Chapter 8
Risk of Transmission of Creutzfeldt–Jakob Disease by Blood Transfusion

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Abstract Early epidemiological studies on sporadic Creutzfeldt–Jakob disease did not identify blood transfusion as a risk factor for the disease. However, the emergence of variant Creutzfeldt–Jakob disease (vCJD) in 1996 and the identification of PrP^{Sc} in lymphoid tissues in this novel disorder led to concerns that transmission of infectivity by blood transfusion might be a possibility. These concerns were fully realised in 2004, when the first case of vCJD associated with transmission by blood transfusion was identified in a recipient who was a methionine homozygote at codon 129 in the prion protein gene, as in all other vCJD patients. Other similar cases have subsequently emerged, along with cases of asymptomatic vCJD infection in a blood transfusion recipient and a plasma product recipient, both of whom were heterozygous at codon 129 of the prion protein gene. This chapter reviews the experimental evidence for the transmission of prion infectivity by blood transfusion in a range of experimental models, discusses the evidence for the transmission of vCJD by blood transfusion and plasma products and considers the future possibilities for the development and potential uses of blood-based screening tests for human prion diseases.

Keywords Blood transfusion • Creutzfeldt–Jakob disease • Prion disease transmission • Prion protein in blood • vCJD

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8.1 Introduction

Despite several decades of research in many different countries, the cause of the commonest form of human prion disease, sporadic Creutzfeldt–Jakob disease (sCJD), remains unclear. sCJD appears to have been transmitted as an iatrogenic infection following a variety of medical and surgical procedures, but evidence to support infection via blood transfusion appears lacking to date. The emergence of variant Creutzfeldt–Jakob disease (vCJD) in the UK 15 years ago and subsequent evidence for the transmission of vCJD infectivity by the transfusion of non-leucodepleted red cell concentrates from donors who were asymptomatic at the time of donation, but who subsequently died from vCJD, have focused attention on the potential for transmission of other forms of CJD by this route (Puopolo et al. 2011).

In this chapter, we review the evidence for the transmission of prions by blood transfusion in experimental models of prion disease and in sCJD and vCJD in humans, describe recent and developing methods to detect prions in blood and discuss the prospects of a blood screening test for prions and the issues surrounding the implementation of such a test.

8.2 Experimental Evidence for Prion Disease Transmission by Blood Transfusion

8.2.1 Cellular Prion Protein in Blood

Expression of the cellular prion protein (PrP^C) is thought to be an absolute requirement for the development of prion infection. PrP^C is widely expressed in different tissues and cell types, including neurones in the central nervous system and follicular dendritic cells in lymphoreticular tissues. It is also present in blood, in which the distribution and cellular physiology of PrP^C has been intensively studied. PrP^C is present in plasma and is also found to be cell associated in human blood (MacGregor et al. 1999). Platelets contribute the greatest amount of cell-associated PrP^C to blood with lesser amounts contributed by white blood cells (WBC) and lower levels still by red blood cells (RBC) (MacGregor et al. 1999; Choi et al. 2009). The highest levels of PrP^C (on a per cell basis) in normal human blood are in specific WBC subpopulations (MacGregor et al. 1999; Durig et al. 2000; Choi et al. 2009). Platelets act as a dynamic reservoir for PrP^C in that it is stored in their α-granules, being recruited to the cell surface or released during platelet activation and storage (Perini et al. 1996; MacGregor et al. 1999; Bessos et al. 2001; Holada et al. 2002a). The activation-dependent upregulation of expression in, or release of PrP^C from leucocytes, dendritic cells, and mast cells, has been interpreted as indicative of the normal cellular functions for PrP^C in blood and suggestive of a role for these cells in prion disease pathogenesis (Durig et al. 2000; Burthem et al. 2001; Lee et al. 2001; Haddon et al. 2009). In so far as PrP^C expression and function in blood might relate

to prion disease pathogenesis, it should be noted that clear differences in PrP^C expression between different blood components are evident when human blood is compared with blood of species that are commonly used as models of prion disease, such as rodents and sheep (Barclay et al. 2002).

