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Original article

vCJD and blood transfusion in the United Kingdom

Variant de la maladie de Creutzfeldt-Jakob et transfusion sanguine au Royaume-Uni

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Abstract

A study was set up in the UK in 1997 to examine whether there is any evidence that variant Creutzfeldt–Jakob disease (vCJD) is transmitted by blood transfusion. To date, the study has identified three probable cases of vCJD transmission by blood transfusion, including two clinical cases and one pre- (or sub-) clinical case.
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Résumé

Une étude a débuté en 1997 au Royaume-Uni, afin de déterminer si le variant de la maladie de Creutzfeldt-Jakob pouvait être transmis par transfusion sanguine. Actuellement, cette étude a permis d'identifier trois cas probables de transmission, incluant deux cas cliniques et un cas pré- ou subclinique.
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Keywords: Blood transfusion; Transmission; vCJD

Mots clés : vMCJ ; Transfusion sanguine ; Risque

1. Introduction

Although rare, prion diseases have caught the public attention in recent years due to the emergence of new forms, such as bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD). BSE occurred as an epidemic in UK cattle in the 1980s and 1990s, and during that time a very large number of BSE-infected cattle carcasses would have been likely to enter the human food chain, possibly giving rise to human infection with BSE (vCJD) through contaminated meat products.

The commonest form of human prion disease is the sporadic form of Creutzfeldt-Jakob disease (sCJD), which occurs most commonly in the seventh decade of life. sCJD occurs as a worldwide disorder affecting around one patient per million of the population each year. The clinical features are those of a rapidly progressive dementia with a range of other neurological abnormalities including visual abnormalities and movement disorders (particularly ataxia and myoclonus), resulting in death around 4 months after the disease onset. Until 2004, it was generally accepted that CJD had not been transmitted by blood transfusion. Small studies, both case–control studies and lookback investigations, had not detected any link between sCJD and blood transfusion.

2. CJD surveillance

In 1990 the National CJD Surveillance Unit (NCJDSU) was established in the UK with the aim of identifying all cases of CJD. Suspected cases of CJD are referred to the NCJDSU from

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targeted professional groups, including neurologists and neuropathologists. A neurologist from NCJDSU reviews the case, the investigation results, and the neuropathological material when available. Cases are classified according to standard diagnostic criteria. Onset of clinical symptoms for CJD cases are estimated to the nearest month by NCJDSU after reviewing the case notes. Details of past medical history, including blood donation or transfusion, are obtained from the family of suspected cases.

As of August 2006, there have been 162 cases of vCJD in the UK: the majority have been confirmed by neuropathological examination. Six of the cases are alive. Modeling by statisticians suggests that the peak of the epidemic occurred in 1999, and confirms that cases continue to decline year-on-year since then. It is possible, however, that there could be further peaks due to cases in individuals of different genetic make-up, or to secondary spread through other routes such as blood transfusion or exposure through surgery.

The median age at onset of the vCJD cases is 26 years and the median age at death is 28 years. The illness has a median duration of 14 months, which is very different from sCJD. Although most cases are in the younger age group, and the youngest case was age 12 at diagnosis, there have been cases in those over the age of 60, and the oldest diagnosed case was age 74.

Risk factors for vCJD, determined by detailed epidemiological investigation, include a young age, being homozygous for methionine at codon 129 of the prion protein gene, and having had surgical treatment in the past. There is a higher prevalence of the disease in the northern half of the UK (Scotland and northern England) compared with the rest of the UK (Northern Ireland, Wales and southern England).

3. Surveillance of vCJD and blood transfusion

Following the identification of vCJD a collaborative study was established in 1997 between the NCJDSU and the four UK blood services (UKBS). The study (the Transfusion Medicine Epidemiology Review, TMER) was designed to investigate whether there is any association between any type of CJD (sporadic, familial and variant) and blood transfusion. The study was granted ethical approval by the local Research Ethics Committee and aims to include all cases of CJD reported in the UK. For the purpose of this paper, only vCJD will be considered.

All patients with vCJD (probable or confirmed) 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. This decision was made on public health grounds, because information obtained from third parties (family or friends) is less reliable than that obtained direct from the individual. Upon receipt of notification from the NCJDSU, the UKBS searches for donor records relating to that case, using name, date of birth and previous addresses as identifiers. For cases reported as blood donors, information on dates and places of donation is also used to help locate past donor records. Where donor records are found, all components issued to hospitals are identified and their fate determined, as recorded in hospital blood transfusion laboratory records. At this stage, no check is made of the medical records relating to identified recipients and it is assumed that the blood component was transfused to that recipient. Details of the recipients (name and date of birth) are checked against the NCJDSU register to establish if there is a match between these individuals and patients who have developed CJD. Checks continue to be made at three-monthly intervals, to identify any cases which develop subsequently. Recipients details are also flagged with the Office of National Statistics (ONS), which provides a copy of the death certificate to NCJDSU for checking to establish date and certified cause of death.

