	Volume inoculated (mL)	Total animals inoculated	Total animals infected	Titre in ID/mL (SD)	Fractional distribution of infectivity
Whole blood	5·2	104	50	13·1 (1·6)	1
Leucoreduced blood	5·4	108	34	7·6 (1·2)	0·58

Titre and SD calculated from the Poisson distribution as described in the text.

Table 2: Concentration of TSE infectivity in whole and leucoreduced blood

approached, but did not fully achieve all specifications; furthermore, because more than one filter is involved, more titrations would have been required to evaluate the removal of infectivity.

For the infectivity study, 448·5 mL of CP2D-anticoagulated whole hamster blood was pooled into the whole-blood receiving bag of a Leukotrap WB collection set and processed within the 8-h time limit specified by the AABB. Filtration was done at room temperature under gravity with a 60-inch pressure head on the in-line WBF2 filter, and was completed in 30 min. After removal of a 19 mL sample of the leucoreduced whole blood for subsequent testing, the remainder was centrifuged at 4150 rpm (about 5000 g) for 8 min at room temperature in a Sorvall RC-3C centrifuge. The plasma fraction was expressed into a satellite in-line bag. A preservative and stabiliser, AS3, was added to the red blood cells. Samples of the pre-filtration whole blood, post-filtration whole blood, red blood cells, and plasma were removed for analysis of cell composition and for titration in animals.

Cellular composition of the blood was assessed with a HemaVet five-part differential cell counter calibrated for hamster blood cells (Drew Scientific, Oxford, CT, USA). The residual white blood cell concentrations in the leucoreduced samples were measured by manual count and flow cytometry.

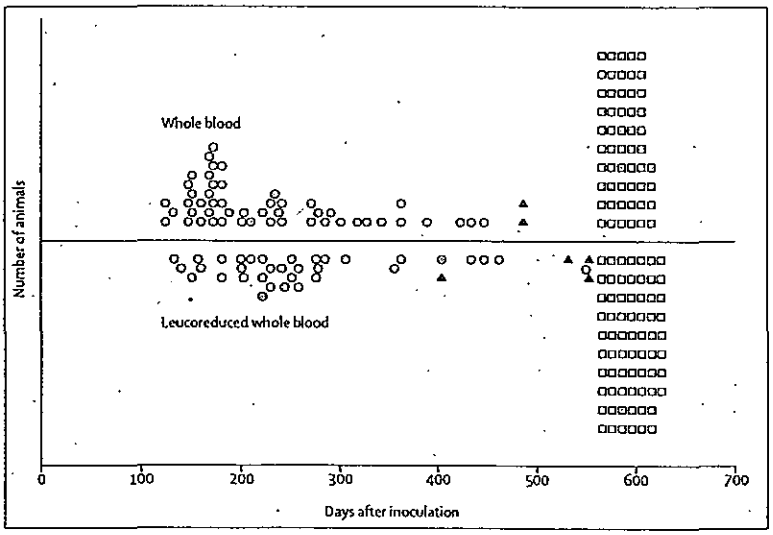

Figure: Incubation times of infections from whole and leucoreduced blood
Results of inoculations of whole blood are represented by data above the horizontal line; those from inoculations of leucoreduced blood are shown below the line. Circles represent infected animals. Squares represent uninfected animals that survived to the end of the experiment. Triangles represent animals that died intercurrently of causes other than the inoculum.

Infectivity of whole and leucoreduced blood was quantified by limiting dilution titration, a method developed in the Rohwer laboratory. The two samples were processed and inoculated separately and sequentially. Each sample of blood was sonicated with a separate sterile probe to lyse cells and disperse infectivity. It was then immediately inoculated intracranially, 50 µl at a time, into about 100 weanling golden Syrian hamsters that were deeply anaesthetised with pentobarbital. Animals were maintained for 566 days; those that contracted scrapie were killed when the clinical diagnosis was conclusive, and animals still alive at the end of the study were killed. All brains were tested for the presence of the proteinase K-resistant form of prion protein by western blot using 3F4 antibody.

The limiting dilution of an endpoint dilution titration is that at which not all of the inoculated animals become infected. At limiting dilution, the distribution of infectivity into individual inoculations is described by the Poisson distribution, where P(0)=probability of no infections at that dilution and inoculation volume, or (1–probability of infection). From the Poisson distribution $P(0)=e^{-(titre)}$ and titre=–ln(P[0]) expressed as ID/(inoculation volume). SD of the limiting dilution titre is the square root of the titre in ID/mL divided by the total volume inoculated in mL.

