

Case Report

The first Japanese case of variant Creutzfeldt-Jakob disease showing periodic electroencephalogram

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In February, 2004, a 50-year-old Japanese man was referred to our Creutzfeldt-Jakob disease (CJD) surveillance committee. In the first half of 1990, the patient had spent about 24 days in the UK, 3 days in France, and 2 weeks in other European countries where variant CJD (vCJD) has not been reported. He had no history of surgery or blood transfusion, or a family history of prion disease. In June, 2001, aged 48 years, he had difficulty in writing Chinese characters. In October, 2001, he showed mental symptoms, such as irritability, personality changes, and memory impairment, followed by painful dysaesthesia in the legs, ataxia, dementia, and abnormal behaviour. Retrospective review of an MRI taken in August, 2002, showed slight hyperintensity in the thalamus. In January, 2003, he showed dementia, ataxia, and hyperreflexia. Brain MRI at that time showed symmetrical hyperintensity of the thalamus. Electroencephalogram (EEG) showed diffuse slowing, but no periodic synchronous discharges (PSD). The cerebrospinal fluid was positive for 14-3-3 protein. Analysis of the prion protein (PrP) gene showed no mutation, methionine/methionine at codon 129, and glutamic acid/glutamic acid at codon 219. He showed rapid deterioration of both motor and cognitive function.

In December, 2003, he developed akinetic mutism, myoclonus, and pyramidal signs. Brain MRI showed hyperintensity in the caudate, putamen, thalamus, and cerebral cortex, with higher intensity in the caudate and putamen than the thalamus. EEG suggested the presence of PSD (figure, A). The diagnosis of probable sporadic CJD was supported by EEG and MRI findings.¹ He died of pneumonia in December, 2004. Autopsy showed findings characteristic of vCJD, including florid plaques (figure, B) and the Parchi type 2B or Collinge type 4 pattern of protease-resistant PrP (not shown).

This is the first Japanese case of definite vCJD. The progressive neuropsychiatric disorder was consistent with vCJD, although the illness duration was unusually long

compared with most vCJD cases reported to date (median 14 months).² The findings 19 months after onset of symptoms, showing the pulvinar sign on MRI and the absence of PSD on EEG, together with the clinical features, fulfilled the criteria of probable vCJD.³ However, 30 months after onset, PSD appeared on EEG, and the pulvinar sign on MRI disappeared following an increase in intensity of other grey matter nuclei, fulfilling the criteria of probable sCJD.³ There have been no previous reports of PSD on EEG in vCJD, although conversion of a positive to negative pulvinar sign on MRI has been described in a few vCJD patients.⁴ Our case shows that PSD does not exclude the possibility of vCJD. We suggest revision of the WHO vCJD case definition³ to prevent missing cases of vCJD. It is unclear when our patient was exposed to the infective agent. The BSE outbreak in the UK was still increasing when he visited the UK, and it was confirmed that he ate food containing mechanically recovered meat that may be associated with contamination with BSE agent from nervous tissue;⁵ however, exposure in France, other European countries, and Japan cannot be excluded. If he was exposed to the BSE agent in the UK (exposure just once would be sufficient to cause vCJD), we calculate the incubation period between such pinpoint exposure and onset of vCJD to be 11.5 years.

Contributors

Following the identification of this first Japanese case of vCJD by the CJD Surveillance Committee, Japan, we constituted the vCJD Working Group in the Committee. To protect the patient's privacy, we have decided to publish this report under the name of the vCJD Working Group, CJD Surveillance Committee, Japan. The corresponding author is the chair of both the vCJD Working Group and the CJD Surveillance Committee.

Acknowledgments

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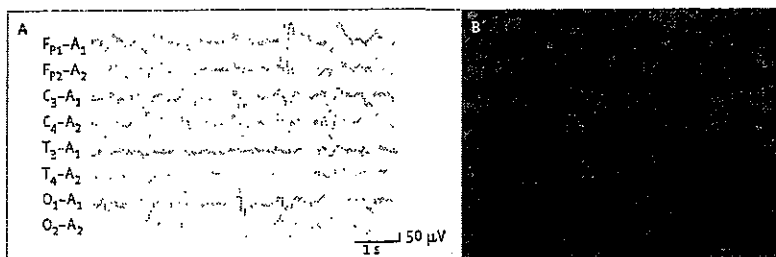
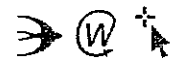


Figure: (A) EEG in August, 2004, 39 months after onset, showing PSD typical of sporadic CJD. (B) The frontal cortex showing florid plaques, severe spongiform changes, and neuronal loss (HE, bar=100 μm). PrP immunohistochemistry showed many PrP-positive plaques and PrP deposits with a pericellular pattern (not shown).

