CREUTZFELDT-JAKOB DISEASE

James W. Ironside
National Creutzfeldt-Jakob Disease Surveillance Unit
School of Molecular and Clinical Medicine, University of Edinburgh
Western General Hospital
United Kingdom

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Address for correspondence

Dr. James W. Ironside
Professor of Clinical Neuropathology and Honorary Consultant Neuropathologist
National Creutzfeldt-Jakob Disease Surveillance Unit
Division of Pathology, School of Molecular and Clinical Medicine
University of Edinburgh
Western General Hospital
EH4 2XU
United Kingdom
Tel: +44 131 537 1980
Fax : +44 131 343 1404

Email: james.ironside@ed.ac.uk

Table of Contents

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James W. Ironside

Introduction

Creutzfeldt-Jakob disease (CJD) is a member of a group of diseases known as **transmissible spongiform encephalopathies** (TSEs) or **prion** diseases [1, 2]. These are rare, fatal disorders that result in the progressive destruction of the nervous system. In animals, the best known examples are scrapie in sheep and bovine spongiform encephalopathy (BSE, also known as mad-cow disease) in cattle. Human prion diseases are a unique group of neurological disorders that includes CJD, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia.

Human prion diseases present with progressive neurological symptoms and result in death, usually after a period of several months. There is no cure, although widespread efforts at finding a form of treatment or **palliation** for prion diseases are underway.

Recently, an outbreak of a new form of CJD in humans (known as variant CJD) was linked to the consumption of BSE-infected meat. In the U.K. a handful of cases were later found to have resulted from blood transfusions from infected donors. The bleeding disorders community has therefore been concerned with the potential of transmission of vCJD through contaminated blood products. Though no such clinical cases have been identified so far, continued vigilance is required.

The Prion Hypothesis

What causes prion diseases?
The nature of the transmissible agent responsible for TSEs has been the subject of intense debate for many decades. Early studies on the agent that causes scrapie indicated that it was small in size and remarkably resistant to most conventional forms of decontamination (including fixation in formaldehyde, exposure to high temperatures, and surprisingly, to degradation by **enzymes** that destroy nucleic acids, the building blocks of DNA). Nucleic acids are found in all living organisms and are necessary for survival and reproduction. Despite many attempts, no one has yet found unequivocal evidence of a DNA component of the infectious agent in TSEs.

In 1982, Stanley Prusiner first proposed his **prion hypothesis,** which suggested that the transmissible agent responsible for TSEs was composed entirely of a protein (the "prion" protein) [3]. His theory was extremely controversial because it went against one of the fundamental laws of molecular biology: that all infectious agents had to be made up of DNA in order to replicate.

A few years later, the gene that codes for the prion protein was discovered in both normal and infected cells. Prusiner concluded that the transmissible agent was actually a misfolded version of the prion protein, which is normally found on the outer surface of cells. Prion diseases seem to result from a build-up of this abnormally folded protein in the cells of the central nervous system.

Molecular biology of prions
In humans, the prion protein gene (*PRNP*) is located on the short arm of chromosome 20. It codes for a 253-amino acid **precursor protein** [2, 4] that is normally cut, **glycosylated**, and anchored to the external cell membrane. The normal cellular form of the prion protein (PrP^C) is produced in many tissues, including skeletal muscle, **follicular dendritic cells**, **platelets**, and **endothelial cells**, but it is found at its highest levels in neurons (nerve cells) within the central nervous system [2].

PrP^C has a short **half-life**, dissolves in water, and is easily broken down by **proteolytic** enzymes [5]. The precise function of PrP^C is unknown, but a number of possibilities have been proposed, including roles in the transmission of nerve signals and protection against **oxidative stress**. In prion diseases, an abnormal form of PrP (designated PrP^{Sc}) accumulates in the central nervous system. This abnormal protein has an identical amino acid sequence to PrP^c, but it has a different three-dimensional shape that makes it relatively resistant to degradation by proteolytic enzymes [2] and insoluble in water. PrP^{Sc} is closely associated with infectivity in prion diseases and

appears to be the major (if not the sole) component of the transmissible agent.

Exactly where and how the misfolding of the prion protein occurs in prion diseases is not fully understood. It is clear, however, that the normal PrP^C must be present for prion diseases to develop. Genetically modified mice in which the prion gene was removed are resistant to prion infection [6].

Prion Detection and Decontamination

Detection
The only way to confirm a clinical diagnosis of prion disease in humans is to conduct a neuropathological examination of the brain, usually at autopsy. Brain **biopsy** is not widely performed because it often leads to a worsening of symptoms. On examination of the diseased brain, several characteristic changes are visible in the grey matter of the cerebrum and cerebellum, including a loss of nerve cells (neurons) that leaves several tiny holes in the brain, making it look like a sponge (hence the term "spongiform" encephalopathy). An increase in **astrocytes** and, in some cases, **amyloid plaques,** can also be detected [1].

The detection of PrP^{Sc} in the brain is an important diagnostic aid. However, most currently available **anti-PrP antibodies** recognize both the normal and abnormal forms of the prion protein. Therefore, any normal PrP^c in the brain sample must first be degraded by proteolysis, leaving only PrP^{Sc} as a partially cleaved protein (which can exist as di-, mono-, and unglycosylated forms) (Figure 1) [7]. The presence of protease-resistant PrP (PrP^{Sc}) provides a means of molecular diagnosis for prion diseases [2].

There is substantial interest in developing new techniques to detect PrP^{Sc} with greater sensitivity than current methods can achieve [8]. Some recently developed techniques are discussed below in terms of their potential to detect PrP^{Sc} in blood and blood products.

