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ORIGINAL PAPER

Creutzfeldt–Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study

P. E. Hewitt,[1] C. A. Llewelyn,[2] J. Mackenzie[3] & R. G. Will[3]

[1]National Blood Service, Colindale Centre, London, UK
[2]National Blood Service, Cambridge Centre, Cambridge, UK
[3]National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK

Background and Objectives This paper reports the results to 1 March 2006 of an ongoing UK study, the Transfusion Medicine Epidemiological Review (TMER), by the National CJD Surveillance Unit (NCJDSU) and the UK Blood Services (UKBS) to determine whether there is any evidence that Creutzfeldt–Jakob disease (CJD), including sporadic CJD (sCJD), familial CJD (fCJD), and variant CJD (vCJD) is transmissible via blood transfusion.

Materials and Methods Sporadic CJD and fCJD cases with a history of blood donation or transfusion are notified to UKBS. All vCJD cases aged > 17 years are notified to UKBS on diagnosis. A search for donation records is instigated and the fate of all donations is identified by lookback. For cases with a history of blood transfusion, hospital and UKBS records are searched to identify blood donors. Details of identified recipients and donors are checked against the NCJDSU register to establish if there are any matches.

Results CJD cases with donation history: 18/31 vCJD, 3/93 sCJD, and 3/5 fCJD cases reported as blood donors were confirmed to have donated labile components transfused to 66, 20, and 11 recipients respectively. Two vCJD recipients have appeared on the NCJDSU register as confirmed and probable vCJD cases. The latter developed symptoms of vCJD 5.5 years and 7.8 years respectively after receiving non-leucodepleted red blood cells (RBCs) from two different donors who developed clinical symptoms approximately 40 and 21 months after donating. A third recipient, given RBC donated by a further vCJD case approximately 18 months before onset of clinical symptoms, had abnormal prion protein in lymphoid tissue at post-mortem (5-years post-transfusion) but had no clinical symptoms of vCJD. CJD cases with history of transfusion: Hospital records for 7/11 vCJD and 7/52 sCJD cases included a history of transfusion of labile blood components donated by 125 and 24 donors respectively. Two recipients who developed vCJD were linked to donors who had already appeared on the NCJDSU register as vCJD cases (see above). No further links were established.

Conclusion This study has identified three instances of probable transfusion transmission of vCJD infection, including two confirmed clinical cases and one pre- or post-clinical infection. This study has not provided evidence, to date, of transmission of sCJD or fCJD by blood transfusion, but data on these forms of diseases are limited.

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Correspondence: Patricia Hewitt, National Blood Service, Colindale Centre, London, UK
E-mail: patricia.hewitt@nbs.nhs.uk

Introduction

Until 2004, it was generally accepted that Creutzfeldt–Jakob disease (CJD) had not been transmitted by blood transfusion.

Preliminary findings from sheep studies indicate that bovine spongiform encephalopathy (BSE) and scrapie can be transmitted by blood transfusion [1,2]. It is vital to find out whether this also applies to human transmissible spongiform encephalopathies (TSEs) and, in particular, variant CJD (vCJD). The UK is the only country where a significant outbreak of vCJD has occurred and is in a unique position to study this question which has important implications for public health policy. The results reported in this paper are from a study which is being carried out, with ethical approval, to investigate whether or not there is any evidence for the transmission of any type of CJD (sporadic, familial and variant) by blood transfusion.

Materials and methods

CJD surveillance

A surveillance system for CJD, the National CJD Surveillance Unit (NCJDSU), was established in the UK in 1990 with the aim of identifying all cases of CJD in the UK. The methodology of this study has been described previously [3]. In brief involves referral of suspected cases to the Unit by targeted professional groups, including neurologists, neuropathologists, review of suspects by a neurologist in the Unit and review of investigation results and neuropathological material when available. Cases are classified according to standard diagnostic criteria [4,5]. Onset of clinical symptoms for vCJD cases are estimated to the nearest month by NCJDSU on the basis of available clinical information. Details of past medical history, including blood donation or transfusion, are obtained from the family of suspected cases. Following the identification of vCJD in 1996 a collaborative study, the Transfusion Medicine Epidemiological Review (TMER), was established between the NCJDSU and UK Blood Services (UKBS) to search for evidence of transfusion transmission of CJD. The study was granted ethical approval by the local Research Ethics Committee.

Notification of CJD cases with a history of donation

Sporadic CJD (sCJD) and familial CJD (fCJD) cases with a history of blood donation are notified to UKBS retrospectively. For vCJD, all patients who are old enough to have donated blood (> 17 years of age) are notified to UKBS at diagnosis whether or not there is a known history of blood donation. Upon receipt of notification from the NCJDSU, a search is made for donor records. Current computer databases and archived records (computerized and paper-based records where appropriate) based at individual blood centres are searched using name, date of birth, and previous address as identifiers. For CJD cases reported as blood donors, information on dates and places of donation is also used to ide

Table 1 Recipients of blood donated by variant Creutzfeldt–Jakob disease cases by year and blood component transfused ($n = 66$)

Year of transfusion	Blood component transfused	Number of recipients
1980–1984	Whole blood	1
	Red blood cells	1
1985–1989	Red blood cells	2
1990–1994	Red blood cells	9
1995–1999	Whole blood	1
	Red blood cells	15
	Red blood cells – buffy coat depleted[a]	2
	Red blood cells – leucodepleted[b]	2
	Fresh frozen plasma	3
	Cryo-depleted plasma	1
	Cryoprecipitate	1
	Platelets (pooled)	1
2000–2004	Red blood cells – leucodepleted	23
	Fresh frozen plasma – leucodepleted	2
	Platelets (pooled, leucodepleted)	2

[a]Red cells with buffy-coat (containing most of the platelets and white cells) removed by centrifugation and physical separation.
[b]Red cells leucocyte-depleted by pre-storage filtration to $< 5 \times 10^6$/unit according to UK guidelines [6].