4. Evidence from animal studies

After the start of the study, in 2002, findings from sheep studies were published, indicating that BSE and scrapie can be transmitted by blood transfusion [1]. It would be reasonable to assume that these findings could also apply to human TSEs, and in particular vCJD, even though there is no evidence that sCJD has been transmitted by blood transfusion. 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.

5. Identification of donors

As of 1st September 2006 there were a total of 162 vCJD cases on the NCJDSU register. Cases who were old enough to have been potential blood donors (150) have been notified to UKBS. In 31 cases families reported a probable history of blood donation 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 20/31 vCJD cases reported by relatives as blood donors and for four additional cases with no reported donation history. Of the 24 found, 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 for a variety of reasons.

The search for donor records was negative in 11/31 (35%) vCJD cases reported as putative donors, three of whom allegedly donated well before the onset of the BSE epidemic in the 1980s. In most of these cases the information provided 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 and no explanation has been found for the lack of records, although discrepancies in some of the details suggest that the history was not as certain as initially thought.

5.1. Labile components issued to hospitals

Over the period 1981–2004, 66 labile components originating from 18 donors were issued to UK hospitals 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. 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 which had been leucocyte-depleted by pre-storage filtration to<5 × 10^6 leucocytes per unit (in 99% of units with 95% statistical confidence according to UK guidelines) after the introduction of universal leucocyte depletion of the UK blood supply in 1999.

5.2. 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 1st January 1980 as a vCJD risk reduction measure. As would be expected in a recipient population, 41 (62%) recipients were aged over 60 years at the time of transfusion and were not eligible to donate under the donor selection guidelines in place before 2004. All living recipients (N=24) 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 sub-clinical infection. All three of these recipients have died. Figs. 1,2 show the survival period for dead (transfusion to death) and live recipients (transfusion to 2006) of vCJD components, respectively, according to the interval between transfusion and onset of clinical symptoms in the donor.

5.3. Dead recipients

Forty-two recipients are known to be dead: mean age at death 66±19 years. Around half (N=21) of these recipients died within a year of receiving their transfusion, with only seven surviving for more than 5 years. Two recipients, who died 4 and 11 months after transfusion, had "Dementia" recorded on the death certificate, but neither case had features to suggest vCJD. All other recipients were certified as dying of causes unrelated to vCJD, except for the two cases who are now known to represent probable transfusion transmission of vCJD. In the first case, the cause of death was recorded as "1A Dementia and II. Prostate Cancer". This person had received a non-leucodepleted red cell component from a donor later con-

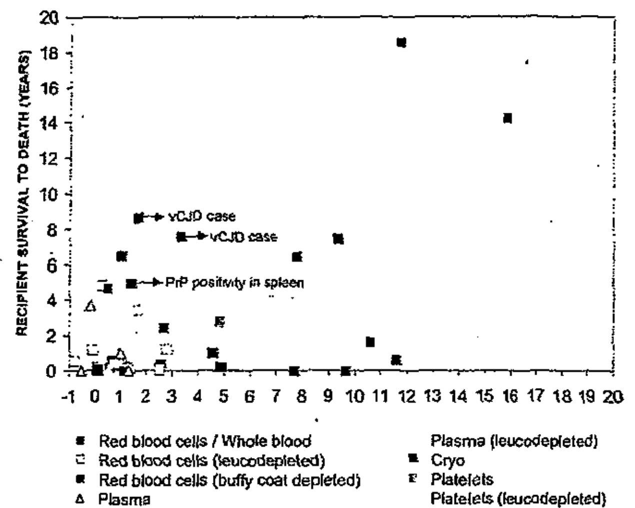

Fig. 1. Survival period (transfusion to death) for recipients of vCJD components according to interval between blood donation and onset of clinical symptoms in the donor.

