

A report on transmissible spongiform encephalopathies and transfusion safety

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Vox Sanguinis

The incidence of BSE in Europe is in continued decline. At present, iatrogenic transmission from person to person is considered a serious threat to public health. This report of the International Society of Blood Transfusion Working Party on Transmissible Spongiform Encephalopathy will focus on the state of the art in relation to blood components and plasma safety. Latest information on the pathogenicity of the infectious agent, the frequency and dynamics of infection in blood and transfusion transmissibility will be documented. Preventive measures including donor deferral policies, technologies for prion removal from labile blood components and for prion detection in plasma, the absence of a sensitive and rapid reference assay able to confirm the positive results from any putative blood screening assay will be updated. At last, as many uncertainties remain and a number of assumptions await confirmation, the areas to continue to explore are listed.

Key words: Prion, transmissible spongiform encephalopathies, transfusion safety, prion detection, prion removal.

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Background

Transmissible spongiform encephalopathies (TSEs or prion diseases) include a number of fatal human and animal disorders, characterized by long incubation periods, central nervous system degeneration, and the accumulation of abnormal prion protein (PrP^{TSE}), a derivative of normal cellular prion protein (PrP^C). Prion-associated diseases in animals include scrapie in sheep and goats, chronic wasting disease in deer and elk, and bovine spongiform encephalopathy (BSE) in cattle. In humans, the disease manifests itself as sporadic Creutzfeldt–Jakob disease (sCJD), Gerstmann–Sträussler–Scheinker disease (GSS), fatal familial insomnia (FFI), kuru and variant CJD (vCJD). Variant CJD has only emerged recently [1], and is thought to be linked to oral exposure to BSE.

Prion protein is a highly conserved surface sialoglycoprotein that attaches to the cell membrane through a glycosylphosphatidylinositol anchor [2]. In mammals, a single-copy chromosomal gene (*PRNP*, or *Prnp* in mice), on the short arm of chromosome 20 in humans, encodes PrP^C, which is expressed during early embryogenesis. PrP^C exists in most adult cells, with the highest levels in neurones. The normal function of PrP^C remains unknown but many investigators believe that PrP^C protects against oxidative stress [3].

We also do not know how PrP^C converts to its abnormal counterpart, PrP^{TSE}, but research has shown that PrP^C can change from a mainly α -helical conformation to a β -sheet structure called PrP^{TSE}, an aggregate more resistant to protease digestion. Extensive cytoplasmic accumulation and aggregation of misfolded PrP^{TSE} may be associated with neuronal cell death. According to the prion hypothesis, PrP^{TSE} is the sole infectious agent. Prion propagation occurs through high-fidelity self-replication of PrP^{TSE}, which recruits endogenous PrP^C with an as yet unidentified factor [4]. Mice without a functioning *Prnp* gene (PrP^{0/0}) show resistance to TSE [5], thus confirming that PrP^C is essential to the spread of infection.

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Transmissible spongiform encephalopathies agents usually accumulate in the central nervous system, although some strains, such as vCJD, accumulate at a lower level in lymphoreticular tissue, including the spleen, lymph nodes, tonsils and appendix [6,7]. Because lymphoid tissue maintains an immediate contact with blood, this may facilitate blood-borne infection [8]. During the last decade, studies have demonstrated that blood from a proportion of animals with both natural and experimentally induced TSE can transmit the infection. Early studies indicated blood-borne infection in rodent animal models [9–11]. Hunter *et al.* used sheep experimentally infected with BSE and scrapie in a long-term study, to confirm the spread of infection by blood and buffy-coat [12,13]. A report noted a case of experimental vCJD transmission by blood transfusion in a *Cynomolgus* macaque in 2007 [14]. Recent epidemiological evidence from the UK indicates that, almost undoubtedly, non-leucocyte-depleted red blood cell (RBCs) transfusions from vCJD patients during the preclinical phase may have transmitted the infection in four instances [15–19].

This report summarizes current knowledge on TSE infection that relates to the safety of blood transfusion and makes recommendations to reduce the possible risk of TSE transmission by blood and blood products.

