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bjh review

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Managing the risk of transmission of variant Creutzfeldt Jakob disease by blood products

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Summary

Whereas plasma-derived clotting factor concentrates now have a very good safety record for not being infectious for lipid enveloped viruses, concern has arisen about the possibility that prion diseases might be transmitted by blood products. There is epidemiological evidence that classical sporadic Creutzfeld Jakob disease (CJD) is not transmitted by blood transfusion. There is now good evidence that the abnormal prion associated with variant CJD can be transmitted by transfusion of fresh blood components and infect recipients. To reduce the risk of the pathological prion in the UK infecting recipients of clotting factor concentrates, these are now only manufactured from imported plasma collected from countries where there has not been bovine spongiform encephalopathy (BSE) in cattle and the risk of variant CJD in the population is, therefore, considered negligible. The safety of these concentrates is also enhanced because prion protein is, to an appreciable extent, excluded by the manufacturing process from the final product. To help reduce the chance of prion transmission by fresh blood products, donations are leucodepleted, there is increasing use of imported fresh frozen plasma (especially for treating children) and potential donors, who have been recipients of blood since 1980 (the beginning of the BSE epidemic in cattle) are deferred.

Keywords: variant Creutzfeld Jakob disease, transfusion, epidemiology, safety, haemophilia.

Emerging pathogens will always challenge the safety of blood transfusion. Whilst the risk of hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV) transmission by blood components and plasma products is now small (http://www.eurosurveillance.org), new potentially transfusion-transmissible pathogens continue to emerge.

Many challenges were posed by the emergence of variant Creutzfeldt Jakob disease (CJD) in 1996 (Will *et al*, 1996). Whereas there has been little evidence of transmission of sporadic CJD by blood components or plasma products, it was recognised that variant CJD represented a different strain of prion disease, with a different distribution of peripheral disease (*vide infra*) and that absence of evidence of transmission could not be considered to equate with evidence of absence of risk (Ricketts, 1997; Ricketts *et al*, 1997). A number of precautionary measures were therefore taken to manage the uncertain risk. The description of two cases of transmission of variant CJD prions by red-cell concentrates over the last 18 months (Llewelyn *et al*, 2004; Peden *et al*, 2004), coupled with continuing concerns over the prevalence of subclinical disease in the UK population (Clarke & Ghani, 2005), has led to the introduction of further precautionary measures. The Departments of Health have considered it a high priority to prevent secondary spread of variant CJD by transfusion as this could lead to the infection becoming endemic in the UK population (Fig 1). However, these measures are likely to be of limited efficacy. The development of prion reduction filters and/or of peripheral blood screening assays pose significant challenges but hold the possibility of achieving better control over the risk of transmission of this disease by blood products. In the meantime, public health policy and medical practice will have to continue to balance the risks and benefits associated with human blood and tissue products (Flanagan & Barbara, 1996).

This review considers the unique aetiological and pathophysiological features of prion diseases, the measures that have been taken or might be taken in future to manage that risk, and the implications for those who prescribe and receive blood components and plasma products.

The biochemistry of prions

Prion protein (PrP^c) is a 30–35 kDa glycoprotein that is widely expressed by many cells and tissues in animals and man. It has two N-glycosylation sites and its secondary structure includes three alpha-helices and one beta-pleated sheet in the membrane-proximal carboxy-terminal of the protein. The membrane-distal amino-terminal of the molecule is largely unstructured. The protein is encoded by a single gene (*PRNP*) and with no spliced isoforms. In man, 20 different single nucleotide polymorphisms have been described that predispose to familial disease (de Silva, 1996a) (*vide infra*). In

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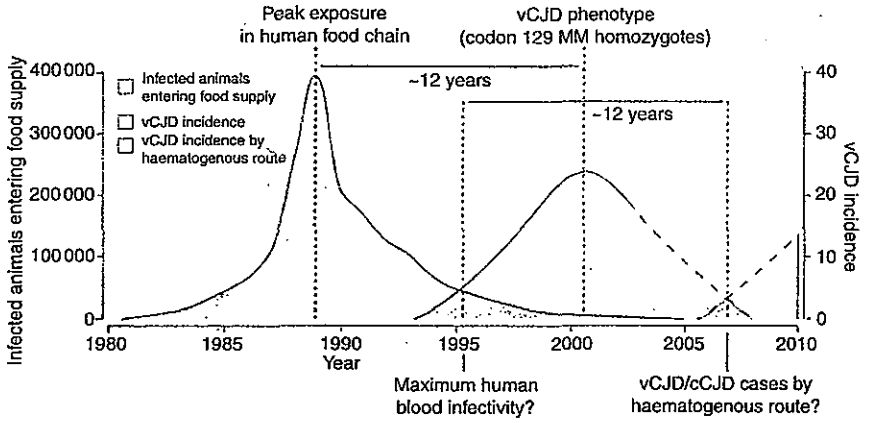