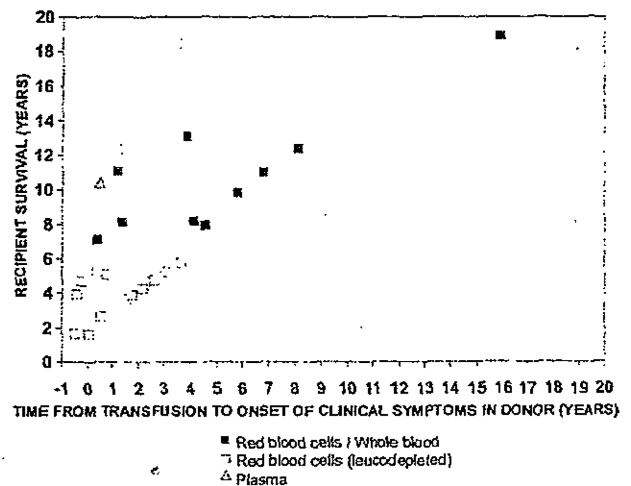

Fig. 2. Current survival period (since transfusion to June 2006) for recipients of vCJD components according to interval between blood donation and onset of clinical symptoms in the donor.

firmed neuropathologically as suffering from vCJD [2]. The recipient received the transfusion of red cells 61/2 years before onset of clinical symptoms, and was a methionine homozygote at codon 129 of the human prion protein gene (*PRNP*). The donor to this individual also had a neuropathological diagnosis of vCJD, with clinical onset approximately 40 months after donating. A second red cell recipient received non-leucodepleted red cells from a different donor who developed clinical symptoms approximately 18 months after donating and was later diagnosed with neuropathologically confirmed vCJD. This recipient died 5 years after transfusion without any clinical symptoms of vCJD, and was found to have protease-resistant prion protein (PrP^{res}) in the spleen and one lymph node (but not in the brain) at post mortem [3]. The recipient was a codon 129 *PRNP* heterozygote and the case is thought to

represent pre- or sub-clinical infection. The third case of probable transmission of vCJD infection by blood transfusion involved a recipient who received a transfusion of red cells 7 years and 10 months before onset of clinical symptoms [4] of vCJD. The recipient had received a large number of donor exposures for a major bleeding episode during surgery, but one of the red cell donors developed vCJD approximately 21 months after the donation, and the recipient was a codon 129 *PRNP* methonine homozygote.

5.4. Live recipients

Twenty-four recipients (%) are alive: mean age of 63 ± 19 years. Half of the live recipients were transfused with components from donors whose donations were made within 20 months of clinical onset of vCJD, in some cases around the time of development of the first signs of clinical illness. These donors would have appeared healthy and passed the normal medical checks as being fit to donate. Sixteen recipients have survived longer than 5 years, with six surviving > 10 years. These patients, mean age currently 61±19 years, were given blood from donors who developed vCJD symptoms at intervals ranging from around 5 to 191 months after making the donation.

5.5. Plasma for UK fractionation

Twenty-five units of plasma originating from 11 different donors, bled between 6 months and 18 years 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, is still being established. The fate of batches of products has not been traced to individual recipients as part of this study. It is known, however, that Hemophilia Centers 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 Hemophilia in the UK.

6. vCJD cases with history of transfusion

Eleven vCJD cases were reported to have received past blood transfusions between 1962 and 1999. A further case, excluded from further analysis, received a blood transfusion after onset of illness. 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 181 donors (169 identified), ranging from 2 to 103 per case. The latter case (103 donor exposures) also received a solid organ transplant and another case (the third case of probable transmission), received 59 donor exposures. To date, two donors 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). No additional linkages between recipients and donors were identified through investigation of the cases in blood recipients, and no infected donor has been identified in the remaining five cases.

7. Discussion

This study has identified three instances in which a recipient of a transfusion derived from a 'vCJD' donor have developed infection with vCJD, including two clinical cases and one pre- or sub-clinical infection [2–4]. 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 per million per 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 deceased recipients; a significant proportion of these individuals may not have survived long enough to express clinical disease even if infected. 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, 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 129 of PRNP, but the individual with 'pre-clinical' transfusion transmitted infection was heterozygous at this locus [3], indicating that individuals with this genotype are susceptible to secondary infection with vCJD. Except for the three cases infected through blood transfusion, the codon 129 genotypes of the recipient population are not known. Risk may vary according to genetic background, but it cannot be assumed that some recipients will possess an absolute genetic barrier to infection.

The analysis of vCJD cases with a history of blood transfusion has identified over 100 donors to these cases, although the

great majority were linked to only two vCJD cases. A risk assessment has suggested that these donors are themselves at significant risk of developing vCJD and these individuals have been informed of this risk and have been advised not to act as blood or organ donors. To date none of these individuals have developed vCJD, with the exception of the two donors already linked to the two clinical cases of vCJD described above.

