Transmission of prion diseases by blood transfusion

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Attempts to detect infectivity in the blood of humans and animals affected with transmissible spongiform encephalopathies (TSEs or prion diseases) have often been inconclusive because of the limitations of cross-species bioassays and the small volumes of blood that can be injected by the intracerebral route. A model has been developed for the experimental study of TSE transmission by blood transfusion using sheep experimentally infected with bovine spongiform encephalopathy (BSE) or natural scrapie as donors and susceptible scrapie-free sheep as recipients. Donors and recipients of the same species greatly increase the sensitivity of the bioassay and in sheep large volumes of blood can be injected by the intravenous (i.v.) route. Transmission of BSE to a single animal using this approach was reported recently. This study confirms this result with a second transmission of BSE and four new cases of transmission of natural scrapie. Positive transmissions occurred with blood taken at pre-clinical and clinical stages of infection. Initial studies indicate that following such infection by the i.v. route, deposition of the abnormal prion protein isoform, PrPSc, in peripheral tissues may be much more limited than is seen following oral infection. These results confirm the risks of TSE infection via blood products and suggest that the measures taken to restrict the use of blood in the UK have been fully justified.

Introduction

Creutzfeldt–Jakob disease (CJD) is one of a group of related diseases known as prion diseases or transmissible spongiform encephalopathies (TSEs), a group that also includes scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle. A new variant of CJD (vCJD) in human beings in the UK (Will et al., 1996) is thought to have been the result of infection with the same agent that causes BSE in cattle (Bruce et al., 1997). The numbers of vCJD-infected people remain unknown, although, to date, over 100 clinical cases have been recorded in the UK. Amongst many sources of concern, one major question relates to the safety of blood transfusions and blood products – especially when inadvertently sourced from individuals during the long pre-clinical phase of vCJD, a time at which these individuals may act as asymptomatic carriers of the infectious agent. There is no epidemiological evidence to indicate that iatrogenic CJD has ever occurred via blood or blood products but vCJD is a new disease with a different pathogenesis and may present different risks.

The TSE disease-associated form of the prion protein (PrPSc) of the neuronal glycoprotein PrPC is often used as a marker for infectivity. Using a sensitive Western blotting technique, no PrPSc was detected in the buffy coat from one vCJD patient (Wadsworth et al., 2001). Although a novel method for detection of PrPSc in scrapie sheep blood has been described (Schmerr et al., 1997), the study was limited by a low number of samples and the technique requires further validation. Other investigators using the more conventional method of immunocytochemistry failed to demonstrate PrPSc in peripheral blood leucocytes of scrapie-infected sheep (Herrmann et al., 2002).

An alternative to PrPSc detection is direct bioassay of infectivity by inoculation of material into hosts of the same or different species. In laboratory rodents experimentally infected with TSE, a number of investigators have demonstrated infectivity in blood and blood components during the pre-clinical and clinical phases of infection (Brown et al., 1998; Diringer, 1984; Manuelidis et al., 1978). However, infectivity has not been isolated, so far, from blood components of natural animal hosts of TSEs (Hadlow et al., 1982; Marsh et al., 1973). Isolated reports of transmission of CJD to laboratory rodents by whole blood or buffy coat from human patients have been questioned for a variety of reasons (Brown, 1995). A large-scale study conducted by the National Institutes of Health (1998) found no evidence of transmission of CJD to laboratory rodents by blood or blood products. Unfortunately, the extensive analysis of blood products was not pursued with the same intensity as the analysis of brain tissue.