Fig 1. Incidence of bovine spongiform encephalopathy and variant Creutzfeldt Jakob disease in the UK (- - - - -, predicted cases). The right hand peak illustrates the potential for secondary spread by haematogenous spread. Reprinted from Collins *et al* (2004) with permission from Elsevier.

addition, a critical polymorphism at codon 129 coding for methionine or valine leads to significant variation in the susceptibility to, and incubation period of, human prion diseases. In the UK, 37% of the general population are homozygous for methionine at this locus, 11% are homozygous for valine and 52% heterozygous. Methionine homozygosity is much more common than expected amongst patients with CJD (*vide infra*). PrP is inserted into the cell membrane predominantly via a glycosylphosphatidyl inositol (GPI) anchor, although transmembrane and soluble forms have also been described. The glycoprotein is predominantly located in calveolar zones in the cell membrane and is estimated to have a half-life of around 6 h, being internalised into endosomes with a proportion recycling to the cell surface (Shyng *et al*, 1993). The function of the protein remains unclear, it has been shown to bind to a laminin receptor precursor protein (Martins *et al*, 1997; Rieger *et al*, 1997) and act as a copper metalloproteinase (Brown *et al*, 1997a). PrP null mice appear to develop normally although some strains show subtle neurological abnormalities (Tobler *et al*, 1996). Prion formation involves changes in the secondary and tertiary conformations of the PrP molecule: up to 40–50% of the molecule can be in the form of beta-pleated sheet, mainly at the expense of the membrane-distal unstructured region. This changes the physicochemical properties of the molecule and engenders relative resistance to proteinase digestion. Prion protein aggregates (PrP^{Sc}) are deposited in cells and tissues leading to the formation of amyloid-like plaques and in the nervous system to neuronal death, astrogliosis and spongiform change.

The mechanism by which PrP^{c} is converted to PrP^{Sc} remains unclear, as does its precise role in the aetiology of the disease. The prion hypothesis (Prusiner, 1998) proposes that the PrP^{Sc} molecule itself converts PrP^{c} to the abnormal conformation, either through a process of heterodimerisation or through nuclear polymerisation (Aguzzi & Weissmann, 1997). PrP^{Sc} is relatively resistant to proteinase-K digestion and different molecular strains of disease can be identified by the balance of di-glycosylated, mono-glycosylated and non-glycosylated species. Several molecular strains of PrP^{Sc} occur in sporadic CJD; however, only a single strain of PrP^{Sc} is found in variant CJD, which is similar to that seen in naturally occurring bovine spongiform encephalopathy (BSE) in cattle, and BSE transmitted naturally and experimentally to other animals (Collinge *et al*, 1996; Hill *et al*, 1997a). Evidence that variant CJD and BSE represent the same strain of prion disease also stems from infectivity studies in a prion disease strain typing panel of inbred experimental mice, where the patterns of incubation period and neuropathological targeting were similar and differed from those seen in sporadic CJD, scrapie and other prion diseases (Bruce *et al*, 1997).

Prion diseases in other species

A range of prion disorders have been described including those involving the SuP35p and Ure2p proteins in yeast, which appear to be non-pathogenic and convey a survival advantage under certain circumstances (Burwinkel *et al*, 2004).

Scrapie was first described as a disease of sheep and goats over 250 years ago and demonstrated to be experimentally transmissible 50 years ago (Aguzzi & Polymenidou, 2004). There is no evidence that scrapie has ever transmitted to man. The only other known self-sustaining animal prion disease is chronic wasting disease in mule deer and elk in several states of the USA. Again there is no current evidence that this disease has transmitted to man.