Table 1 shows the distribution of cells in each component of the scrapie-infected blood. Leucofiltration reduced the number of white blood cells by 2·9 log, thereby meeting the AABB standard. White cell contamination of the red blood cell fraction and red blood cell recovery were within AABB specifications of less than 5×10^6 and greater than 85%, respectively. Hamster platelets are not removed by the WBF2 filter, and partition with the red cells during centrifugation.

The incubation times of infections in each measurement are shown in the figure. At limiting dilution, incubation times begin at the end of the predictable dose-response seen in endpoint dilution titrations (about 140 days) and rarely extend beyond 500 days. All clinical and western blot results were consistent.

The limiting dilution titre of the whole blood pool (table 2) was close to the values from titrations of similar pools of whole blood by this method (unpublished data). Leucofiltration of whole blood removed only 42% (SD 12) of the initial TSE infectivity (table 2); of the 5900 ID present in the original unit of blood, 3400 ID were recovered in the leucofiltered blood.

Ideally, leucoreduction would be validated by measuring infectivity concentrations before and after leucoreduction of full units of vCJD-infected human blood. However, it is not currently possible to assay

either infectivity or the infection-specific form of the prion protein in human blood. By contrast, limiting dilution titration of rodent blood can detect less than 1 ID/mL of TSE infectivity and can readily show a difference of less 20% between samples. With this technique we did a study that: avoided the issue of spikes by using endogenously infected blood; avoided the question of scale by using a human-sized unit of fresh hamster blood obtained within the time limits specified for human blood; minimised the possibility of artefact by using a commercial blood collection set with integral filtration unit and a blood centre centrifuge and expressor; and achieved precision in the infectivity measurements by limiting dilution inoculation of 5 mL of each fraction. We assessed the performance of the filter by measuring the level of white blood cell reduction obtained and the cell recoveries of each component. The leucoreduction met or exceeded AABB specifications for all relevant variables.

Leucoreduction removed only 42% of the initial TSE infectivity from whole blood. This distribution is consistent with that obtained in a centrifugal separation of TSE-infected hamster whole blood, in which the buffy coat contained 70% of the total white cells but only 45% of the total whole blood infectivity (unpublished data). Both methods showed that a substantial proportion of the TSE infectivity was not associated with white cells. We have shown previously[7] that TSE infectivity is not associated with highly purified platelets, and we are currently testing purified red blood cells. We presume that the majority of blood-borne infectivity is plasma-associated.

Although leucoreduction is a necessary step for removing white-cell-associated TSE infectivity from blood, this process is insufficient to remove the risk from an infected transfusion unit. Due to the low concentration of TSE infectivity in blood and the absence of screening or inactivation alternatives, removal is an attractive strategy. However, the feasibility of removal depends upon the actual associations and distributions of TSE infectivity in blood itself, which can only be ascertained by assessment of endogenous blood-borne infectivity.

Contributors

The overall design and execution of the experiment, including management of the logistics and all the infectivity work, was by L Gregori and R G Rohwer with the assistance of the staff of the Molecular Neurovirology Laboratory. A Giulivi, N McCombie, D Palmer, and P Birch supplied expertise on blood centre operations, blood collection, component separation, leucoreduction, and quantitation of white blood cells. D Palmer and P Birch undertook and interpreted flow cytometry. S Coker supplied expertise on the use of the collection set and leucofilter.

Conflict of interest statement

R G Rohwer is a cofounder and part owner of Pathogen Removal and Diagnostics Technologies, which is developing technologies for the removal of TSE infectivity from blood and other materials. L Gregori receives contract support from Pathogen Removal and Diagnostics Technologies for studies on TSE removal. S Coker is an employee of Pall Corporation, which produces leucofilters and is developing TSE removal strategies for blood. The remaining authors declare that they have no competing financial interests.