Plasma derived from vCJD cases has been used in the production of plasma derived products, including clotting factors and immunoglobulin. To date, there is no evidence that vCJD has developed in a recipient of these products. A risk assessment carried out in the UK suggested that, on worst case assumptions, some plasma products could be associated with an additional risk of developing vCJD in relation to the background population risk through exposure to BSE and since 1999 plasma for the production of plasma products has been imported to the UK from other countries. The risks from plasma products are probably much lower than the risks from transfusion of labile blood components.

The TMER study has provided evidence that vCJD is transmissible through blood transfusion. The implications for public health have led to measures to minimize the risk from blood transfusion [5] and plasma products derived from cases incubating vCJD and many of these actions were taken years in advance of the evidence for transfusion transmission, both in the UK and many other countries. Although there is uncertainty about the potential for transfusion transmission of vCJD to lead to a self-sustaining epidemic, the introduction of a policy of deferring transfusion recipients as blood donors in the UK has minimized this possibility. The identification of vCJD cases with a history of blood donation in France, Ireland, Spain and Saudi Arabia indicates that this issue has an international dimension. Although the population prevalence of vCJD infection is almost certainly highest in the UK, the identification of secondary transmission of vCJD underlines the importance of international surveillance systems both for human and animal prion diseases.

Acknowledgements

This paper is based on a full review of the TMER study, which has been published in Vox Sanguinis [6]. I am grateful to my colleagues in the TMER study: Professor Robert Will and Ms. Jan MacKenzie (NCDSU) and Dr. Charlotte Llewelyn (National Blood Service), and to our colleagues at the NCJDSU for their support for this project. The study would not have been possible without the collaboration of the National Blood Service (NHS Blood and Transplant), Scottish National Blood Transfusion Service, the Welsh Blood Service and the Northern Ireland Blood Transfusion Service; also the relatives of CJD cases who provided information, and the many clinicians and other staff at UK hospitals and UKBS who have helped to trace records. I thank Dr. Nicky Connor and Dr. Anna Molesworth of the HPA for the data on plasma product batches. The study was funded by the NBS and the Department of Health.

References

[1] Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, et al. Transmission of prion diseases by blood transfusion. J Gen Virol 2002;83: 2897–905.

[2] Llewelyn CA, Hewitt PA, Knight RSG, Amar K, Cousens S, Mackenzie J, et al. Possible transmission of variant Creutzfeldt–Jakob disease by blood transfusion. Lancet 2004;363:417–21.

[3] Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 2004;364:527–9.

[4] Health Protection Agency. New case of transfusion-associated variant-CJD. CDR Weekly 2006;16(6):1.

[5] Ludlam CA, Turner ML. Managing the risk of transmission of variant Creutzfeldt-Jakob disease by blood products. Br J Haematol 2005;132: 13–24.

[6] Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sang 2006;91:221–30.

別紙 3-4

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一般的名称			研究報告の公表状況	Detection of miss-folded prion protein in blood with conformationally sensitive peptides. Pan, T. et al., Transfusion, 47, 1418-1425 (2007).	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
折りたたみ異常プリオン蛋白（PrP^{TSE}）の存在下においてαヘリックスからβシートへの構造の配座変換を行う、高度に保存されたプリオン蛋白（PrP）のN末端領域に由来する小規模なフルオロフォア標識ペプチドの使用について報告した。これは、ペプチドモノマーと凝集ポリマーの区別を放射蛍光波長を変化により可能とする。また、配座の変化は他のペプチド分子に伝播され、それによってシグナル増幅が起こる。著者らは、この PrP^{TSE} の診断的（MDP）アッセイは脳ホモジネート及び伝達性海綿状脳症（TSE）感染マウスの血漿プール内に存在する PrP^{TSE} を特異的に検出可能であるが、正常なマウス又はPrPノックアウトマウスの検体を検討した時にはシグナルは得られなかったことを示している。これらの結果はウェスタンブロット法に完全に一致する。感度閾値は約 1 感染用量／mL と推定される。血漿又は血清に対して適用した場合には、MPD アッセイは未感染の対照と比較して、種々の実験 TSE 感染の検体と自然 TSE 感染の検体を区別した。したがって、本アッセイは、ヒト及び動物の双方においてプリオン病の前臨床診断及び臨床診断にあたり有用であると考えられる。	BYL-2007-0291

