is uncertain and it also currently remains unclear whether this could be translated into the setting of human clinical and preclinical disease and whether an appropriate differential exists between patients incubating variant CJD and normal individuals.

Infectivity has not thus far been detected in the peripheral blood of patients with clinical variant CJD by intracerebral inoculation into rodents despite the evidence of clinical transmission, reflecting the limitations of infectivity bioassays due to the species barrier and the small amounts of blood inoculated.

A central difficulty in the development of molecular assays is the differentiation of PrP^{Sc} from PrP^{c} (Minor, 2004). There are currently no monoclonal antibodies or other reagents of sufficient analytical specificity to differentiate between the normal and abnormal isoforms. Most assays therefore depend on differential physicochemical characteristics, such as resistance to proteinase-K digestion or display of additional or novel PrP epitopes following treatment with chaotropic agents, such as guanidine hydrochloride. The level of sensitivity required is challenging. Brown *et al* (1999) has estimated that in the order 1 pg of PrP^{Sc}/ml may be present in the peripheral blood of individuals in the pre- or subclinical phases of disease, in the context of around 100 ng/ml of PrP^{c}, i.e. a ratio of 1 PrP^{Sc} molecule:1 million PrP^{c} molecules. There are also significant challenges in validating such assays. This would normally be undertaken using samples from individuals with the disease in question. However, there are very few patients alive at any one time with variant CJD and large amounts of blood cannot be drawn for ethical reasons. As it is not currently possible to determine who may, or may not, be incubating the disease, the assays will therefore need be validated on brain homogenate-spiked human blood or animal endogenous infectivity samples posing questions around the extrapolation of the data to the human setting. Finally it should be borne in mind that it will not be possible to determine which of the donors with positive assays are actually incubating variant CJD and which of these are likely to go on to develop clinical disease. There is no treatment available at the present time to offer such individuals. There is concern, therefore, over the number of donors who may need to be deferred due to positive assay results and the potential impact of the introduction of such assays on the willingness of donors to donate (Blajchman *et al*, 2004; McCullough *et al*, 2004).

Blood component processing

In October 1997, the UK Spongiform Encephalopathy Advisory Committee advised that universal leucodepletion be considered. The UK Departments of Health commissioned an independent risk assessment by Det Norske Veritas Consulting (DNV) and asked the Blood Services to consider the feasibility (Comer & Spouge, 1999). Implementation was recommended in July 1998 and completed by the autumn of 1999 (Department of Health, 1998a,b). The measure was predicated on studies suggesting that B lymphocytes were likely to be involved in the initial phases of disease and that leucocytes were an important locus of infectivity in the peripheral blood. Subsequently, it has become apparent that in animal studies leucodepletion does not reduce infectivity in plasma and is likely to reduce the prion concentration in blood by only about 40% (Prowse & Bailey, 2000; Gregori *et al*, 2004; St Romaine *et al*, 2004). Universal leucodepletion is also considered to offer a number of additional benefits, e.g. reduction in transmission of cell-based viruses such as cytomegalovirus and human T-cell lymphotropic virus, rates of alloimmunisation, immunomodulatory effects and transfusion-mediated graft *versus* host disease (Roddie *et al*, 2000)

Other approaches under consideration in the UK to reduce infection risk include the greater use of apheresis platelets from a single donor (rather than a pool from four individual donors), extension of imported fresh frozen plasma and cryoprecipitate to all patients under the age of 16 years and further reduction in residual plasma in red cell and platelet concentrates.

Two companies are developing filtration devices, which may reduce the prion concentration in blood by several orders of magnitude. Such a reduction could significantly reduce the likelihood of transmission of variant CJD from a donor with sub- or preclinical disease. However, validation is likely to pose a significant challenge as studies cannot be carried out on naturally infected human peripheral blood and data will therefore have to be extrapolated from studies using homogenised infected brain spikes in human blood and endogenously infected rodent blood, raising issues around the relevance of these models.

Plasma product manufacture

In 1997, the Committee for Proprietary Medicinal Products (Committee for Propriety Medicinal Products, 1998), as the licensing authority, recommended recall of two batches of in date, factor VIII concentrate that had been manufactured from a plasma pool containing donations from two patients who had subsequently developed variant CJD. The UK Haemophilia Centre Doctors Organisation (UKHCDO) in November 1997 recommended that as 'variant CJD occurs almost exclusively in the UK, it is likely that any risk of transmission would be reduced by using concentrate prepared from blood donor plasma collected in other countries, e.g. USA, where there are no cases of variant CJD or BSE' (Ludlam, 1997). The UK government was keen to try to evaluate the risk of blood transmission of variant CJD so that other rational safety measures could be taken and, in 2004, the original DNV risk assessment was reviewed using further estimates of quantitative risk that had emerged from animal studies and fresh guidance offered.

