Table 1. Outcome of transfusions from BSE-exposed donor sheep

Donor sheep details								Recipient sheep details				
Donor sheep ID	Donor genotype	Clinical status at donation	Percentage of actual or average incubation period at donation*	Clinical outcome	IHC result	Incubation period, d	Component transfused	Recipient sheep ID	Recipient PrP 168 codon genotype	Clinical outcome	IHC result	Incubation period, d
58x51	ARQ/ARQ	Preclinical	12	+	+	2131	WB	D529	PP	+†	–	–
60x43	ARQ/ARQ	Preclinical	22	–	+/–	–	WB	D433	PL	–	–	–
			44		(DRG)‡		WB	F14	PL	–	–	–
J2747	ARQ/AHQ	Preclinical	42	–	–	–	BC	F182	PP	–	–	–
			44				WB	F181	PP	–	–	–
61x24	ARQ/AHQ	Preclinical	42	–	–	–	BC	F238	PP	+†	–	–
			43				WB	F234	PP	–	–	–
J2746	AHQ/AHQ	Preclinical	45	–	–	–	WB	F19	PP	+	+	536
J2553	AHQ/AHQ	Preclinical	51	+	+	629	WB	D505	PP	+	+	610
58x81	ARQ/AHQ	Preclinical	61	–	+/– (IPP)‡	–	BC	D358	PP	–	–	–
58x26	ARQ/AHQ	Preclinical	61	–	–	–	WB	D421	PP	–	–	–
			61				BC	D384	PP	–	–	–
58x27	AHQ/AHQ	Preclinical	61	–	–	–	WB	D452	PP	–	+§	–
			61				BC	D318	PP	–	–	–
58x33	ARQ/AHQ	Preclinical	62	–	–	–	WB	D337	PP	–	+‖	–
			62				WB	D386	PP	–	–	–
J2409	AHQ/AHQ	Preclinical	86	+	+	761	WB	D341	PP	–	–	–
J2771	AHQ/AHQ	Clinical	100	+	+	561	BC	G61	PL	–	+	–
J2775	AHQ/AHQ	Clinical	100	+	+	589	WB	G74	PP	+	+	594
60x63	AHQ/AHQ	Clinical	100	+	+	660	WB	G78	PP	+	+	556
							BC	G49	PP	+	+	531
D389	ARQ/ARQ	Clinical	100	+	+	671	WB	G92	PL	–	+	–

WB indicates whole blood; BC, buffy coat; DRG, dorsal root ganglion; IPP, ileal Peyer patch; +, positive; and –, negative.

*Calculated from the days after infection at the time of donation, as a percentage either of the final incubation period (in sheep kept alive until the development of clinical signs) or of the average incubation period in orally infected donors (640 days), excluding the outlying incubation period of 2131 days (58x51).

†No evidence of infection was found on postmortem examination of tissues from these clinical suspects; therefore, it is most likely they were clinically misdiagnosed.

‡These tissues were initially scored weakly positive by IHC, but the results were not reproducible in two laboratories and can therefore be considered as inconclusive.

§This sheep died of unrelated causes (ie, without showing clinical signs of BSE) at 1139 days after transfusion but was positive by IHC.

‖This apparently healthy sheep was culled 3018 days after transfusion and found to be positive by IHC; however, further analysis suggested this was a case of "atypical" scrapie and therefore unlikely to be transfusion related.

buffy coat ($n = 8$) collected from 8 uninfected VRQ/VRQ donors. There were 2 intercurrent deaths at 397 days and 464 days after transfusion, and the other 14 animals were culled between 2052 and 2409 days after transfusion. None of the negative controls for the BSE or scrapie experiments showed clinical signs of TSEs, and all were IHC negative for PrP^{Sc}.

PrP^{Sc} detection by immunohistochemistry

Tissue samples from the brain, spleen, mesenteric lymph node, and palatine tonsil of the sheep under study were fixed in formaldehyde and processed according to standard procedures. Sections were immunolabeled for PrP^{Sc} detection by IHC with primary antibody R145, which recognizes the 222-226 amino acid sequence of ovine PrP,[11] as described previously.[12,13]

Results

BSE transfusion experiment

A total of 5 transfusion recipients showed clinical signs of TSEs and were confirmed positive by IHC and/or Western blot (Table 1; Figure 2). These included 2 (F19 and D505) of 12 sheep transfused with whole blood from donors in the preclinical phase of infection (at 45% and 50% of estimated IP, respectively), as reported previously.[5,9] Two of 3 recipients of whole blood and one of 2 recipients of buffy coat from donors clinically affected by BSE developed clinical BSE. The IPs in the 5 clinically positive recipient sheep ranged from 531 to 610 days after transfusion (mean ± standard deviation [SD] = 565 ± 35 days), and there was no obvious difference in the IPs of those that received blood from preclinical or clinical donors.

One recipient (D452) of whole blood from a preclinical donor died of unrelated causes at 1139 days after transfusion but had PrP^{Sc}-positive IHC labeling in brain and other tissues. One of 3 recipients of whole blood (G92) and one of 2 recipients of buffy coat (G61) from clinical donors showed weak PrP^{Sc} deposition in the brain and lymphoid tissues after being culled at 2003 and 2497 days after transfusion, respectively, in the absence of clinical signs. Full sequencing of the *PrP* gene of these sheep revealed that they carried an additional proline (P) to leucine (L) substitution at codon 168,[14,15] which appears to be associated with the prolonged survival of these infected sheep. The polymorphism was also identified in 2 recipients of blood from a preclinical BSE-challenged donor, neither of which showed evidence of infection.

Taking the results for all 22 recipients of blood from BSE-exposed donors, 5 clinical cases and 3 sheep showing evidence of infection in the absence of clinical signs were identified, giving an overall transmission rate of 36%.