報告企業の意見	今後の対応
ここで説明されている折りたたみ異常蛋白質の診断的（MPD）アッセイは PrP の立体配座の変化を検出する非常に有用なアッセイである。著者らは散発性クロイツフェルト・ヤコブ病患者の血漿検体を試験し、その検体が最高で20年以上も凍結されていたものであるにもかかわらず、対象となる健康者からの血漿検体と比べ統計学的有意差が認められた。本試験は将来非常に有用となり得る。	現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報、特にスケールアップした際の検出可能性についての収集に努める。

Detection of misfolded prion protein in blood with conformationally sensitive peptides

Tao Pan, Jasmeet Sethi, Craig Nelsen, Alan Rudolph, Larisa Cervenakova, Paul Brown, and Cindy S. Orser

BACKGROUND: The long-standing goal of a preclinical diagnostic test for transmissible spongiform encephalopathy (TSE) has recently become urgent because of the discovery that humans with variant Creutzfeldt-Jakob disease can transmit disease via blood transfusions.

STUDY DESIGN AND METHODS: The misfolded protein diagnostic (MPD) assay employs a pyrene-labeled palindromic sequence of prion peptides that undergoes a cascade of coil to β-sheet conversion in the presence of the misfolded prion protein (PrP^{TSE}). The ability of the assay to detect PrP^{TSE} in brain, serum, and plasma was tested. The basic protocol involved a several-hour incubation of 200-μL sample volumes with the peptide reagent in 96-well plates, after which fluorescence was monitored by a fluorescence plate reader with an excitation wavelength of 350 nm and emission scanning wavelength range of 365 to 600 nm.

RESULTS: Target specificity for PrP^{TSE} was documented by correlation of assay signal with Western blot signals in brain tissue from TSE-infected, normal, and knockout mice and negative assay signals by use of reagents with different peptide sequences. When applied to plasma or serum, the assay discriminated between samples from a variety of experimental and natural TSE infections compared to uninfected controls, with a sensitivity threshold of approximately 1 infectious dose per mL in pooled plasma from TSE-infected mice.

CONCLUSIONS: The MPD assay is a sensitive and specific test for the detection of PrP^{TSE} that may be useful in both preclinical and clinical diagnosis of TSE diseases of animals and humans.

Prions are normal host proteins that may also exist in a misfolded proteinase-resistant conformation (PrP^{TSE}) responsible for disease in animals and humans.[1] The considerable public health consequence of these diseases, which include bovine spongiform encephalopathy, sheep scrapie, chronic wasting disease in deer and elk, and Creutzfeldt-Jakob disease in humans, has led to significant efforts to develop methods of preclinical detection. The transmissibility of transmissible spongiform encephalopathy (TSE) via blood has been established in animal models for Gerstmann-Sträussler-Scheinker (GSS) disease and variant Creutzfeldt-Jakob disease (vCJD) in mice,[2-5] scrapie in hamsters,[6] and bovine spongiform encephalopathy in sheep.[7,8] Blood is also infectious in endemic sheep scrapie[9] and in human infections with vCJD.[10-12]

There are several challenges to detecting PrP^{TSE} in blood. The level of PrP^{TSE}, especially early in disease, is below the detection limits of standard immunoassay methods, and the coexistence of a large amount of normal cellular form of the protein (PrP^{C}) compromises detection

ABBREVIATIONS: CTI = C-terminal inversion; GSS = Gerstmann-Sträussler-Scheinker; ID(s) = infectious dose(s); MPD = misfolded protein diagnostic; PrP = prion protein; PrP^{C} = cellular form of prion protein; PrP/KO mouse = PrP knockout mouse; PrP^{TSE} = misfolded form of prion protein; vCJD = variant Creutzfeldt-Jakob disease.

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specificity without the use of proteinase enzyme to digest interfering species. PrP^{TSE} does not elicit a humoral immune response in the infected host, preventing detection of a serologic antibody.[13,14] The absence of a foreign nucleic acid component in the infectious particle eliminates the use of PCR; however, immuno-polymerase chain reaction has been used to detect both recombinant PrP and PrP^{TSE} from scrapie-infected brain homogenate at a level 1 million-fold more sensitive than antibody-based detection methods, positioning immuno-polymerase chain reaction as capable of detecting PrP^{TSE} in the preclinical phase of disease in blood.[15]

These challenges have restricted diagnostic tests to the use of postmortem tissue and, in some cases, antemortem lymphoreticular tissue, but there is an urgent need for a preclinical screening test to prevent secondary transmission of vCJD disease from blood donation. We have developed a method of detecting PrP^{TSE} in blood that utilizes a conformationally sensitive pyrene-labeled peptide from the N-terminal region of PrP that contains conserved amino acid sequences[16] (Pronucleon ligands).

