Short Communication

Chronic wasting disease and atypical forms of bovine spongiform encephalopathy and scrapie are not transmissible to mice expressing wild-type levels of human prion protein

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The association between bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (vCJD) has demonstrated that cattle transmissible spongiform encephalopathies (TSEs) can pose a risk to human health and raises the possibility that other ruminant TSEs may be transmissible to humans. In recent years, several novel TSEs in sheep, cattle and deer have been described and the risk posed to humans by these agents is currently unknown. In this study, we inoculated two forms of atypical BSE (BASE and H-type BSE), a chronic wasting disease (CWD) isolate and seven isolates of atypical scrapie into gene-targeted transgenic (Tg) mice expressing the human prion protein (PrP). Upon challenge with these ruminant TSEs, gene-targeted Tg mice expressing human PrP did not show any signs of disease pathology. These data strongly suggest the presence of a substantial transmission barrier between these recently identified ruminant TSEs and humans.

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of fatal infectious neurodegenerative diseases that include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in cervids and Creutzfeldt–Jakob disease (CJD) in humans. TSEs are characterized by the accumulation in the brain of PrP^{TSE}, which is a protease resistant conformational variant of the normal host-encoded cellular prion protein (PrP^{c}). Due to the infectious nature of TSEs, these diseases can be transmitted via a number of different routes. While TSEs tend to transmit more readily within species they are also able to transmit between species, although efficiency is dependent on both the TSE agent and host. Often transmission to a new species may initially present low transmission rates; however, further passage within the new species may result in increased transmission rates and

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shorter incubation periods. The transmission of BSE to humans through contaminated food is thought to be the cause of the variant form of Creutzfeldt–Jakob disease (vCJD) (Bruce *et al.*, 1997; Hill *et al.*, 1997). This relationship reveals a potential risk of transmission of other ruminant TSEs to humans. In the present study, we aimed to assess this risk by using gene-targeted transgenic (Tg) mice expressing human PrP as a model system for investigating transmissibility of several atypical ruminant TSE agents (atypical BSE, atypical scrapie and CWD).

Until recently, TSE disease in cattle was believed to be caused by a single TSE strain, classical BSE (BSE-C). However, two atypical BSE agents have recently been reported (Biacabe *et al.*, 2004; Casalone *et al.*, 2004; Jacobs *et al.*, 2007; Stack *et al.*, 2009), and are identified as H-type BSE (BSE-H) and bovine amyloidotic spongiform encephalopathy (BASE, also named BSE-L). Given the association of classical BSE with vCJD, in the present study we investigated the potential risk of transmission of these atypical forms of BSE to humans. CWD is a fatal, endemic TSE disease affecting free-ranging and captive cervids, including mule deer, white-tailed deer, Rocky Mountain elk and moose. Although CWD has not been reported in Europe, cases have been found in 14 USA states, two Canadian provinces and in South Korea. CWD has been shown to spread via a variety of routes (Denkers *et al.*, 2010; Mathiason *et al.*, 2009; Miller & Williams, 2003; Miller *et al.*, 1998; Sigurdson *et al.*, 1999; Trifilo *et al.*, 2007), and transmission between cervids is highly efficient. In addition to brain, spinal cord and lymphoid tissues (Race *et al.*, 2007; Sigurdson *et al.*, 1999; Spraker *et al.*, 2002), PrP^{TSE} has also been found in muscle, saliva, urine, fat, blood and antler velvet of CWD-infected cervids (Angers *et al.*, 2006, 2009; Haley *et al.*, 2009, 2011; Mathiason *et al.*, 2006; Race *et al.*, 2009a). Due to hunting of deer and elk, the possible consumption of CWD-infected meat raises concern over the risk to humans. Furthermore, previous studies have shown the intracerebral and oral transmission of CWD into squirrel monkeys (Race *et al.*, 2009b). Atypical scrapie, also known as Nor98, was first identified in 1998 in sheep in Norway (Benestad *et al.*, 2003) and can be distinguished from classical scrapie and BSE by the biochemical features of PrP^{TSE}, and its pathology and transmission characteristics. Despite the fact that no evidence of transmissibility of classical scrapie to humans has ever been obtained, atypical scrapie is a newly identified TSE, and is now known to have been present throughout the BSE epidemic (Benestad *et al.*, 2003, 2008); thus, the risk to humans warrants investigation.