BSE was first described in UK cattle in 1985 (Wells *et al*, 1987) and is thought to have spread through oral consumption of ruminant-derived meat and bone meal (Wilesmith *et al*, 1988; Brown, 1998). The disease spread widely, peaking in 1992 with over 180 000 clinical cases in the UK, although mathematical estimates suggest that 1–2 million cattle could have been infected but slaughtered and entered the human food chain before they were old enough to demonstrate evidence of clinical disease (Fig 1) (Anderson *et al*, 1996). BSE has crossed into up to 20 other species, including domestic and exotic cats (Wyatt *et al*, 1991; Kirkwood & Cunningham,

1994) and exotic ungulates in British zoos. In July 1988, the spread of BSE led the UK Government to restrict the use of ruminant-derived meat and bone meal as an animal feed and in November 1989 specified that bovine offals were banned for human consumption.

Sporadic Creutzfeldt Jakob diseases

Sporadic CJD was the first described human prion disease, is of uncertain aetiology, has a worldwide distribution and an incidence of around one per million population per year (Will *et al*, 1998). The median age at onset is around 68 years and the disease is characterised by a rapidly progressive dementia leading to death in around 4–6 months. The incidence of the disease varies with the codon 129 genotype of the *PRNP* gene, with 83% of patients homozygous for the expression of methionine at this locus (Deslys *et al*, 1998). Molecular strain typing suggests that six forms of disease are dependent on codon 129 phenotype and strain of prion disease. One of the pathological hallmarks of sporadic CJD is the restriction of accumulation of plaques of prion protein to the central nervous system (CNS). However, with recently developed, more sensitive techniques, prion accumulation has also now been reported to be present in peripheral nerve (Favereaux *et al*, 2004) as well as in muscle, lymphoid tissue and olfactory epithelium (Glatzel *et al*, 2003) at an advanced stage of clinical disease.

Although there are a small number of reports claiming transmission of sporadic CJD by inoculation of blood from patients with clinical disease into experimental rodents (Manuelidis *et al*, 1985; Tateishi, 1985), these results have not been supported by further studies in primates (Brown *et al*, 1994). Similarly, although there are a handful of reports of sporadic CJD arising after blood or plasma product transfusion (Klein & Dumble, 1993; Creange *et al*, 1995, 1996; de Silva, 1996b; Patry *et al*, 1998), in none of these has a causal link to a donor with CJD been established. Moreover a series of epidemiological case control (Kondo & Kuroiwa, 1982; Davanipour *et al*, 1985; Harries-Jones *et al*, 1988; Will, 1991; Wientjens *et al*, 1996; Van Duijin *et al*, 1998; Collins *et al*, 1999), lookback (Esmonde *et al*, 1993; Heye *et al*, 1994; Operskalski & Mosley, 1995) and surveillance (Evatt, 1998; Evatt *et al*, 1998; Lee *et al*, 1998) studies carried out over almost 25 years have failed to demonstrate evidence of transmission of sporadic CJD by blood components or plasma products. It seems likely therefore that the preclinical incubation period in sporadic CJD is sufficiently short, or peripheral blood infectivity is sufficiently low, as to make transmission of the disease by blood components and/or plasma products at worst a very rare event (de Silva & Mathews, 1993; Brown, 1995; Ricketts *et al*, 1997; Will & Kimberlin, 1998).

Thus, although individuals suspected of having sporadic CJD are permanently deferred from blood donation, no other precautions, such as withdrawal of plasma products if the donor has contributed to the plasma pool, are undertaken. This is because although sporadic CJD is a rare disease, the large number of donations contributed to a plasma pool leads to frequent withdrawal and product shortages.

Familial human prion diseases

Familial human prion disorders are uncommon, which include Gerstmann–Straussler–Scheinker disease (GSS), fatal familial insomnia and familial CJD, and are associated with mutations in the prion gene (*vide supra*). Although there is no evidence of familial human prion disease transmission via blood products, individuals with two or more blood relatives with prion disease, or who have been advised that they are at risk of prion disease as a result of *PRNP* gene sequencing, are deferred from blood donation as a precautionary measure.

Acquired human prion diseases

The transmission of human prion disease (Kuru) was first reported in the Fore people of Papua New Guinea in the late 1950s (Gajdusek & Zigas, 1957) and is thought to have been transmitted during ritual cannibalistic or sacrificial funeral rites. The clinical features differ from those of sporadic CJD with more prominent ataxia and a longer clinical course. At one time Kuru was a leading cause of death amongst the Fore people and interestingly, despite abandoning these practices around 1960, there are still occasional people presenting with clinical disease – testimony to the fact that the incubation period in prion diseases can be very long.