Current transmissible spongiform encephalopathy epidemiology

Approximately 85% of all human TSE cases result from sCJD, which has a relatively even worldwide incidence of 1–1.5 cases per million per year, with equal frequency in males and females. Approximately 10% of human prion diseases have a genetic cause due to mutations in the *PRNP* gene and phenotypically they manifest as GSS or FFI. The remaining cases constitute the subgroup of acquired diseases, which include iatrogenic CJD, Kuru and vCJD.

The infectious agent responsible for vCJD is identical to the BSE agent and we accept that human infection occurred through the consumption of BSE-contaminated bovine products. The first case of vCJD occurred in the UK in 1996. It affects a younger age group than sCJD (median age 28, range 12–74) with a more prolonged clinical course (median 14 months, range 4–48 months) [20]. Subsequently, six other European countries and Saudi Arabia reported cases. UK residence-associated infection occurred in the USA, Canada and Japan. As of September 2008, a total of 208 primary cases of vCJD have occurred in 11 countries, with the vast majority (164 or 79%) in the UK (www.eurocjd.ed.ac.uk). France reported 23 primary cases, Ireland (four), Spain and the USA (three), the Netherlands and Portugal (two). Italy, Saudi-Arabia, Canada and Japan each reported one case. The UK reported four secondary cases associated with RBC transfusions: three clinical and one subclinical. France did not find any such cases, although three of the 23 French patients had donated blood.

A retrospective study in the UK examined 14 674 surgically removed tonsillectomy and appendectomy specimens for PrP^{TSE} – 60% of which were from patients aged 20–29 years at operation. Three appendectomy samples showed PrP^{TSE} prion protein accumulation, giving an estimated prevalence of 3/12 674 [21] or 237 asymptomatic carriers per million (95% CI: 49–692 per million) in the UK. These results suggest that the prevalence of infection may be far more common than indicated by the small number of clinical cases seen. In the UK, two out of the three positive asymptomatic individuals were Valine/Valine homozygous at *PRNP* codon 129. Interestingly, all the UK clinical vCJD cases, including the three potential secondary clinical cases, have occurred in methionine/methionine (M/M) homozygous individuals at *PRNP* codon 129. Approximately 40% of the population carry this genotype. However, the secondary case with subclinical evidence of infection was methionine/valine (M/V) heterozygous. As 50% of the population are M/V heterozygous, this finding raises the possibility that other genotypes may develop clinical symptoms much later or remain in some asymptomatic carrier state after exposure.

Blood infectivity

Blood can transmit infection in naturally occurring scrapie, BSE induced in sheep and in vCJD in humans. Even so, infectivity levels remain undetermined. Rodent models of TSEs show low infectivity levels in buffy-coat and plasma compared to brain, both preclinically and at the end stage of the disease. However, we do not clearly understand the source of blood-borne transmission. Infection probably targets the lymphoid tissues first before reaching the brain through haematogenous and neuronal routes. Quantitative data from rodent studies suggest that blood infectivity occurs via the intravenous route around 10 infectious doses per ml (10 ID/ml) and that it may be split roughly equally between plasma and leucocytes, with negligible levels associated with RBCs or platelets [22]. The plasma and leucocyte amounts within each transfused component, therefore determining risk assessment [23].

Based upon these assumptions, the UK adopted five strategies: (i) to reduce exposure to donor leucocytes; (ii) to reduce exposure to donor plasma; (iii) to minimize the number of donors contributing to each therapeutic dose of product; (iv) to defer donors who had received a transfusion, to reduce the risk of secondary infection; and (v) to promote an evidence-based approach to prescribing donor-derived blood components and to encourage the use of alternatives.

To monitor blood safety, we need to determine when infectivity appears in blood. Primary vCJD has an incubation period of 16 or 17 years in the UK (95% confidence interval 12–23 years) [24]. Research predicted a few scenarios: (i) infectivity would be detectable at least in the second half of

the incubation period [25]; (ii) infectivity would be detected in the early presymptomatic phase and increase linearly towards the clinical stage [26,27], and (iii) an early increase in the incubation period would be followed by a disappearance and a secondary rise in infectivity in the symptomatic phase [27].