One recipient was culled for health reasons at 1444 days after transfusion, 2 were culled with suspected TSE clinical signs at 2480 and 2160 days after transfusion, respectively, and the remaining clinically negative sheep were culled between 2239 and 3068 days after transfusion. With one exception, examination of the tissues by IHC did not find evidence of infection. The exception (D337) was culled at 3018 days after transfusion and showed

Table 2. Outcome of transfusions from scrapie-exposed donor sheep

Donor sheep details						[illegible]
Donor sheep ID	Donor genotype	Clinical status at donation	Percentage of actual or average incubation period at donation*	Clinical outcome	IHC result	[illegible]
67x42	VRQ/VRQ	Preclinical	17	–	–	[illegible]
			19			
66x45	VRQ/VRQ	Preclinical	17	–	–	
			19			
67x23	VRQ/VRQ	Preclinical	18	–	+	[illegible]
			20			
65x13	VRQ/VRQ	Preclinical	28	+	+	[illegible]
			30			
65x02	VRQ/VRQ	Preclinical	34	[illegible]	+	
			37			
65x03	VRQ/VRQ	Preclinical	34	–	+	
			37			
61x75	VRQ/ARQ	Preclinical	53	+	–	[illegible]
			57			
61x68	VRQ/VRQ	Preclinical	64	[illegible]	+	[illegible]
			63			
61x66	VRQ/VRQ	Preclinical	62	–	ND	–
			64			
59x27	VRQ/VRQ	Preclinical	73	+	+	[illegible]
			77			
59x28	VRQ/VRQ	Clinical	100	+	+	[illegible]

+ indicates positive; and –, negative.

*Calculated from the age at the time of donation, as a percentage either of the final [illegible] the average incubation period (1295 days) for sheep that died or were culled before deve[illegible]

†No evidence of infection was found on postmortem examination of tissues from this [illegible]

positive PrP^{Sc} labeling in the brain, but with a pattern distinct from that observed in other BSE-infected sheep. The brain PrP^{Sc} distribution involving major white matter tracts and sparing the dorsal motor nucleus of the vagus was similar to that of Nor98 (or "atypical" sheep scrapie) and therefore doubtful to be transfusion-related. No other sheep in the present study showed evidence of being infected with atypical scrapie.

Of the 10 sheep that were infected intravenously with BSE as positive controls, 8 developed clinical signs confirmed by IHC, with an average IP of 702 days (± 61 days, SD). The remaining 2 animals were culled at 2591 days after infection and, although not demonstrably clinically affected, IHC showed PrP^{Sc} deposition in the brains and lymphoid tissues of both animals. These 2 sheep were heterozygous (PL_{168}) for the PrP polymorphism P168L, whereas the other 8 were homozygous (PP_{168}).

The PrP^{Sc} profile obtained by IHC from BSE-positive recipients was the same as that found in the orally inoculated donors and in the positive controls.[16] In addition, characteristic BSE glycoform patterns were obtained by Western blot analysis of PrP^{Sc}-positive donor and recipient sheep (data not shown),[9] and inoculation of brain homogenates from infected donors and recipients into a panel of inbred mouse strains produced IPs and lesion profiles characteristic of BSE (data not shown). Taken together, these results confirm that the strain characteristics were not altered after transmission via blood.

Scrapie transfusion experiment

Four of 10 recipients of whole blood and 4 of 10 recipients of buffy coat from donors in the preclinical phase of scrapie infection developed clinical signs of scrapie, which were confirmed by positive IHC results. One sheep transfused with buffy coat from the single clinical donor was also clinically affected and IHC positive

[illegible]

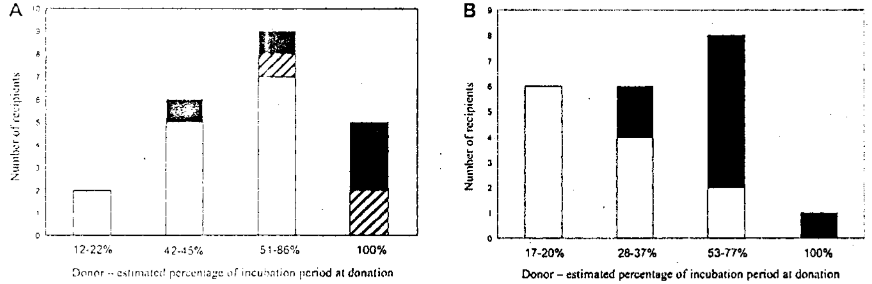

Figure 2. Outcome of transfusions as a function of the stage of disease incubation in the donor. (A) BSE-infected donors. (B) Scrapie-infected donors. For each stage of infection in the donor sheep, the number of uninfected (□), clinically positive/ IHC-positive (■), and clinically negative/IHC-positive (▨) recipients are shown.

transmitted between sheep by blood transfusion, using volumes similar to those used in human transfusions. The overall transmission rates (percentage of all recipients that became infected) were 36% for BSE and 43% for scrapie. For BSE, the figure was much higher than anticipated because 3 of the 8 BSE-infected recipients survived for long periods without showing clinical signs, whereas all the scrapie-infected recipients identified by IHC were also clinically positive. The greater probability of subclinical infection in recipients of blood from BSE-exposed donors is largely the result of variability in the genetic susceptibility to infection among sheep used in the BSE experiment, which will be discussed in "Effect of genetic variation in susceptibility." The results are consistent with the known facts about transmission of vCJD by blood transfusion in humans.[17] Sixty-six patients known to have received labile blood products from 18 donors who subsequently developed vCJD were followed up in an ongoing study. Three of these recipients have been confirmed clinically and pathologically as vCJD cases, with intervals between transfusion and the development of clinical signs ranging from approximately 6.5 years to 8.5 years.[18-20] Another patient, who died of unrelated causes 5 years after transfusion, showed PrP^{Sc} deposits in lymphoid tissues but not brain postmortem, and is thought to represent preclinical or subclinical infection.[21] These 4 patients represent 6% of the total recipients, or 12.5% of recipients surviving longer than 5 years.

Various factors influence the transmission rate by transfusion in both sheep and humans, including: (1) the interval between blood donation and the onset of clinical signs in the donors, (2) genetic variation in susceptibility of donors and recipients, and (3) the blood component transfused.

Stage of incubation period of the donors at the time of blood donation

The effect of the stage of incubation can best be deduced from the results of the scrapie transfusion experiment because the *PrP* genotype of the sheep used (VRQ/VRQ) renders them almost 100% susceptible to natural and experimental infection.[22] The stage of incubation of the donor has a strong influence on the probability of transmission to the recipient (Figure 2). When donations were made at less than or equal to 20% of the estimated IP, there was no disease transmission, whereas donations made at more than 50% of the estimated IP produced an 80% transmission rate, with a mean IP of 729 days (± 99, SD) in the recipients. Blood collected at 28% to 37% of the estimated IP transmitted infection at a lower rate of approximately 33%, and with longer IPs in the recipients of more than 1000 days. The data are consistent with a gradual increase in infectivity in the blood, from approximately 30% to 50% of IP until the clinical phase.