To address the transmissibility of these recently recognized ruminant TSEs to humans, we performed inoculations of two forms of atypical BSE (BASE and H-type), one isolate of CWD (from white-tailed deer), six field isolates of atypical scrapie and one sheep passaged isolate of atypical scrapie into a panel of gene-targeted Tg mice expressing human PrP under the same spatial and temporal controls as wild-type PrP (Bishop *et al.*, 2006). Previously, three lines of Tg mice (HuMM, HuMV and HuVV) were generated (Bishop *et al.*, 2006) that represent the genetic diversity in the human population, due to the PrP codon 129-methionine/valine polymorphism. Interestingly, this polymorphism correlates with human susceptibility to TSE, and all confirmed clinical cases of vCJD to date have occurred in individuals who are methionine homozygous at PrP codon 129. In addition, we also inoculated these ruminant TSEs into gene-targeted Tg mice expressing bovine PrP (named Bov6 mice) and wild-type 129/Ola mice (which have the same genetic background as the human and bovine PrP Tg mice) as controls.

For experimental setup at The Roslin Institute, groups ($n=24$) of gene-targeted Tg mice expressing human (HuMM, HuMV and HuVV) or bovine PrP (Bov6) and 129/Ola controls were inoculated intracerebrally (i.c.) with 0.02 ml 10^{-1} brain homogenate (BASE, BSE-H, CWD or atypical scrapie) into the right cerebral hemisphere under halothane anaesthesia. As inocula were sourced from field cases they were treated with gentamicin (0.25 mg ml^{-1}) prior to inoculation to remove bacterial contamination. In complementary studies, groups of the same HuMM, HuMV and HuVV mice were also inoculated i.c. (20 µl) and intraperitoneally (i.p.) (100 µl) with BASE inoculum at 'Carlo Besta' Neurological Institute, Milan, and i.c (20 µl) with two different cases of BASE and BSE at the Istituto Superiore di Sanità, Rome, Italy. Mice were scored each week for clinical signs of disease and killed by cervical dislocation or carbon dioxide (Rome, Italy) at a predefined clinical end point, or due to welfare reasons (Dickinson *et al.*, 1968). Brains and spleens were recovered at post-mortem. To assess the abundance and location of TSE-associated vacuolation in grey and white matter of the brain, sections were cut (6 µm) from each mouse brain and stained using haematoxylin and eosin. TSE-related vacuolation was assessed at nine grey-matter regions (medulla, cerebellum, superior colliculus, hypothalamus, thalamus, hippocampus, septum, retrospinal cortex, cingulated and motor cortex) and three regions of white matter (cerebellar white matter, midbrain white matter and cerebral peduncle) as described previously (Fraser & Dickinson, 1967). Sections of brain tissue were also examined for abnormal PrP deposition, which is a key pathological marker of TSE infection, by immunohistochemistry and Western blot analysis following phosphotungstic acid (PTA) precipitation using mAb6H4 (Prionics) as described previously (Bishop *et al.*, 2006). Although some mice in these experiments exhibited clinical signs of disease, following analysis of all mice in this study for vacuolar pathology and PrP deposition, no signs of TSE pathology were detected in any of the gene-targeted human PrP Tg mice (Table 1). Transmission of BASE and BSE-H in Bov6 and 129/Ola mice was detected as described previously (Wilson *et al.*, 2012); however, no transmission of atypical scrapie was observed in these two control mouse lines.

Recent studies of TSE inoculations in mice that result in inefficient disease transmission have identified that lymphoid tissues were more permissive to TSEs than brain (Béringue *et al.*, 2012). Tg338 (ovine PrP) mice inoculated

Table 1. Transmission of BASE, BSE-H, CWD and atypical scrapie to human and bovine PrP Tg mice

>*n*, Represents the survival in days of the oldest mouse in groups where pathological signs of disease were not observed in any animals. NA, Not applicable.