Transmissible spongiform encephalopathy and transfusion

Several experimental animal models have shown that prion diseases can be transmitted by blood: BSE, GSS, vCJD in mice and Macaques, sCJD in guinea pigs, scrapie in hamsters, BSE in sheep and GSS in chimpanzees [11]. Epidemiological evidence indicates that human sCJD cannot readily be transmitted through blood and blood components. An ongoing study by the American Red Cross, together with the US Centers for Disease Control and Prevention, tracked 368 known recipients of blood components from persons who later died of CJD. From this group, 125 have been followed for at least 5 years after transfusion. None have died of CJD. In addition, to date, no CJD cases have been reported among more than 20 000 haemophilia patients who have repeatedly received coagulation factors over the past 10 years [28,29]. However, evidence supports natural scrapie transmission by the intravenous route [10,12,13]. Work recently revealed that sCJD of a particular subtype (M/V-2) shares molecular and pathological features with an atypical form of BSE, in which the glycoform ratio of PrP^{TSE} resembles that of human sCJD [30,31]. The unexpected similarities of blood infectivity data in experimental models of vCJD and sCJD emphasizes the need for continued vigilance with regard to the potential infectivity of blood from patients with sCJD.

In the UK, of the patients who died of vCJD, 22 had donated blood before their clinical symptoms appeared. Transfusion records identified a total of 64 recipients, 26 of whom are still alive. Of these recipients, three developed vCJD and one had evidence of subclinical infection at post-mortem. The first case of vCJD transmission through transfusion was reported in December 2003. A patient with *PRNP* codon 129 M/M homozygosity developed vCJD 7 years after having received non-leucocyte-depleted RBCs donated by a 24-year-old donor 3 years prior to illness. In the second transmission case, the patient died from a non-vCJD-related disorder 5 years after receiving the transfusion from a young adult who became ill 1.5 years after donating the implicated blood. Post-mortem revealed PrP^{TSE} in the lymph nodes and spleen of the recipient, who had been asymptomatic and tested as M/V heterozygous at codon 129. The third case also received non-leucocyte-depleted RBCs and developed the disease approximately 8 years after transfusion from a donor who developed vCJD almost 2 years after the donation. This case was confirmed to be a homozygous M/M carrier at codon 129. These three cases provide almost indisputable evidence

that blood of vCJD infected persons can be infectious during the preclinical period. The second case also indicates that genetic susceptibility to vCJD is not limited to *PRNP* codon 129 M/M homozygosity and that individuals with M/V and even V/V genotypes might be infected as well. France reported no such cases, but three out of the 23 patients who developed signs of vCJD in 2004 had donated blood. Records identified a total of 42 recipients and 12 are still alive. Interestingly, nearly 80% of the transfused RBCs had undergone leucocyte-depletion [32]. The latest risk analysis in France from 2007 lowered the risk of vCJD transmission by blood from 1 in 120 000 (in 2000) to 1 in 360 000 (in 2005) [33]. The UK proposes three scenarios: 1 in 1000, 1 in 4000 or 1 in 20 000 of the population carrying vCJD.

So far, reports do not indicate prion disease transmission, including vCJD, by plasma products, although we cannot totally discount the risk of such transmission. A major concern is that plasma product manufacture requires large volumes of plasma pools, and many patients receive plasma products from the same, potentially infectious, pool. Experimentally, exposing animals to multiple small doses of the TSE agent can cause the disease in rodent models. Currently, UK plasma is not fractionated to produce plasma products. Available data indicate that the separation processes involved in manufacturing plasma products significantly remove prion agents. Consequently, the risk of vCJD transmission by plasma products seems low. However, most of the experiments evaluated removal efficiency in multiple steps rather than evaluating the whole process. Furthermore, these tests employed highly infectious exogenous brain homogenates as spiking materials, creating higher and probably artificial conditions compared to those normally found in blood [34,35]. Therefore, further research is required to define the safety of plasma products.