In the BSE transfusion experiment, the correlation between stage of infection and transmission is not clear-cut but shows the same general trend of increasing probability of transmission to recipients as infection progresses in the donors (Figure 2). Possible explanations for the lower transmission rates from preclinical BSE-infected blood donors compared with preclinical scrapie-infected donors include the following:

(a) Variation in susceptibility to infection of both donor and recipient sheep.

(b) Differences in the pathogenesis of natural scrapie and experimental BSE. VRQ/VRQ sheep naturally infected with scrapie have detectable PrP^{Sc} deposits in lymphoid tissues early after infection (ie, < 50% estimated IP).[23,24] Time course studies of ARQ/ARQ sheep orally infected with BSE showed that PrP^{Sc} was not consistently detected in lymphoid tissues before at least 65% of the average IP.[7] If infectivity in blood correlates with its presence in lymphoid tissues, this could explain the differences observed in the 2 transfusion experiments.

The probability of transmission from preclinical donors is of greatest relevance to the human situation. In the case of the 4 transfusion-related transmissions of vCJD, the donors developed clinical signs between 17 and 42 months after donation. The mean IP for vCJD has been estimated to be 16.7 years, with a lower 95% confidence interval of approximately 12.4 years.[25] Therefore, it is probable that the transfusion-related vCJD cases resulted from donations made at least halfway through the IP, which is in agreement with the data from the sheep experiments. In vCJD cases, the timing of detectable lymphoid replication in the preclinical stages of disease is unknown; therefore, it is not clear whether the peripheral pathogenesis more closely resembles BSE or natural scrapie in sheep.

Effect of genetic variation in susceptibility

A small proportion of sheep with $A_{136}Q_{171}/A_{136}Q_{171}$ PrP genotypes do not die of infection after natural or experimental exposure to scrapie and BSE, or have very prolonged incubation periods.[26-28] The reasons for this variability in response are not clearly understood, but it can be predicted to reduce infection rates in both donor and recipient sheep in the BSE transfusion experiment. The majority of preclinical donor sheep (8 of 11) in the BSE transfusion experiment were killed at, or shortly after, the time of donation, and none showed conclusive evidence of infection, although 2 transmitted infection to their respective transfusion recipients. It is potentially significant that donors that failed to transmit infection were heterozygous at *PrP* codon 154, whereas those that did transmit infection were homozygous. Thus, variable susceptibility to infection among the donor sheep may be the result of a protective effect of codon 154 heterozygosity to oral challenge with BSE, although more data are required to confirm this association.

A novel polymorphism, resulting in a proline to leucine substitution at codon 168 of the *PrP* gene, was identified in 4 BSE transfusion recipients and 2 positive control sheep inoculated intravenously with BSE.[14] All 6 survived more than 2000 days without developing clinical signs of BSE, but on postmortem examination 4 showed PrP^{Sc} deposition in brain and lymphoid tissues. This suggests that the P168L polymorphism can protect against clinical disease but does not prevent infection by the intravenous route. This polymorphism has not been identified in the Edinburgh NPU Cheviots used as donors in the BSE experiment or in sheep with the VRQ/VRQ genotype.

Although the genetic basis of susceptibility to BSE infection in sheep and humans is not directly comparable, the variability in response to BSE found in ARQ/ARQ sheep provides a more realistic reflection of the situation with vCJD in the human population than the very uniform susceptibility of VRQ/VRQ sheep to scrapie infection. In addition, the survival of BSE-infected transfusion recipients for up to 7 years without clinical signs demonstrates that prolonged secondary incubation periods and/or a subclinical/"carrier" state are possible after transfusion in sheep. The existence of such subclinical or prolonged preclinical infection states in humans is recognized as one of the important factors influencing the probability of onward transmission, and thus the potential size of the vCJD epidemic.[29] Susceptibility to human TSEs has been linked to codon 129 of the *PrP* gene, which can encode either methionine (M) or valine (V). Until recently, all clinical cases of vCJD (including the 3 transfusion-related cases) that have been tested have been homozygous for methionine at 129 (129MM). Interestingly, the "preclinical" patient thought to have been infected by transfusion was heterozygous (129MV).[21] There is accumulating evidence to suggest that all human 129 genotypes may be susceptible to vCJD infection, with apparently greater likelihood of subclinical infection in 129MV and 129VV persons.[30-32]

Effect of blood component

The 4 transfusion-related vCJD infections occurred in patients who received transfusions of red cells that had not been leukodepleted. Leukodepletion was introduced in the United Kingdom in 1999 to control the risk of transmission of vCJD by blood transfusion because previous studies in rodents had shown that infectivity appeared to be concentrated in the buffy coat, which contains most of the blood leukocytes.[3] Subsequently, leukodepletion of blood from scrapie-infected hamsters was shown to remove up to 72% of infectivity.[33,34] In the sheep experiments, only whole blood and buffy coat were transfused because we were seeking to establish proof of principle of transmission of TSEs by blood transfusion, and assessing whether infectivity appeared to be concentrated in the buffy coat. The effect of leukodepletion was not investigated but is being addressed in a follow-up study, along with estimates of the distribution of infectivity among other blood components, including plasma, platelets, and red cells.

In our experiments, transmission rates did not appear to be significantly different in recipients receiving whole blood compared with recipients transfused with buffy coat. The number of sheep transfused with buffy coat in [illegible] allow statistical analysis. In the scrapie [illegible] recipients were transfused with buffy [illegible] similarity in transmission rates for [illegible] contain approximately equivalent [illegible]

We have shown that, for sheep [illegible] high transmission rates can be [illegible] particularly when donors are at [illegible] period. The results also revealed [illegible] incubation periods and/or subclinical [illegible] of BSE-infected blood, which is [illegible] variation in the sheep PrP gene. The [illegible] titers of infectivity in blood is [illegible] need for ultrasensitive methods of det[illegible] It may be that, in blood, infectivity [illegible] levels of protease-resistant PrP, but [illegible] and blood-borne infectivity in sheep [illegible] define the relationship. The results [illegible] experiments are consistent with what [illegible] associated vCJD transmission in hum[illegible] sheep as an experimental model in which [illegible] with different blood products, the effects [illegible] the development of diagnostic and screening [illegible]

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Authorship

Contribution: F.H. designed the study, [illegible] postmortems on recipient sheep, [illegible] paper; A.C. performed Western blots; [illegible] reviewed the report; [illegible] coordinated [illegible] tems on donor sheep; A.G. analyzed [illegible] and reviewed the report; S.S. and [illegible] IHC results, analyzed data, and reviewed [illegible] the interpretation of IHC results [illegible] design of the study, analyzed data, and [illegible]

Conflict-of-interest disclosure: The [illegible] financial interests.