8.2.2 Animal Models

The study of human prion diseases, such as CJD, continues to be informed by analogous diseases of animals, specifically sheep scrapie and bovine spongiform encephalopathy (BSE) and the establishment of experimental animal models of those animal diseases and of the human diseases themselves. The adaptation of sheep scrapie isolates to rodents has been of fundamental significance to the field, providing a series of well-characterised meta-stable strains in both hamsters and mice, but the modelling of blood transfusion has recently been particularly well served by the development of an experimental blood transfusion paradigm using the BSE agent experimentally transmitted to sheep.

8.2.3 Rodent Models

Reports of the existence of a "viraemia" associated with prion disease have a long history and quite naturally these observations raised fears of transfusion-related transmission of CJD. Guinea pig-adapted CJD, serially transmitted by intracerebral (i.c.) inoculation was reported to have infectivity detectable throughout the incubation period in buffy coat samples, as determined by further i.c. challenge (Manuelidis et al. 1978). This finding was supported by a study using a different human prion disease, a mouse-adapted Gerstmann–Straussler–Scheinker disease isolate, termed Fukuoka-1. When challenged with Fukuoka-1 by the i.c route, mice showed detectable infectivity in circulating whole blood from around half way through the incubation period onwards, as determined by intraperitoneal (i.p.) challenge of further susceptible mice (Kuroda et al. 1983). Direct (but limited and poorly documented) testing of blood and buffy coat specimens from CJD patients also indicated the presence of infectivity in human blood during the clinical illness, when inoculated into guinea pigs and hamsters (Manuelidis et al. 1985; Deslys et al. 1994).

The further development of high titre, well-characterised rodent scrapie models has provided more consistent, reliable and perhaps more relevant data. A sustained low level of infectivity was found to characterise blood throughout the incubation period in the 263K hamster scrapie model, following i.p. inoculation (Diringer 1984; Casaccia et al. 1989). At the clinical stage, the infectivity was reported to be associated with the mononuclear leucocyte fraction and not with platelets (Holada et al. 2002b). The hamster 263K model has been used extensively in the development and evaluation of prion reduction filters (Gregori et al. 2004a, 2006a, b; Sowemimo-Coker et al. 2005, 2010) and to investigate partitioning during plasma

protein manufacture (Lee et al. 2000; Foster et al. 2000; Li et al. 2001; Gregori et al. 2004b; Hartwell et al. 2005; Burdick et al. 2006).

Similar results to those obtained with the 263K scrapie strain hamster model have also been obtained using the Fukuoka-1 mouse model. Following i.c. inoculation, blood was found to contain ~10 infectious units per ml (IU/ml) during the pre-clinical phase, rising to ~100 IU/ml during the clinical phase and largely associated with the buffy coat fraction, as measured by bioassay using the same (i.c.) route (Brown et al. 1998, 1999). Infectivity levels in plasma were found to be low and further reduced by plasma processing (Brown et al. 1998, 1999). When comparisons were made between the blood-borne infectivity levels in the Fukuoka-1 GSS model and RIII mouse-adapted vCJD, the latter was found to contain 20–30 ID/ml at both the pre-clinical and clinical phase, primarily in buffy coat and plasma, with lower levels in platelets and no infectivity detectable in red blood cells (Cervenakova et al. 2003).

These experiments demonstrated clear proof of principle of blood-borne prion infectivity and they also provided information on infectivity levels, on which risk assessments could be based. However, direct extrapolation to blood transfusion and the risk posed by vCJD is difficult due to the possible effects of route and agent/host interaction. Consequently, the use of large animal models offers distinct advantages over rodents where blood transfusion is concerned.

8.2.4 Primate Models

Early attempts to transmit human spongiform encephalopathy by transfusion of unit quantities of blood to chimpanzees were reported to be negative (Brown et al. 1994). Nevertheless, non-human primates experimentally infected with the BSE/vCJD agent have been used to model vCJD (Lasmezas et al. 2001, 2005 Herzog et al. 2005; Williams et al. 2007). Both brain and buffy coat from a clinically affected lemur (previously exposed by the i.c. route) were found to transmit disease when inoculated i.c. into naive lemurs (Bons et al. 2002). Conversely, brain tissue from clinically affected macaques (previously exposed by the i.c. route) was shown to transmit disease when further macaques were exposed orally or intravenously (Herzog et al. 2004). Reported use of primate models to directly mimic transfusion practice has been surprisingly limited in scope and has recently been complicated by the finding of a novel myelopathic syndrome in macaques exposed intravenously (i.v.) to blood components from vCJD infected macaques and from a vCJD patient (Comoy et al. 2012; Lescoutra-Etchegaray et al. 2012).