TSE isolate	Mouse line									
	HuMM		HuMV		HuVV		Bov6*		129/Ola*	
	Survival time	No. affected	Survival time	No. affected	Survival time	No. affected	Survival time	No. affected	Survival time	No. affected
BASE (Roslin)	>687	0/24	>672	0/24	>763	0/24	547±18†	24/24‡	>687	1/24‡
BASE (Milan)	>753	0/23	>700	0/23	>726	0/19	NA	NA	NA	NA
BASE#1 (Rome)	>633	0/19	>680	0/14	>707	0/17	NA	NA	NA	NA
BASE#2 (Rome)	>604	0/16	>854	0/29	>740	0/20	NA	NA	NA	NA
BSE-C (Rome)	>592	0/14	>856	0/15	>509	0/13	NA	NA	NA	NA
BSE-H	>722	0/24	>708	0/24	>708	0/24	561±15†	17/23‡	675±19†	5/23‡
CWD	>680	0/24	>730	0/24	>722	0/24	>716	0/23	457, 707	2/24‡
Sheep passaged atypical scrapie	>693	0/24	>693	0/24	>693	0/24	>693	0/24	>693	0/24
Atypical scrapie ARR/ARR1	>651	0/23	>724	0/21	>829	0/24	>781	0/24	>753	0/24
Atypical scrapie AHQ/AHQ1	>822	0/24	>718	0/24	>682	0/22	>757	0/23	>710	0/11
Atypical scrapie ARR/ARR2	>722	0/24	>744	0/24	>841	0/23	>756	0/22	>673	0/12
Atypical scrapie AHQ/AHQ2	>786	0/22	>768	0/23	>700	0/24	>805	0/24	>779	0/21
Atypical scrapie AFRQ/AFRQ1	>815	0/24	>717	0/23	>759	0/23	>757	0/23	>772	0/24
Atypical scrapie AFRQ/AFRQ2	>750	0/23	>722	0/23	>756	0/24	>726	0/24	>756	0/12

*Results for BASE and H-type BSE inoculations into Bov6 mice and 129/Ola mice have previously been published (Wilson *et al.*, 2012).
†Measured as days±SEM and calculated from mice showing pathological signs of disease (vacuolation and/or PrP deposition).
‡Number of mice showing pathological signs of disease (vacuolation and/or PrP deposition)/number of mice inoculated.

with CWD and Tg650 (human PrP) mice inoculated with cattle BSE did not develop high rates of clinical disease or significant PrP^{TSE} in brain, but a large proportion of inoculated mice had PrP^{TSE} detectable in the spleen. Sixty mice inoculated at Roslin with the atypical TSE agents (either showing clinical signs or a selection of the oldest mice, ranging from 321 to 730 days post-inoculation) were analysed for the presence of peripheral agent replication using the IDEXX HerdChek Bovine Spongiform Encephalopathy Antigen Test kit, which is an antigen capture enzyme immunoassay used to detect aggregated PrP in post-mortem tissues. Spleens derived from human PrP Tg mice challenged with BASE, BSE-H, CWD and atypical scrapie were homogenized in sterile saline in a Rybolyser (Hybaid) to achieve a 30 % homogenate and processed in the IDEXX HerdChek assay. All assay readouts were negative for the presence of disease-related PrP. Hence, there was no evidence of increased cross-species transmission in lymphoid tissues of gene-targeted human Tg mice inoculated with these atypical TSE agents.

Interestingly, several human PrP Tg mice were scored as showing positive clinical signs of disease despite the lack of disease-associated pathology, most notably in those mice inoculated with atypical scrapie (Table 2). Indeed, out of a total of 662 mice inoculated with six atypical-scrapie-field isolates, 25 had clinical signs of TSE (10 × HuMM, 9 × HuMV, 4 × HuVV, 1 × Bov6, 1 × 129/Ola). If the data were simply due to scoring errors we would expect similar numbers of cases in all groups. However, on the assumption that mice responded and were scored independently of one another (i.e. all mice had an equal chance of being scored as showing clinical signs) the distribution of clinical cases between the 30 groups of atypical scrapie-inoculated Tg mice is statistically significant at $P \leqslant 0.003$. The relevance of this observation is unclear. It is possible that the clinical signs observed in these mice are due to non-TSE intercurrent illnesses encountered because of the extended nature of these transmission experiments. However, scoring protocols are robust and do not usually yield high numbers of false-negative results when compared with disease pathology post-mortem. It is possible that these clinical signs indicate a different TSE disease phenotype whereby the pathological signs associated with disease cannot be detected using our conventional methods of tissue analysis. This hypothesis is being further investigated by subpassage from selected cases to identify any evidence of subclinical disease or low-level agent replication. While control ovine Tg mice were not available to include in the original transmission panel at The Roslin Institute, five of six of the atypical-scrapie-field isolates were inoculated into Tg338 ovine transgenic mice at Animal Health and Veterinary Laboratory Agency (Griffiths *et al.*, 2010) (sample numbers 2 and 5 in Griffiths *et al.* 2010, and J. Spiropoulos, personal communication). All five isolates transmitted efficiently to Tg338 transgenic mice (incubation times ~200 days post-inoculation), proving the infectivity of the source material.