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一般的名称	①ポリエチレングリコール処理抗破傷風人免疫グロブリン ②乾燥抗破傷風人免疫グロブリン		研究報告の 公表状況	The Lancet Neurology 2006; 5: 393-398	公表国 イギリス	
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研究報告の概要	<p><背景> 輸血を介した vCJD 感染の可能性が確認されたことによって、ヒトの間でこの疾患が蔓延することへの懸念が起きている。我々が目指したのは、vCJD 及び BSE の感染効率を比較し、人の感受性に関するコドン 129 遺伝的多型の影響の評価することを可能にするための医原的伝播のモデルを作成することであった。</p> <p><方法> マウス PrP 遺伝子の交換によってヒト又はウシの PrP 蛋白を発現するマウスを作製した。ヒトの PrP 遺伝子はコドン 129 に MM, VV, MV の遺伝型の多様性を有することから、コドン 129 の MM, VV, MV 遺伝型を有するヒト PrP を発現するために、同じ遺伝背景を有する 3 つの近交系 (inbred line) を作製した。マウスに BSE 又は vCJD を接種し、疾患の臨床的及び病理学的な徴候を観察した。</p> <p><結果> BSE はウシの系には感染したが、ヒトの系には感染しなかった。対照的に、vCJD はヒトの 3 つの系全てに感染し、各々の遺伝型で病理学的特徴が異なり、感染効率は MM>MV>VV の順であった。</p> <p><解釈> BSE からヒトへの感染はおそらく、種の壁の存在によって制限を受けている。しかし、vCJD のヒトからヒトへの感染には、実質的に壁が低くなっているように見える。さらに、全ての個人は、コドン 129 の遺伝型に関係なく、輸血のようなルートによって、vCJD の 2 次感染が起りやすい可能性がある。潜伏期間の長い疾患はこれらのモデルによって予測ができるが、このモデルは、病気の感染を更に拡げる危険性があることを示しており、これにより重大な公衆衛生の問題を提示している。</p>					<p>使用上の注意記載状況・ その他参考事項等</p> <p>代表としてテタノプリン-IH の記載を示す。</p> <p>2. 重要な基本的注意 (1)略 1)略 2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病 (vCJD) 等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。</p>
	報告企業の意見				今後の対応	
<p>トランスジェニックマウスを用いた vCJD 感染実験により、vCJD はコドン 129 が MM, VV, MV の全ての遺伝型が感染し、VV 及び MV は MM 型よりも潜伏期間が長いために、輸血のようなルートによって、vCJD の感染を更に拡げる危険性を示唆した報告である。</p> <p>これまで血漿分画製剤によって vCJD を含むプリオン病が伝播したとの報告はない。しかしながら、万一 vCJD 感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程における TSE 感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。</p>				<p>本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。</p>		

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.

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 hundreds² 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.^{5,6} 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.⁸⁻¹¹ 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,^{9,10} 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.¹² 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.¹³

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

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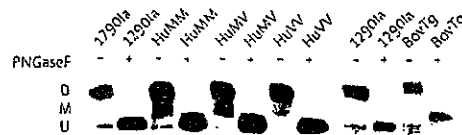


Figure 1: Western blot of brain extract from uninoculated mice showing that PrP^{Sc} is detected with equivalent electrophoretic mobility and glycoform ratio in all three human transgenic lines. D=diglycosylated PrP^{Sc} band; M=monoglycosylated PrP^{Sc} band; U=unglycosylated PrP^{Sc} band. In the BovTg 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 (1290la). 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 NHBV0/0003). 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

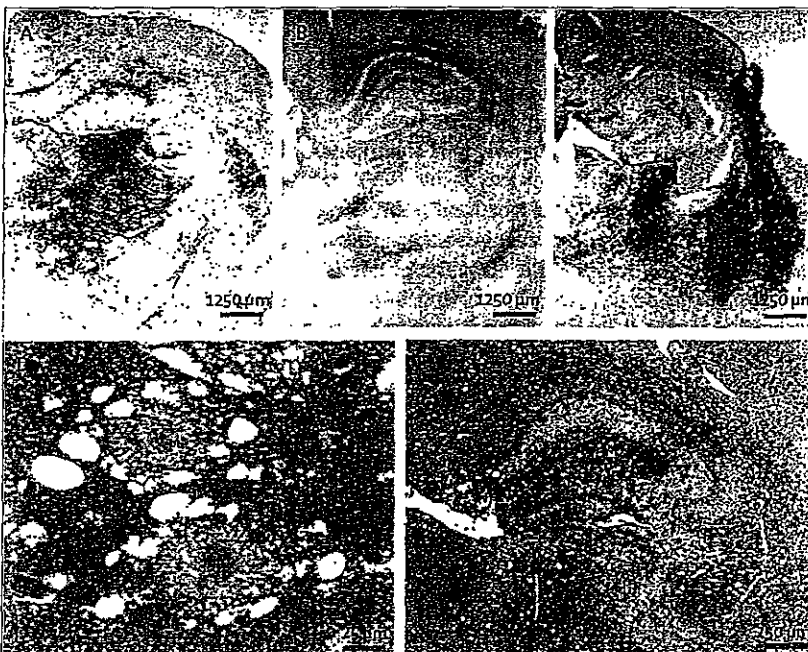


Figure 2: Immunocytochemistry of histological sections with anti-PrP antibody 6H4 showing the cortex, hippocampal, and thalamic regions of the mouse brain with PrP detection (brown). A-D: Human transgenic mice with vCJD inoculum. A: HuMM mouse 693 days post inoculation. B: HuMV mouse 707 days post inoculation. 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.

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.

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.^{2,18} 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 polyvinylidene 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,

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⁺ 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

	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

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

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

	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

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

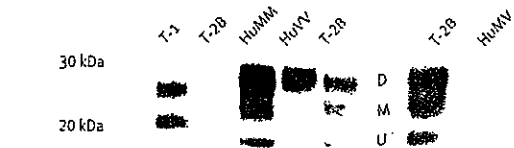


Figure 3: Western blots of brain extract from three transgenic lines inoculated with vCJD

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.

(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 haematoxylin 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.^{15,23,26} 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.¹⁷

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

biochemical features of all human prion diseases. Our findings indicate that for human-to-human vCJD infection it should be assumed that all codon-129 genotype individuals (not just MM) can be infected, that long incubation times can occur, and that a significant level of subclinical disease might be present in the population.

Contributors

MTB, PH, and CP did immunocytochemical and western blot analysis; JCM, NT, HNB, and LA produced the transgenic mouse lines; JW1 supplied vCJD case material and reviewed the neuropathology; VT did the mouse inoculations; and MTB, PH, MWH, RGW, JW1, and JCM prepared the manuscript.

Conflicts of interest

We have no conflicts of interest.

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