2006. Of these, 31 of 150 (21%) were reported to have been blood donors at various times in the past, although there is variation in the details of available information and the confidence of families in donation history.

Donor records were found for 24 vCJD cases, comprising 20 reported by relatives as blood donors and four additional cases with no reported donation history. Of these, 18 vCJD cases (12% of the total eligible to donate blood) were confirmed to have donated labile blood components, with the number of components made and issued for use in UK hospitals ranging from 1 to 14 per donor. Six vCJD cases were registered as donors, but had not donated labile blood components. Two of these had never attended sessions, three were deferred (due to past medical history, low haemoglobin value and illness, respectively) and one case had donated plasma for fractionation only (made from a single donation from which the red cells were discarded).

The search for donor records was negative in 11 of 31 (35%) vCJD cases reported as putative donors (three of whom allegedly donated well before the onset of the BSE epidemic in the 1980s). The information provided in these negative cases was minimal, except in one case where relatives were confident that regular donations (up to 50) had been made in the years leading up to 1993. Despite extensive searches no records were found; moreover, blood collection sessions had never been made at the purported venue. No explanation has been found for the lack of records, although discrepancies in some of the details given suggest that the history was not as certain as initially thought.

Labile components issued to hospitals

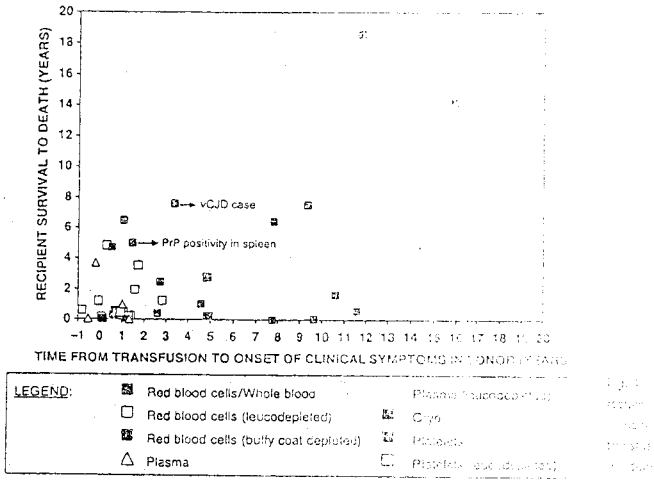
Sixty-six labile components originating from 18 donors were issued to UK hospitals over the period 1981–2004 and transfused to patients according to blood transfusion laboratory records. A further nine components issued between 1982 and 1996 could not be traced by the relevant hospital. Table 1 gives the number of recipients transfused by year and the type of blood component transfused. Fifty-six recipients (85%) received red cells or whole blood, seven (11%) were transfused with labile plasma components or derivatives and three (4%) received pooled platelets made according to UK specifications in which the buffy-coat preparation containing platelets from the implicated vCJD donor was pooled with buffy coats from three other donors and resuspended in plasma from one of the four donations. Nearly half of the red cell recipients received red cells that had been leucocyte-depleted by pre-storage filtration to $< 5 \times 10^6$ leucocytes per unit (in 99% of units with 95% statistical confidence according to UK guidelines [6]) after the introduction of universal leucocyte depletion of the UK blood supply in 1999.

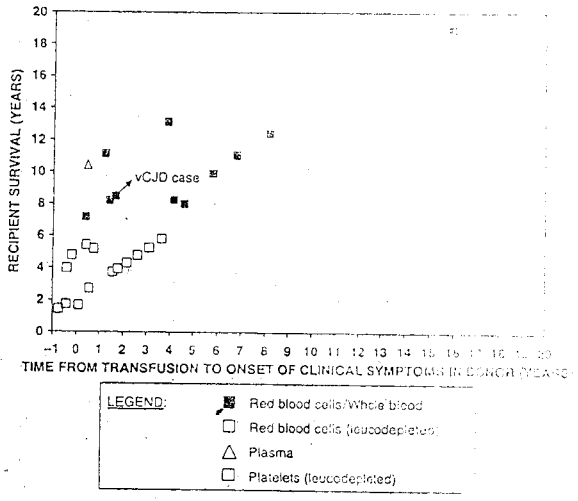
Recipients of blood components

Patient identifiers are available for 66 recipients who received blood from 18 different donors who went on to develop vCJD. None of the 66 recipients had themselves donated blood between receiving their transfusion and early 2004 when the UKBS implemented a policy of excluding all donors transfused in the UK since 1 January 1980. It is of note that 41 (62%) recipients were aged over 60 years at the time of transfusion and were not eligible to donate. All living recipients ($n = 26$) have been informed of their risk and advised not to donate blood, tissues or organs. Three instances of probable transfusion transmitted vCJD infection have occurred, including two confirmed clinical cases and one pre- or subclinical infection. Of these, two cases have died, and one is still alive (see succeeding discussion). Figures 1 and 2 show the survival period for dead (transfusion to death) and live recipients (transfusion to 1 March 2006) of vCJD components, respectively, according to the interval between transfusion and onset of clinical symptoms in the donor.