Donor deferral policies

The UK Department of Health made an announcement in March 1996, acknowledging a possible link between BSE and vCJD. They then rapidly implemented several precautionary measures to reduce the risk of infected meat being sold for human consumption. Over the following years, these included protecting against transmission from surgical instruments and guidelines on the handling of animal-derived materials for pharmaceutical use, including vaccines. In terms of blood-borne transmission, a range of precautionary measures have been introduced to safeguard the blood supply against the risk of vCJD transmission. These include deferral of subjects at risk for CJD (France, 1992), withdrawal and recall of any blood components, plasma products or tissues obtained from any individual who later develops vCJD (1997); plasma sourcing from outside the UK (UK: 1998) and preparing products, such as clotting factors, from US-imported plasma

(UK: 1999); removal of white blood cells (leucocyte-depletion) from all blood components (France: 1998 and UK: 1999) from all cellular blood components and in 2001 from plasma); deferral of donors who have spent more than a cumulative period of 1 year in the UK between 1980 and 1996 (France: 2000); import of fresh-frozen plasma from the USA for neonatal patients and for older children as appropriate (UK: 2004); refusing donations from people who have received a blood transfusion after January 1980, including apheresis donors, and those who are unsure whether or not they have received a blood transfusion (UK: 2004 and France: 1997); selectively notifying patients who are considered to be at risk as a result of receiving plasma products (2004); and distributing information about better transfusion practices and the appropriate use of blood (2002).

In 2002, the US Food and Drug Administration issued revised guidelines in order to reduce the risk of TSE transmission from blood and blood products. In terms of donor deferral, it recommended that donors who are diagnosed with or are suspected of having CJD or vCJD, or are at risk of CJD, because they received pituitary-derived growth hormone or dura mater transplants, or have a family history of CJD, should be deferred indefinitely. In addition, anyone who has resided in the UK for 3 months or longer between 1980 and 1996, or who has resided in Europe for 5 years or longer since 1980, or who received a transfusion in the UK since 1980, are indefinitely deferred. In August 2006, the Food and Drug Administration issued draft guidelines proposing the indefinite deferral of donors who have been transfused in France since 1980.

Technologies for infectivity detection and removal

Currently, TSE diagnoses cannot be confirmed until the disease enters its clinical phase. Laboratory testing targets brain tissue from suspected subjects, using techniques such as immunohistochemistry, enzyme immunoassays and immunoblotting (Western blot), all of which require protease treatment. We believe infectivity in blood to be four or even six orders of magnitude lower than that in brain, even lower during the preclinical period. Recent studies indicate that 1 ID/ml of blood obtained from scrapie – afflicted hamsters is equivalent to 0.1–1 pg/ml of PrP^{Sc}, an amount that would be very difficult to detect with current methods or those at different stages of development. Several techniques aim to detect PrP^{TSE} in blood, but none have yet reached the licensing stage for human use. They all use PrP^{TSE} detection as a marker of infectivity, although attempts have been made to develop surrogate assays [36].

Detection assays

Immunocapillary electrophoresis (CE) was the first method that successfully detected PrP^{TSE} in sheep blood with natural

scrapie and in experimentally infected hamsters. However, its performance has not been confirmed with human or monkey samples. The assay is based on competitively inhibiting the binding of specific antibody to the antigen (PrP^{TSE}) by a synthetic peptide analogous to the PrP fragment used to produce the antibody. While efforts continue to develop this test, reports conclude that CE is difficult to optimize and, while specific, may lack sufficient sensitivity [37].

A few antibodies distinguish PrP^{TSE} from PrP without protease treatment. This could increase the sensitivity of some assays to the point of detecting PrP^{TSE} in blood, although currently only the Prionics monoclonal antibody 15B3 is being actively developed for this purpose.

Conformation-dependent immunoassay uses the binding differences of (3F4) monoclonal antibody to PrP^C and PrP^{TSE} molecules [38]. The antibody-binding site is fully accessible in the normal protein but largely inaccessible in its pathologic form. Thus, PrP gives a similar signal intensity whether in its native or denatured state, whereas the PrP^{TSE} signal increases after denaturation. CDI is up to two times more sensitive than immunoblot assays on the same samples. Reports claim that this method approaches the sensitivity of the bioassay in transgenic mice.