Acknowledgments

We thank the staff of the BSL-3 animal facility at the VA Medical Center, Baltimore for their excellent animal care. This study was funded by Health Canada and by the US National Heart, Lung and Blood Institute Award # HL-63930. Health Canada participated in the study design, assisted with leucofiltration, and facilitated flow cytometry analysis. They had no role in the infectivity measurements, their analysis, or interpretation. Health Canada reviewed and approved the final submission without changes. The National Heart Lung and Blood Institute participated only as a source of funding. Pall Corporation supplied a blood centrifuge, plasma expressor, and tube sealer, and served as consultants on the use of their collection sets and filters.

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Predicting susceptibility and incubation time of human-to-human transmission of vCJD

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Summary

Background Identification of possible transmission of variant Creutzfeldt-Jakob disease (vCJD) via blood transfusion has caused concern over spread of the disease within the human population. We aimed to model iatrogenic spread to enable a comparison of transmission efficiencies of vCJD and bovine spongiform encephalopathy (BSE) and an assessment of the effect of the codon-129 polymorphism on human susceptibility.

Methods Mice were produced to express human or bovine prion protein (PrP) by direct replacement of the mouse *PrP* gene. Since the human *PrP* gene has variation at codon 129, with MM, VV, and MV genotypes, three inbred lines with an identical genetic background were produced to express human PrP with the codon-129 MM, MV, and VV genotypes. Mice were inoculated with BSE or vCJD and assessed for clinical and pathological signs of disease.

Findings BSE was transmitted to the bovine line but did not transmit to the human lines. By contrast, vCJD was transmitted to all three human lines with different pathological characteristics for each genotype and a gradation of transmission efficiency from MM to MV to VV.

Interpretation Transmission of BSE to human beings is probably restricted by the presence of a significant species barrier. However, there seems to be a substantially reduced barrier for human-to-human transmission of vCJD. Moreover, all individuals, irrespective of codon-129 genotype, could be susceptible to secondary transmission of vCJD through routes such as blood transfusion. A lengthy preclinical disease is predicted by these models, which may represent a risk for further disease transmission and thus a significant public-health issue.

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Introduction

After the identification of variant Creutzfeldt-Jakob disease (vCJD) in 1996,[1] there have been many attempts to estimate the extent of the UK epidemic. Many individuals are likely to have been exposed to bovine spongiform encephalopathy (BSE) material through their diet; however, there have been only 161 cases of the disease in the UK. The predicted total number of future cases has ranged from the low hundreds[2] to hundreds of thousands.[3] However, findings from a retrospective immunocytochemical study that aimed to detect prion protein (PrP) in appendix and tonsil specimens suggested a prevalence of BSE infection of 237 per million people in the UK.[4] DNA sequence analysis of the PrP gene (*PRNP*) in vCJD has shown that 100% of tested cases are homozygous for methionine at the codon-129 polymorphism compared with about 40% of the general white population and about 70% of sporadic CJD cases. The methionine homozygous genotype (MM) has been included as a limiting variable in most mathematical predictions of the size of the epidemic.[2,3] Identification at autopsy of preclinical vCJD infection in a methionine/valine (MV) heterozygous individual who had received a transfusion of red cells from a donor who later died of vCJD, was the first indication that MM might not be the only susceptible genotype.[5]

Polymorphisms and mutations in *PRNP* in various species can affect disease susceptibility, although the precise mechanisms by which these effects are mediated have not been established.[6,7] Codon 129 of the human *PRNP* gene has been shown to affect the clinicopathological phenotype of disease in CJD and fatal familial insomnia.[8–13] Heterozygosity at *PRNP* codon 129, when compared with homozygous individuals, has been reported to lengthen incubation times in iatrogenic CJD cases associated with growth hormone treatment, and in kuru,[9,14] whereas valine homozygosity (VV) has been proposed to be protective for both BSE and vCJD transmission in studies that used murine models overexpressing human PrP.[15] At a molecular level, the biophysical properties of PrP refolding into the disease associated form (PrP^{Sc}) have been shown to be affected by the codon-129 genotype, with the methionine variant having an increased propensity to form PrP^{Sc}-like structures.[16]

We sought to analyse the transmission characteristics of BSE and vCJD to four inbred lines of transgenic mice after intracerebral inoculation with brain homogenate from cases of vCJD and BSE. We then aimed to use these models to address the apparent low level of vCJD in the human population resulting from exposure to BSE and to predict the potential for human-to-human spread of vCJD and the susceptibility of different genotypes in the human population.