Decontamination
The methods conventionally used to destroy bacteria and viruses are largely ineffective for prions. Current recommendations for infection control in human prion diseases include the use of high-temperature

Figure 1
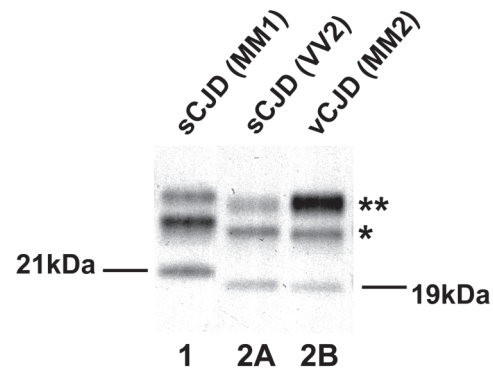
Western blot analysis of protease-resistant prion protein (PrP^{Sc}) in two cases of sporadic CJD (with MM and VV *PRNP* codon 129 genotypes) and one case of variant CJD. The size of the non-glycosylated (bottom) band is either 21kDa (termed type 1) or 19kDa (termed type 2). Diglycosylated PrP^{Sc} (**) predominates in the variant CJD and the pattern is termed type 2B to distinguish it from type 2 cases in which the monoglycosylated form (*) predominates (type 2A).

autoclaving, sodium hydroxide in molar concentrations, and concentrated formic acid [9]. None of these methods, however, is guaranteed to remove all prion infectivity, particularly from tissues such as the brain that are highly infectious. Furthermore, these methods can not be used to decontaminate blood or plasma products as they would render the products ineffective and/or unsafe. The need to develop a screening test for human prion diseases is a high priority in attempts to reduce the chances for person-to-person spread of disease.

Human Prion Diseases

Human prion diseases can be categorized as sporadic, genetic, or infectious (Table 1) [1, 2]. Sporadic cases occur in irregular, scattered, and unpredictable patterns. Genetically transmitted cases are passed down from parent to child, while infectious forms are acquired either from contaminated material (such as infected meat or blood) or from person to person contact.

Sporadic CJD
The most common human prion disease is the sporadic form of CJD. It has been found in all regions of the world with a remarkably uniform incidence of around 1.5 per million of the population per year

[10]. The causes of sporadic CJD are unknown, but a number of possibilities have been suggested, including an (unknown) environmental effect or random events that result in the spontaneous production of PrP^{Sc} in the brain [2, 10].

Table 1. Classification of human prion diseases

Sporadic: Sporadic Creutzfeldt-Jakob disease
           Sporadic fatal insomnia

Genetic: Familial Creutzfeldt-Jakob disease
           Fatal familial insomnia
           Gerstmann-Sträussler-Scheinker syndrome and variants

Infectious: Bovine source: Variant Creutzfeldt-Jakob disease
            Human source: Kuru
            Iatrogenic source: Creutzfeldt-Jakob disease

Genetics may play a role in the development of sporadic CJD. There is a naturally occurring genetic variation (**polymorphism)** at codon 129 in the *PRNP* gene [2, 4], which can code for either methionine (M) or valine (V). The majority of the normal Caucasian population is **heterozygous** (MV) at this codon (Table 2) [11]. Among cases of sporadic CJD, however, there is a predominance of **homozygotes**, particularly methionine homozygotes (MM) (Table 2). The biological significance of this observation is uncertain, but it does appear to indicate a genetic risk factor for sporadic CJD.

Sporadic CJD occurs across a wide age range, but the majority of cases occur in the 7th decade of life [10, 11]. Men and women are equally affected. Most patients present with a rapidly worsening dementia accompanied by other neurological symptoms, particularly **ataxia**, muscle spasms, visual abnormalities, and **pyramidal** and **extrapyramidal** signs. Unlike other more common dementing diseases such as Alzheimer's, sporadic CJD progresses rapidly and the majority of patients die within six months of disease onset [10, 11].

There have been a number of case control studies to investigate whether blood transfusion is a risk factor for sporadic CJD, and the evidence appears reassuring that this is not the case [12, 13]. Despite occasional claims that the blood of patients with sporadic CJD is infectious (reviewed by Brown [14]), neuropathological studies of individuals dying with hemophilia have not revealed any cases of undiagnosed or pre-clinical prion disease, and there is no epidemiological evidence to indicate so far that individuals receiving large amounts of blood products or blood transfusions are at increased risk of developing **iatrogenic** CJD [15, 16].

Genetic prion diseases
The inherited human prion diseases include the familial form of CJD, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia [1-4]. All of these are inherited as **autosomal dominant** disorders with a high degree of **penetrance**. Each is associated with pathogenic mutations in the *PRNP* gene (point mutations or octapeptide repeat region insertions) that appear to affect the stability of PrP^{C}, making it more likely to misfold into PrP^{Sc}. There are over 30 pathogenic *PRNP* gene mutations and insertions currently identified that are associated with a variety of characteristics and symptoms, encompassing a wide range of neuropsychiatric disorders [17]. Pathological studies in these rare

Table 2. Prion protein gene polymorphisms in the normal population and in prion diseases

	Prion protein gene codon 129 polymorphisms (%)		
	MM	**MV**	**VV**
Normal population	39	50	11
Sporadic CJD	71	13	16
Variant CJD	100	-	-

(M=methionine, V=valine)

disorders (that together account for only around 10% of all human prion diseases) are more limited than in sporadic CJD, but there is little evidence to indicate that infectivity or PrP^{Sc} is present outside the central nervous system [17].

Infectious prion diseases

The first infectious human prion disease to be identified was kuru, a disorder that reached epidemic proportions in the 1950s within the Fore population of Papua New Guinea. The disease was transmitted through ritualistic **endocannibalism** [18]. Since these practices were abandoned, the disorder has markedly declined in incidence, but a very small number of cases have been recorded over recent years. This suggests that the disease could have an **incubation period** of over 40 years [19].