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failed to demonstrate infectivity in blood from 13 patients with CJD, using either highly susceptible primates or rodents as bioassay hosts (Brown et al., 1994). With vCJD, no infectivity was detected in blood from two patients using mouse bioassays (Bruce et al., 2001). Many of these studies could have failed to reveal low levels of infectivity in blood because of the use of rodents as bioassay hosts, thus limiting the sensitivity by crossing a species barrier. Also, in most cases, the intracerebral (i.c.) route of inoculation was used, because it is the most efficient, but this severely limits the volume of blood that can be assayed. Thus, where transmission from blood has been successful, infectivity was usually concentrated in some way, for example, by the use of buffy coat fractions. Transmission by the intravenous (i.v.) route has been shown to be up to seven times less efficient than following i.c. infection (Brown et al., 1999), but there have been very few attempts to transmit TSEs by whole blood transfusion. Units of whole blood from three CJD cases were transfused into chimpanzees with negative results (Brown et al., 1994) and pooled blood from three terminally ill TSE-infected mice produced disease in 1 of 20 transfusion recipients (Brown et al., 1999).

Sheep infected orally with BSE show widespread deposition of PrPSc in the lymphoreticular system (LRS) (Foster et al., 1996a, 2001b), similar to that seen in human vCJD patients. In contrast, in cases of sporadic human CJD and cattle BSE, peripheral pathogenesis does not appear to involve the LRS (Hill et al., 1999; Wells et al., 1998). Sheep were chosen as a model in which to study transmission of TSEs by blood transfusion because of the similarity of the pathogenesis with vCJD and because large volumes of blood can be transferred in the absence of a species barrier.

We have transfused whole blood and buffy coat from BSE-infected sheep and natural scrapie-infected sheep into susceptible but scrapie-negative recipient animals. In the first report on these experiments (Houston et al., 2000), we described a single case of BSE infection via blood transfusion. The significance of this finding in a single animal has been questioned. However, the present report gives details of further successful transmissions from BSE and natural scrapie cases, the latter being the first conclusive demonstration of infectivity in blood of naturally infected individuals. Although still incomplete, our study indicates a frequency of transmission of TSEs in at least 10% of the transfusion recipients. We have decided to provide an update of our results because of the potential importance of the study for human health. In addition, in the two BSE transfusion cases examined so far, deposition of PrPSc in peripheral tissues appears rather limited when compared with sheep infected by the oral route. The potential implications of this observation for pre-clinical diagnosis and screening are discussed.

Methods

Sheep. The sheep were all Cheviots and were (as indicated) either from the Edinburgh NPU Cheviot flock (blood donor animals) or from the DEFRA scrapie-free (DEFRA/SF) flock of New Zealand (all blood recipients and scrapie-negative control donors). DEFRA/SF animals are exactly the same as those known previously as MAFF/SF and simply reflect the change of name of the Ministry of Agriculture, Fisheries and Food (MAFF) to Department of Environment, Food and Rural Affairs (DEFRA) in 2001. This flock was established from sheep imported from New Zealand in 1998 and is maintained in strict isolation from UK livestock and any other potential source of infection on land formerly used exclusively for growing crops. A large spectrum of PrP genotypes are produced and maintained in the flock, with some sheep of the sensitive genotypes being kept to older age as biological sentinels. Cull animals are routinely screened for evidence of TSE infection and there have been no recorded cases of scrapie since the flock was established. Recipient sheep were housed in a purpose-built isolation unit on a separate site (Compton, UK) around 400 miles away from the infected blood donors and completely isolated from other UK sheep. BSE and scrapie transfusion recipients, and positive and negative control animals, were housed continuously in separate pens so that they never came into direct contact with one another. Precautions were taken to minimize the risks of cross-contamination between the groups: for example, use of dedicated equipment for each group. The animals were fed on a diet of dried lucerne pellets and hay, with free access to a mineral supplement. Care was taken to ensure that the diet did not contain any animal protein.

Sheep have one of the most complex blood grouping systems known (Tizard, 1992) and therefore exact cross-matching of donors and recipients was not possible. Single transfusions only were made between animals that were checked for compatibility using a simple agglutination test (mixing recipient serum and donor erythrocytes and vice versa).

Genotypes of all sheep were confirmed by sequencing the region coding of the PrP gene (Foster et al., 2001a) and are presented in three-codon format with each codon (136, 154 and 171) in turn for each allele in turn. The single letter amino acid code is used as follows: A, alanine; H, histidine; R, arginine; Q, glutamine; V, valine; X, any of the known polymorphisms at that position.