Dead recipients

Forty recipients (61%) are known to be dead, with mean age at death 66 ± 19 years. Table 2 gives the time and cause of death as stated on death certificates for the recipients known to have died. Around half ($n = 21$) of the dead recipients died within a year of receiving their transfusion, with only seven surviving for more than 5 years. Two recipients, who died 4 months and 14 months, respectively, after transfusion had





'dementia' recorded on the death certificate, but examination of case notes indicated that neither case had features to suggest vCJD. All the other recipients were certified as dying of causes unrelated to vCJD, except for a recipient whose cause of death on the death certificate was recorded as '1A dementia and II. prostate cancer' and was later confirmed neuropathologically as suffering from vCJD [7]. This patient,

Table 2 Cause of death of variant Creutzfeldt–Jakob disease recipients [illegible] have died, n = 40

Interval from transfusion to death	Number of recipients	Cause of death
[illegible] month	7	Acute [illegible] cortical necrosis
		Cancer (2)
		Myocardial infarction
		Septicaemia (2)
		[illegible]
[illegible] months	[illegible]	[illegible] pneumonia
		[illegible]
		Cancer [illegible]
		[illegible]
		[illegible]
		[illegible]
		Stroke/diabetes mellitus/dementia
6 – < 12 months	3	Cancer (3)
1 – < 5 years	12	Acute myeloid leukaemia (2)
		Bronchopneumonia/senile dementia
		Cancer
		Ischaemic heart disease (3)
		Chronic obstructive airways disease (COAD)
		Hypertensive heart disease, chronic renal failure
		Myelodysplasia
		Disseminated sepsis
		Spinal haemangioblastoma
5 – < 10 years	6	Cerebrovascular accident
		Ischaemic heart disease
		Acute lymphoblastic leukaemia
		Dementia/prostatic cancer
		Ruptured aortic abdominal aneurysm/severe atheroma/COAD[b]
≥ 10 years	2	Bronchopneumonia
		Ischaemic heart disease

[a][illegible]

[b][illegible] tissue, pre- or subclinical vCJD infection [8].

[illegible] months after donating and was later diagnosed with neuropathologically confirmed vCJD. [illegible]-resistant prion protein (PrP^{res}) was detected in the spleen and one lymph node (but not in the brain) at post-mortem [8]. This recipient, who died 5 years after transfusion without any clinical symptoms of vCJD, was a codon 129 *PRNP* heterozygote and is thought to represent pre- or subclinical infection.

Live recipients

Twenty-six recipients (39%) are alive as of 1 March 2006 with a mean age of 63 ± 19 years. Table 3 shows the number of live recipients according to the time elapsed since transfusion, along with their current age, component transfused and the interval between donation and onset of clinical symptoms of vCJD in the donor. Fifty per cent of live recipients were transfused with components from vCJD donors whose donations were made within 20 months of clinical onset, in seven cases around the time of development (n = 3) or shortly after (n = 4) the first signs of clinical illness. These cases would have appeared healthy when attending donor sessions and passed the normal medical checks as being fit to donate. Sixteen recipients have survived longer than 5 years, with six surviving > 10 years (one for over 18 years). These patients, mean age currently 61 ± 19 years, were given blood from donors who developed vCJD symptoms at intervals ranging from around 5 months to 191 months after making the donation (see Table 3). Recently, a diagnosis of probable vCJD has been made in one of these surviving recipients who had received a transfusion of red cells 7 years and 10 months before onset of clinical symptoms [9]. The donor of this third probable transfusion-transmitted vCJD infection developed vCJD approximately 21 months after the donation, and the recipient is a codon 129 *PRNP* methonine homozygote.

Plasma for UK fractionation

Twenty-five units of plasma originating from 11 different donors, bled between 6 months and 17 years, 11 months before onset of clinical vCJD symptoms, were supplied for UK fractionation during the period 1986–1998. Product batches manufactured from 23 plasma units derived from nine donors have been traced. The fate of batches of product derived from the two remaining plasma donations, from two different donors, has not yet been traced, and this search is still ongoing. Table 4 lists the plasma products derived from the 23 traced donations and the number of batches implicated, divided into risk categories as used in the plasma product notification exercise (www.hpa.org.uk/infections/topics_az/cjd/Recommendations.pdf). The fate of batches of products has not been traced to individual recipients as part of this study. It is known, however, that haemophilia centres have traced the ultimate fate of the batches of factor VIII. It is also known that no case of vCJD has been identified in a patient with haemophilia in the UK.

sCJD cases with history of blood donation

Ninety-three cases of sCJD identified between 1980 and 2000 were reported to have been blood donors, with only 38 reported to have donated from 1980 onwards. Donation records for most sCJD cases were untraceable since most dated back many years before 1980, in some cases to the 1940s. Donation records were found for eight sCJD cases, but only three had actually donated labile blood components for hospital use (one with 18 recipients, and one each with one recipient) which could be traced to recipients. A total of 20 recipients were transfused between 1995 and 1999 with components from these three donors who went on to develop

Table 3 Live recipients of labile blood components donated by variant Creutzfeldt–[illegible]

Time elapsed since transfusion[a]	Current age of recipient (years)[a]	Blood component transfused	Interval [illegible]
1 – < 2 years	48	Platelets [illegible]	[illegible]
	49	[illegible]	[illegible]
	83	[illegible]	[illegible]
2 – < 3 years	33	[illegible]	[illegible]
3 – < 4 years	58	[illegible]	[illegible]
	83	[illegible]	[illegible]
	93	[illegible]	[illegible]
4 – < 5 years	59	[illegible]	[illegible]
	67	[illegible]	[illegible]
	83	[illegible]	[illegible]
5 – < 6 years	33	[illegible]	[illegible]
	52	[illegible]	[illegible]
	64	[illegible]	[illegible]
	71	[illegible]	[illegible]
6 – < 7 years	–		–
7 – < 8 years	42	[illegible]	[illegible]
8 – < 9 years	31[c]	[illegible]	[illegible]
	74	[illegible]	[illegible]
	76	[illegible]	[illegible]
	87	[illegible]	[illegible]
9 – < 10 years	75	[illegible]	[illegible]
> 10 years	33	[illegible]	[illegible]
	49	[illegible]	[illegible]
	67	[illegible]	[illegible]
	70	[illegible]	[illegible]
	75	[illegible]	[illegible]
	87	[illegible]	[illegible]

[a]As at 1 March 2006.