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TRANSFUSION

Transfusion

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REVIEWS

From mad cows to sensible blood transfusion: the risk of prion [illegible] labile blood components in the United Kingdom and in France

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ABSTRACT

Transfusion transmission of the prion, the agent of variant Creutz[illegible]
Subjects infected through food may transmit the disease through [illegible]
to date by this threat are the United Kingdom (UK) and France. [illegible]
the UK over the past 5 years. In France, a few individuals who developed [illegible]
leading to a risk of transmission to recipients, some of whom could [illegible]
large-scale screening test, it is impossible to establish the prevalence of [illegible]
and transfused patients. This lack of a test also prevents specific screening of [illegible]
transfusion transmission essentially relies at present on deferral of "at-risk" [illegible]
in both white blood cells and plasma, leukoreduction is probably insufficient to [illegible]
In the absence of a screening test for blood donations, recently developed [illegible]
Furthermore, while the dietary spread of vCJD seems efficiently controlled [illegible]
the spread of prions through blood transfusion and other secondary route[illegible]

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DIGITAL OBJECT IDENTIFIER (DOI)

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ARTICLE TEXT

The first case known in the history of medicine as "mad-cow disease" [illegible]
United Kingdom (UK),[1] where the epidemic spread widely: by 200[illegible]
by bovine spongiform encephalopathy (BSE). Most likely, the epidemic [illegible]
livestock with animal food prepared from residues from slaughtering [illegible]
which died from scrapie and cattle affected by a sporadic form of [illegible]
in 1988 in the UK and in 1994 in France. On March 20, 1996, the [illegible]
BSE agent was transmissible to man. A new human pathology [illegible]
Creutzfeldt-Jakob Disease (CJD), transmitted by a variant of the [illegible]

Unlike other transfusion-transmissible agents, the prion (Proteinaceous in[illegible]

and is composed purely of protein.[2,3] The "normal" prion or PrP^C ("proteinaceous particle") is a protein expressed on the cellular membrane by a number of tissues, but the greatest amount is found on the neurons in the brain. Sensitive to the action of proteolytic enzymes, PrP^C has a half-life of a few hours. Despite having an identical amino acid sequence to that of the normal form, the BSE agent is a prion of different conformation, designated by the abbreviation PrP^{Sc} (sc for scrapie) and is derived from the isoform of the normal protein by a posttranslational structural modification and conversion to a richly beta-pleated sheet.[4] The abnormal prion has a tendency to aggregate and above all a resistance (from which is derived its other abbreviation, "PrP^{res}") to proteolytic enzymes (notably proteinase K), resistance lying in the majority conformation in beta-pleated sheets.[5] The prion itself plays a role of cofactor in this conformational change. In infected subjects, PrP^{Sc} induces, on native PrP molecules, a conformation that confers on them their pathologic character, and the phenomenon of amplification is self-propagated.[6] Because it affects the accumulation of a protein present in its natural state in the body, there is no immunologic response: neither the production of antibodies nor a specific cellular response. Accumulation of abnormal prions generates vacuoles in the cerebral tissue, giving eventually a spongiform appearance and hence the title "spongiform encephalopathy."

HUMAN PRION DISORDERS

The human transmissible spongiform encephalopathies (TSEs) generally follow a long incubation period, but with subsequent rapid evolution and death. Various forms are described:

- The idiopathic disorders, principally the sporadic form of CJD (sCJD), which continues to be the commonest form. It appears predominantly in the seventh decade, with an annual incidence of 1 to 1.5 cases per million population.[7] Death follows within 6 months. This form, which is associated with a pathologic prion, has unknown etiology. It probably results from a spontaneous conformational modification of PrP to PrP^{Sc}.
- The genetic forms: familial CJD (fCJD), Gerstmann-Strausler-Scheinker syndrome, and fatal familial insomnia.
- The acquired forms were, until 1966, of human origin: kuru, found in New Guinea and linked to funeral practices, and the iatrogenic form of CJD, seen after use of contaminated neurosurgical instruments, corneal grafts, dura mater, and intramuscular injection of pituitary hormones, obtained from cadavers. Acquired disease of bovine origin, that is, variant CJD (vCJD) is seen in subjects infected with the BSE agent.[8,9] This is the only human prion disorder that has crossed the species barrier. The first series of 10 patients was described in 1996 by the UK National CJD Surveillance Unit (NCJDSU) based in Edinburgh. The disease was found chiefly in adults under 40 years of age, contrasting with the mean age for sCJD. The disease is fatal in a mean of 14 months, which is slower than the sporadic form.[10] Nuclear magnetic resonance imaging scanning shows hypersignals situated in the posterior thalamus ("pulvinar sign"). Unlike other human prion disorders, notably sCJD, in which the accumulation of abnormal prion protein affects the central nervous system with a minimal peripheral involvement, vCJD progresses with invasion, by the abnormal protein, of the central nervous system, the peripheral nervous system, and other tissues, notably lymphoid: tonsils, appendix, Peyer's patches, and thymus.[11] Tonsillar biopsy may reveal the presence of PrP^{Sc}, but a negative result does not categorically exclude the diagnosis.

GENETIC INFLUENCES

The prion protein gene is situated on the short arm of chromosome 20 and codes for 254 amino acids, with either valine (V) or methionine (M) at position 129. With two copies of the gene, an individual can be MM (39% of the normal population), MV (50%), or VV (11%). This polymorphism is fundamentally important in the development of the variant type of the disease, since there is a host susceptibility linked to the genetic type.[12,13] Homozygosity for M (MM) appears to confer susceptibility to clinical expression and to influence the incubation period of the disease: all vCJD cases, to date, in whom codon 129 typing has been performed, are MM homozygotes.[14] In one case of infection, where the individual died 5 years after an implicated blood transfusion but did not have any clinical symptoms of vCJD at the time of death, the genotype was MV. Furthermore, PrP^{Sc} has been detected in the appendix of two VV cases.[15] The MV genotype and perhaps more the VV genotype could confer a protective effect, but this remains true only until a symptomatic case of vCJD is described in a MV or VV subject. In fact, we know that individuals with all three genotypes can accumulate PrP^{Sc} in vCJD-specific tissues, but we do not know whether symptomatic cases will develop in all genotypes.