BSE blood transfusions. The experimental BSE incubation period is controlled in sheep by the PrP gene codons 136 and 171, with no major effect of codon 154. BSE has the shortest incubation period in AXQ/AXQ sheep and extremely long incubation periods in heterozygote animals expressing AXQ with the ARR or VRQ alleles (Foster et al., 2001a and unpublished observations). Of the 18 donor sheep (all genotype AXQ/AXQ), 17 were taken from the NPU Cheviot flock ‘negative’ line (not affected by natural scrapie) and were infected with 5 g cattle BSE brain homogenate by the oral route as part of a separate series of experiments. Five of the donor animals developed BSE at 559–761 days (mean ± SD = 640 ± 78 days) post-challenge; three are still alive at more than 1500 days post-challenge; however, the other donors (n = 9) were culled during the pre-clinical phase as part of a serial cull pathogenesis experiment.

Due to a shortage of clinical BSE cases in the orally infected group, an ARQ/ARQ Cheviot sheep (DEFRA/SF) that was showing clinical signs following intracerebral inoculation of 0.05 g cattle BSE brain homogenate (incubation period 671 days) was used to provide blood for the final transfusion in the group.

The 24 recipients for the BSE blood transfusions were sheep of ARQ/ARQ genotype from the DEFRA scrapie-free flock of New Zealand origin (DEFRA/SF sheep). Six donors were used to provide both whole blood and buffy coat transfusions but these were transfused into separate recipient sheep.

Natural scrapie transfusions. Natural scrapie occurs in the NPU Cheviot flock positive line in carriers of the VRQ allele (Hunter et al., 1996) and the 11 donors from this group were of VRQ encoding
PrPSc detection by immunocytochemistry and Western blot. 

Blood transfusions and prions

PrPSc detection by immunocytochemistry and Western blot. PrPSc immunocytochemistry was carried out as described previously (Foster et al., 2001b). The primary antibody, BG4, was a monoclonal antibody (mAb) raised in mice against recombinant bovine PrP, binding to epitopes in the regions of aa 47–57 and 89–99 (courtesy of TSE Resource Centre, IAH, Compton, UK). The secondary antibody was biotinylated rabbit anti-mouse IgG, which was linked to streptavidin peroxidase with 3-amino-9-ethylcarbazole as the chromogen. Comparisons of PrPSc distribution were made with sheep infected with BSE (by oral or i.c. routes) and with animals naturally infected with scrapie in the NPU Cheviot flock, using methods published previously (Foster et al., 2001a, b).

For Western blotting to detect PrPSc, extraction procedures were used as described previously (Hope et al., 1986; Towbin et al., 1979). PNGase-treated PrPSc was prepared using the method described by Collinge et al. (1996). For Fig. 1(A, B), mAb 6H4 (Prionics) was used to detect of PrPSc and was visualized with a chemiluminescence substrate (Roche). For Fig. 1(C), mAb 8H4 was used (Zanusso et al., 1998).

Results

Blood transfusions

To date, 24 transfusions from BSE-challenged sheep have been carried out, including seven withuffy coat preparations and 17 with whole blood. Two of the 24 recipients have so far been culled after showing clinical signs typical of TSE in sheep. The first of these (D505) was reported previously (Houston et
al., 2000) and occurred 610 days following transfusion with whole blood taken from a donor animal (J2559) during the pre-clinical stage of infection (50% of incubation period). The second case (F19) is new and also received whole blood that was taken from the donor (J2746) during the pre-clinical stage of disease, at 282 days following oral BSE challenge. The donor was culled at this time as part of another experiment; however, 282 days represents 45% of the mean BSE incubation period in this study. The recipient (F19) developed BSE with an incubation period of 538 days. Two additional animals are currently showing early signs of BSE, having been transfused with whole blood taken when the donors were themselves at the clinical phase of BSE. Of the remaining transfused sheep, there has been one intercurrent death (not TSE) at 1108 days post-transfusion and the other 19 animals are between 68 and 1243 days post-transfusion and remain healthy at the time of writing. If the BSE suspect cases are confirmed, this brings the minimum rate of infection by transfusion in this part of the study to 4 of 24, roughly 17%. Four sheep that received blood or buffy coat from confirmed BSE cases are still alive and free from clinical signs at the time of writing (at 68, 499, 562 and 1216 days post-transfusion).