[b]A negative interval denotes that donation was made by individual [illegible]

[c]Probable variant Creutzfeldt–Jakob disease case [9].

sCJD between 1 and 5 years after donation. Of these, [illegible] received red cell components, eight recipients (40%) [illegible] platelets and one (5%) received fresh frozen plasma. [illegible]

As of 1 March 2006, 12 recipients are confirmed dead [illegible] a mean age at death of 74 ± 15 years. Of these, five died [illegible] after transfusion (four within a week, and one 2 months [illegible]) and seven survived for between 1 and 5 years after [illegible] their transfusion before dying of a variety of [illegible] related causes (cerebrovascular accident/stroke, n = [illegible] myeloid leukaemia, n = 3, general debility/old age, [illegible] Seven recipients are not known to be dead from [illegible] to date, and are therefore presumed to be alive. The [illegible] of these seven recipients is 58 ± 19 years. The time [illegible] since their transfusion ranges from 7 to 9 years. The [illegible] a further recipient is unknown. None of the sCJD recipients [illegible] identified as having received blood from donors who [illegible] on to develop sCJD have appeared on the NCJDSU register [illegible] to date. [illegible]

Table 4 Product batches made by UK fractionators derived from plasma donated by individuals who later developed variant Creutzfeldt–Jakob disease[a,b]

Infectivity Classification[c]	Plasma product	Number of implicated batches
Low	Factor VIII (excipient[d])	77
	Albumin 20%	21
	i.m. immunoglobulin	12
Medium	Albumin 4·5%	28
	i.v. immunoglobulin	11
High	Factor VIII	16
	Factor IX	8
	Anti-thrombin	1
	TOTAL	174

[a]Twenty-three plasma donations from nine variant Creutzfeldt–Jakob disease donors, data courtesy of Health Protection Agency.
[b]Excludes fate of two plasma units from two further vCJD cases (see text for explanation).
[c]Risk categories as used in plasma product notification exercise.
[d]Albumin from implicated plasma donation used as excipient (inert substance added to provide bulk) in preparation of batch of Factor VIII.

Three recipients are not known to be dead from ONS flagging to date, and are therefore presumed to be alive. The mean age of these three recipients is 44 ± 20 years. The time elapsed since their transfusion ranges from 13 to 21 years. The fate of a further three recipients is not known. None of the fCJD recipients identified as having received blood from donors who went on to develop fCJD have appeared on the NCJDSU register to date.

vCJD cases with history of transfusion

Eleven vCJD cases were reported to have received past blood transfusions between 1962 and 1999. A further case received a blood transfusion after onset of illness. This case is excluded from further analysis. For two cases, hospital records showed that they had not been transfused. No hospital records could be found for another two cases reported to have been transfused in 1962 and 1971, respectively. Hospital transfusion records were found for seven vCJD cases (64% of those reported as transfused) who had been transfused with components donated by 125 donors (121 identified), with one vCJD case, who also received a solid organ transplant, receiving components from 103 donors. The identity of four donors who donated red cell/whole blood components to two cases (case 2 and case 7, see Table 5) is unknown. Table 5 shows the transfusion date, number of donors and blood components donated, and the interval from transfusion to onset of clinical symptoms of vCJD in these seven recipients. These cases had been exposed to between two and 103 donors, respectively (NB search for donors to case 6 is incomplete). To date, one donor who gave red cells to case 5 and another donor who gave red cells to case 6 are also registered on the NCJDSU database as vCJD cases. These are the donors of the two clinical cases of transfusion-transmitted vCJD referred to previously (see vCJD cases with history of donation).

sCJD cases with history of transfusion

Fifty-two cases of sCJD identified between 1980 and 2000 were reported to have received a blood transfusion, of which 28 received a transfusion after 1980. Transfusion records were found for seven sCJD cases transfused between 1984 and 1997. Donor details were found for 24 donors who donated components transfused to these seven sCJD cases. One of these donors is known to have died, with a cause of death not related to CJD. Twenty donors are not known to have died from ONS flagging to date, and are therefore presumed to be alive. The fate of a further three donors is not known. The mean age of the donors presumed still alive is 51 ± 9 years. None of the traced donors who gave blood to patients who were subsequently diagnosed with sCJD have appeared on the NCJDSU register to date.

fCJD cases with history of transfusion

One case of fCJD identified in 1992 was reported to have received three blood transfusions in 1965, 1970, and 1987 none of which could be traced.

Discussion

This study has identified three instances in which a recipient of a transfusion derived from a 'vCJD' donor has developed infection with vCJD, including two clinical cases and one pre- or subclinical infection [7–9]. These are three different donor/recipient pairs. In view of the small size of the total at-risk recipient population ($n = 66$) and the background mortality rate for vCJD in the general UK population (0·24/million/annum), these observations provide strong evidence that vCJD can be transmitted from person to person through blood transfusion. This finding has had important implications for public health policy nationally and internationally.