EPIDEMIOLOGY OF vCJD

To December 2007, a cumulative total of 166 cases of vCJD have [illegible] accounts for the majority of cases worldwide. Mean age of affected [illegible] with a slight male predominance. The mean duration of the symptomatic [illegible] months). All tested patients were homozygous MM. The most probable [illegible] dietary: no risk factor of other kinds of CJD was observed.[16,17] A [illegible] transfusion, and some of these have been linked with a known infected [illegible]

In France, vCJD incidence was, as in the UK, proportional to dietary exposure [illegible] contaminated beef into France increased regularly from 1985 to 1995, while [illegible] decreased in the UK over the same period.[18] However, the level of exposure [illegible] been 10 to 20 times lower than that of the UK, with moreover a difference [illegible] dates of occurrence: the comparison between the number of French cases and [illegible] account the year of the beginning of the symptomatic phase) indicates that [illegible] occurred 5 years after the peak of the epidemic in the UK, where the number [illegible] decreased since 1999.[17] This temporal gap in the epidemic in the UK and in France is attributa[illegible] maximal exposure of the general population in the two countries.

Between 1996 and 2007, 23 vCJD cases have been registered in France, with [illegible] 58 years) and an equal sex ratio (12 males, 11 females). The clinical and [illegible] to those of the British vCJD patients. The mean duration of the symptomatic [illegible] months). All the analyzed cases were homozygous MM, without any risk [illegible] stays in the UK (less than 10-day periods) were mentioned in 3 patients [illegible] the UK, for long periods, between 1987 and 1996.

In other countries, vCJD cases remain exceptional. A small number of these cases [illegible] the UK, but there remain a number which appear to have been acquired outside [illegible] in Ireland, the Netherlands, Saudi Arabia, Spain, and Portugal and one in each of [illegible]

Several studies have been conducted to estimate the extent of the vCJD [illegible] British retrospective study revealed the presence of the abnormal [illegible] 12,674 individuals without clinical vCJD:[22] this rate appears higher than [illegible] recorded in the general population. From these results, a prevalence of [illegible] was proposed (95% confidence interval, 49-792). In the most pessimistic hyp[illegible] highest range of this interval), 41,250 of 60 million individuals would be infected [illegible] the latest count of vCJD cases in the UK is more in agreement with the [illegible]

In France, where the level of exposure was lower than in the UK, estimates [illegible] 60 years have been suggested in one study,[19] and 205 cases in another.[20] In [illegible] prediction suggested a total number of 33 cases (0-100), with 14 cases (2-30) over the 200[illegible] 11 (1-20) over the 2006-2010 period.[18] These data are compatible with the [illegible] A recent study predicted 39 (6-99) subsequent cases.[21] The worst case [illegible] years is, however, maintained in the epidemiologic estimations, in particular to estimate the pre[illegible] infection in the blood donor population.

Measures taken against the vCJD epidemic, with screening for the BSE agent in [illegible] animals from the food chain, and the latest epidemiologic observations suggest that a vCJD [illegible] origin is unlikely in the coming years. The unknowns now reside in other sources of contamina[illegible] cellular vectors: blood transfusion, grafts of tissues or organs, or use of medical [illegible] contaminated with the abnormal prion. Transfusion transmission is especially [illegible] Nowadays, since food contamination, which was the main source of infection, [illegible] transmission by blood components taken its place? In the UK, as in France, [illegible] eliminated from beef, they are likely to be present in the blood of asymptomatic human car[illegible] to the recipients of their blood donations. The fear of human-to-human [illegible] interspecies contamination.

EXPERIMENTAL BASIS OF vCJD TRANSMISSION BY TRANSFUSION

Until about 1996 it was acknowledged that the CJD agent was not transmitted [illegible] failed to show any association between the occurrence of sCJD and a past transfusion.[illegible]

higher in vCJD than in sCJD,[27] the number of infectious particles in blood and/or their distribution in individuals affected by sCJD are presumably too low to cause transmission through a blood transfusion. Thus, before the first vCJD cases, the two main circumstances of prion transmission between humans had been kuru and iatrogenic contamination by injection of growth hormones of pituitary origin. It should be noted that transmission of the kuru agent belongs to the past since the prohibition of certain rituals in New Guinea and that the exclusive use of growth hormones of recombinant origin put an end to iatrogenic transmissions through this route. Though no cases of human transmission of vCJD had yet been described, the possibility of transmission by blood transfusion remained a theoretical risk.[28-31] Unlike major transfusion-transmitted viruses observed in the past decades (hepatitis B virus, human immunodeficiency virus [HIV], hepatitis C virus), the vCJD agent did not immediately enter into the family of blood-borne agents.

In experimental models, invasion of lymphoid tissue by abnormal prion has been observed rapidly after infection, with persistence throughout the whole incubation period. It has been suggested (but not demonstrated) that the lymphoid infiltration is brought about by circulating cells, which led to the hypothesis that infected lymphocytes could transmit the prion to recipients of blood components containing lymphocytes.[32] Intracerebral injection in mice of buffy coats and plasma collected from patients with vCJD has not shown such transmissibility,[33] but these experiments only involved a small number of cases and the sensitivity of the technique may have been insufficient to detect low level of infectivity. Subsequently, transfusion transmission of prions was shown in rodents,[34] in particular in mice made susceptible to vCJD.[35,36] However, the turning point was the result of experiments aiming to show transmissibility through blood from orally infected sheep to healthy sheep:[37] it was then found that the abnormal prion was present in circulating blood and that blood could be a vector of transmission. Blood infectivity being thus demonstrated, at least in certain circumstances, French and British Health Authorities, as a precaution, considered the possibility of transmission of the vCJD agent by transfusion.

In another experiment, transfusion of healthy sheep with blood from infected sheep led to transmission rates of 17 percent for BSE and 19 percent for scrapie.[38] A more recent animal experiment was based on detection of PrP^{sc} in blood of hamsters experimentally contaminated by the scrapie agent through intraperitoneal inoculation of infected brain tissue.[39] In both cases, the infectious agent was present in circulating blood during a part of the incubation phase of the disease, and the transmission rate was shown to be quite high. However, it is important to distinguish the studies conducted with a Western blot assay detecting the amplified amyloid protein and those involving a titration of endogenous infectivity.