More recent cases of iatrogenic transmission of CJD have occurred via medical treatments and surgical procedures, particularly following the administration of human **pituitary growth hormone** (used to stimulate growth in children who are lacking the hormone) and the use of human **dura mater** grafts (Table 3) [20, 21]. *PRNP* gene analysis in individuals who received contaminated human growth hormone and later developed iatrogenic CJD has also indicated a predominance of homozygotes at codon 129 (which in the U.K. is valine homozygotes, in contrast to sporadic CJD) [22]. Again, there is no convincing evidence that iatrogenic CJD has occurred by blood transfusion or in the recipients of blood products, including plasma products [13-16].

Variant CJD

In 1990, surveillance of CJD was resumed in the U.K. in order to investigate the possible effects of BSE ("mad cow disease") on humans. BSE occurred as an epidemic in U.K. cattle, with over 180,000 clinical cases identified since the 1980s, but it has since been estimated that as many as 3 million infected cattle may have entered the human food chain in the U.K. [23]. Smaller outbreaks of BSE have occurred in several other countries, particularly in Portugal, Spain, and Switzerland, with isolated cases in a more widespread distribution, including the U.S.A. and Japan. In 1996, a new form of human prion disease, now known as variant CJD (vCJD), was reported in a series of ten patients from the U.K. [24].

Convincing evidence for a link between BSE and vCJD came from the results of experimental studies in mice, which showed that the transmissible agent in vCJD has identical biological properties to the agent that causes BSE [25, 26]. vCJD is the only example of a human prion disease acquired from another species, and is therefore unique within the spectrum of human prion diseases (Table 1).

A case control study in the U.K. has indicated that humans were most likely exposed to BSE through the consumption of contaminated meat products, although other possibilities (such as occupational exposure) cannot be excluded [27]. As of March 2009, there had been 168 cases of vCJD in the U.K., with 44 additional cases in ten other countries

Table 3. Iatrogenic transmission of Creutzfeldt-Jakob disease

Source of infection	Number of reported cases	Incubation period (months)
Neurosurgical instruments	4	12-28
Intracerebral electrodes	2	16-20
Corneal graft	2	16-320
Dura mater graft	192	18-216
Human growth hormone	198	55-456
Human **gonadotropin**	5	144-192

Table 4. Variant CJD cases worldwide (as of March 2009)

Country	Number of primary cases	Number of secondary cases: transmission by blood transfusion
U.K.	165	3
France	23	-
Spain	5	-
Ireland	4	-
U.S.A.	3	-
Netherlands	3	-
Portugal	2	-
Canada	1	-
Italy	1	-
Japan	1	-
Saudi Arabia	1	-

(Table 4). Some of the cases outside the U.K. represent individuals who had previously visited or lived in the U.K. and therefore were presumably infected there, while the majority represent **endogenous** infections.

Clinical and molecular features of vCJD
There are several notable differences between the sporadic and variant forms of CJD. First, the average age at disease onset in vCJD is considerably younger than in patients with sporadic CJD. vCJD also has a unique clinical, neuropathological, and biochemical profile (Table 5) [28]. All patients affected with vCJD to date are methionine homozygotes at the codon 129 polymorphism in the *PRNP* gene, indicating a genetic susceptibility to vCJD in this subset of the population.

Another major difference between vCJD and other human prion diseases is the widespread presence of PrP^{Sc} and infectivity within lymphoid tissues, including the spleen, thymus, lymph nodes, and gut-associated lymphoid tissue (e.g. the appendix) (Figure 2) [29-32]. PrP^{Sc} accumulates in follicular dendritic cells within the **germinal centres** in these

tissues, and tonsil biopsy has been used as a diagnostic test in some living patients [21]. Studies with inbred mice have shown that spleen and tonsil tissue samples are infectious when injected directly into the brain (though the infectivity of these tissue samples is approximately 2-3 logs lower than brain tissue) [33].

PrP^{Sc} was not found in **buffy coat** samples of vCJD and injection of buffy coat from vCJD patients into the brain of susceptible mice failed to demonstrate infectivity [31, 33]. However, the sample numbers in these studies were small and the sensitivity of the techniques that were used may have been insufficient to detect low levels of PrP^{Sc} and infectivity. The presence of PrP^{Sc} and infectivity in lymphoid tissues in vCJD has led to the suggestion that circulating **lymphocytes** may carry infectivity in blood [34]. Studies on experimental models of prion diseases indicate that lymphoid tissues can become infected soon after exposure and may remain infectious throughout the incubation period [35].

The incidence of vCJD has declined in the U.K. since 1999-2000; however, a retrospective study of the

Table 5. Comparison of sporadic and variant Creutzfeldt-Jakob disease

	Sporadic CJD	Variant CJD
Mean age at death (years)	67	29
Mean duration of illness (months)	4	13
Psychiatric symptoms at onset	Rare	Common
Rapidly progressive dementia	Common	Rare
Sensory symptoms	Rare	Common
Electroencephalogram	Periodic triphasic Complexes in 66%	Periodic triphasic Complexes rarely in final illness
MRI brain scan	High signal in basal ganglia in 70%	High signal in the **pulvinar** nuclei in the thalamus in >90%
Cerebrospinal fluid 14.3.3 protein	Elevated in 90%	Elevated in 50%
Neuropathology	Spongiform change Amyloid plaques in 15%	Spongiform change Florid plaques in 100%
PrP^{Sc} distribution	Central nervous system	Central nervous system and lymphoid tissues
PrP^{Sc} isotype on Western blot	Several forms identified (types 1A and 2A)	A single form (type 2B) identified with a predominant diglycosylated band

prevalence of vCJD infection in the U.K., found an accumulation of disease-associated PrP in surgically resected appendix and tonsil tissues in three out of 12,674 samples [36]. This figure is higher than the current number of vCJD cases in the U.K. and would suggest that the cases of vCJD identified so far may represent only those with the shortest incubation periods. Another possible explanation is that BSE infection may not always result in clinical disease, but may produce a prolonged **asymptomatic carrier** state in certain individuals.