Methods

Transgenic mice

Details of how the gene-targeted transgenic lines were created are supplied as supplementary information

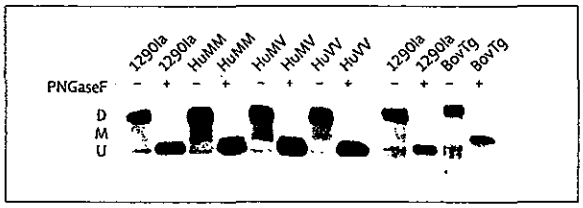

Figure 1: **Western blot of brain extract from uninoculated mice showing that PrP^{C} is detected with equivalent electrophoretic mobility and glycoform ratio in all three human transgenic lines**
D=diglycosylated PrP^{C} band; M=monoglycosylated PrP^{C} band; U=unglycosylated PrP^{C} band. In the BovTg line, a deglycosylated band is detected of increased molecular weight due to the additional N-terminal octapeptide repeat motif. Protein levels are similar to the wildtype line used in generating the transgenics (129Ola). Glycosylation is confirmed by the reduction to a single band after deglycosylation with the enzyme PNGaseF. The anti-PrP antibody 7A12 was used for the HumTg blot as it will react with both murine and human PrP, and 8H4 was used for the BovTg blot.

See Online for webappendix

(webappendix). Transgenic mice were anaesthetised with halothane and then injected with 0·02 mL of brain homogenate into the right cerebral hemisphere. The vCJD tissue homogenate (at 10^{-2} dilution) was supplied by the UK National Institute for Biological Standards and Control (Code NHBY0/0003). BSE-infected cattle brain (Veterinary Laboratories Agency, reference BBP 12/92) was prepared by maceration of the tissue in sterile saline to a dilution of 10^{-1}. From 100 days they were scored each week for signs of disease.[17] Mice were killed by cervical dislocation whether they had clinical signs of transmissible spongiform encephalopathy (TSE) or another non-specific disorder. The brain was recovered at post mortem. Half the brain was snap-frozen in liquid nitrogen for biochemical analysis and the remaining half was fixed for histology.

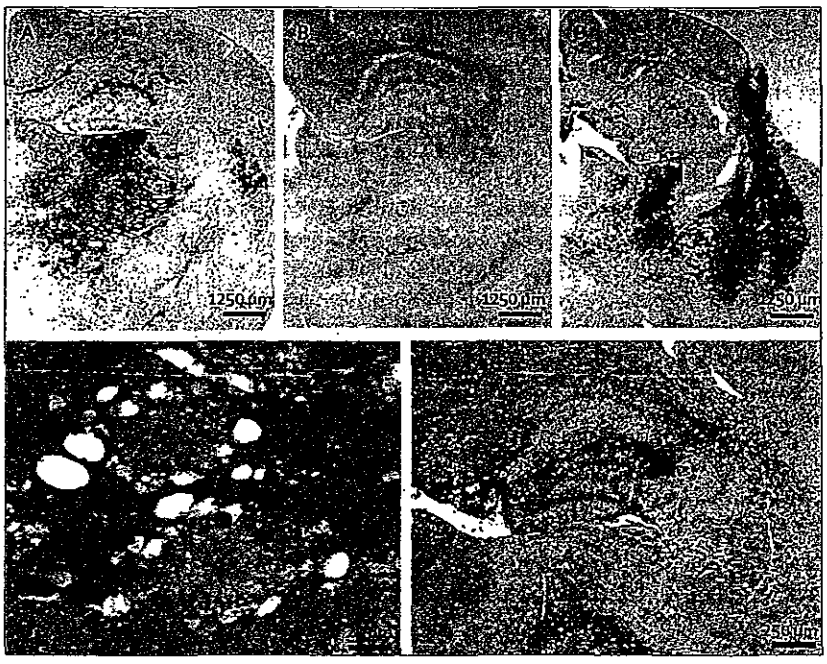

Figure 2: **Immunocytochemistry of histological sections with anti-PrP antibody 6H4 showing the cortex, hippocampal, and thalamic regions of the mouse brain with PrP detection (brown)**
A–D: Human transgenic mice with vCJD inoculum. A: HuMM mouse 693 days post inoculation. B: HuMV mouse 707 days post inoculation. C: HuVV mouse 693 days post inoculation. D: Florid plaques found in the hippocampus of the HuMM mouse in panel A. Each plaque has an eosinophilic core with a paler halo and is surrounded by a ring of vacuolation (haematoxylin and eosin stain). E: Hippocampal region of a BovTg mouse inoculated with BSE. PrP is deposited in a more diffuse/granular form with occasional plaques.