Although prions are highly resistant to physical and chemical degradation and methods for their inactivation would be too severe to be used on plasma products, their physicochemical features suggest that they will partition selectively during the

plasma fractionation process (Foster, 1999). Studies with blood from endogenously infected animals (Brown *et al*, 1998; Foster, 2004) and blood spiked with high-titre brain homogenates (Foster *et al*, 2000, 2004; Tateishi *et al*, 2001; Reichl *et al*, 2002; Stenland *et al*, 2002; Vey *et al*, 2002), suggest that a number of steps in existing plasma fractionation processes should contribute individually to reduction in infectivity, including cryoprecipitation and cold ethanol fractionation, depth filtration, adsorption chromatography and nanofiltration. Some of these steps have also been studied in sequence, where it has been shown that, in general, the overall degree of prion removal exceeds that of any one individual step but is less than the sum of the individual steps (Foster, 2004).

Other measures

For the past 5 years the UK Transfusion Services have had an active policy of trying to optimise the use of all blood and blood products. An important component of this policy has been to ensure the appropriate use of red cell concentrates. The aim has been to prevent unnecessary red cell use as exemplified by Sirchia *et al* (1994). Such a policy not only reduces the risk of all transfusion-transmitted infections to each individual patient but it allows more patients to be treated with a scarce red cell resource.

Non-blood transfusion related strategies to prevent secondary spread of variant CJD horizontally in population

Between 1996 and 2004 several attempts were made to assess the risk of horizontal spread of variant CJD transmission by mechanisms other than blood products and make rational recommendations on appropriate safety measures (Bird, 2004). There has been concern about transmission in health care settings by invasive medical and surgical procedures. The second 2004 DNV risk assessment was informed by animal studies, which provided some measure of risk related to prion load in the inoculum. The aim was to try and identify the patients and procedures for which specific safety precautions should be instituted. Clearly some level of precaution was appropriate for patients who had clinical variant CJD, but for what other groups of individuals should precautions be taken? It was proposed that precautions should be taken for individuals who could be identified as having more than a 1% risk of exposure to an infectious dose of variant CJD prions (two ID_{50} extrapolated from experimental rodent studies).

The UK CJD Incidents Panel and Health Protection Agency offered advice based on the 2004 DNV risk assessment in relation to recipients of blood components and plasma products. Precautions were to be taken with all identified recipients of fresh blood components from donors who went on to develop variant CJD. For those who received fractionated plasma products, the risk from each was calculated on a product-by-product basis, dependent on the size of the donor pool, detail of the manufacturing process, and the dose of product that would give a 1% risk of exposure to an infectious dose (as defined above) was estimated. The products were divided into three groups based on the assessed risk. Those that were considered to pose a high risk were factors VIII/IX and antithrombin concentrates, where less than one injection of a therapeutic dose for an adult would exceed the risk threshold. Products in the medium risk group were those where the risk threshold would be exceeded if several or more treatments were given and included intravenous immunoglobulin and high doses of albumin. The low risk group consisted of products where very high doses, far in excess of those used in normal medical practice would be required to exceed the risk threshold, e.g. albumin used as an excipient in other products, intramuscular immunoglobulin.

Having defined the threshold dose of 'implicated' product it was necessary to identify which patients were likely to have received such a dose. For those with haemophilia and antithrombin deficiency, it would have been possible in principle to have identified all those patients known to have received implicated concentrates. But this was likely to represent a significant proportion of all UK haemophiliacs as, by September 2004, 16 batches of factor VIII and eight batches of factor IX were implicated and furthermore, it is likely that more batches used in treatment several years ago will become implicated as further former blood donors develop variant CJD in the future. It was therefore decided to use a 'population' approach and consider all haemophiliacs who had received clotting factor concentrate manufactured from UK plasma between 1980 (the beginning of the BSE epidemic) and 2001 (the expiry date of the last batch of product prepared from UK plasma) as being 'at risk of variant CJD for public health purposes'. Such a policy strongly advocated by UKHCDO, was seen as the simplest and least threatening way to categorise those for whom extra precautions would need to be taken for certain invasive procedures. For other groups, e.g. those with immunodeficiency, patients are being reviewed individually and a decision made as to whether they would fall into the 'at additional risk of exposure to variant CJD for public health purposes' category (Hewitt, 2004).