The risk of developing vCJD infection in the surviving recipient population is significant but cannot be precisely estimated because of variables including the timing of blood donation in relation to clinical onset in the donor, the influence of the codon 129 genotype of donor and recipient and the effect of the introduction of leucodepletion in 1999. Furthermore, the currently observed number of infections in the recipient population may be an underestimate as some surviving recipients may yet develop vCJD and there is limited available information on the outcome in the cohort of

Table 5 Donors ($n = 125$) of labile blood components given to variant Creutzfeldt–Jakob disease [illegible] with identifi[illegible]

Case	Transfusion date	Number of donors of labile blood components transfused	Blood components donated to vCJD patient
1	1993	38	Cryoprecipitate [illegible] Fresh frozen plasma [illegible] Platelets [illegible] Red cells [illegible] Whole blood [illegible]
1	1993	65	Cryoprecipitate [illegible] Fresh frozen plasma [illegible] Platelets [illegible] Red cells [illegible]
2	1983	2[b]	Red cells
2	1993	3	Fresh frozen plasma
3	1994	4	Red cells [illegible]
4[c]	1999	5	Red cells [illegible] [illegible] platelets (3)
5[a]	1996	5[d]	Platelets [illegible]
6[a]	1997	1+[e]	Red cells [illegible]
7	1982	2[b]	Whole blood

[a]Two of these cases linked to donors already on the National CJD Surveillance Unit [illegible] (12).
[b]Component details traced, but donors not identifiable.
[c]Timing of clinical illness excludes blood transfusion as the source of infection in this case.
[d]One of the donors already on NCJDSU register as vCJD case, others presumed [illegible].
[e]One donor already on NCJDSU register as vCJD case. Search for 40+ donors to Case 6 [illegible] of 1 March 2006.

deceased recipients; a significant proportion of these individuals may not have survived long enough to express clinical disease even if infected. The minimum incubation period in CJD transmitted from person to person by a peripheral route is 4·5 years in kuru and growth-hormone-related CJD [10,11] and only nine deceased recipients survived for longer than this period. An investigation of the hospital records of the deceased recipients is underway, and to date, none had clinical features of vCJD pre-mortem. However, the identification of the individual with 'preclinical' vCJD infection was dependent on post-mortem examination of peripheral lymphoreticular tissues, and, to date, no equivalent tissues have been available in the deceased transfusion recipients. Extrapolating from the three observed infections in the total recipient population is likely to lead to an underestimate of the overall risk of transfusion transmission of vCJD, although the introduction of leucodepletion in 1999 may have reduced the risk to recipients transfused after this date.

A further important variable in estimating individual risk is the time from blood donation to clinical onset in the donor and, although evidence from animal studies in relation to this issue is conflicting [12–14], it is likely that an extended gap between blood donation and clinical onset in the donor will reduce the risk of transfusion transmission. All tested clinical cases of vCJD have been methionine homozygotes at codon

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5 [illegible]

Effectiveness of leucoreduction for removal of [illegible] transmissible spongiform encephalopathi[illegible] from [illegible]

Luisa Gregori, Nancy McCombie, Douglas Palmer, Paul Birch, Samuel O Sowemimo-Coker, An[illegible]

In 1999, the UK implemented universal leucoreduction as a precaution against transmission [illegible] Jakob disease by transfusion of domestic blood or red blood cells. We aimed to assess how [illegible] reduced infectivity of transmissible spongiform encephalopathies (TSEs) in [illegible] 450 mL [illegible] and pooled from scrapie-infected hamsters was leucoreduced with a commercial filter. [illegible] were quantified, and infectivity titres measured. Blood cell recovery and white blood cell [illegible] American Association of Blood Banks standards. Leucofiltration removed [illegible] ID 12) of the [illegible] endogenously infected blood. Leucoreduction is necessary for the removal of [illegible] from blood; however, it is not, by itself, sufficient to remove all blood-borne TSE infectivity.

Transmissible spongiform encephalopathies (TSEs) are fatal CNS infections that can incubate asymptomatically for a decade or more in human beings before the appearance of clinical disease. People in the asymptomatic phase of variant Creutzfeldt-Jakob disease (vCJD) appear healthy and donate blood with the same frequency as any healthy person. Transmission of vCJD by transfusion was recently recognised in Great Britain.[1] To reduce the risk of transfusion transmission of such diseases in human beings, the UK implemented universal leucoreduction of donated blood in 1999. This measure was based on the expectation that infectivity would be associated with white blood cells.[2] However, findings in blood from infected mice and hamsters suggested otherwise; at least 40% of the infectivity was plasma-associated, suggesting that leucoreduction would not eliminate infectivity (Rohwer laboratory, unpublished).[3] Other investigations showed no loss of infectivity when small amounts of TSE-infected plasma were passed through scaled-down filters.[4] Similarly, no significant removal of abnormal prion protein was detected when units of human whole blood, spiked with a microsomal fraction from TSE-infected brain, were passed through leucoreduction filters from any of the four major suppliers.[5] Because of reservations about the relevance of these experiments, none of these findings aroused concern.