Procedures

Immunocytochemical detection of disease-associated PrP (PrP^{Sc}) deposits in the brain is a key pathological marker of TSE transmission, and variation in location and morphology of PrP^{Sc} deposits can be affected by both the strain of TSE agent and by the host PrP.[7,18] After fixation in 10% formal saline, brains were treated for 1·5 h in 98% formic acid (to reduce the titre of infectivity for safety reasons), cut transversely into four sections, and embedded in paraffin. We used the Vectastain Elite ABC Kit (Vector Labs, UK) with overnight primary antibody incubation (6H4 at 1:2000; Prionics, Switzerland) for PrP detection. Identification of antibody binding was through deposition of 3,3′-diaminobenzidine chromogen via a horseradish peroxidase reaction. The BSE-inoculated human transgenics were also studied using the Catalysed Signal Amplification kit (DAKO K1500). This kit uses the same principles as the Vector Labs kit, but has an additional step, which amplifies the final detected signal and therefore improves sensitivity.

Scoring of the abundance and location of TSE-associated vacuolation in grey and white matter of the brain is routinely used for diagnosis and strain classification in non-transgenic mice[17,19] and was used to assess all the mice in this study. TSE-related vacuolation was assessed at nine grey-matter regions and three white-matter regions to produce a lesion profile, as previously described.[20,21]

Analysis

Frozen brain samples from the human transgenic mice were homogenised in 0·9% saline to give a 10% suspension. This material was cleared by centrifugation and the supernatant treated with 0·05 g/L proteinase K for 1 h at 37°C, as previously described in detail.[22] The digested product was denatured then loaded onto a 10% Bis/Tris NuPAGE Novex gel (Invitrogen, UK). After electrophoresis the gel was blotted onto polyvinylidine difluoride (PVDF) membrane. We used the ECL+ technique (Amersham Biosciences, UK) with primary antibody 6H4 (Prionics, Switzerland) at 1:40000 and an anti-mouse IgG peroxidase-linked secondary (Amersham Biosciences, UK) at 1:40000 for the detection of PrP. Chemiluminescence was captured on radiographic film. Samples prepared for figure 1 were digested overnight at 37°C with 500 units of PNGaseF (New England Biolabs, UK) and not with proteinase K; the primary antibody was 7A12.[23]

Frozen brain samples from the bovine transgenic mice were homogenised in an NP40 buffer (0·5% v/v NP40,

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0·5% w/v sodium deoxycholate, 0·9% w/v sodium chloride, 50mM Tris-HCl pH 7·5) to give a 10% suspension. This material was cleared by centrifugation and the supernatant digested with PNGaseF. The products were denatured then loaded onto a 12% Novex Tris/Glycine gel (Invitrogen, UK). After electrophoresis the gel was blotted onto PVDF membrane. PrP was identified with the SuperSignal West Dura chemiluminescence detection kit (Pierce, UK) with primary antibody 8H4[24] at 1:20000 and an anti-mouse IgG peroxidase-linked secondary (Jackson Immuno Research Laboratories, UK) at 1:10000. Images were captured on radiographic film and with a Kodak 440CF digital imager (figure 1).

Role of the funding source

The sponsors of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We first investigated the potential effects of the species barrier between BSE and human beings and any alteration in that barrier once BSE had passed through people in the form of vCJD. We then investigated the effect of the codon-129 polymorphism on human-to-human transmission of vCJD using gene-targeted inbred mice developed by direct replacement of the murine *PrP* gene for the human gene. These mice produce PrP under the control of the normal regulatory elements for PrP and thus express physiological concentrations of PrP with the correct tissue distribution (figure 1). Three inbred lines with an identical genetic background were produced to express human PrP with the codon-129 MM, MV, and VV genotypes (designated HuMM, HuMV, and HuVV, respectively). Each line differs by only a single codon in *PRNP* and in all other respects the mice were genetically identical. Additionally, in an identical manner, we produced mice that express bovine PrP to enable direct comparisons to be made not only between transgenic and wild-type mice, but also between each of the transgenic lines.