For those considered to in the 'at additional risk of exposure to variant CJD for public health measures' group, either on the basis of population or individual assessment, the arrangements to prevent horizontal transmission have been laid out by the Advisory Committee on Dangerous Pathogens (http://www.hpa.org.uk/infections/topics_az/cjd/blood_products.htm). In such individuals CNS tissue constitutes a high risk of tissue infectivity and therefore potential contamination of surgical instruments. Surgery on lymphoid tissue or olfactory epithelium and the anterior chamber of the eye, e.g. cataract surgery, involved tissue of medium risk infectivity. Instruments for all these procedures should either be disposable or 'quarantined' after surgery and not reused. It has been suggested that some of these could profitably be used for research studies into decon-

tamination techniques. All other surgeries, including dental and orthopaedic, were not considered to pose a significant risk of contaminating instruments with prions as the tissues were considered at low risk of infectivity and therefore no special precautions were advised.

With the publication of the primate study (Herzog *et al*, 2004), in which, following infection of Macaques with BSE prion both orally and intravenously, PrP^{Sc} was clearly demonstrated in the gut subepithelial neural plexuses as well as Payer's patches, it became clear that endoscopic biopsies of the gut mucosa could potentially contaminate the biopsy forceps and its channel in the instrument with PrP^{Sc}. Whilst the current recommendation is that endoscopes used for non-invasive procedures be cleaned and reused in the normal way, those used for invasive procedures, e.g. colonic biopsies, should be 'quarantined' and not reused. This has had major financial implications for hospitals.

Concluding remarks

Management of the risk of transmission of variant CJD and indeed, other prion diseases by blood and plasma products remains highly problematic (Wilson & Ricketts, 2004a,b). Although the relatively small and falling number of clinical cases in the UK is reassuring, data indicating that up to 90% of infected individuals may sustain long-term preclinical or subclinical disease and that most such individuals are likely to be currently in the 20–40 years age group suggests a significant pool of potentially infectious blood donors. Blood donor selection criteria are a blunt instrument for risk management and current measures, such as universal leucodepletion, seem likely to be only of limited efficacy. Blood donor screening assays and prion reduction filters offer a better chance of control, but much of the validation will need to be based on animal experimentation, the extrapolation of which to the human setting is problematic. Most new risk reduction measures are likely to be highly expensive and engender the possibility of alternative risks, including critical blood shortages. In this context, it is of increasing importance that health services work to ensure prescription of blood products only where they are required (Hart *et al*, 2004; McClelland & Contreras, 2005).

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Vaccines, Blood & Biologics

Questions and Answers on "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products"

Why do we recommend new blood donor deferrals for possible exposure to BSE and vCJD?

FDA is taking this step as a prudent measure to assure the safety of the blood supply by further reducing the theoretical risk from vCJD. In 1999, we recommended the first donor deferral for people who may have been exposed to the vCJD agent, which is believed to be the same as the agent of bovine spongiform encephalopathy (BSE, or "mad cow" disease). We recommended deferral of donors who resided in the United Kingdom (U.K.) for 6 months or more between 1980 and 1996. At this time, we are recommending new blood donor deferrals for possible exposure to BSE and vCJD for the following reasons:

1. Since 1999, the rate of vCJD cases in the U.K. has been on the rise.
2. Significant exposures to potentially contaminated U.K. beef occurred in France and cases of vCJD have appeared in France.
3. Significant exposures to potentially contaminated U.K. beef occurred at U.S. military bases in Europe
4. In Europe, outside the U.K., the BSE epidemic has been increasing.
5. Particularly in the U.K., transfusion recipients may have been exposed to donors already infected with vCJD.

What are the new donor deferrals for possible exposure to vCJD?

1. Residence in the U.K. for 3 months or more, between 1980 and 1996.

Rationale: The U.K. has experienced the largest epidemic of BSE, and also has the largest number of cases of vCJD (over 100). However, in 1996, the U.K. instituted and enforced rules to prevent contaminated cattle from entering the human food chain (www.defra.gov.uk/animalh/bse/public-health/public-health-index.html). Due to these effective food chain protections, the risk of exposure to the BSE agent has been greatly reduced. For this reason, the donor deferral extends only through 1996.

2. Military personnel (current and former), and their dependents, who spent time in military bases in northern Europe 1980-1990, or southern Europe (1980-1996), for 6 months or more.