Our results indicate that BASE, H-type BSE, CWD and atypical scrapie do not transmit to gene-targeted Tg mice expressing wild-type levels of human PrP; however, subpassage experiments are currently in progress to assay for any possible subclinical infection in mice that received these agents. The lack of BASE transmission to HuMM Tg mice has been confirmed following independent transmissions to mice in three different laboratories (Roslin Institute, 'Carlo Besta' Neurological Institute and Istituto Superiore di Sanita). Surprisingly, other studies have shown the transmission of BASE into microinjection-derived human PrP Tg mice (Tg40) (Kong *et al.*, 2008), which were reported to also express human PrP-129M at wild-type levels. Despite the apparent similarities in

Table 2. Cases of clinically positive (Clin +ve) signs of disease in human and bovine PrP Tg mice inoculated with atypical scrapie

	HuMM		HuMV		HuVV		Bov6		129/Ola	
	Clin +ve	*n*	Clin +ve	*n*	Clin +ve	*n*	Clin +ve	*n*	Clin +ve	*n*
Atypical scrapie ARR/ARR-1	0	23	1	21	0	24	0	24	0	24
Atypical scrapie AHQ/AHQ-1	1	24	3	24	3	22	0	23	0	11
Atypical scrapie ARR/ARR-2	2	24	2	24	0	23	0	22	0	12
Atypical scrapie AHQ/AHQ-2	1	22	2	23	1	24	0	24	1	21
Atypical scrapie AFRQ/AFRQ-1	6	24	1	23	0	23	1	23	0	24
Atypical scrapie AFRQ/AFRQ-2	0	23	0	23	0	24	0	24	0	12
Total Clin +ve atypical scrapie cases	10	140	9	138	4	140	1	140	1	104

expression levels between these lines, previous studies have produced other conflicting results between the Tg40 line and our targeted HuMM Tg line. While Tg40 mice were reported to be highly susceptible to sporadic CJD (sCJD)(MM2) (Kong *et al.*, 2008), HuMM mice inoculated with sCJD(MM2) showed no clinical signs of disease (Bishop *et al.*, 2010). The reasons for this discrepancy are not clear, but may be due to different mouse genetic background, or a more subtle difference in PrP expression levels in each Tg line. Other studies have shown the transmission of BASE into overexpressing human PrP Tg mice (Tg650; ~sixfold overexpression), with prolonged incubation times of 600–700 days. However, similarly to our findings they did not achieve transmission of H-type BSE into Tg650 mice (Béringue *et al.*, 2008). Previous studies have shown CWD TSEs do not transmit to mice overexpressing human PrP (Sandberg *et al.*, 2010; Tamgüney *et al.*, 2006). Furthermore, other studies investigating transmissibility of elk CWD TSEs, did not observe transmission into Tg40 mice (human PrP Tg) (Kong *et al.*, 2005). Studies have shown levels of PrP^{TSE} in lymphoid tissues are much higher in CWD-infected deer compared with elk (Race *et al.*, 2007), suggesting deer may be more likely to transmit disease to other cervids and non-cervids. In the present study, we challenged our human PrP Tg mice with CWD-infected white-tailed deer, but did not observe any signs of disease. However, it may be possible that CWD can be caused by multiple strains (Angers *et al.*, 2010) and as distinct cervid TSE strains become recognized and characterized, further studies will be required to assess human risk.

In this study, we examined, for the first time, the transmissibility of BASE, BSE-H, CWD and atypical scrapie into gene-targeted Tg mice expressing human PrP and we show that these mice are highly resistant to infection with these animal TSEs. In contrast to recently published research (Béringue *et al.*, 2012), we did not find any evidence of disease within lymphoid tissue of gene-targeted HuTg mice inoculated with these atypical TSE agents. While other studies have conducted similar experiments using overexpressing human PrP Tg mouse lines, the Tg mice used in this study are produced by gene replacement and do not suffer from any adverse phenotypes that can be associated with overexpression or ectopic expression of the transgene in standard Tg lines. While overexpression may increase sensitivity of these models by reducing incubation times, these levels of expression do not occur in host species. Gene-targeted models may therefore more closely represent infection and disease progression in nature. Indeed, previous studies have shown transmission of sCJD, vCJD and sheep BSE into gene-targeted human PrP Tg mice, demonstrating that these mice do live long enough to show signs of infection, supporting the use of targeted mouse models to analyse TSE disease transmission (Bishop *et al.*, 2006, 2010; Plinston *et al.*, 2011). In conclusion, the results presented here strongly suggest the presence of a significant transmission barrier between these ruminant TSEs and humans. However, while TSEs are still present in the environment, the potential for cross-species transmission and emergence of novel TSE isolates remains, thus supporting the need for continued surveillance of these agents.

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