Infectivity of Blood in Prion Diseases

The potential for transmission of vCJD through contaminated blood and blood products is of particular concern for the bleeding disorders community. Though there have been no reported cases to date, the presence of infectivity in blood, coupled with the lack of a sensitive screening test, and the fact

that PrP^{Sc} is not affected by viral inactivation procedures used on blood products mean that continued vigilance is warranted.

Experimental studies
Studies in animal models (including both a hamster 263K scrapie model and a mouse-adapted vCJD model) have demonstrated that prion diseases can be transmitted through blood during both the incubation period and the **clinical phase** of the disease [37, 38]. In the hamster model, whole blood appears to contain around 10 infectious doses (ID)/mL. Most of this infectivity seems to be associated with white blood cells and not platelets; however, infectivity may also be present in the plasma and in red blood cells [37].

Recently, an experimental BSE model in sheep has been used to investigate infectivity in blood. A preliminary report from this study indicated transmission of BSE by transfusion of whole blood from one

Figure 2. Detection of PrP in lymphoid tissues in vCJD

A.

B.
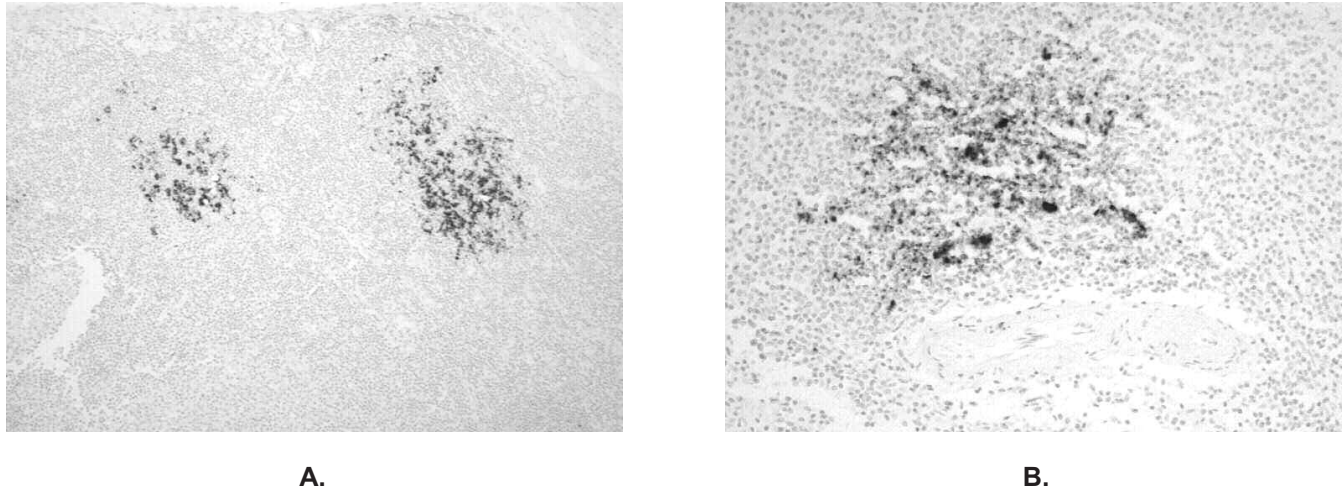
Immunocytochemistry for PrP in lymphoid tissues in vCJD shows staining of follicular dendritic cells and macrophages within germinal centres in the tonsil (A) and spleen (B). Anti-PrP antibody (KG9) with hematoxylin counterstain.

infected sheep in the pre-clinical stage of the disease [39]. Subsequent transfusions of whole blood from BSE-infected animals (during both the pre-clinical and clinical stages of infection) have resulted in a rate of infection of at least 36% [40]. Scrapie transmission by blood transfusion has also been investigated in this model, with a resulting transmission rate of at least 40%. Transmission was also reported in a sheep transfused with a relatively small volume of a buffy coat preparation made from blood taken from the donor at the clinical endpoint of disease [40]. This model has the potential to be used to study the distribution of infectivity in the different components of BSE and scrapie-infected sheep blood, and could also be used to study the effect of various interventions (such as **leucodepletion**) on infectivity in blood, blood components, and blood products.

Transmission of vCJD by blood transfusion
A collaborative study known as the Transfusion Medicine Epidemiology Review was established between the various national blood authorities in the U.K. and the National CJD Surveillance Unit to investigate the possibility of transmission of vCJD by blood transfusion. This study has so far identified three cases of vCJD in patients who received one unit of non-leucodepleted red blood cells from donors who were asymptomatic at the time of the donation but who died from vCJD years later [41, 42]. The recipients developed symptoms of vCJD five to eight years after the transfusions.