Typical clinical signs of TSE disease were seen in more than half (15/22) the BovTg mice inoculated with BSE material with a mean incubation period of 551 days (SD 47). These clinical cases were confirmed by a positive test for the presence of TSE vacuolation or PrP^{Sc} deposition by immunocytochemistry. The lesion profiles generated for targeting and degree of vacuolation showed similar patterns for all positive mice. Immunocytochemical data showed PrP^{Sc} deposition mainly in a diffuse and synaptic form, and also as plaque-like structures, frequently associated with areas of spongiform change (figure 2). Deposition was most abundant in the thalamus and hippocampus, but was recorded throughout other regions of the brain. The cerebral cortex showed only occasional plaque-like structures and the cerebellum had only a few areas of PrP^{Sc} deposition limited to the granule cell layer. Further pathological analysis was undertaken on mice that were culled for reasons other than clinical TSE (intercurrent deaths). This analysis showed that all the brains had pathological signs of TSE disease in terms of vacuolation or PrP deposition. Thus, all the bovine transgenic mice (22/22) seemed to be susceptible to BSE infection, although not all developed clinical signs of infection (tables 1 and 2).

HuMM, HuMV, and HuVV mice were inoculated with BSE material and after extensive pathological analysis all were confirmed as negative for TSE transmission (table 1). Mice of each genotype line were inoculated with vCJD material. Two pathologically confirmed clinically positive mice were seen in the HuMM line (at 497 and 630 days post inoculation), one in the HuMV line (at

	Clinically positive	Vacuolation positive	PrP positive*	Negative†
BovTg (n=22)				
0–400	0	3	6	0
401–500	1	1	0	0
501–600	10	11	5	0
>600	4	4	2	0
HuMM (n=18)				
0–400	0	0	0	4
401–500	0	0	0	5
501–600	0	0	0	2
>600	0	0	0	7
HuMV (n=23)				
0–400	0	0	0	3
401–500	0	0	0	6
501–600	0	0	0	4
>600	0	0	0	10
HuVV (n=22)				
0–400	0	0	0	9
401–500	0	0	0	4
501–600	0	0	0	7
>600	0	0	0	2

*Because most mice were positive by both clinical and vacuolation scoring not all mice were tested by immunocytochemistry for PrP deposition. †Negative by clinical or pathological analysis, or positive by clinical scoring but not confirmed by pathology.

Table 1: Clinical and pathological scoring of BovTg and human transgenic mice, by number of days after BSE inoculation

	BSE				vCJD		
	BovTg	HuMM	HuMV	HuVV	HuMM	HuMV	HuVV
Susceptibility	22/22	0/18	0/23	0/22	11/17	11/16	1/16

Positives confirmed by immunocytochemistry or lesion profile.

Table 2: Susceptibility to TSE disease comparison of BovTg and human transgenic mice inoculated with BSE or vCJD

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	Clinically positive	Vacuolation positive	PrP positive	Negative*
HuMM (n=17)				
0–400	0	0	2	2
401–500	1	1	1	2
501–600	0	1	3	2
>600	1	4	5	0
HuMV (n=16)				
0–400	0	0	0	0
401–500	0	0	0	0
501–600	0	0	4	3
>600	1	1	7	2
HuVV (n=16)				
0–400	0	0	0	0
401–500	0	0	0	1
501–600	0	0	0	5
>600	0	1	1	9

*Negative by clinical or pathological analysis, or positive by clinical scoring but not confirmed by pathology.

Table 3: Clinical and pathological scoring of human transgenic mice, by number of days after vCJD inoculation

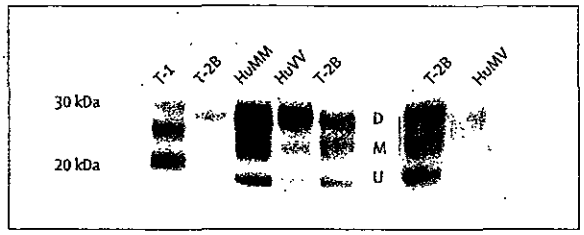

Figure 3: Western blots of brain extract from three transgenic lines inoculated with vCJD

D=diglycosylated PrP^{Sc} band; M=monoglycosylated PrP^{Sc} band; U=unglycosylated PrP^{Sc} band. T-2B corresponds to human vCJD brain homogenate showing the typical PrP^{Sc} type 2B and T-1 corresponds to human sCJD brain homogenate showing the typical PrP^{Sc} type 1 signature. Type 2B and 1 differ in mobility of the unglycosylated band (~19 kDa and ~20 kDa respectively) and the degree of glycosylation (diglycosylated dominant and mono/unglycosylated dominant respectively). All samples were treated with proteinase K. The anti-PrP detection antibody was 6H4. The HuMV and T2-B control blot had to be overexposed as the signal from the HuMV was weak, due to the low levels of PrP^{Sc} seen by immunocytochemistry.