8.2.5 Sheep Models

To date, only in sheep models have relevant agents (principally BSE) been used to infect animals using the relevant route (orally, to model zoonotic transmission to humans)

to produce donors of blood (at clinical and pre-clinical time points) that can be used to transfuse recipients, using protocols that closely mimic human transfusion practice. The report of one successful transmission by intravenous administration of a unit of whole blood from a pre-clinical BSE orally exposed donor sheep to a naive recipient (Houston et al. 2000, and see an accompanying commentary by Brown 2000) was confirmed and has been fully justified by subsequent publications describing the whole study (Hunter et al. 2002; Siso et al. 2006; Houston et al. 2008; McCutcheon et al. 2011). The overall BSE transfusion transmission rate was 36% and included blood from donors throughout the second half of the (asymptomatic) incubation period, suggesting that either the titre of the infectious agent in blood is higher than anticipated or that transfusion of blood is a very efficient mode of transmission (Houston et al. 2008). Using the same experimental paradigm, components separated from orally exposed pre-clinical BSE sheep blood have shown infectivity to be present in red cell concentrates, plasma and platelet units, even when the blood has first been leucoreduced (McCutcheon et al. 2011). Interestingly, efficient transfusion transmission is not a property restricted to the BSE agent. Similar transmission rates (43%) were seen in parallel experiments conducted using clinical and pre-clinical sheep scrapie (Houston et al. 2008). The neuropathological phenotype of experimental ovine BSE is largely unaffected by route (Siso et al. 2006), whereas that of scrapie appears to differ between natural infection and transfusion transmission (Siso et al. 2009). The efficiency of transfusion mediated transmission has been further explored using a different sheep scrapie model system in which transfusion-mediated transmission rates approach 100% (Andreoletti et al. 2012; Lacroux et al. 2012). The results using this model system demonstrate a marked discrepancy between prion titres in sheep blood as defined by i.c. challenge of susceptible (ovinised) transgenic mice and the efficiency of disease transmission following intravenous transfusion of viable cells between sheep. This may not be surprising from a biological perspective, but it does provide an important caveat for calculations previously based on blood infectivity measurements obtained by i.c. inoculation of rodents (Andreoletti et al. 2012).

Each of the above rodent, primate and sheep experimental systems is at one or more removes from the events they seek to model, and not all of the evidence accumulated to date, such as the kinetics of accumulation or cell types involved, is entirely consistent. Titre is a key case in point. Rodent studies have previously supported an estimate of 10IU/ml of blood, whereas this has now been revised downwards to less than 1IU/unit of blood (~400ml) based on examination of existing ovine and human data (Gregori et al. 2011). However, when taken together three conclusions can be drawn: first that low levels of infectivity in blood occur during the pre-clinical phase in these acquired prion diseases. Second, that some of this infectivity is cell associated, and third, that intravenous delivery, especially the transfusion of fresh blood and its components is an efficient mode of prion disease transmission.

8.3 Evidence for vCJD Transmission by Blood Transfusion and Plasma

8.3.1 Secondary Transmission of vCJD by Blood Transfusion

There have been four known cases of vCJD in recipients of blood components from asymptomatic donors who subsequently developed vCJD (Llewelyn et al. 2004; Peden et al. 2004; Wroe et al. 2006; Health Protection Agency 2007) and a fifth case in which only circumstantial evidence implicates blood transfusion as the cause (Chohan et al. 2010). These individuals were all members of a cohort identified by the Transfusion Medicine Epidemiology Review (TMER), a collaboration of the National CJD Research & Surveillance Unit (NCJDRSU) and the UK Blood Services (Hewitt et al. 2006; http://www.cjd.ed.ac.uk/TMER/TMER.htm). Figure 8.1 summarises information on the time of the relevant transfusions and the