Of the 21 animals transfused with blood taken from natural scrapie-infected animals, four animals have been culled with clinical signs of scrapie to date. Blood was collected from the donors (61x75, 61x68, 59x27 and 59x28) at time-points of 761, 764, 1138 and 1080 days of age. The donors developed clinical signs of scrapie at 1324, 1112, 1484 and 1080 days of age, respectively, making the blood donations at 57, 69, 77 and 100% (clinical phase) of donor survival time, respectively. Scrapie developed in recipients (F144, F153, F141 and F143) at 614, 624, 571 and 737 days post-transfusion, respectively. Of the 17 remaining recipients, one died of unrelated causes (not TSE) at 364 days post-transfusion and the others remain healthy at 660 days post-transfusion. The other 15 recipients are alive and healthy at two groups at 38–103 (n = 6) and 516–537 (n = 9) days post-transfusion. It should be noted that both the BSE- and scrapie-negative controls are at a relatively early stage of the possible incubation time and we must wait until the study is complete before it can be confirmed that the controls are free of TSEs.

Positive controls were given a dose of 0.2 g BSE-affected cattle brain homogenate by the i.v. route to provide evidence that the i.v. route allows infection by TSE challenge in sheep, not established previously. Of ten animals (AXQ/AXQ genotype) challenged by the i.v. route, two animals were culled with clinical signs of BSE at 605 and 655 days post-challenge and three additional animals are showing early clinical signs at the time of writing, at 626–702 days post-challenge.

**PrPSc detection by Western blot**

Glycoform analysis of PrPSc by Western blot is one means by which strains of TSEs may be identified (Collinge et al., 1996). PrPSc samples were prepared from the brains of affected sheep for analysis and comparison with animals known to be infected with BSE or scrapie. Fig. 1(A, lanes 5 and 6) shows PrPSc extracted from the BSE transfusion cases D505 and F19, respectively. The glycoform pattern is dominated by the high molecular mass isoform of PrPSc, as expected for BSE. For comparison, proven transmissions of BSE to sheep in Fig. 1(A, lanes 2 and 9) (i.c. challenge) and Fig. 1(A, lanes 3 and 11) (oral challenge) show characteristic predominance of high molecular mass glycoformas. The donor for D505 survived until clinical signs of BSE developed. The Western blot also shows the BSE glycoform pattern in the donor (J2599) at clinical phase (Fig. 1A, lane 8). The donor for F19 (J2746) was culled at the time of blood donation, 282 days post-challenge, in the absence of clinical signs of BSE. A brain sample taken from J2746 at the same time as the blood donation showed no sign of PrPSc (Fig. 1A, lane 12). In contrast, natural scrapie PrPSc (Fig. 1A, lanes 1, 4, 7 and 10) shows a clearly different glycoform pattern typical of UK natural scrapie (Hope et al., 1999).

An additional characteristic of BSE is the ‘band shift’ seen in the lowest, unglycosylated isoform of PrPSc, which is of lower apparent molecular mass than that seen in natural scrapie (Hope et al., 1999; A. Chong, unpublished observations). As this feature was not clear from the blots in Fig. 1(A), PNGase treatment was used to remove the carbohydrates from the PrPSc protein. As shown in Fig. 1(B), in all BSE cases, the resulting band sizes have a slightly lower apparent molecular mass than is seen in natural scrapie.