PeopleBio (Seoul, South Korea) has developed a platform technology named Multimer Detection System that can detect only multimers from plasma samples. The method is based on a specific epitope-overlapping antibody system that differentiates the aggregated PrP^{TSE}/PrP (multimer) from PrP (monomer). Multimer Detection System's procedure is simple and does not only rely on the protease resistance of PrP^{TSE}.

Other tests use PrP^{TSE}-binding ligands with high affinity for PrP^{TSE}. Sulphated polyanionic compounds, such as pentosan sulphate or dextran sulphate, bind PrP^{TSE} *in vitro*. Plasminogen has been also shown to bind predominantly PrP^{TSE}.

Amorfix Life Science (Toronto, Canada) has developed a chemical means of differentiating between PrP and PrP^{TSE}, called epitope protection assay (EP-vCJD). PrP^C is selectively modified with highly reactive chemicals (peroxynitrite) that block immunological PrP epitopes, leaving them unrecognizable to many PrP antibodies. The same epitopes in aggregated PrP^{TSE} are 'protected' from chemical modification, and can then be revealed by disaggregating the sample and performing a conventional immunoassay.

Seprion (Microsens Biotechnologies, London, UK) uses a ligand from the polyanionic family that specifically captures aggregated PrP^{TSE}, without requiring protease treatment. It can be used in a variety of animal species with appropriate species-specific anti-PrP antibodies. The USA has approved this test for BSE, scrapie and chronic wasting disease. However, given the limited data with blood, it is too early to know whether this test will function as a sensitive and specific blood-screening test.

BioMerieux (Marcy l'Etoile, France) has developed a different ligand, calyx-(6)-arene, to capture aggregated oligomers of protease-digested PrP^{TSE}, precipitated by streptomycin. Labelled monoclonal antibody recognizes macromolecular aggregates. The method detects BSE and CJD PrP^{TSE} in brain homogenates, as well as small numbers of plasma samples from scrapie-infected sheep, BSE-infected cattle, and humans with the sporadic, iatrogenic and variant forms of CJD. All 200 normal human plasma samples were tested negative.

Adlyfe (Rockville, MD, USA) is another ligand-based approach, which uses a peptide analogous to the narrow region of the PrP protein that may mediate the conformational transition from PrP to PrP^{TSE}. This palindromic polypeptide, labelled at each end with a fluorophore, is mixed with a test specimen containing PrP^{TSE}. Upon contact, the ligand causes it to fold into a β -sheet, thus changing the fluorescence wavelength. The folded ligand induces more ligand molecules to adopt the same conformation. From these kinetic changes, the ratio of relative excimer to monomer fluorescence emission provides a measurement of PrP^{TSE}. The assay is neither protease digestion nor antibody dependent. It demonstrates broad reactivity across different species and claims to have a threshold sensitivity at a femtomolar level.

Alicon Priomax (Schlieren, Switzerland) has developed a prion protein-specific resin, which is incubated with body fluids. After trapping prion protein molecules, a concentrated resin-bound protein is produced by centrifugation. Western blot or enzyme-linked immunosorbent assay then detects the eluted protein, with an alleged detection limit of less than 1 pg/ml.

Finally, some assays have developed novel methods to amplify the amount of PrP^{TSE}.

Protein misfolding cyclic amplification (PMCA) emerged from the concept that under optimal conditions, with enough PrP molecules, even small amounts of PrP^{TSE} initiate the conversion of PrP into PrP^{TSE} [39]. As a result, repeated cycles of conversion and fragmentation by sonication could amplify PrP^{TSE} to detectable levels. Theoretically, with enough PrP, the amplification process can proceed indefinitely. Thus, amplifying brain homogenates from scrapie-infected hamsters with 140 PMCA cycles increases sensitivity 6600-fold over standard detection methods [26]. It is also claimed that the method can amplify not only PrP^{TSE} but the infectivity as well. Applying PMCA to buffy-coat samples from clinically affected scrapie-infected hamsters, 16 out of 18 samples tested positive, while none of 12 samples from uninfected hamsters gave positive results. More recent work demonstrated the presence of PrP^{TSE} in buffy-coat samples as early as 20 days (1/4 way through the incubation period) after inoculation in 50% of animals, while no samples tested positive at 14 days. Surprisingly, the number of positive samples started to decrease after 40 days, with no samples testing positive at day 70. However, 80% of samples then tested positive 80 days after infection, during the clinical stage of