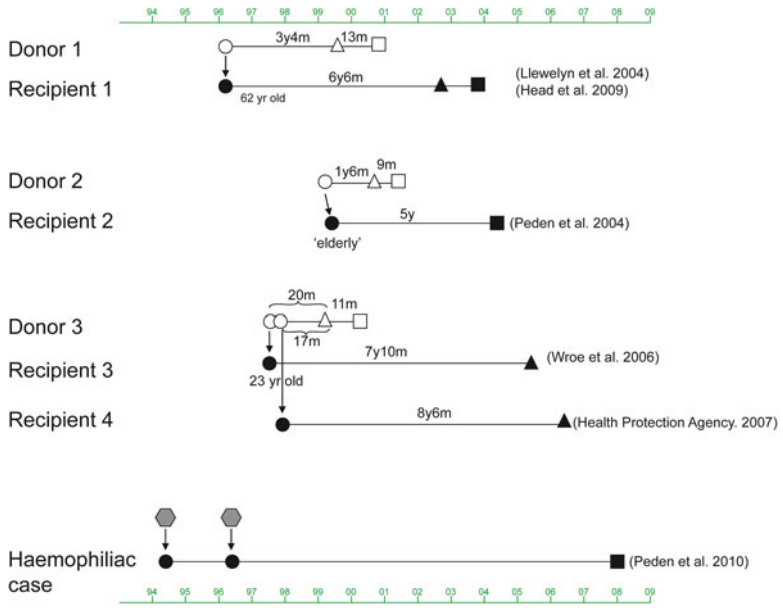

Fig. 8.1 Time lines for the donors and recipients of blood in the four known cases of blood transfusion-associated vCJD infection. The interval between donation/transfusion (*circles*) and death (*square*) or vCJD disease onset (*triangles*) are represented by *lines* drawn to a scale indicated at the *top* and *bottom* of the figure. The donations/transfusions are indicated by *open symbols* for the blood donors and *filled symbols* for the recipients. The recipients' ages at the time of transfusion (where published) are shown. The asymptomatic haemophiliac patient that showed evidence of vCJD infection in the spleen had been a recipient of two identified vCJD-implicated batches of Factor VIII (*indicated by hexagons*). Key references are shown on the *right*; the data are also reviewed in Hewitt et al. (2006). Recipient 2 and the haemophiliac patient died of non-neurological disorders and recipients 3 and 4 shared a common donor

deaths or onsets of vCJD in both the donors and the recipients. In all four cases, secondary vCJD infection in the recipient appears to have resulted from the transfusion of a single unit of non-leucodepleted red cells from a pre-clinical vCJD donor. These transfusions occurred prior to the phasing in of leucodepletion of all blood for transfusion in the UK during 1998–1999; to date there have been no secondary vCJD cases in patients receiving leucodepleted blood.

The clinical reports of recipients 1, 3 and 4 were typical for vCJD and genotype analysis showed they were all methionine homozygous (M/M) at codon 129 of the prion protein gene *PRNP*. All neuropathologically confirmed clinical cases of vCJD to date have also been homozygous for methionine. The neuropathological findings for recipients 1 and 3 were typical for vCJD (Head et al. 2009; Wroe et al. 2006) (Fig. 8.2a–d). In both of these recipients, Western blotting analysis of brain homogenate following treatment with proteinase K revealed the presence of disease-associated protease-resistant PrP (PrP^{res}) with a banding pattern of type 2B, characteristic of vCJD.

Mice inoculated with cerebral frontal cortex samples from recipient 1 became infected with incubation times and brain lesion profiles that were consistent with previous transmissions of vCJD to mice of the same lines, suggesting that there had been no alteration of agent strain (Bishop et al. 2008). Therefore, in *PRNP* codon 129M/M individuals, the strain properties and clinicopathological features of secondary vCJD following blood transfusion are currently indistinguishable from those in patients with vCJD resulting from exposure to BSE.

The second case of blood transfusion-associated vCJD infection differed from the other three in that the transfused recipient (recipient 2) died 5 years after transfusion from a non-neurological disorder and was methionine/valine (M/V) heterozygous at *PRNP* codon 129 (Fig. 8.1) (Peden et al. 2004). Evidence for vCJD infection in this recipient was obtained when autopsy tissues were examined for the presence of PrP^{Sc} by sodium phosphotungstate precipitation/Western blotting (NaPTA/WB) (Fig. 8.2g), paraffin embedded tissue blotting (PET) and immunohistochemistry (IHC). PrP^{res} was found to be restricted to the spleen (NaPTA/WB, PET and IHC) and a cervical lymph node (IHC). The PrP^{res} banding pattern in spleen was type 2B. No pathological signs of vCJD were detected in the central nervous system. Recipient 2 thus provided the first evidence that *PRNP* codon 129M/V individuals might be either susceptible to vCJD or capable of incubating this disease.