665 days post inoculation), and none in the HuVV line (table 3). HuMM mice were more likely to show disease-associated vacuolation, beginning at around 500 days post inoculation. Six were scored positive and showed similar distribution of vacuolation in the brain, with the highest levels found in the dorsal medulla, thalamus, and cerebellar white matter. By contrast, only a single mouse in each of the HuMV and HuVV groups scored positive for vacuolation at approximately 700 days post inoculation.

Most of the HuMM mice (11/15) showed PrP^{Sc} deposition in most areas of the brain at a relatively early stage (from around 370 days post inoculation), before the vacuolar pathology became evident. From 500 days post inoculation the appearance of vacuolation was accompanied by a significant increase in PrP^{Sc} deposition. By contrast, although PrP^{Sc} deposition was identified in many HuMV mice (11/13), they had little deposition restricted to only a few areas (including the ventrolateral and ventromedial thalamic nuclei and the red nucleus of the mid-brain), even after 700 days post inoculation (figure 2, table 4). Although PrP^{Sc} deposition was clearly present at 581 days, the timing of initial onset of deposition in this line was not established.

Significant levels of PrP^{Sc} deposition were noted in the brain of the subclinical HuVV case. Indeed, these were similar in intensity to those observed in the clinical HuMM cases. Patterns of PrP deposition and plaque formation show differences among the three genotypes, including the presence of florid plaques only in the HuMM mice (table 4).

PrP^{Sc} found in vCJD brain is characterised by a 19 kDa non-glycosylated fragment and the predominance of the diglycosylated form (type 2B).[22] Both biochemical properties of PrP^{Sc} are maintained when vCJD is transmitted to the human transgenic mice, irrespective of their codon-129 genotype (figure 3). Preliminary densitometric analysis suggested that there was an increase in the diglycosylated form in the HuVV mouse compared with the HuMM mouse. Additionally, comparison of PrP^{Sc} from the BSE inoculum and brain material from BovTg mice also confirmed propagation of the predominantly diglycosylated glycoform signature of PrP^{Sc} associated with the BSE/vCJD agent strain (data not shown).

	HuMM	HuMV	HuVV
Vacuolation*	Thalamus (severe); cerebral cortex and hippocampus (mild); cerebellar cortex (minimal)	Thalamus, cerebral cortex, hippocampus, and cerebellar cortex (minimal)	Thalamus and cerebral cortex (severe); hippocampus (mild); cerebellar cortex (minimal)
Plaque formation*	Fibrillary amyloid plaques; florid and non-florid plaques in cerebral cortex and hippocampus; no evidence of plaques in cerebellum	No evidence of amyloid plaques	Amorphous non-fibrillary structures often forming into clusters in cerebral cortex and thalamus
PrP deposition†	Intense staining of plaques in hippocampus and cerebral cortex; plaque-like, pericellular, and amorphous deposits in the hippocampus; synaptic, peri-neuronal, and diffuse perivascular deposits in the thalamus	Occasional small plaque-like deposits and pericellular deposits in the thalamus	Strongly positive large amorphous deposits and clusters of plaques, small plaque-like structures, perivascular aggregates, and sub-pial deposits in the cerebral cortex and thalamus

*Analysed with haematoxilin and eosin staining. †Analysed with immunocytochemical techniques.

Table 4: Comparison of TSE-associated neuropathology in human transgenic mice inoculated with vCJD

Discussion

Although the cattle BSE epidemic in the UK has amounted to more than 180 000 cases since the 1980s, the extent of the human vCJD epidemic has so far remained limited with the total number of cases worldwide currently at 190. One explanation for this apparent discrepancy is that there exists a significant species barrier between cattle and human beings, which limits the susceptibility of the human population to BSE. The data shown here suggest that this could indeed be the case since BSE was readily transmissible to the bovine transgenic mice but not to the human transgenic mice. However, once BSE has passed through human beings in the form of vCJD, the transmissibility of this TSE strain is altered for the human population.