Iatrogenic transmission of CJD has been well documented by direct inoculation of the CNS through contaminated neurosurgical instruments, stereotactic intracerebral electrodes, dura mater and corneal grafts. Iatrogenic transmission has also occurred via cadaveric human pituitary growth hormone and gonadotrophins administered by intramuscular injection (Buchanan *et al*, 1991; Brown *et al*, 1992). The clinical presentation varies depending on the route of infection; centrally transmitted cases tend to have a shorter incubation period of around 2 years and develop a rapidly progressive dementia reminiscent of sporadic CJD, whilst peripherally transmitted cases tend to have a much more prolonged incubation period of around 13–15 years and present with ataxia and sensory disturbance (Table I) (Brown *et al*, 2000).

Variant CJD

Variant CJD was first described almost 10 years ago (Will *et al*, 1996) as a result of systematic monitoring of the incidence and clinical phenotype of CJD in the UK by the National CJD Surveillance Unit in Edinburgh. Clinically, the disease is unusual in that it presents with neuropsychiatric symptoms, such as anxiety or depression, dysaesthesia and ataxia. Patients develop progressive dementia, myoclonus and choreoathetosis with an average clinical course to death of 6 months–2 years (Will, 2004; Will & Ward, 2004). Non-specific electroence-

Table I. Iatrogenic transmission of Creutzfeldt Jakob disease.

	Number	Incubation period (months)
Neurosurgical instruments	5	12–28
Intracerebral electrodes	2	16–20
Dura mater graft	120	18–216
Corneal graft	4	16–320
Human growth hormone	142	550–456
Human gonadotrophin	5	144–192

The incubation period for infections transmitted by peripheral inoculation is shorter than that when infection is directly in the brain (from Ironside and Head, 2003, with permission from Blackwell Publishing).

phalogram changes are observed, but magnetic resonance imaging (MRI) is more informative, with changes in the pulvinar (posterior thalamus) in the majority of cases.

Neuropathologically, the disease is characterised by neural cell loss, astrogliosis and spongiform change with particularly florid amyloid plaques as a pathognomic feature (Fig 2) (Ironside & Head, 2003; Peden & Ironside, 2004). To date all clinical cases of variant CJD have occurred in methionine 129 homozygous individuals; it seems likely that valine homozygous and methionine/valine heterozygous individuals are more resistant to infection or, if infected, to the development of clinical variant CJD. In this context it may be relevant that methionine 129 human prion protein oligomises more rapidly with beta-sheet formation whereas 129 valine tends to form alpha-helix rich monomers (Tahiri-Alaoui *et al*, 2004). Furthermore it is of interest that following inoculation with prions, mice homozygous for human methionine developed 'typical' variant CJD, whilst those that were homozygous for valine appeared more resistant to infection and when this occurred, the clinical and pathological features were more similar to sporadic CJD (Wadsworth *et al*, 2004). It is noteworthy, in this context that the second case of probable variant CJD prion transmission by blood transfusion was recorded in a methionine/valine heterozygous patient who did not develop clinical features of the disease despite surviving 5 years after transfusion (Peden *et al*, 2004). This patient had been identified as part of the variant CJD lookback process and postmortem examination was requested following death from unrelated causes (*vide infra*).