The clinical and neuropathological features of the illness in these three patients were closely similar to those of other cases of vCJD, and all three recipients were methionine homozygotes at codon 129 in the *PRNP* gene (as are all other patients with vCJD). In 2004, the U.K. National CJD Surveillance Unit reported the autopsy findings in an elderly patient who had received a transfusion of one unit of non-leucodepleted red blood cells from another asymptomatic donor who developed vCJD 18 months after donation [43]. The recipient never developed any symptoms of vCJD and died five years later of an unrelated illness. No pathological changes consistent with vCJD were found upon examination of the brain, and **immunohistochemistry** and **Western blot** investigations for PrP^{Sc} in the brain were negative. However, immunohistochemistry revealed evidence of PrP^{Sc} in the spleen and a cervical lymph node, but not in the tonsil or the appendix. Western blot analysis confirmed the presence of PrP^{Sc} in the spleen [43]. Unlike all clinical cases of vCJD, this transfusion recipient was heterozygous at codon 129 (methionine/ valine). This case raises a number of important questions, in particular the influence of the *PRNP* codon 129 **genotype** on the incubation period of the disease and the potential for other *PRNP* genotypes to be infected by the BSE agent.

The identification of vCJD infection in four individuals who received blood transfusions from vCJD-infected donors is highly unlikely to have occurred by chance, and indicates that blood is infectious in

asymptomatic individuals in the pre-clinical stage of the illness. As a result, a number of precautionary steps have been taken to reduce the likelihood of transmission from blood and blood products in the U.K. [44]. None of these are likely to remove all risks of vCJD transmission by blood transfusion, although it seems likely that leucodepletion is capable of reducing levels of infectivity in blood [45]. These findings have also renewed concerns that vCJD might be transmissible by blood products, including plasma products, since it is known that some of the donations from the asymptomatic donors who subsequently died from vCJD were used for plasma processing.

Potential for transmission of vCJD by plasma products

Several experimental prion disease models have demonstrated infectivity in blood or blood components during both the incubation period and the clinical phase of the disease (see Brown [14] for review). This has been shown in both animals with endogenous prion infectivity in blood, and in "spiking" studies, in which a sample of normal blood or plasma is "spiked" with highly infectious prion-infected tissue, usually brain. The latter method has also been used to study the effects of various processing steps used in the production of **plasma** components. However, spiking studies may not ultimately be very useful, since it seems unlikely that the physical and/or chemical properties of the abnormal prion protein in the brain (where it is found in large clusters) is necessarily the same as in blood.

A spiking study of normal human blood with tissue from a scrapie-infected hamster brain found the highest levels of infectivity in the cellular blood components (red and white blood cells, platelets), with lower levels in plasma, **cryoprecipitate**, and **Cohn fractions** I-III, and almost none in fractions IV and V [46]. In a mouse model of Gerstmann-Sträussler-Scheinker syndrome, endogenous infectivity at levels between 6-12 IU/mL was detected in the buffy coat, and was also occasionally present in plasma. Infectivity increased in the clinical phase of the illness in this model to around 100 IU/mL in buffy coat, 20 IU/mL in plasma, 2 IU/mL in cryoprecipitate, and less than 1 IU/mL in Cohn fractions IV and V [47]. Similar findings were subsequently reported in a mouse-adapted strain of vCJD [38].

The low levels of infectivity in the Cohn fractions IV and V in these studies suggest that the processing steps used in the manufacture of these plasma components, which include **alpha-1 antitrypsin** and **albumin**, may be effective in reducing prion infectivity. A number of process steps (including ethanol precipitation and ion exchange chromatography) have been reported to potentially reduce levels of PrP^{Sc} and, by implication, prion infectivity, during plasma fractionation of "spiked" blood. This would indicate that at least some plasma products (particularly **immunoglobulins** and albumin) are likely to be safe with regard to their potential to transmit prion diseases [48, 49].

In the U.K., a risk assessment of exposure to vCJD infectivity in blood and blood products was commissioned by the Department of Health [50]. Clotting factor concentrates VIII and IX were judged likely to carry sufficient levels of vCJD infectivity to warrant the implementation of public health measures in recipients to minimize the possible risk of onward transmission. Although it was recognized that many of the assumptions in this risk assessment were pessimistic, on the advice of the U.K. Haemophilia Centre Doctors' Organisation, all patients with bleeding disorders who had been treated with U.K.-sourced pooled factor concentrates between 1980 and 2001 were informed that they may be at an increased risk of infection with vCJD, and were required to take precautionary measures to prevent the possibility of secondary spread of infection, for example by surgical instrument [51]. The U.K. Haemophilia Society endorsed this approach.

Asymptomatic vCJD infection in a hemophilic patient in the U.K.

In February 2009, the Health Protection Agency in the U.K. announced the identification of a case of asymptomatic vCJD infection in a hemophilic patient in the U.K. [52]. The patient was part of a study investigating the prevalence of vCJD infection in people with bleeding disorders in the U.K., and had been entered into the study following his death from an unrelated cause. The patient had not shown any signs or symptoms of vCJD (or any other neurological disorder) during his life. Biochemical analysis of the spleen found evidence of PrP^{Sc} in a very localized distribution, while other tissues studied were negative. The patient was known to have received factor VIII prepared from a plasma pool that

included donations from an individual who subsequently died from vCJD. The investigations on this case are incomplete; the hemophilic patient had been a U.K. resident, and had also received several blood transfusions. Whether the biochemical finding in this case represents the result of primary exposure to BSE by the dietary route, i.e. the consumption of BSE-infected meat, or secondary infection via blood transfusion or from contaminated plasma products, is unclear. Further data are required to perform a statistical analysis of the relative likelihood of each of these possibilities. However, this case reinforces the need for vCJD surveillance in patients potentially exposed to vCJD from blood or plasma products.