Finally, even before the description of the first human transfusion cases, these animal experiments had shown blood transmissibility of prions and the possibility of a short incubation period of the disease through this transmission route.

SURVEILLANCE OF TRANSFUSION RISK OF CJD IN THE UK

The first UK epidemiologic studies did not suggest transfusion as a mode of transmission of the vCJD agent, and the first descriptions of recovery of abnormal prions within the body had indicated that the blood route would be an improbable source of contamination. Subsequently, experiments into blood-borne transmission of the BSE agent in sheep and the observation of a wider distribution of PrP^{sc} in the body of subjects infected by the variant agent compared with subjects infected with sCJD led to reconsideration of this view.

In 1990, the UK, the country most exposed to the BSE risk, put in place a national surveillance system named "The National Creutzfeldt-Jakob Disease Surveillance Unit" or NCJDSU, charged with identifying and monitoring all cases of CJD.[40,41] All suspected cases were to be reported by health professionals (principally neurologists and neuropathologists) and then confirmed and categorized according to the defined diagnostic criteria. As far as transfusion is concerned, the medical history of each patient was examined and family members were interviewed, looking for history of blood donation or of receipt of transfusion. A collaborative study between the NCJDSU and the UK Blood Transfusion Services, called "TMER" (Transfusion Medicine Epidemiology Review), was set up in 1997 to examine all cases of CJD, including sCJD, fCJD, and vCJD, who had either donated or received blood in the past. On December 1, 2007, among the 166 UK cases of vCJD, 150 were old enough to have been blood donors and, among these, 31 (21%) had, at least according to their families, donated their blood at least once.[42] Records were checked and the dates and places of the donations were established. The fate of the donations was traced, including whether they were used for blood component preparation and/or for fractionated plasma products, and the fate of recipients of blood components was established. These enquiries identified donor records relating to 24 individuals who later developed vCJD: 18 of whom had donated blood that had been used to prepare components issued for hospital use. A total of 66 recipients were identified from these 18 donors: 23 of these are still alive. Blood donor records were identified for only 3 of 93 individuals who later developed sCJD and were reported to have been donors in the past, with 20 recipients identified, of whom 12 are known to be dead: 5 died within 1

year of the blood transfusion and 7 between 1 and 7 years after [illegible] dead and have survived 7 to 9 years after transfusion. Three cases [illegible] fCJD [illegible] donors) were associated with 11 identified recipients, of whom [illegible] 3, 10, and 17 years after transfusion. Three recipients not known to [illegible] transfusion. Among the 97 recipients thus identified, 4 have developed [illegible] died of the disease: these all belong to the first group, those exposed [illegible] evidence that either sCJD or fCJD has been transmitted by blood transfusion, [illegible] informed and none were tested for evidence of infection.

THE FIRST UK CASES OF vCJD IN RECIPIENTS OF BLOOD COMPONENTS

All four transfusion-associated vCJD infections occurred in patients transfused in the UK with [illegible] blood cells (RBCs). There have been no transfusion-associated cases of sCJD or of fCJD; no [illegible] described in these two latter groups, even in retrospective lookbacks or case control studies. [illegible] infections have been detected in the blood in experimental animal studies, even in transgenic [illegible] susceptible to the disease, although it is not possible to formally exclude transfusion cases [illegible] have passed unnoticed if they possessed exceptional features and/or a particularly long incubation [illegible]

The first of the four patients infected with vCJD through blood transfusion was [illegible] in the [illegible] group, who developed the illness in 2002 and died the next year. During surgery in 1996, he [illegible] nonleukoreduced RBCs, one of which was donated by a young donor who developed vCJD in the following year. Both donor and recipient were MM homozygous. Infection of dietary origin [illegible] excluded in this case (as in the others), but the transfusion was the most plausible explanation [illegible] the recipient which was greater than the median for cases believed exposed through the diet [illegible] association of this rare disease in both donor and recipient: statistical analysis demonstrated [illegible] these two observations of vCJD would have happened independently if transfusion was not the [illegible] infection, was in the order of 1:15,000 (and rose to 1:30,000 taking into account the age of [illegible] first transfusion-associated case in world literature was reported in December 2[illegible]

The second case was an elderly recipient, who died of cardiovascular causes without developing [illegible] of vCJD. Asymptomatic infection with PrP^{sc} was established by postmortem examination, [illegible] presence of abnormal prion protein in lymphoid tissue (the spleen and one cervical lymph node [illegible] tonsils or appendix), but not in the brain. This patient had been identified as "at risk" since, [illegible] 1999, a nonleukoreduced RBC component had been provided from a donor who [illegible] of vCJD [illegible] donation. The PrP^{sc} isolated from the spleen had an isoform identical with that observed in [illegible] donor was an MM homozygote, but the recipient was a heterozygote (MV), which may explain [illegible] nature of this case, assigned as "preclinical" or "subclinical" vCJD. Alternatively, [illegible] recipient [illegible] vCJD at a later date, if survival had been longer. This second case of possible transfusion tra[illegible] infection was reported in July 2004.[49]

The third case, reported in 2006,[42,50,51] was also one of the cohort of [illegible] recipients who [illegible] nonleukoreduced RBCs and had been notified of their risk. This recipient [illegible] had received transfusion support during a surgical intervention [illegible] developed vCJD in 2005, 7 years after the transfusion episode, and died [illegible] years later. [illegible] presented with vCJD in 1999 (18 months after the donation) and died the following year (2[illegible] blood donation). Both donor and recipient were MM homozygotes.

The fourth and last case to date was a recipient who developed vCJD [illegible] years after transfusion [illegible] donor who presented with vCJD 17 months after donation. This donor was [illegible] three. The recipient, genotype MM, died 1 year after presentation.[illegible]

These cases reported in professional journals (and subsequently [illegible] have led [illegible] transfusion-associated vCJD moving progressively from "theoretical [illegible]" then finally "demonstrated." There are a number of unknowns in the [illegible] but the combination of the low prevalence of vCJD in the general population (the [illegible] individual unit varies between 1:15,000 and 1:30,000 in the UK)[illegible] and of the high prevalence [illegible] the small group of recipients who have been rendered at risk (and [illegible] that a [illegible] these at-risk recipients have died without surviving long enough to develop an [illegible] vCJD [illegible] tested for the presence of infection) makes highly probable a transfusion [illegible] than a [illegible] these cases reinforces the theory that the blood of a donor in the asymptomatic phase of the [illegible] infective for recipients. This evidence of the transfusion transmissibility of vCJD [illegible] largely [illegible] preventative measures previously applied in the UK and in France.