PrP^{res} was not detected in tonsil tissue from recipient 2 (Peden et al. 2004). This finding highlights a potential caveat in the use of resected tonsil for estimating the prevalence of vCJD in the population and the use of tonsil biopsies for the pre-mortem diagnosis of secondary vCJD. Interestingly, PrP^{res} was detected in tonsil tissue taken at autopsy from recipient 3, but a pre-mortem tonsil biopsy had not been performed on this recipient (Wroe et al. 2006).

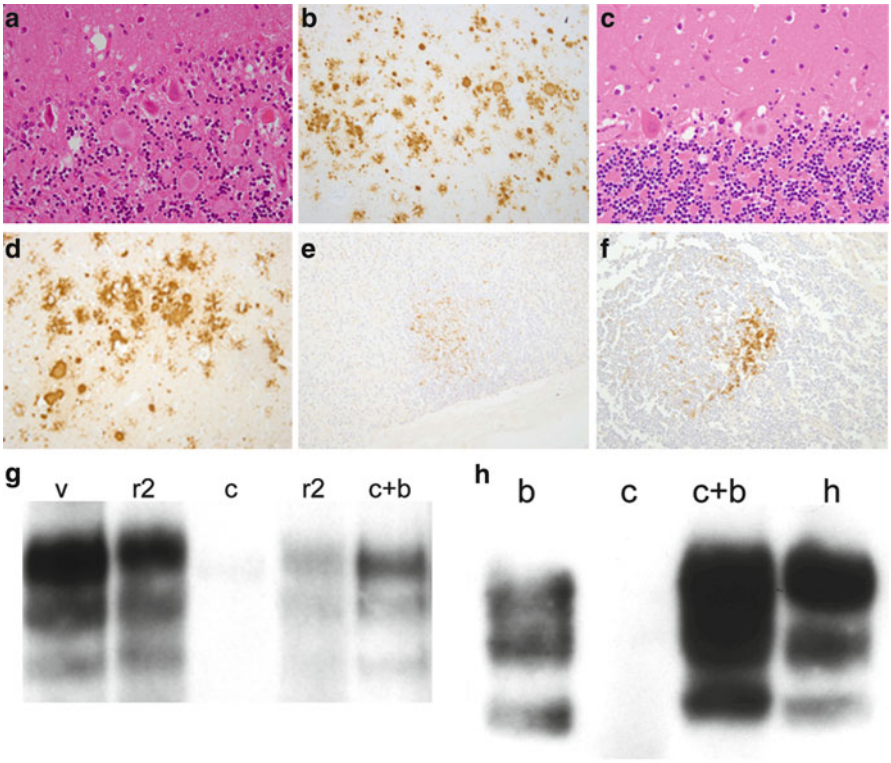

Fig. 8.2 Pathology and biochemistry of autopsy tissues from symptomatic and asymptomatic vCJD infected individuals following blood transfusion and plasma product administration. Haematoxylin and eosin stained sections of the cerebellum from blood donor 1 (A) and the corresponding blood transfusion recipient 1 (C) show spongiform change and florid plaques. Corresponding immunohistochemistry for PrP in sections of cerebral cortex (B for donor 1 and D for recipient 1) shows florid plaques, cluster plaques and other deposits of disease-associated PrP. E and F show PrP-labelling of germinal centres of the spleen (E) and the cervical lymph node (F) from the asymptomatic blood transfusion recipient (recipient 2). Panels G and H show the presence of protease resistant PrP by NaPTA/WB analysis in spleen from blood transfusion recipient 2 (marked r2) and the case of asymptomatic vCJD infection in a plasma product recipient with haemophilia (marked h). These samples have been run alongside spleen from a clinical case of vCJD ('v'), non-CJD control spleen ('c') and vCJD brain homogenate alone ('b') or spiked into control spleen ('c + b') for comparison

8.3.2 Evidence for vCJD Transmission by Plasma Products

There has been one case of vCJD infection detected at autopsy in a patient who had been treated with large doses of UK-produced Factor VIII (Peden et al. 2010). The patient was a haemophiliac who died of a non-neurological disorder in 2008, aged 73. This patient was heterozygous (M/V) at *PRNP* codon 129. PrP^{res} was detected by NaPTA/WB in only one sample of spleen with a banding pattern of type 2B (Fig. 8.2h). All other tissues tested from this patient, including brain and tonsil, were negative.