The structural features of the peptide have been previously reported, including circular dichroism studies documenting the thermodynamic conditions for its transition from a coiled structure to a β-sheet structure that occurs in the presence of PrP^{TSE}.[17] This conformational change induces an excimeric signal from the conjugated pyrenes when the conformational change to β-sheet brings the pyrene pair into close proximity. The conformational change is propagated to other peptide molecules, serving to amplify the signal for the presence of PrP^{TSE}. We have previously shown that these peptides can detect PrP^{TSE} in brain during the preclinical and clinical stages of disease in hamsters infected with the 263K strain of scrapie.[16] We here extended these studies to the detection of PrP^{TSE} in the blood of animals and humans with various forms of TSE.

MATERIALS AND METHODS

PrP^{TSE} peptides and reagents

The misfolded protein diagnostic (MPD) peptide, formerly referred to as 33-H1,[16] was used for the detection and amplification of PrP^{TSE} in all tissue and plasma samples in this report. Two additional peptides were also used to evaluate specificity of the reaction: a C-terminal inversion (CTI) and a randomly scrambled version of the MPD peptide The CTI peptide sequence was VVAGAAAAGAVHKLVVAGAAAAGAVHKLNTKPK, and the scrambled sequence was LKVAHVAPAGGAKGGAHLAAVAVKAATKVAAVNK. All peptides had pyrene conjugated to both ends. The peptides were synthesized with solid-support FMOC synthesis methods and provided as a desiccated powder with greater than 95 percent purity as determined by amino acid sequence and mass spectroscopy (New England Peptide, Gardner, MA). The peptides were aliquoted from a dry powder and stored in dimethyl sulfoxide (or hexafluoroisopropanol) at –80°C as a 1 mg per mL stock reagent until use in the PrP^{TSE} assay.

The stock peptide reagent was diluted to a 2 μmol per L solution in 50 percent trifluoroethanol (Sigma Aldrich, St Louis, MO), 50 percent 10 mmol per L Tris, pH 7.4, and allowed to equilibrate for 1 hour at room temperature before the addition of the test sample. The trifluoroethanol and peptide concentrations were previously optimized to provide the maximum separation between background peptide misfolding and self-aggregation and substrate-induced conformational change in the peptide as well as to minimize fluorescence quenching.[17] Test samples were either used as such or partially purified with methods described for a given experiment. Twenty-microliter sample volumes were added to 180 μL of the equilibrated peptide solution. Reactions were allowed to incubate at ambient temperature with fluorescence readings taken at 1, 3, and 5 hours. A sample containing only the peptide was included in each test run as a control on peptide stability and self-induced conformational change during the incubation. For the standardized MPD reaction, the signal of the peptide only control reaction was subtracted from the signals of test sample reactions. Reactions were monitored in 96-well plates in a fluorescent plate reader (Safire II, Tecan, Mannedorf, Switzerland) with an excitation wavelength of 350 nm and emission scanning from 365 to 600 nm. The resulting PrP^{TSE} signals were analyzed as the natural log (ln) of the integrated excimeric fluorescence from 430 to 530 nm in all cases and for Fig. 4 as the ratio of pyrene $excimeric_{max}$ fluorescence to pyrene $monomeric_{max}$ fluorescence as well. Integrated peak values were from 370 to 385 nm and from 430 to 530 nm that correspond to monomer and excimer emission, respectively.

GSS murine model

Brains from a normal Swiss mouse, a PrP knockout (PrP/KO) mouse (gift of C. Weissman to L. Cervenakova) or a GSS (Fukuoka-1 strain)-infected mouse were homogenized in 10 percent phosphate-buffered saline (PBS). The brain homogenates were analyzed by Western blot according to previously published methods[5] and precipitated with phosphotungstic acid according to a previously published method for evaluation in the MPD assay.[16] For Western blot analysis, 10 μL of 1 percent GSS or 10 percent Swiss or PrP/KO brain homogenates were mixed with 10 μL of Laemmli sample buffer containing 5 percent β-mercaptoethanol, heated at 95°C for 10 minutes, and all loaded into 1-mm, 10-well, 4 to 12 percent Bis-Tris gel (Invitrogen, Carlsbad, CA), which after electrophoresis was transferred onto a 0.45-μm nitrocellulose membrane (Invitrogen).