In fact, despite the small number of reported transfusion cases, many observations have been proposed or are already known:

1. The possibility of a relatively short incubation period with a transfusion source: 6½ years between the transfusion and the first clinical signs in Case 1, 6 years in Case 3, 8½ years in Case 4. This short incubation period demonstrates the efficacy of the transfusion route. It might suggest a particular pathogenic character of the abnormal prion circulating in the blood and transmitted by this route, even if it is established that intraspecies transmission is usually accompanied by a shorter incubation than interspecies transmission. Indeed, the shortest incubation period has been observed in kuru, in the iatrogenic form after injection of growth hormone,[53] and in transfusion-associated vCJD.[54]
2. The rate of transmission in the population of at-risk recipients is high, even though it is not inevitable in the relatively short follow-up period.[55] A review of the UK's TMER study published in 2006 gave an indication of the transfusion risk of vCJD and of the incubation period of the first observed cases:[42] among the 66 blood component recipients transfused from donors who later developed vCJD, 37 died within the first 5 years posttransfusion with a cause of death linked to the existing illness. Apart from the one case shown to have evidence of infection, none of the other deceased recipients were tested for evidence of infection because their deaths predated the information that their donors had developed vCJD. Furthermore, no postmortem tissue was available for retrospective testing.

Among the 29 who survived over 5 years, 20 are still alive and have no signs of vCJD, and 9 are now deceased. Among these 9, 6 died of pathology not linked to vCJD (although only 1 of these had a postmortem to look specifically for infection, which was demonstrated) and 3 developed (and died from) vCJD.

3. The influence of codon 129 genotype is not refuted in the context of the transfusion route: the sole recipient known to be infected but asymptomatic was a heterozygote (MV), although it should be noted that the observation period was the shortest of the series of infected recipients, since this recipient died 5 years after transfusion.
4. All the infected recipients had received nonleukoreduced RBCs between 1996 and 1999. Routine leukoreduction was introduced in the UK by October 1999.
5. The four recipients who developed evidence of infection had been transfused respectively with components from 5, approximately 8-10 (figure uncertain), 56, and 23 blood donors. [Correction added after online publication 2-Jan-2009: Number of donors has been updated.]
6. In the UK and France, no case of vCJD has been reported in recipients of fractionated plasma products. As indicated in the title of this article, we have limited our review to labile blood components, aware of the additional procedures that contribute to the safety of plasma products with respect to prions.

SURVEILLANCE OF TRANSFUSION RISK IN FRANCE: FIRST CASES OF vCJD WITH PREVIOUS BLOOD DONATIONS AND FIRST MEASURES TAKEN WITH REGARD TO THE RECIPIENTS

Although epidemiologic investigations conducted in France have not revealed previous blood transfusions during the "risk period" for vCJD (one case had received a blood transfusion, but in 1971, before the epidemic), some patients had been blood donors, as would be predicted, in the same period. In 1992, a national surveillance network for cases of CJD was set up in France, coordinated by Inserm Unit U708 and including representatives of various medical specialities and the health services: neurologists, neuropathologists, reference laboratories, and the "Institut de Veille Sanitaire" (InVS). The aim of this network was to collect and investigate reports of suspected cases of CJD, follow their progress, classify the type (sporadic, familial, iatrogenic) and the degree of probability (distinguishing confirmed cases from probable cases), and establish epidemiologic characteristics. In cases with previous history of blood donation, InVS was charged with informing the French Blood Service ("Etablissement Français du Sang") so that a transfusion investigation could be started. It appeared that three of the French vCJD cases, who had developed the disease in 2004, had a history of blood donation.

The first case (eighth in the series, reported in February 2004) was a 32-year-old female who donated blood between 1993 and 2003. The components prepared from these donations were 13 concentrated RBCs (of which 10 were leukoreduced) and one platelet (PLT) concentrate. Fourteen recipients, of whom 10 were still alive, were traced. Ten plasma donations were used for fractionated plasma products.

The second case (ninth in the series, reported in April 2004) was a 52-year-old man who had donated blood since 1984, chiefly between 1996 and 2002. No investigations were carried out into donations which preceded the vCJD epidemic. The blood components were 5 concentrated RBC units (all leukoreduced, and [illegible] leukoreduced). For donations made after 1994, 7 recipients were traced, of which 2 were [illegible] donations were used for fractionation.

The third case (13th in the series, reported in October 2004) was a 48-year-old man who [illegible] 1991 and 2004. The components were one fresh-frozen plasma (FFP) and 15 concentrated [illegible] them leukoreduced). All 16 recipients were identified, of which 6 were alive.

In total, these 3 donors account for 42 recipients of RBCs or PLTs, of whom 16 were alive at [illegible] investigation: 2 of these, transfused before 1984, were not informed, but 14 were notified [illegible] transfusions between 1991 and 2004. To date, none has presented with symptoms of vCJD [illegible] recipients were tested for evidence of infection, because all died several years before the [illegible] donor. There were clearly more recipients of fractionated plasma products prepared from plasma [illegible] affected donors. Two of the donors had given plasma destined for fractionation in the period [illegible] donations in one case, 12 in the other). These 2 donors accounted for around 50,000 recipients [illegible] treatment of chronic disorders (hemophilia, immunodeficiency), the rest for occasional treatment [illegible] immunoglobulins).

In response to the first three cases of blood donors who later developed vCJD, the following [illegible] into place in France:

- Immediate recall of in-date fractionated plasma products and labile blood components [illegible] donors. When the illness was discovered in the donor, blood products had almost always [illegible] transfused, but this strategy allowed the following actions.
- Information to the prescribers of the labile blood components implicated in the investigation.
- Direct and personal information to the recipients of blood components (except those [illegible] epidemic); exclusion of all recipients as donors of organs, tissues, and cells (they were [illegible] blood donation because of their history of transfusion); and finally, putting in place long-term [illegible] up.
- A decision to not inform individual recipients of fractionated plasma products, except [illegible] received Factor (F) VIII or F IX produced from the affected donations.
- Information aimed at the general population and at health professionals about the [illegible] transmission of vCJD.