We investigated the effectiveness of leucoreduction in removal of TSE infectivity from a human-sized unit of pooled hamster blood. To ensure that the 150 hamsters needed for a 450 mL blood pool were at the same symptomatic stage of disease (wobbling gait and head bobbing) for each of two separate experiments, 400 weanling golden Syrian hamsters (Harlan, Madison, WI, USA) were [illegible] brain homogenate containing [illegible] dose$_{50}$ (ID$_{50}$) of hamster-adapted [illegible] low dose of infectivity was used [illegible] of the inoculum [illegible] blood. The [illegible] approved by the University of [illegible] Animal Care and [illegible] Committee.

We [illegible] one at 106 days and one at 121 [illegible] Under carbon dioxide [illegible] drawn from the [illegible] ventricle [illegible] anticoagulant. [illegible] tissue. Only [illegible] no visible clots were [illegible]

Two [illegible] Corporation, [illegible] Washington, [illegible] for [illegible] [illegible] complete [illegible] of [illegible] complied with [illegible] [AABB] [illegible] and [illegible] for [illegible] The [illegible]

[illegible]

	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.

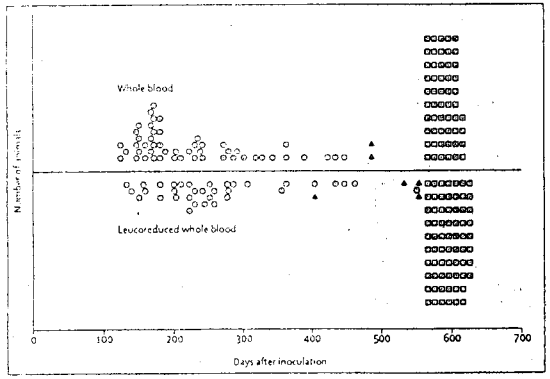

Figure: Incubation times of infections from whole and leucoreduced blood
Results of inoculations of whole blood are represented by data above the horizontal line; those from inoculations of leucoreduced blood are shown below the line. Circles represent infected animals. Squares represent uninfected animals 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 $P(0)=e^{-titre\times vol}$ 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[1] 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 [illegible] management of the logistics and all [illegible] by L Gregori and R G Rohwer with the [illegible] of the staff of the Molecular Neurovirology Laboratory. A [illegible] N McCormick, D Palmer, and P Birch supplied expert [illegible] blood collection, component separation [illegible] and quantitation of white blood cells. D [illegible] and P Birch in [illegible] interpreted flow cytometry. S Coker [illegible] experience in the use [illegible] collection set and leucofilter.

Conflict of interest statement
R G Rohwer is a cofounder and part owner [illegible] Diagnostics Technologies, which is developing technologies for the removal of TSE infectivity from blood [illegible] L Gregori receives contract support from Pathogen [illegible] Technologies for studies on TSE removal. Neither [illegible] Pall Corporation, which produces leucofilters and is developing TSE removal strategies for blood. The remaining authors declare that they have no competing financial interests.

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

M T Bishop, P Hart, L Aitchison, H N Baybutt, C Plinston, V Thomson, N L Tuzi, M W Head, J W Ironside, R G Will, J C Manson

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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National CJD Surveillance Unit, Bryan Matthews Building, Western General Hospital, Edinburgh, UK (M T Bishop BSc, M W Head PhD, J W Ironside FRCPath, R G Will FRCP); and Institute for Animal Health, Neuropathogenesis Unit, King's Buildings, Edinburgh, UK (P Hart PhD, L Aitchison MSc, H N Baybutt PhD, C Plinston BSc, V Thomson BA, N L Tuzi PhD, J C Manson PhD)

Correspondence to:
Prof J C Manson, Institute for Animal Health, Neuropathogenesis Unit, Ogston Building, King's Buildings, West Mains Road, Edinburgh EH9 3JF, UK
Jean.Manson@bbsrc.ac.uk

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.[5] 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.[6]

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.[8–12] 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

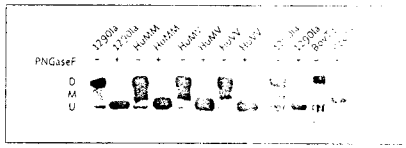

Figure 1: Western blot of brain extract from uninoculated mice showing that PrP^{C} is detected with equivalent electrophoretic mobility and glycosylation ratio in all three human transgenic lines
D=diglycosylated PrP^{C} band; M=monoglycosylated PrP^{C} band; U=unglycosylated PrP^{C} band. In the BovTg line, a deglycosylated band is detected of increased molecular weight due to the additional N-terminal octapeptide repeat. Protein levels are similar to the wild-type line used in generating the transgenic (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 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 10% 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 NIHBY0/0003). BSE-infected cattle brain (Veterinary Laboratories Agency, reference 12/92) was prepared by maceration of the tissue in phosphate 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 or not.

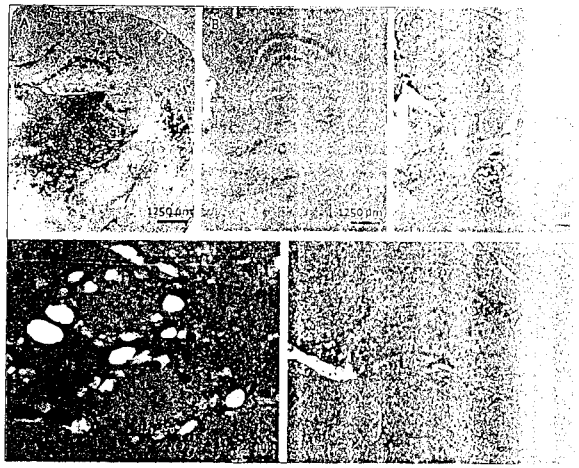

Figure 2: Immunocytochemistry of histological sections with anti-PrP antibody 6H4 showing the cortical, hippocampal, and thalamic regions of the mouse brain with PrP detection (brown)
A–D: Human transgenic mice with vCJD inoculum. A: HuMM mouse 693 days post inoculation. B: HuMM mouse 707 days post inoculation.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