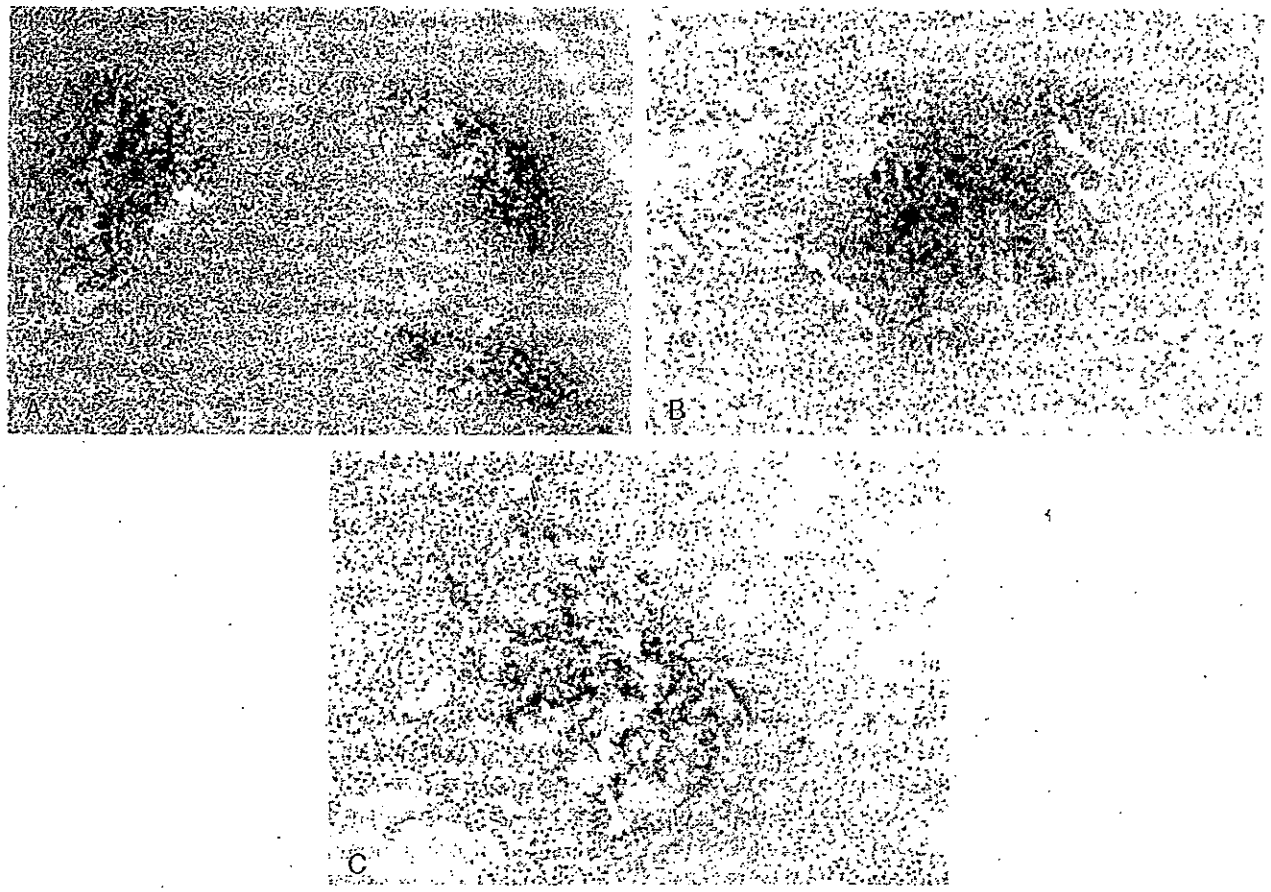

Fig 2. Immunocytochemistry for the prion protein (PrP) in lymphoid tissues in variant Creutzfeldt Jakob disease shows staining of follicular dendritic cells and macrophages in (A) the tonsil, (B) spleen and (C) lymph node. Anti-PrP antibody (KG9) with haematoxylin counterstain [from Ironside and Head (2003) with permission from Blackwell Publishing].

Unlike sporadic and familial forms of CJD, patients with variant CJD show evidence of abnormal prion accumulation in follicular dendritic cells in peripheral lymphoid tissue including tonsils (Hill *et al*, 1997b; Kawashima *et al*, 1997), appendices, spleen (Hilton *et al*, 1998) and lymph nodes (Hill *et al*, 1999). In two patients, appendices removed 8 months and 2 years prior to the onset of clinical disease have also shown evidence of prion accumulation, although a sample removed 10 years prior to onset of clinical disease did not (Glatzel *et al*, 2004).

The median age at death is 29 years (range 14–74 years) and has not altered over the first 10 years of the outbreak, suggesting an age-related susceptibility or exposure (Ghani *et al*, 1998a; Boelle *et al*, 2004). At the time of writing there have been 154 definite and probable cases of variant CJD in the UK, nine in France, two in Ireland and one in each of the USA, Canada, Italy, Saudi Arabia and Japan. In the UK, the incidence of clinical disease appears to have peaked around 2000 and has since fallen significantly (http://www.cjd.ed.ac.uk). However, although the outbreak thus far has been very much less than that which was initially feared (Cousens *et al*, 1997; Ghani *et al*, 1998b), with an upper boundary of around a further 70 new cases now predicted based on the pattern of clinical disease (Will, 2003; Smith *et al*, 2004; Sneath, 2004), a recent retrospective study of tonsil and appendix samples demonstrated three of 12 500 samples positive for abnormal prion accumulation, suggesting that up to 3500 people could be infected with a prevalence of pre- or subclinical disease amongst the 10 to 30-year-old UK population of one of 10 000 (Hilton *et al*, 2004). Ghani *et al* (1998a) have suggested that up to 90% of individuals infected may have prolonged preclinical or true subclinical disease and that this could be related to codon 129 genotypes encoding valine homozygosity or methionine/valine heterozygosity. If transmissible prion infectivity is present in the peripheral blood of such asymptomatic individuals, the concern is that blood-derived products could provide a route to long-term persistence of variant CJD within the population.