Future Prospects

Many of the concerns over the possible iatrogenic transmission of vCJD by blood and plasma components could be reduced if a sensitive and specific screening test were available. As yet, such a test is not available, although a number of approaches are currently under investigation. Most of these are based on the detection of PrP^{Sc} in blood, which has been achieved in an experimental scrapie model using the **protein misfolding cyclical amplification** (PMCA) **assay** in both symptomatic and asymptomatic animals [53, 54]. The PMCA assay can be applied to human tissues to amplify PrP^{Sc} in both sporadic and vCJD, but there are some limitations that must be considered in the assay design [55, 56]. This technique has not yet been reported on human blood, but there is considerable interest in its application. The PMCA technique, however, is rather lengthy to be used for a screening test. The use of **peptides** sensitive to the conformational changes that characterize PrP^{Sc} may be more appropriate [57].

At present, no case of human prion disease transmission by plasma products has been reported. However, in view of the potentially lengthy incubation period of these diseases, it is premature to conclude that such a case will never arise, at least in the case of vCJD. Surveillance for human prion diseases, particularly in countries where cases of vCJD have been recorded, is required in order to assess this possibility and to investigate any potential risk factors in future cases. The potential use of a screening test for vCJD (since it seems possible that one may become available in the foreseeable future)

should also be considered, including the potential need for testing of individuals at the time of blood donation. The need for **informed consent** for such tests (particularly in individuals) is currently under debate, since the present lack of any preventative or therapeutic treatment for prion diseases questions the potential benefits for the individual undergoing testing. The development of such a treatment from the wide range of substances currently under investigation is not inconceivable [58, 59], in which case the cost/benefit analysis and ethics for screening will need to be re-evaluated.

Conclusion

vCJD is a new form of human prion disease that is most likely caused by exposure to the BSE agent in the diet. Infectivity of vCJD in the body, including in the blood, is more widespread than with other forms of CJD. To date, four cases of vCJD infection have been recorded following blood transfusion from asymptomatic donors who subsequently died from vCJD. The risks of transmission through plasma products is likely to be low, but at present this possibility cannot be entirely discounted in view of the lengthy incubation periods associated with human prion diseases. Continued surveillance is required to investigate this possibility. ❦

Glossary

albumin: protein found in the blood that helps maintain the volume of blood in the body

alpha-1 antitrypsin: enzyme that protects proteins from being broken down by proteases

amyloid plaque: an insoluble accumulation of an abnormal protein found in the space between nerve cells in the brain. Amyloid plaques are a characteristic feature of certain forms of human prion diseases, including vCJD and the Gerstmann-Sträussler-Scheinker syndrome

anti-PrP antibodies: antibodies are proteins produced by the body's immune system to recognize and neutralize foreign particles. Antibodies can also be produced in the laboratory to react selectively against a specific molecule, in this case the prion protein (PrP)

astrocytes: cells that support, provide nutrients to, and help repair nerve cells

asymptomatic carrier: person who has contracted an infectious disease or who carries a gene that causes disease, but who displays no symptoms. Although unaffected by the disease themselves, carriers can potentially transmit it to others

ataxia: inability to coordinate voluntary muscle movements. Signs include clumsiness, inaccuracy, or instability

autoclaving: sterilization method in which materials are subjected to high temperatures and steam

autosomal dominant: 'autosomal' refers to any chromosome other than the sex chromosomes (X and Y). In genetics, dominance is when one particular form of a gene dominates over other forms, and is always expressed when present in an individual. A disease inherited as an autosomal dominant disorder will develop in any person who carries even a single copy of the disease-causing gene

biopsy: the removal and examination of a sample of cells or tissue for diagnostic purposes

buffy coat: portion of a blood sample that that contains most of the white blood cells and platelets

clinical phase: stage of a disease in which symptoms are present and supportive therapy or treatment is necessary

Cohn fractions: the Cohn process is a method used to separate the different components of blood. Each of the five fractions separates out from the rest under specific laboratory conditions

cryoprecipitate: component of blood obtained by freezing and thawing plasma. Cryoprecipitate is used to treat diseases, such as hemophilia, in which a given clotting factor is missing or following an operation or trauma

dura mater: the outermost, toughest and most fibrous of the three membranes covering the brain and spinal cord

endocannibalism: practice of eating dead members of one's own culture, tribe or social group

endogenous: produced, originating or growing from within

endothelial cells: cells that make up the inside lining of a tissue or organ

enzyme: molecule that speeds up a particular biochemical reaction in an organism

extrapyramidal: subdivision of the nervous system that regroups the pathways outside the **pyramidal tract**. The extrapyramidal system is involved in the coordination of movement. Extrapyramidal symptoms are disturbances of facial or body movements, such as a shuffling walk or sudden stiffness

follicular dendritic cells: cells of the immune system found in small cavities (follicles) of lymph nodes. Lymph nodes are found throughout the body, and act as filters or traps for foreign particles, including bacteria and viruses

genotype: the genetic makeup of an organism, or of a specific characteristic, such as hair colour

germinal centres: areas within lymph nodes where immune cells (B lymphocytes) divide rapidly

glycosylated: biochemical modification of a substance (usually a protein) by the addition of sugar molecules

gonadotropin: hormone that acts on the gonads (ovaries in the female, testes in the male) to promote the production of sex hormones and either eggs or sperm

half-life: the time required for half the amount of a substance to be eliminated from the body

heterozygous: each individual has two copies, or alleles, of each gene: one from the mother and one from the father. If both copies are different, then the individual is said to be heterozygous for that gene

homozygote: individual in whom both copies, or alleles, of a particular gene (one inherited from the mother and one from the father) are identical

iatrogenic: resulting from the action of a physician or surgeon or from a medical treatment or diagnostic procedure

immunoglobulins: also called antibodies. Antibodies are proteins produced by the body's immune system to recognize and neutralize foreign particles

immunohistochemistry: laboratory technique of using labelled antibodies to look for a specific protein in a tissue section (examined under the microscope). *See also* **anti-PrP antibodies**

incubation period: the period of time between infection and the appearance of symptoms of a disease