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 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 560 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

	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**

	HuMM	HuMV	HuVV
Vacuolation*	Thalamus (severe), cerebral cortex and hippocampus (mild), cerebellar cortex (minimal)	Thalamus, cerebral cortex and cerebellar	
Plaque formation*	Fibrillary amyloid plaques, florid and non-florid plaques in cerebral cortex and hippocampus; no evidence of plaques in cerebellum	No evidence of	
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	

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

Table 4: **Comparison of TSE-associated neuropathology in human transgenic mice**

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 lifespan of the experimental (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 *Prnp* gene and may not therefore give a true reflection of the species barrier between BSE and human beings.[16,17] 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^{Sc} 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,[4] 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 biochemical features of all human prion diseases. [illegible] findings indicate that for human-to-human [illegible] infection it should be assumed that all [illegible] genotype individuals (not just MM) can be infected, [illegible] 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 analyses. JCM, NT, HNB, and LA produced the transgenic mouse lines. JWI supplied vCJD case material and reviewed the neuropathology. NT did the mouse inoculations; and MTB, PH, MWH, RGW, JWI, and JMS prepared the manuscript.

Conflicts of interest

We have no conflicts of interest.

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Prion diseases are efficiently transmitted by blood transfusion in sheep

Fiona Houston,[1] Sandra McCutcheon,[1] Wilfred Goldmann,[2] Angela Chong,[2] James Foster,[2] Silvia Sisó,[3] Lorenzo González,[3] Martin Jeffrey,[3] and Nora Hunter[2]

[1]Neuropathogenesis Division, [illegible], Compton, United Kingdom; [2]Neuropathogenesis Division, Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom; and [3]Lasswade Laboratory, Veterinary Laboratories Agency, Penicuik, United Kingdom

The emergence of variant Creutzfeld-Jakob disease, following on from the bovine spongiform encephalopathy (BSE) epidemic, led to concerns about the potential risk of iatrogenic transmission of disease by blood transfusion and the introduction of costly control measures to protect blood supplies. We previously reported preliminary data demonstrating the transmission of BSE and natural scrapie by blood transfusion in sheep. The final results of this experiment, reported here, give unexpectedly high transmission rates by transfusion of 36% for BSE and 43% for scrapie. A proportion of BSE-infected tranfusion recipients (3 of 8) survived for up to 7 years without showing clinical signs of disease. The majority of transmissions resulted from blood collected from donors at more than 50% of the estimated incubation period. The high transmission rates and relatively short and consistent incubation periods in clinically positive recipients suggest that infectivity titers in blood were substantial and/or that blood transfusion is an efficient method of transmission. This experiment has established the value of using sheep as a model for studying transmission of variant Creutzfeld-Jakob disease by blood products in humans. (Blood. 2008;112: 4739-4745)

Introduction

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases, which include Creutzfeld-Jakob disease (CJD) in man, scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle. A new variant of CJD (termed vCJD) was recognized in the United Kingdom in the mid-1990s, apparently as a result of transmission of BSE to humans.[1] To date, there have been 165 cases of vCJD recorded in the United Kingdom, as well as several cases in other countries. Human TSEs are characterized by long asymptomatic incubation periods (usually several years), and there is no reliable test for detecting infection before the onset of clinical disease. It is not known how many people in the United Kingdom harbor vCJD, although estimates based on screening of tonsil and appendix samples suggest there could be up to 4000.[2] These infected persons pose a risk of human-to-human transmission via blood transfusion or contaminated surgical instruments.

In patients with vCJD, there is widespread replication of the infectious agent and deposition of PrP^{Sc} (disease-associated form of prion protein) in lymphoreticular tissues, such as the tonsil, spleen, and lymph nodes, in contrast to sCJD, where lymphoreticular involvement is minimal.[3] The fact that lymphocytes continuously recirculate between blood and lymphoreticular tissues strongly suggests that the blood of vCJD patients is probably infectious. Data from rodent TSE models had shown that the highest levels of infectivity in blood were associated with leukocytes and, to a lesser extent, plasma.[4] As a result, costly control measures such as leukodepletion (filtration of blood and blood products to remove leukocytes) and importation of plasma were introduced to protect United Kingdom blood supplies, despite the limited data that were then available to judge the size of the risk and the efficacy of the control measures.

The potential for using sheep as a model for studying the risks of vCJD transmission by blood transfusion was highlighted by the similarity between the distribution of infectivity and PrP^{Sc} in sheep infected with TSEs and humans infected with vCJD.[5-7] One factor limiting the successful transmission of TSEs by blood in rodent models was the small volumes of blood that could be injected. In contrast, the relative similarity in size of sheep and humans means that volumes of blood comparable with those used in human transfusion practice can be collected from and transfused into sheep. Using this model, we previously reported preliminary results showing that both BSE and natural scrapie could be transmitted between sheep by blood transfusion.[8,9] Although scrapie is not thought to be transmissible to humans, it was included as a representative of infection acquired under field conditions, which may give different results to those obtained from experimentally infected animals. Our blood transfusion experiment in sheep is complete after 9 years, and this paper presents the full data from the study. The overall transmission rates for both scrapie and BSE are surprisingly high when factors such as the stage of infection and genetic background are taken into account, suggesting that blood transfusion represents an efficient route of transmission.