Fig. 1(C) shows the glycoform analysis of one of the scrapie blood transfusion cases (F141) (Fig. 1C, lane 3) and the corresponding donor sheep (59x27) at clinical phase (Fig. 1C, lane 2), compared with another natural scrapie case (Fig. 1C, lane 1) and an experimental sheep BSE case (Fig. 1C, lane 4).
The transfusion case clearly shows a PrP<sup>Sc</sup> pattern typical of scrapie rather than that of BSE. Murine transmissions are being set up to confirm the identity of the TSE agent in the transfusion cases but the genotypes of affected animals (ARQ/ARQ for BSE and VRQ/VRQ for scrapie) and the PrP<sup>Sc</sup> Western blot patterns strongly support the conclusion of successful transmission of both BSE and scrapie by blood transfusion.

**Immunocytochemistry**

The distribution and intensity of PrP<sup>Sc</sup> immunostaining in two of the BSE transfusion cases has been compared with our extensive data on sheep infected with BSE by other routes (oral and i.c.) and with natural scrapie. For simplicity, only two sheep from each group are described (Table 1). Selected areas of the brain were scrutinized for PrP<sup>Sc</sup> immunostaining, including the dorsal motor nucleus of the vagus (DMNV) in the medulla oblongata and thalamic nuclei of the diencephalon, which we have shown previously to stain positively in natural scrapie (Foster et al., 1996b) and in sheep orally dosed with BSE (Foster et al., 2001b). Peripheral lymphoid tissues were also collected, although reporting here has been restricted to the tonsil, spleen, mesenteric lymph node and Peyer’s patches of the gut.

The DMNV showed strong PrP<sup>Sc</sup> immunostaining in six sheep, two infected with BSE by blood transfusion, two infected with BSE by the oral route and two infected with natural scrapie (Table 1), but staining was reduced slightly in both of the BSE cases inoculated by the i.c. route. It was especially prominent as perineuronal and extracellular punctate staining, with the cell body clearly outlined (Fig. 2). In contrast, the thalamus demonstrated fairly weak immunostaining in both transfusion cases but more pronounced staining was seen in the natural scrapie cases and also in one each (Table 1, sheep 4 and 6) of the oral and i.c. infected BSE cases.

PrP<sup>Sc</sup> immunostaining was variable in peripheral lymphoid tissues. For example, both BSE blood transfusion cases showed nil or marginal staining of the tonsil (Fig. 3) and mesenteric lymph node (Fig. 4 and Table 1), as well as retropharyngeal, submandibular and ileo–caecal lymph nodes (data not shown), in contrast to the BSE oral and natural scrapie cases. However, Peyer’s patches in the proximal colon stained strongly in one transfusion case (Table 1, sheep 1) and less so in the other. Jejunal Peyer’s patches only stained lightly (data not shown) in both sheep.

Both orally dosed BSE cases (Table 1, sheep 5 and 6) demonstrated clearly observable immunostaining in all peripheral lymphoid areas tested, with sheep 6 having strong tonsillar staining (Fig. 5). Both natural scrapie cases (Table 1, sheep 7 and 8) had intense immunostaining in the mesenteric lymph node (Fig. 6) and tonsil, as well as retropharyngeal, ileo–caecal, prescapular and submandibular lymph nodes (data not shown).
Fig. 2. Transfusion BSE case. PrPSc immunostaining in the DMNV shown in sheep 2 at an incubation period of 536 days post-transfusion. Magnification, ×10.

Fig. 3. Transfusion BSE case. No PrPSc immunostaining in lymphoid tissue from the palatine tonsil of sheep 2 at an incubation period of 536 days post-transfusion. Magnification, ×10.

Fig. 4. Transfusion BSE case. Marginal PrPSc immunostaining in lymphoid tissue from the mesenteric lymph node of sheep 2 at an incubation period of 536 days post-transfusion. Magnification, ×20.

Fig. 5. Oral BSE case. PrPSc immunostaining in lymphoid tissue from the palatine tonsil of sheep 6 at an incubation period of 935 days post-infection. Magnification, ×20.