This case of vCJD infection was identified through a United Kingdom (UK) Department of Health funded study to undertake active surveillance of haemophiliac patients for vCJD infection. All haemophiliacs undergoing surgery on tissues from the central nervous system and lymphoid tissues were invited to participate and give consent for analysis of tissue samples at NCJDRSU for PrP^{res}. In addition, consent was sought for the analysis of samples from autopsy tissues from relatives of patients who died during this study. A variable range of biopsy and autopsy specimens from 17 patients have been analysed by NaPTA/WB, PET and IHC. All tissues tested negative for PrP^{res} apart from one spleen sample from the patient described above (Peden et al. 2010).

A number of possibilities have been considered to explain how this haemophiliac patient became infected with vCJD. Prior to 1998 in the UK, blood products such as Factor VIII and Factor IX were manufactured from blood plasma sourced in the UK. Units of blood from asymptomatic donors, who went onto develop vCJD, contributed to pooled plasma for the manufacture of batches of clotting factor concentrates (Hewitt et al. 2006). The patient described above had been treated with two of these "vCJD-implicated" batches of Factor VIII, totalling 9,025 units, in 1994 and 1996. However, this person's medical history also included treatment with approximately 400,000 units of non-implicated Factor VIII between 1980 and 2001, four blood transfusions and multiple endoscopic procedures. An assessment of all risk factors, including dietary exposure to BSE, concluded the most likely route of exposure for this patient was non-implicated batches of Factor VIII (Bennett and Ball 2009). This conclusion was based on (1) the large number of units of Factor VIII received by this patient, (2) an estimated prevalence of vCJD in the UK population of 1/10,000 (Spongiform Encephalopathy Advisory Committee 2008) and (3) the routine pooling of around 20,000 units of plasma to make a single batch of clotting factor concentrate (Clarke and Ghani 2005; Clewley et al. 2009; Hilton et al. 2004).

8.4 Methods to Detect Prions in Blood and the Prospect of Implementation of a Blood Screening Test for vCJD

8.4.1 The Challenge

The development of a workable blood screening test for vCJD faces a series of formidable obstacles. Some of these are biochemical in nature: if prions are equated with abnormal forms of the prion protein (PrP^{Sc}), then a prospective blood test must be able to detect extremely low levels of PrP^{Sc} in the analyte (whole blood, plasma or buffy coat), in which the normal precursor protein, PrP^{C} is more abundant by orders of magnitude. The property of PrP^{Sc} being measured must be unique to the disease-associated or infected state. Whilst brain PrP^{C} and PrP^{Sc} are well characterised, both PrP^{C} and PrP^{Sc} are now recognised as being biochemically heterogeneous with protease-resistant forms of PrP^{C} being found in normal brain and protease-sensitive

forms of PrP^{Sc} being found in CJD brain (Safar et al. 2005; Yuan et al. 2006). Moreover, the exact biochemical form of PrP^{Sc} in blood is unknown. This may result in a practical problem for test development, in that an assay developed with, and optimised for brain PrP^{Sc}, even if spiked into blood or plasma at high dilution, may not be applicable for the detection of endogenous blood PrP^{Sc}. Blood from analogous animal diseases or animal models may therefore appear an attractive option, especially since blood from pre-clinical stages can be taken to mimic screening for asymptomatic vCJD infection, but translation may be complicated by differences in the prion strain and host species involved. Given all of these difficulties, a framework for CJD blood test evaluation has been developed by the UK National Institute for Biological Standards and Control (http://www.nibsc.ac.uk/spotlight/cjd_resource_centre/cjd_tests.aspx).