the disease [27]. No control sample tested positive. Although the method shows promising results, it takes days to perform, diminishing its potential for routine blood screening. However, it may provide a useful confirmatory assay and the Edinburgh group has recently demonstrated that PrP from platelets acts as well as that from uninfected brain in the PMCA assay [40].

Recently, Atarashi *et al.* [41] developed a new prion assay, quaking-induced conversion. This uses recombinant PrP as a substrate and automated tube shaking instead of sonication. It detects about one lethal prion dose within a day.

Other methods, such as intensely fluorescent targets, immunopolymerase chain reaction, flow cytometry of seeded aggregates and different capturing methods (magnetic beads) followed by sandwich enzyme-linked immunosorbent assay formats detect PrP^{TSE} in brain homogenates, cerebro spinal fluid (CSF), plasma and spleen homogenates. However, none of these methods successfully detect PrP^{TSE} in blood.

Therefore, the challenge remains to detect the small PrP^{TSE} concentrations in blood, estimated to be as low as the femtogram range during the preclinical phase. Although significant progress in improving sensitivity has been made, the levels are still insufficient to permit blood transfusion screening. Moreover, the incidence of vCJD is declining, and many laboratories seem to have lost interest in competing to develop a screening assay. At present, fewer than six companies research detection assays for PrP^{TSE} in human blood. The combination of amplification and sensitive detection assays may enhance sensitivity enough to allow early detection and shorten the assay time for blood transfusion screening.

Another concern is the lack of reference vCJD-positive plasma panels, which impairs the performance analyses of the detection assays. For this reason, studies perform evaluations with dilutions in plasma pools of brain and spleen homogenates, although this creates uncertainties regarding their relevance to the infectious agent naturally present in blood. Specificity raises another concern. In the UK, which collects up to 2.5 million donations per year, the prevalence of subclinical vCJD may be as high as 1 in 10 000. An assay with 99% specificity and sensitivity would generate therefore around 25 000 false-positive results per year [42]. Developing sensitive confirmatory assays, such as the PMCA, quaking-induced conversion or a cell culture-based infectivity assay, will be extremely important to confirm the data from any putative blood screening assay without relying on lengthy and expensive animal models. Current cell lines are of limited use, because their sensitivity is currently restricted to scrapie agents only. Recently, however, a study reported the first successful generation of spleen-derived murine stroma cell cultures, which propagate two mouse-adapted isolates of human TSE agents. This new cell culture may aid diagnostic and therapeutic developments, as well as forming a rapid *ex vivo* assay for TSE inactivation/removal procedures [43].

In the UK, the National Institute of Biological Standards and Controls, the Health Protection Agency and National Blood Service assess potential assays by checking their ability to detect blinded samples prepared from dilutions of infected brain or spleen added to human plasma. If this stage achieves promising results, they then test assays on infected animal plasma samples and then on the few samples available from infected human patients.

Pending suitable assays, defining the number of 'preclinical' cases in an affected population is also important. In addition to an ongoing prevalence study looking at 100 000 anonymized tonsil samples, a prospective study in the British population aims to determine the prevalence of abnormal prions in blood once a screening assay becomes available [44]. The study plans to test 50 000 blood donors from all parts of the UK, using 5000 non-British plasma samples as a negative control panel. These results will reflect the prevalence of PrP^{TSE}, and describe any variations in blood-borne PrP^{TSE} prevalence by year of birth, gender or region of the UK.

Prion removal devices

As well as detecting prions, a different but complementary approach will be to remove prion infectivity from blood and blood components. At least three companies are developing prion-removal filtration-type technology for this purpose [45].