Animal studies of peripheral blood infectivity and transmissibility

The route by which the prions disseminate and replicate following peripheral inoculation is of importance in understanding the likely distribution of infectivity and has been recently reviewed (Mabbott & Turner, 2005). Studies in knockout mice with deficiencies in PrP expression, or lacking various cellular compartments of their immune systems, have led to the conclusion that initial accumulation or replication in follicular dendritic cells is essential to peripheral transmission (McBride *et al*, 1992; Bueler *et al*, 1993; Fraser *et al*, 1996; Brown *et al*, 1997b; Klein *et al*, 1997, 1998; Mabbott *et al*, 1998). Indeed, infection and abnormal prion accumulation can be demonstrated in the lymphatic tissues of scrapie-infected rodents and sheep prior to the stage at which it can be detected in the nervous system (Diringer, 1984; Casaccia *et al*, 1989; Farquhar *et al*, 1994; Mabbott *et al*, 1998). The mechanism by which prions are transmitted from a site of initial exposure, such as the intestinal lumen, to the lymphoid germinal centres where they replicate is uncertain. It is possible that follicular dendritic cells (FDCs) trap circulating cell-free prion or that agent is transported by lymphocytes, macrophages or migratory dendritic cells. Once prion replication has occurred within FDCs, retrograde neuroinvasion occurs via sympathetic and parasympathetic peripheral nerves to the CNS. Again it is unclear whether direct FDC–neuronal interaction or dendritic cell-mediated spread is responsible for this step. These observations suggest that it is quite plausible that the peripheral blood also harbours infectivity from an early stage in the preclinical phase of the disease.

As a generalisation, peripheral blood infectivity has been shown to be detectable in rodents experimentally infected with scrapie, BSE and variant CJD and in experimentally infected sheep and goats. Peripheral blood infectivity has not been demonstrated in the peripheral blood of sheep naturally infected with scrapie or BSE-infected cattle. The reason for these differences is unclear but may relate to the route and size of the primary infectious inoculum.

In mice infected with the Fukuoka 1 strain of GSS, 100 infectious units (i.u.)/ml have been demonstrated in the peripheral blood during the clinical phase of disease and 10 i.u./ml during the preclinical phase of disease. Around 70% of the infectivity is associated with the buffy coat (inclusive of the leucocyte and platelets) and the remainder with the plasma (Brown *et al*, 1998, 1999). Similar findings have been demonstrated in the 293K hamster model. The latter also suggested an efficiency of transmission via the intravenous route of approximately 5–10% of that of the intracerebral route of inoculation.

In sheep naturally infected with scrapie or experimentally infected by an oral dose of BSE, peripheral blood drawn during the clinical and preclinical phases of disease has been shown to transmit infection to 20–25% of secondary recipients. This study amounted to proof of principal that prion diseases are transmissible by transfusion (Hunter *et al*, 2002).

Transmission of variant CJD by blood transfusion

The UK has established surveillance to assess the transmissibility of CJD by blood components (http://www.cjd.ed.ac.uk/). For each individual who develops CJD, close relatives are questioned and Blood Transfusion Service records searched to try to establish whether he/she was a blood donor. If so, the recipients of blood components manufactured from those donations are traced and notified. They themselves are subject to public health restrictions around blood, tissue and organ donation and invasive medical and surgical procedures and are flagged to the UK Office of National Statistics.

The reverse arm of the surveillance scheme addresses the question as to whether any of the patients who have developed variant CJD could have become infected via a previous blood transfusion. The transfusion history of all patients developing variant CJD is assessed and the donors are traced and also flagged to the UK Office of National Statistics.