Implementation presents a further series of challenges: the actual prevalence of vCJD infection in the UK population can only be estimated with very wide confidence intervals (Hilton et al. 2004; Clewley et al. 2009; de Marco et al. 2010; Garske and Ghaini 2010) but the most recent prevalence estimate is of 1:2,000 based on retrospective screening of archived tonsil specimens in England (Health Protection Agency 2012). A routine blood screening test with an exceptionally high specificity, if applied routinely to all blood donations, would still generate significant numbers of false positives (Turner 2006; Ludlam and Turner 2006; Peden et al. 2008). One way to mitigate the effects of these unavoidable false positive screening test results (for donors and for the transfusion services alike) would be to implement a second (confirmatory) assay in parallel with a screening assay. Therefore two assays are actually being sought. Ideally the screening assay and confirmatory test would work by different principles, and only one (the screening assay) would need to be high throughput and rapid.

8.4.2 *Approaches to Sensitive Detection of PrP^{Sc}*

A wide variety of approaches have been taken to the development of blood tests for vCJD. We have reviewed these recently (Peden et al. 2008) and a detailed description is beyond the scope of this chapter. In general, they involve a step that distinguishes PrP^{C} and PrP^{Sc}, followed by a sensitive end detection method. Despite considerable scientific and commercial interest none of these conventional approaches have, as yet, delivered a prototype assay for the detection of PrP^{Sc} in human blood.

8.4.3 *PrP^{Sc} Amplification and Current Blood Test Development*

Prion disease pathogenesis is thought to depend on the autocatalytic conversion of PrP^{C} by PrP^{Sc}. Using an in vitro cell-free system to model this process could

effectively amplify PrP^{Sc} from sub-detectable levels to levels readily detectable by conventional means. Capitalising on earlier work by Byron Caughey and co-workers (Kocisko et al. 1994; Caughey et al. 1999), Claudio Soto and colleagues developed a method termed protein misfolding cyclic amplification (PMCA) in which a "seed" of PrP^{Sc} promotes the conversion of PrP^{C} "substrate" supplied by an appropriate (usually brain) tissue homogenate. Accelerated by cycles of sonication and incubation, the amplified PrP^{Sc} product is then detected by protease digestion and Western blotting (Saborio et al. 2001). The sensitivity of detection can be further enhanced by using the product from one PMCA reaction to seed further rounds in a process termed serial PMCA or sPMCA (Bieschke et al. 2004; Castilla et al. 2005). Working with the experimental hamster 263K scrapie model, serial PMCA has been able to distinguish between bloods from infected and uninfected hamsters at the clinical phase (Castilla et al. 2005) and during the asymptomatic preclinical phase (Saa et al. 2006). This general PMCA methodology has been adopted by numerous researchers and has been further developed towards basic science (Deleault et al. 2007), medical (Jones et al. 2007) and veterinary (Thorne and Terry 2008) applications. Blood or plasma appears to require the introduction of additional preparative steps in part to avoid inhibition of the amplification reaction by plasma constituents (Castilla et al. 2005; Saa et al. 2006; Thorne and Terry 2008). Our own approach (in collaboration with the Scottish National Blood Transfusion Service) has been to collect PrP^{Sc} from plasma, perform serial PMCA using out-dated human platelet extracts as substrate, followed by detection using conformation-dependent immunoassay (Jones et al. 2009). Other configurations of the PMCA methodology aimed at human blood testing also appear promising (Tattum et al. 2010).

A third generation amplification method termed QuIC has been described in which recombinant PrP replaces natural PrP^{C} substrates, periodic shaking replaces sonication, and (in the real-time variant, RT-QuIC), amyloid formation is monitored in real time by thioflavin T fluorescence (Atarashi et al. 2007; Atarashi et al. 2008). RT-QuIC is already under evaluation as a clinical diagnostic using cerebrospinal fluid from suspected cases of sporadic CJD (Atarashi et al. 2011; McGuire et al. 2012). Problems with relatively inefficient detection of vCJD brain and CSF samples (Peden et al. 2012) and with inhibitors of QuIC in plasma appear to have been overcome by a further modification of the methodology (termed e-QuIC) that incorporates PrP^{Sc}-specific antibody immunoprecipitation, a chimeric recombinant PrP substrate and a reaction buffer replacement step (Orru et al. 2011). e-QuIC is reported to be able to detect a 10^{14}-fold dilution of vCJD brain or 2 ag/ml of vCJD PrP^{res} making it the most sensitive assay yet reported as judged by limit of detection (LoD) of human CJD brain. However, the method has not yet been tested on clinical vCJD blood specimens and relevant controls. A prospective blood test with a somewhat higher LoD has been tested using whole blood from clinical vCJD patients ($n=21$) against 142 blood specimens from donors ($n=100$) and neurological controls ($n=42$) giving sensitivity and specificities of 71.4% and 100%, respectively (Edgeworth et al. 2011). The novelty and biochemical point of interest of this assay is the use of stainless steel particles to concentrate, modify or present PrP in advance of a sensitive immunoassay. The assay failed to detect PrP^{Sc} in blood taken from sCJD patients ($n=27$).