Methods

Donor and recipient sheep

The animal work was reviewed and approved by internal ethical review procedures at the Institute for Animal Health, United Kingdom, and carried out under the authority of Home Office Project Licences.

PrP genotypes of all sheep were confirmed by sequencing the coding region of the *PrP* gene[10] and are represented by single letter amino acid

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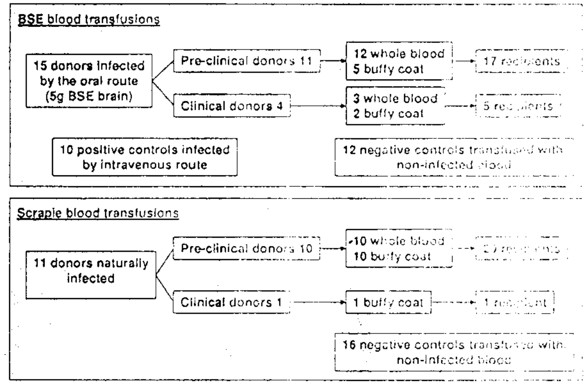
BSE blood transfusions
15 donors infected by the oral route (5g BSE brain)
Pre-clinical donors 11
Clinical donors 4
12 whole blood
5 buffy coat
3 whole blood
2 buffy coat
17 recipients
5 recipients
10 positive controls infected by intravenous route
12 negative controls transfused with non-infected blood
Scrapie blood transfusions
11 donors naturally infected
Pre-clinical donors 10
Clinical donors 1
10 whole blood
10 buffy coat
1 buffy coat
1 recipient
16 negative controls transfused with non-infected blood

code for codons 136, 154, and 171, which have been linked to scrapie susceptibility (eg, ARQ represents alanine, arginine, and glutamine, respectively, at codons 136, 154, and 171).

All donor sheep were from the Edinburgh NPU Cheviot flock, which has endemic natural scrapie. The recipient sheep (including scrapie negative control donors) were Cheviots derived from the DEFRA scrapie-free (DEFRA/SF) flock of New Zealand origin. Transfusion recipients and positive and negative controls were housed in a purpose-built isolation unit on a different site to the donors, with strict procedures in place to minimize the risk of cross-contamination between groups, as described.[9] The sheep were scored at weekly intervals for clinical signs of TSEs and killed when they reached humane endpoints agreed with the Home Office. For experimentally inoculated animals (BSE donors, positive controls, and transfusion recipients), the incubation period (IP) in clinically positive sheep was defined as the period between the date of inoculation and the date of death. For scrapie-exposed donors, the IP in clinically positive sheep was defined as the age at death (ie, they were assumed to have become infected immediately after birth).

Blood collection and transfusion

Procedures for blood collection/transfusion were as previously described.[9] Briefly, venous blood (450-500 mL = 1 unit) was collected into sterile collection bags (NBPI-Fresenius, Emmer-Compascuum, The Netherlands) containing citrate phosphate dextrose adenine solution as anticoagulant. From donors that were about to be killed, 2 units was collected just before postmortem, whereas from donors that were to be left alive, separate collections of 1 unit were made at least 28 days apart. However, for practical reasons, it was not always possible to collect 2 units of blood from every donor sheep. In most cases where 2 units of blood was obtained, one was transfused as whole blood (without leukodepletion) and the other was used to prepare a buffy coat fraction.

BSE blood transfusions

Fifteen sheep experimentally inoculated either orally (n = 14) or intracerebrally (n = 1) with 5 g or 0.05 g, respectively, of BSE-infected cattle brain homogenate were used as blood donors. The donor *PrP* genotypes were ARQ/ARQ (n = 3), ARQ/AHQ (n = 5), or AHQ/AHQ (n = 7), which are resistant to natural scrapie in the NPU flock but produce the shortest IPs after inoculation with BSE. Two sheep previously reported as donors[9] were excluded from the study (along with their recipients) when regenotyping showed them to be ARQ/ARR and VRQ/AHQ, respectively, genotypes that result in relative resistance to oral infection with BSE.

Eleven donor sheep provided blood for transfusion at the preclinical stage of infection. Eight of these were culled at the time of donation as part

of a separate time course [illegible] 3 preclinical donors went on to [illegible] respective IPs of 625, [illegible], and 2131 days [illegible] used as blood donors once they [illegible] 561 to 671 days after infection. PrP^{Sc} [illegible] tissues were confirmed in all clinically [illegible] try (IHC). In 2 donors culled at the preclinical [illegible] found in only one tissue in each sheep [illegible] ganglion [illegible] 49). However, a negative [illegible] tissues were immunostained in another [illegible] recipients of whole blood and 7 [illegible] BSE-infected donors. Figure 1 gives a summary [illegible] whereas details of the donor and recipient sheep [illegible]

Scrapie blood transfusions

The donors for this experiment were [illegible] Cheviot sheep from the Edinburgh NPU [illegible] genotypes [illegible] incidence [illegible] [illegible]

Positive and negative controls

Seven ARQ/AHQ and 3 ARQ/ARQ sheep were [illegible] 0.2 g of the same BSE-infected cattle brain [illegible] the blood donors and served as positive controls [illegible] used in the scrapie transfusion experiment [illegible] transfusion experiment, 12 ARQ/ARQ recipients [illegible] whole blood (n = [illegible]) buffy coat [illegible] (6 ARQ/AHQ, 1 ARQ/ARR). Two recipients [illegible] after transfusion, respectively, and the [illegible] between 2402 and 2556 days after transfusion [illegible] scrapie experiment, 16 VRQ/VRQ sheep [illegible]