To date 17 variant CJD patients are known to have been blood donors (15 in the UK and two in France). Of the 50 recipients of blood components, 17 are still alive. Plasma from 23 donations was fractionated to produce albumin, immunoglobulin and clotting factor concentrates that were used in the UK, France, Belgium, Germany and Italy. In the UK it appears that the incidence of variant CJD peaked in about 2001 and is now declining (Fig 1).

To date there have been two cases of probable transmission of variant CJD prions via non-leucodepleted red cell concentrates. In the first episode, a 24-year-old individual gave a blood donation in 1996 (Llewelyn *et al*, 2004). Three years later he developed variant CJD and died the subsequent year. The recipient of this donation in 1996 was aged 62 years and also received four other units of red cell concentrate to cover a surgical operation. In 2002 he became depressed and developed blurred vision, motor difficulties including a shuffling gait and cognitive impairment. An MRI of his brain was reported as normal. In 2003 he died of dementia. At autopsy, histology of his brain revealed characteristic features of variant CJD, and this was confirmed by proteinase-K resistance and typical features on Western blotting. Analysis of his *PRNP* gene revealed him to be homozygous for methionine at codon 129. A statistical assessment concluded that there was only a 1:15 000–1:30 000 chance of this occurring by coincidence.

A second individual was reported in 2004 as a result of the national surveillance of recipients of transfusions from donors who later developed variant CJD. This patient very likely became infected with variant CJD prions by a unit of red cell concentrate in 1999 from a donor who developed variant CJD 18 months later (Peden *et al*, 2004). Although this patient died 5 years after the transfusion of unrelated causes with no clinical features of variant CJD, analysis of her lymphoid tissue at autopsy revealed that prion accumulation was present in the spleen and one cervical lymph node. There were no histological features or evidence of prion accumulation in her CNS. The other unusual feature as noted above, was that the *PRNP* gene was heterozygous at codon 129 for methionine/valine.

These two cases are therefore of great importance because they have demonstrated that variant CJD prions can be transmitted by blood transfusion from donors who are in a preclinical phase of disease at the time of donation and that methionine/valine heterozygous individuals can also be infected, although whether they are as susceptible to infection and/or the development of clinical disease as methionine homozygous individual remains uncertain (Aguzzi & Glatzel, 2004).

Blood donor selection

Many countries have instituted policies of donor deferral for those who have spent time in the UK, France or more broadly Europe, based on the likely comparative level of risk with their indigenous population, the extent or pattern with which their population visit affected areas and the likely impact on their blood donor base.

In the UK, there are few epidemiological criteria that would allow identification of a 'high-risk' donor population. In response to the blood transfusion related transmissions of variant CJD, in 2004, a policy of deferral of donors who themselves have been recipients of blood components since 1980 was instituted to reduce the risk of tertiary or higher-order transmissions leading to a self-sustaining outbreak. This policy also has the advantage of reducing the risk of other blood borne infectious agents being recycled in the community by transfusion. There was concern that this would lead to a significant reduction in the donor base and that a sometimes precarious blood supply would be further compromised. Whilst about 5–10% donors have been lost from the UK blood donor panels, the impact has been mitigated by proactive recruitment campaigns to enlist more new donors.

Importation of blood components

It is not likely to be feasible to import red cell or platelet concentrates due to the large volumes required, the short shelf life and lability of these components and concerns over the risk of other transmissible agents in some overseas donor populations. To reduce the risk of variant CJD transmission to children, in 2002 the decision was made to only use imported non-UK plasma to treat those born after 31 December 1995. This date was chosen because it was considered that BSE-infected foods had been largely eliminated from the diet by this date, and therefore, children born after this time were unlikely to be infected from food. In addition, with relatively small volumes of plasma, the product can be stored, transported frozen and be virus-inactivated.

Donor screening

No immunological response to prion infection has yet been identified nor has DNA been found associated with disease transmission. Therefore, traditional serological and molecular biological approaches to donor screening are not currently feasible.

Several groups have looked at the possibility of using surrogate markers. The proteins 14·3·3 (Zerr *et al*, 1998) and S100 (Otto *et al*, 1998) are non-specific markers of CNS damage and are therefore likely to be elevated only in the clinical stages of disease. It has been shown that transcription of erythroid differentiation associated factor (EDAF) is depressed in the peripheral blood of animals suffering from prion disease (Miele *et al*, 2001). The cause of this observation

