transmission remained negative after 540 days of an ongoing subpassage experiment. Interestingly, all other lymphatic tissues that were included in this experiment, such as spleen and tonsils, were free of detectable infectivity by both mouse bioassays and in a subpassage experiment in Tgbov XV mice after 540 days. IHC and Western blot results of these experiments are summarized in figures 2 and 3.

Because the cow had been in the late stage of pregnancy when clinical symptoms of BSE became obvious and severe, samples from the reproductive tract (such as samples of amniotic fluid, colostrum, and caruncle) were used in transmission experiments and were also found to be free of detectable infectivity by both the Tgbov XV and the RIII mouse bioassay. Subpassage experiments in Tgbov XV mice revealed no BSE infectivity 600 days after the mice were challenged with colostrum and 540 days after mice were challenged with a caruncle homogenate.

Finally, 2 samples of muscle from this cow—musculus longissimus dorsi (long back muscle) and musculus semimembranosus (hind quarter)—were tested by the Tgbov XV mouse bioassay. None of the Tgbov XV mice died of BSE after challenge with the musculus longissimus dorsi homogenate, and all mice remained healthy after >600 days of subpassage. Surprisingly, of

the 10 Tgbov XV mice challenged with a homogenate of musculus semitendinosus, 1 (which was found dead after 520 days) had accumulated PrP^{Sc} in its brain, as detected by Western blot. Unfortunately, IHC could not be performed for this mouse. Subpassage of brain material from 3 other mice in this group into Tgbov XV mice had not provoked the disease as of the time of manuscript preparation, after >600 days. Subpassage experiments in Tgbov XV mice are summarized in table 3.

DISCUSSION

The data presented here underscore the high value of Tgbov XV mice for the detection of minute concentrations of BSE infectivity in BSE-diseased cattle. Tgbov XV mice overexpress bovine PrP^C, which, according to PrP theory, should render them free of the species barrier for cattle-derived BSE infectivity. To verify this, an end-point titration experiment using a cattle BSE brain-stem pool for which the infectivity titer in cattle was known from previous experiments was conducted in these transgenic mice as well as in conventional RIII mice. Tgbov XV mice proved to be at least 10,000-fold more sensitive to cattle-derived BSE infectivity than RIII mice and even ~10-fold more sensitive than the original host species itself, cattle. Tgbov XV mice, therefore, represent a most valuable tool for all kinds of BSE infectivity studies of cattle tissues and bodily fluids (e.g., for risk assessment of meat products entering the human food supply, of medical products, or of any other consumer goods derived from bovine tissues). Moreover, this mouse bioassay will help to further determine the route of the BSE agent after oral uptake into the CNS of cattle.

This is the first study in which highly BSE-susceptible transgenic mice have been challenged with a variety of tissue samples collected from terminally BSE-diseased cattle. First, we tested a pool of spleens, which is one of the most important organs of the lymphoreticular system (LRS), from 3 clinically diseased British cattle. It is well known that the lymphatic system is involved in TSE pathogenesis in sheep, as it has been demonstrated by the detection of both PrP^{Sc} and infectivity during early preclinical disease stages [12, 25–28]. In contrast to the well-documented transmission and intracorporal spread of TSE infection in small ruminants, TSE pathogenesis in cattle is far less well understood. To date, all previous investigations of bovine lymphatic tissue by IHC or conventional mouse bioassays have given a negative result [6, 29]. However, because these methods are known to be rather insensitive, it was suspected that BSE infectivity was present in these tissues, only at concentrations low enough to be undetectable. Our transgenic mouse bioassay results for 2 different spleen homogenates derived from 4 terminally BSE-diseased cattle (including 1 British spleen pool homogenate) provide more strong evidence that supports the idea that BSE infectivity cannot be found in this organ in cattle. Even after 2 subpassages, no signs of a BSE

infection were observed in any of the inoculated mice, and no PrP^{Sc} accumulation was detected in their brains. Challenge with a homogenate of a mesenteric lymph node also did not provoke disease in Tgbov XV mice, underscoring the hypothesis that the LRS, as an organic system, does not play an essential role in the propagation of BSE infectivity in cattle. The only exception was the Peyer's patches of the distal ileum from a terminally BSE-diseased German cow, in which we detected BSE infectivity by the Tgbov XV mouse bioassay but not by the RIII mouse bioassay. In previous experiments, this tissue had been found to be positive only in cattle that had been experimentally infected with a massive BSE dose (100 g of brain-stem homogenate) but not in cattle that were naturally infected with BSE [7]. These results, therefore, indicate that BSE infectivity propagates poorly only in this part of the bovine LRS and from there spread centripetally to the CNS via nonlymphatic tissues, such as those of the enteric nervous system and the PNS.

When we investigated samples from the CNS and PNS of a clinically diseased cow, BSE infectivity was found in all brain regions, the spinal cord, the optical nerve, the retina (which contains neuronal cells that outgrow from the brain during the embryonic stage), and the facial and sciatic nerves. The radial nerve was negative for BSE infectivity after the first transmission and after 540 days of a subpassage experiment in Tgbov XV mice. Because the facial and sciatic nerves, unlike the radial nerve, are in close proximity to the CNS, there may also be centrifugal spread within the peripheral nerves, with the highest level of infectivity in the neuronal stromata.

Bioassay in Tgbov XV mice of a musculus longissimus dorsi sample from the same cow revealed no infectivity therein. However, 1 of 10 mice challenged with a musculus semitendinosus sample developed BSE, even though a blind passage of 3 other mice from the same inoculation group was negative (600 days after inoculation). Although a technical error should not categorically be excluded, an explanation for this unusual result may be that musculus semitendinosus is innervated by a branch of the sciatic nerve, which the present study has shown to contain infectivity. However, the potential level of infectivity in the examined bovine muscle is lower than that in the brain by at least 6 log steps, as can be deduced from the results of our titration experiment. This result underscores the importance of performing BSE rapid testing on the brain stems of slaughtered cattle, which is obligatory in the European Union and elsewhere, on the basis of its relevance for consumer protection.

Because the cow had been in the late stage of pregnancy, we had the opportunity to also test various samples of the gestating reproductive organs. Interestingly, no infectivity was found in colostrum. It is noteworthy that colostrum has a much higher cell content than does milk collected during later stages of the lactation period and that milk with a high cell content (such as colostrum) is not allowed to enter the human food supply.

The amniotic fluid and caruncle samples that were tested by the Tgbov XV mouse bioassay were also free of detectable infectivity. The results of the transmission experiments with tissue samples from the reproductive system do not point to an intrauterine vertical transmission. These data are, therefore, in line with the general observation that maternal transmission from cow to calf does not play an important role in the epidemiologic profile of BSE [30].

Taken together, our results have demonstrated that Tgbov XV mice effectively lack a species barrier for cattle-derived BSE infectivity. Because incubation times are much shorter and mice are generally much easier to handle, Tgbov XV mice can be used to replace vastly more expensive and impractical BSE bioassays in bovines. Moreover, our Tgbov XV mouse bioassay results for various tissues have indicated that, after the agent has been initially taken up from the alimentary tract via the intestinal lymphatic system, the spread of BSE infectivity in cattle is strictly neurotropic.

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