informed consent: agreement by a patient to undergo a medical or surgical treatment or to participate in an experiment after he/she understands the risks involved

leucodepletion: process by which white blood cells are removed from a sample of blood

lymphocyte: type of white blood cell that is an important component of the immune system

oxidative stress: the accumulation of destructive molecules called free radicals that can damage human cells, including brain cells

palliation: the alleviation of a disease's symptoms without a cure; temporary relief

penetrance: proportion of individuals with a mutation causing a particular disorder who actually develop the disorder. A condition is said to have complete penetrance if symptoms develop in all individuals who have the disease-causing mutation

peptide: small protein

pituitary growth hormone: hormone produced by the pituitary gland that promotes growth in humans. The pituitary gland is pea-sized structure located at the base of the brain

plasma: the clear, liquid part of blood that contains no cells but in which the clotting factors are found

platelets: small disk-shaped particles found in blood that play an important role in the formation of blood clots

polymorphism: common variation in the sequence of DNA among individuals

precursor protein: protein that is modified by one or several biochemical reactions

prion: a protein particle that has been implicated as the cause of various transmissible neurodegenerative diseases (such as scrapie, Creutzfeldt-Jakob disease, and bovine spongiform encephalopathy). It is a disease-causing form of a naturally occurring protein that is both less soluble and more resistant to enzyme degradation

prion hypothesis: scientific theory developed by Stanley Prusiner which states that a misfolded protein, lacking any DNA component, is the causative agent for transmissible spongiform encephalopathies

protein misfolding cyclical amplification assay: technology used to "amplify" or make copies of misfolded proteins in a sample of blood, making it easier to detect and isolate

proteolytic: pertaining to the directed degradation or breaking down of proteins by cellular enzymes called proteases

pulvinar: the back end of the thalamus, a structure in the brain that acts as a relay station for sensory (especially pain and pleasure) information

pyramidal: subdivision of the nervous system. The pyramidal tract is a collection of nerve fibres that travel between the cerebral cortex of the brain and the spinal cord

transmissible spongiform encephalopathies (TSEs): a group of rapidly progressive, fatal neurological disorders affecting both humans and animals that are caused by infection with prions

Western blot: a laboratory test used to identify and separate proteins of different sizes in a complex mixture, such as a blood sample

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References

1. Ironside JW. Creutzfeldt-Jakob disease. *Brain Pathol* 1996; 6: 379-88.

2. Prusiner SB. Prions. *Proc Nat Acad Sci USA* 1998; 95: 13363-83.

3. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982; 216: 136-44.

4. Goldfarb LG, Brown P, Cervenakova L, Gajdusek DC. Molecular genetic studies of Creutzfeldt-Jakob disease. *Mol Neurobiol* 1994; 8: 89-97.

5. Borchelt DR, Scott M, Taraboulos A, Stahl N, Prusiner SB. Scrapie and cellular prion proteins differ in their kinetics of syntheses and topology in cultured cells. *J Cell Biol* 1990; 110: 743-52.

6. Weissmann C, Büeler H, Fischer M et al. PrP-deficient mice are resistant to scrapie. *Ann N Y Acad Sci* 1994; 724: 235-40.

7. Ironside JW, Head MW, Bell JE, McCardle L, Will RG. Laboratory diagnosis of variant Creutzfeldt-Jakob disease. *Histopathology* 2000; 37: 1-9.

8. MacGregor I. Prion protein and developments in its detection. *Transfus Med* 2001; 11: 3-14.

9. WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies. *World Health Organisation* 2000, Geneva.

10. Brown P, Cathala F, Raubertas RF, Gajdusek DC, Castaigne P. The epidemiology of Creutzfeldt-Jakob disease: conclusion of a 15-year investigation in France and review of the world literature. *Neurology* 1987; 37: 895-904.

11. Alperovitch A, Zerr I, Pocchiari M et al. Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. *Lancet* 1999; 353: 1673-4.

12. Kitamoto T, Mohri S, Tateishi J. Organ distribution of proteinase-resistant prion protein in humans and mice with Creutzfeldt-Jakob disease. *J Gen Virol* 1989; 70: 3371-9.

13. Esmonde TFG, Will RG, Slattery JM et al. Creutzfeldt-Jakob disease and blood transfusion. *Lancet* 1993; 341: 205-7.

14. Brown P. Can Creutzfeldt-Jakob disease be transmitted by transfusion? *Curr Opin Haematol* 1995; 2: 472-7.

15. Evatt B, Austin H, Barnhart E, Schonberger L, Sharer L, Jones R, DeArmond S. Surveillance for Creutzfeldt-Jakob disease in patients with haemophilia. *Transfusion* 1998; 38: 817-20.

16. Lee CA, Ironside JW, Bell JE et al. Retrospective neuropathological review of prion disease in UK haemophilic patients. *Thromb Haemost* 1998; 80: 909-11.

17. Kovacs GS, Trabattoni G, Hainfellner JA, Ironside JW, Knight RS, Budka H. Mutations of the prion protein gene: phenotypic spectrum. *J Neurol* 2002; 249: 1156-82.

18. Gajdusek DC, Vigas V. Degenerative disease of the central nervous system in New Guinea: the endemic occurrence of 'kuru' in the native population. *New Engl J Med* 1957; 257: 974-8.

19. Collinge J, Whitfield J, McKintosh E et al. Kuru in the 21st century – an acquired human prion disease with very long incubation periods. *Lancet* 2006; 367: 2068-74.

20. Brown P, Brandel JP, Preece M, Sato T. Iatrogenic Creutzfeldt-Jakob disease: the waning of an era. *Neurology* 2006; 67: 389-93.