Fig. 6. Natural scrapie case. PrPSc immunostaining in the mesenteric lymph node of sheep 7, which developed natural scrapie at 1080 days of age. Magnification, ×20.

Fig. 7. Intracerebral BSE case. No PrPSc immunostaining in the mesenteric lymph node of sheep 3 at an incubation period of 504 days post-infection. Magnification, ×20.
Those resulting from i.v. injection of 0.2 g BSE cattle brain homogenate to its appropriate susceptible genotype (AXQ/AXQ for BSE and VRQ/VRQ for scrapie) and Western blot/glycoform analyses support the conclusion that donors and recipients are infected with the same strains of BSE and scrapie. Our negative controls remain healthy, although still at relatively early stages post-transfusion and our positive controls are developing clinical signs at around, or greater than, 600 days post-challenge, showing incubation periods very similar to the transfusion cases.

Whole blood transfusion (400–450 ml) cases are presenting incubation periods of around 600 days, which is very similar to those resulting from i.v. injection of 0.2 g BSE cattle brain homogenate. The transfusions might be expected to be more efficient because they are a sheep-to-sheep transmission with no species barrier, which contrasts with the i.v. brain infections, which is a cattle-to-sheep transmission. A full titration of the inoculum used in the cattle BSE brain i.v. controls is under way in mice but is incomplete at the time of writing. Accurate estimation of the levels of infectivity in blood will require i.v. titration in any of the lymphoid tissues examined (Fig. 7).

Discussion

With this report we have confirmed and extended our initial observation of a single case of BSE following transfusion of blood from a BSE-infected sheep and have provided the first conclusive evidence of significant levels of infectivity in blood in a naturally occurring TSE (scrapie). The experiment may take up to 5 years to complete; however, so far we have clear evidence of disease transmission by the blood transfusion route in 2 of 24 sheep (8%) with BSE and 4 of 21 sheep (19%) with scrapie, with two additional animals showing clinical signs in the BSE group. If the clinically suspect BSE-transfused sheep progress as expected, this would bring the transmission rate for BSE up to 17%, comparable with the scrapie rate. Positive transmissions have occurred not only with samples taken from sheep at the clinical phase of disease but also with those from apparently healthy donors as early as halfway through the incubation period (Fig. 1, lane 9; no PrP^{Sc} detection in the brain of donor J2746). Each TSE is transmitting to the sheep orally infected with BSE or natural scrapie (Foster et al., 2001). A recent report has shown that a proportion of Romney sheep in the late pre-clinical stages of infection with BSE following oral dosing (22 months post-infection) have PrP^{Sc} deposits in the CNS in the absence of any detectable involvement of peripheral lymphoid tissues (Jeffrey et al., 2001). This study also noted the relatively late and variable onset of PrP^{Sc} accumulation in the lymphoid tissues of BSE-infected sheep. A more detailed study of BSE and scrapie transfusion cases, and positive controls, will be undertaken to determine whether lack of involvement of the LRS is a consistent feature in animals infected by the i.v. route; the results will be published at a later date. If our preliminary observations are confirmed, there may be implications for human patients with the misfortune to have received blood products from vCJD cases, because a negative tonsil biopsy as a means of reassurance might very well be unreliable. On the other hand, it also may mean that if a human patient became infected with vCJD by the i.v. route, then the peripheral tissues and blood of this secondary case may not themselves be highly infectious.

In conclusion, our results so far indicate that, with more than 10% of transfusions resulting in disease in the recipients, blood transfusion represents an appreciable risk for transmission of TSEs in sheep and, by extension, of vCJD in human beings. The relatively short and consistent incubation periods seen in positive cases suggests that levels of infectivity in the blood may be higher than suspected previously, even in the pre-clinical stages of infection, and/or that transmission by the i.v. route is highly efficient. From these preliminary results, it would appear that measures taken to safeguard the blood
supply in the UK are fully justified. However, further work, in particular a thorough investigation of the distribution of infectivity in different blood fractions, is required before a reliable estimate of the risks associated with contaminated blood products can be made.

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References


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