All the human transgenic lines inoculated with BSE were negative for TSE transmission, which suggests that either the human transgenic lines are relatively resistant to transmission of BSE or the incubation time is longer than the length of the experiment (approximately 700 days). BSE transmission previously observed by others, in human transgenic lines overexpressing the human prion protein, could be due to overexpression of the *PrP* gene and may not therefore give a true reflection of the species barrier between BSE and human beings.[15,25,26] This apparent resistance of human transgenic mice to BSE could be explained by a large species barrier and this in turn could explain the low number of vCJD cases in the human population.

vCJD was transmitted to all three human lines with different pathological characteristics for each genotype, and a gradation of transmission efficiency from MM to MV to VV. The greater transmission efficiency in HuMM mice suggests that homozygosity for methionine at codon 129 leads to earlier onset of TSE-related pathological features and clinical disease than for the other two genotypes. The differences in PrP^{Sc} deposition in the HuMM and HuMV lines suggest that the codon-129 polymorphism in human beings is likely to affect the distribution of PrP^{Sc} deposition in the brain. Moreover, the similar numbers that scored positive for PrP deposition in each of the MM and MV groups (11/15 and 11/13 respectively) suggest that the two genotypes might be equally susceptible to vCJD, but with different incubation periods. Titration experiments are needed to fully compare the susceptibility of each line. The single HuVV mouse positive for PrP^{Sc} shows that VV individuals may be susceptible to vCJD with very long incubation times, including a lengthy subclinical phase. Transmission studies from all three genotype mice are now underway to examine the infectious nature of the disease and determine any alterations in the strain characteristics on passage through human transgenic mice. By contrast with published data suggesting that VV individuals cannot propagate the vCJD biochemical phenotype,[15] the data presented here suggest that the PrP^{Sc} type will remain a useful diagnostic feature of secondary vCJD infection irrespective of codon-129 genotype, as has been observed for the two extant cases of transfusion-associated vCJD infection.[5,27]

Transmission of vCJD to the three lines of human transgenic mice indicates that the human population could be at significantly heightened risk of developing disease after iatrogenic exposure to vCJD. Secondary transmission of vCJD has partly removed the cattle-to-human species barrier and has resulted in an agent that can be transmitted from human to human with relative efficiency. Transmission studies in cynomolgus macaques provide further evidence for this agent adaptation as they show reduction in incubation times after serial passage of BSE.[28] Our BSE inoculation at 10^{-1} dilution was compared with vCJD inoculation at 10^{-2} because the latter inoculum was found to be toxic to the mice at 10^{-1}. Use of a higher dose of vCJD inoculum would have maintained or increased the transmission efficiency of vCJD and enhanced the current findings.

Our findings raise concerns relevant to the possibility of secondary transmission of vCJD through blood transfusion, fractionated blood products, or contaminated surgical instruments. For this study mice were injected intracerebrally, whereas the probable human exposure to these agents is by peripheral routes (eg, oral or intravenous), and thus human-to-human exposures might be significantly less efficient. However, it is difficult to know for sure what the practical implications might be in human beings. Peripheral route challenge is in progress; however, BSE transmission studies in primates have shown the intravenous route to be as efficient as the intracerebral route, with an extension of the incubation time.[28]

Although all cases of vCJD up to now have been observed in the MM genotype, this model of human-to-human vCJD transmission suggests that other genotypes are also susceptible. In our experimental setting, all *PRNP* codon-129 genotypes are susceptible to vCJD infection; however, progressive development of pathological TSE features (vacuolation and PrP deposition) is more rapid in the MM-genotype mice. An explanation for this finding might be provided by in-vitro conversion of recombinant human PrP by BSE and vCJD agents, which has shown that PrP with methionine at position 129 is more efficiently converted than PrP with valine, and that conversion by vCJD is significantly more efficient than by BSE.[29] Long incubation periods during which PrP^{Sc} is deposited predicts that, in human beings, infection could be present in all genotypes for a significant period before clinical onset. Incubation periods of more than 30 years have been reported in the human TSE disease kuru.[30]

The possibility that an MV or VV genotype could result in a phenotype distinct from that recognised in vCJD draws attention to the importance of systematic assessment of the clinical, genetic, pathological, and