21. Brown P, Preece M, Brandel JP et al. Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* 2000; 55: 1075-81.

22. Collinge J, Palmer MS, Dryden AJ. Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. *Lancet* 1991; 337: 1441-2.

23. Smith PG, Bradley R. Bovine spongiform encephalopathy (BSE) and its epidemiology. *Br Med Bull* 66:185-98.

24. Will RG, Ironside JW, Zeidler M et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996; 347: 921-5.

25. Bruce ME, Will RG, Ironside JW et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 1997; 389: 498-501.

26. Scott MR, Will R, Ironside J et al. Compelling transgenetic evidence for transmission of bovine

spongiform encephalopathy prions to humans. *Proc Natl Acad Sci USA* 1999; 96: 15137-42.

27. Ward HJ, Everingtom D, Cousens SN et al. Risk factors for variant Creutzfeldt-Jakob disease: a case-control study. *Ann Neurol* 2006; 59: 111-20.

28. Ironside JW, McCardle L, Horsburgh A, Lim Z, Head MW. Pathological diagnosis of variant Creutzfeldt-Jakob disease. *APMIS* 2002; 110: 79-87.

29. Hill AF, Zeidler M, Ironside JW, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet* 1997; 349: 99-100.

30. Hill AF, Butterworth RJ, Joiner S et al. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet* 1999; 353: 183-9.

31. Wadsworth JD, Joiner S, Hill AF et al. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001; 358: 171-80.

32. Hilton DA, Fathers E, Edwards P, Ironside JW, Zajicek J. Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. *Lancet* 1998; 352: 703-4.

33. Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. *Lancet* 2001; 358: 208-9.

34. Turner ML, Ironside JW. New-variant Creutzfeldt-Jakob disease: the risk of transmission by blood transfusion. *Blood Rev* 1998; 12: 255-68.

35. Weissmann C, Raeber AJ, Montrasio F et al. Prions and the lymphoreticular system. *Philos Trans T Soc Lond B Biol Sci* 2001; 356: 177-84.

36. Hilton DA, Ghani AC, Conyers L et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004; 203: 733-9.

37. Holada K, Vostal JG, Theisen PW et al. Scrapie infectivity in hamster blood is not associated with platelets. *J Virol* 2002; 76: 4649-50.

38. Cervenakova L, Yakovleva O, McKenzie C et al. Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. *Transfusion* 2003; 43: 1687-94.

39. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion in sheep. *Lancet* 2000; 356: 999-1000.

40. Houston F, McCutcheon S, Goldmann W et al. Prion diseases are efficiently transmitted by blood transfusion in sheep. *Blood* 2008; 112: 4739-45.

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

42. Wroe SJ, Pal S, Siddique D et al. Clinical presentation and pre-mortem diagnosis of Creutzfeldt-Jakob disease associated with blood transfusion. *Lancet* 2006; 368: 2061-7.

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

44. Ludlam CA, Turner ML. Managing the risk of transmission of variant Creutzfeldt-Jakob disease by blood products. *Brit J Haematol* 132, 13-24.

45. Gregori L, McCombie N, Palmar D et al. Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. *Lancet* 2004; 364: 529-31.

46. Brown P, Rohwer RG, Dunstan BX et al. The distribution of infectivity in blood components and plasma derivates in experimental models of transmissible spongiform encephalopathy. *Transfusion* 1998; 38: 810-6.

47. Brown P, Cervenáková L, McShane LM et al. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. *Transfusion* 1999; 39: 1169-78.

48. Stenland CJ, Lee DC, Petteway SR, Rubenstein R. Partitioning of human and sheep forms of the pathogenic prion protein during the purification of therapeutic proteins from human plasma. *Transfusion* 2002; 42: 1497-1500.

49. Gregori L. Maring JA, MacAuley C et al. Partitioning of TSE infectivity during ethanol fractionation of human plasma. *Biologicals* 2004; 32: 1-10.

50. DH risk assessment for vCJD infectivity in blood products. *Det Norske Veritas* 2003 http://www.dnv.com/binaries/vCJD_Update_Report_tcm4-74414.pdf.

51. Variant CJD and blood products. Health Protection Agency 2004 http://www.hpa.org.uk/webw/HPAweb&HPA webStandard/HPAweb_C/1195733818681?p=11 91942152861.

52. Health Protection Agency. Variant CJD and plasma products. Press Release 2009. http://www.hpa.org.uk/webw/HPAweb&HPA webStandard/HPAweb_C/1195733818681?p=12 25960597236.

53. Castilla J, Saá P, Soto C. Detection of prions in blood. *Nat Med* 2005; 11: 982-5.

54. Saá P, Castilla J, Soto C. Presymptomatic detection of prions in blood. *Science* 2006; 313: 92-4.

55. Jones M, Peden AH, Prowse CV et al. In vitro amplification and detection of variant Creutzfeldt-Jakob disease PrP^{Sc}. *J Pathol* 2007; 213: 21-6.

56. Jones M, Peden AH, Yull H, Wight D, Bishop MT et al. Human platelets as a substrate source for the in vitro amplification of the abnormal prion protein (PrP) associated with variant Creutzfeldt-Jakob disease. *Transfusion* 2008 *in press*.

57. Pan T, Sethi J, Nelsen C et al. Detection of misfolded prion protein in blood with conformationally sensitive peptides. *Transfusion* 2007; 47: 1418-25.

58. Head MW, Ironside JW. Inhibition of prion-protein conversion: a therapeutic tool? *Trends Microbiol* 2000; 8: 6-8.

59. Caughey B, Caughey WS, Kocisko DA et al. Prions and transmissible spongiform encephalopathy (TSE) chemotherapies: A common mechanisms for anti-TSE compounds? *Acc Chem Res* 2006; 39 (9): 646-53.

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