Leukotrap Affinity Prion Reduction Filter (PALL, East Hills, NY, USA) consists of polyester fibers with surfaces modified by a proprietary chemical that bind prions in the residual plasma in leucodepleted RBCs. Two studies assessed the original filter: human RBCs, to which brain homogenate from scrapie-infected hamsters had been added, and RBCs from scrapie-infected hamsters. In the first study, bioassays removed infectivity by 3.7 log ID/ml, confirming initial results obtained by Western blotting. The second study used hamster blood collected during the clinical disease stage, pooled and processed into RBC units then filtered through the LARPF. The team then inoculated samples of pre- and post-filtered RBCs into the brains of healthy hamsters. Six out of 187 animals that received non-filtered blood developed clinical symptoms and carried PrP^{TSE} in their brains [46]. Unfortunately, one animal in the filtered group also became infected. As a result, the company has now redesigned the filter and is currently revalidating it, as a combined leucodepletion and prion-removal filter.

The MacoPharma P-Capt Filter incorporates patented PRDT (Pathogen Removal and Diagnostic Technology, Falls Church, VA, USA) ligand into an established, biocompatible filter from MacoPharma (Tourcoing, France). The ligand binds the abnormal prion proteins from both sporadic CJD and vCJD-infected human brain, familial GSS-infected mouse brain and scrapie-infected hamster brain with a high affinity in solution. The prototype filter removed infectivity

from human RBCs spiked with scrapie-infected brain homogenate to below the detection level of Western blotting, and the *in vivo* assay documented an infectivity reduction of 4 log ID/ml. Researchers tested the filter on leucodepleted whole blood from scrapie-infected hamsters in the clinical phase of the disease. They inoculated pre- and post-filtered blood intracerebrally into a 100 hamster cohort and followed them up for 18 months, examining for signs of the disease. The filter removed 2.8 ID/ml of blood infectivity, to a level below detection by the bioassay.

Combination filter for prion and leucocyte reduction (Asahi Kasei, Medical, Oita, Japan) has modified the leucodepletion filter surface, a Sepacell type, with a polymer, with preliminary results showing a reduction in prion proteins by greater than 3.5 logs and a decrease in leucocyte levels to less than 10⁵ per container. In addition, RBC process recovery seems good, with units greater than 85%.

Leucodepletion alone does not eliminate blood infectivity, at least in hamster-scrapie models [45]. However, such filters do not promote prion release from leucocytes as a result of lysis or microvesiculation [47].

Future prospects

Epidemiological evidence demonstrates that blood from vCJD-infected individuals is infectious and can transmit the disease upon transfusion. Experimental evidence indicates that blood from TSE-infected animals contains infectivity during both the incubation period and the clinical phase of the disease. However, many key questions remain and a number of assumptions await confirmation. We must therefore continue to expand our knowledge in the following areas:

- basic research into prion protein chemistry, conformational change and conversion mechanisms, the natural history of infection in humans, the distribution of infectivity in blood, genetic susceptibility and the risk of transmission from transfusion;
- epidemiological surveillance studies, including closer monitoring, targeted look-back studies, and prevalence studies in different donor populations, once an effective and reliable test becomes available;
- development of effective and reliable blood assays for *in vitro* detection of TSE infectivity in samples collected during the preclinical stage; the specificity of these assays will be critical;
- establishment of libraries of blood samples, particularly during the preclinical stage, from infected patients and animals in order to create effective panels for evaluating detection assays;
- improvement of prion removal and/or inactivation technologies without compromising product quality;
- development of simple and sensitive prion amplification or cell culture infectivity assays as research tools and

confirmatory tests for blood screening; establishing faster animal models using end-points other than death to quantify infectivity in blood and related samples may form an alternative here;

- establishment of uniform and practicable assessment criteria for the acceptability of prion removal procedures and blood screening assays; this will require participation from regulatory authorities;
- improvement of the current donor deferral policies to reduce the risk of transfusion but yet minimize the unnecessary deferral of qualified donors; and
- continue of promoting the avoidance of inappropriate blood transfusion.

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