Table 8.1 Measures to reduce the risk of transmission of vCJD taken by UK blood services

Withdrawal and recall of blood components, plasma derivatives, cells or tissues obtained from any individual who later develops variant CJD (1997).
Importation of plasma from countries other than the UK for fractionation to manufacture plasma derivatives (1999).
Leucodepletion of all blood components (1999).
Importation of clinical fresh frozen plasma for patients born after January 1996 (2004). Extended to all patients under the age of 16 by 2005.
Exclusion of whole blood and apheresis donors who may have received a blood component transfusion in the UK since 1980, any donors who have been treated with UK plasma derived intravenous immunoglobulin or have undergone plasma exchange (2004). Extended in November 2005 to transfusions anywhere in the world.
Exclusion of blood donors whose blood has been transfused to recipients who later developed vCJD, where blood transfusion cannot be excluded as a source of the vCJD infection and where no infected donor has been identified (2005).
Promotion of appropriate use of blood and tissues products and alternatives throughout the NHS.

8.4.4 Future Perspectives

The above assays, in addition to a further blood test under development by Prionics AG, all have a considerable distance to go before they could be considered validated as vCJD blood screening tests. Moreover, none of these tests currently meet the assay time requirements demanded by blood donation testing. At present, e-QuIC (Orru et al. 2011) and the assay of Edgeworth et al. (2011) appear most promising. The serial format of PMCA involved in achieving the appropriate analytical sensitivity makes assays such as sPMCA/CDI better suited to development as a confirmatory blood test. It is tempting to speculate that these technologies could be in implementable forms within the next 2 or 3 years. However, the track record in this area indicates that this is far from certain and the potential benefits of implementation of any test will need to be weighed carefully against the costs and potential consequences.

8.5 Conclusions

The emergence of vCJD has had a major impact on blood transfusion in the UK and other affected countries. It is greatly to the credit of the UK transfusion services that several precautionary measures to protect the blood supply were put into place even before the first cases of transfusion-associated vCJD were identified. The measures taken to reduce the risks of vCJD transmission by blood and blood products in the UK are summarised in Table 8.1. It remains to be seen whether any further measures will be implemented, for example the introduction of “prion filters”. The cases of transfusion-associated vCJD infection all occurred prior to the full introduction of leucodepletion in the UK. The most recent data from sheep models indicate that

whilest leucodepletion alone does not prevent disease transmission completely (McCutcheon et al. 2011), it does have a pronounced effect, and that it is the leucoreduction component of combined leucodepletion/prion reduction filters that is responsible the prion removal (Lacroux et al. 2012). Both of these sheep studies also show that all blood components may be considered potential vectors for prion transmission (McCutcheon et al. 2011; Lacroux et al. 2012). These findings reinforce the need for multiple control measures to reduce the risk of vCJD transmission by blood and blood products and raise the possibility that future cases of transfusion-associated vCJD may be identified in recipients of blood products other than non-leucodepleted red blood cell concentrates. In view of the uncertainties over the prevalence of asymptomatic vCJD infection in the UK, it seems likely that these control measures will continue to be required, perhaps until an effective test for asymptomatic vCJD infection is available.

The evidence for the transmission of other forms of CJD by blood transfusion is far less clear-cut: although data from experimental models indicate that different strains of prions can be transmitted by blood, the epidemiological evidence in humans to support these findings is largely absent. The recent contention that blood transfusion may be a risk factor for sCJD (Puopolo et al. 2011) has renewed interest in this field of research and will hopefully generate new research that is better designed to answer this problem than many of the previously published studies. Until these new studies are concluded, continued surveillance and analysis of risk factors for all forms of human prion disease is required.

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