The information given to the blood transfusion recipients by their doctor proved more difficult [illegible] more than 20 years previously, to the first blood donors to be found "LAV positive," who were [illegible] the large number of uncertainties at the time about the prognosis of infection by the agent [illegible] Those who supported not informing recipients of the risk of prion transmission through blood [illegible] following arguments: it is not possible to quantify the absolute risk, because of a number of [illegible] absence of a diagnostic test; the existence of preventive measures applied in recent years [illegible] to labile blood components; the major psychological harm resulting for such information, which [illegible] major anxiety; absence of any diagnostic or prognostic tests (except for codon 129 status) [illegible] prophylaxis or treatment. On November 4, 2004, the National Ethical Consultative Committee [illegible] confirmed its position expressed in 1997: to not worry without benefit, notably where no [illegible] available, and to take into account the risk of excluding a patient from health care in the name [illegible] precautionary principle. Finally, the CCNE insisted on the need for complete traceability of [illegible] donors who had subsequently developed vCJD.

Those in favor of informing recipients of the risk pointed out the need to inform them that [illegible] donate (in principle, because they had been transfused, the subjects were already excluded [illegible] blood donation), but also that the patients had "the right to know," imposed by French law [illegible] which puts an obligation on the doctor to alert the patient to all "newly identified risks," even [illegible] individual risk is not quantifiable and there is no available diagnostic procedure and no means [illegible] Another factor favoring informing recipients is to reduce the risk of secondary spread to [illegible] dentists, and other patients. The French circular number 138 of March 14, 2001, [illegible] principles of the risks of transmission of "nonconventional transmissible agents" during medical [illegible] procedures and had classified the recipients of labile blood components in the category of [illegible] risk of contamination by the vCJD agent. For all these reasons, in France, it was ultimately [illegible] patients at high risk of prion infection.

PRECAUTIONARY MEASURES FOR DONORS AND LABILE BLOOD COMPONENTS IN [illegible] FRANCE

Since the removal of infected beef products from the food chain, a public health measure taken to protect the general population, precautionary measures to reduce the risk of transfusion transmission of prions were implemented in the UK and France in line with advances in epidemiologic knowledge. Some were put in place before the emergence of the first case of transfusion-associated vCJD, primarily to reduce the risk of transmission of other forms of CJD and in particular the iatrogenic forms. The first case of transfusion transmission of vCJD provoked the health authorities in the UK and France to take new and complementary risk reduction measures. Along with the exclusion of at-risk donors, the introduction of leukoreduction has contributed to the reduction of the infectious load in prion transmission by blood[56] (it has been shown that this could reduce the infectivity of whole blood by almost 50%[57]). Despite this, as the cases of vCJD transmission by blood transfusion observed in the UK were all due to nonleukoreduced blood components, it could be concluded that the decrease is by definition accounted for by leukoreduced blood components, and above all that it has been established that the white blood cell (WBC) layer does not contain all the infectivity: an equal amount of infectivity exists, we now know, in plasma. Leukoreduction therefore appears a necessary measure, but certainly not sufficient.

Table 1 lists, in chronological order, the precautionary measures, specific or nonspecific, put in place in the UK[58,59] against the risk of transfusion transmission of vCJD. In France, the precautionary measures followed the same pattern in a number of complementary actions. The circular of September 23, 2005,[60] concerning the reports of the first probable British cases of transfusion transmission of vCJD and the first case of a French donor who developed the illness, raised the issue of secondary transmission by transfusion of labile blood components or by use of surgical instruments or endoscopes on patients who had received transfusions of blood components originating from donors who later developed vCJD. The successive measures instituted in France and including those taken for the other forms of CJD before the emergence of vCJD, are shown in Table 2.

TABLE 1. Preventive measures in the UK against the transfusion risk of vCJD

1997	Recall and discard of labile blood components and of plasma derivatives obtained from donors who later developed vCJD
1998	Importation of plasma destined for fractionation from non-UK sources.
1999	Leukoreduction of all labile blood products.
2000	Importation of FFP for recipients born after January 1, 1996.
2004	Permanent donor deferral in case of transfusion after January 1, 1980.
2005	Importation of FFP for recipients age less than 16 years.
	Permanent donor deferral in case of transfusion anywhere in the world after January 1, 1980.
	Permanent deferral and notification of donors whose donations have been transfused to recipients who later developed vCJD.
	Progressive replacement of PLT pools with apheresis (single-donor) PLTs. Apheresis PLTs recommended for children age less than 16 years.

TABLE 2. Preventive measures in France against the transfusion risk of vCJD

1992	Permanent donor deferral in case of treatment by injection of growth hormones of pituitary origin.
1996	Permanent donor deferral in case of history of neurodegenerative disease.
	Recall and discard of labile blood components and batches of plasma products containing plasma from donors who later developed sCJD, fCJD, or iatrogenic CJD; having a history of fCJD; or having been treated with hormones of pituitary origin.
	Permanent donor deferral in case of history of neurosurgery.
1997	Tracing of recipients of labile blood components collected from donors who later developed CJD.
	Permanent donor deferral in case of transfusion of graft.
	Recall and discard of labile blood components and plasma products obtained from donors who later developed vCJD.
1998	Leukoreduction of cellular blood products for a residual level <1 × 10^6/unit.
2000	Permanent deferral of donors who lived in UK for 1 year or more between 1980 and 1996.
2001	Leukoreduction of all plasma (FFP or plasma destined for fractionation) to a residual level <1 × 10^6/unit.
	Residual WBC level <1 × 10^6/unit for plasma not destined for fractionation.
	Reduction of volume of plasma in PLT components through use of PLT additive solution, potentially reducing an infectious load.

One difficulty with the current situation is that individuals incubating vCJD [illegible] they are at risk and may be donating their blood. This is relevant as they [illegible] relatively young and could donate their blood several times per year. [illegible] only specific preventive measure against prion contamination of blood transfusion [illegible] blood test for qualification of donors, or a general measure which could [illegible] for prion filters), or both.

In the prevention of any transfusion risk, an equilibrium between the [illegible] blood components is necessary. Being the most exposed country, the UK has [illegible] transmission by blood transfusion, such as the importation of all plasma [illegible] the exclusion of numerous blood donors,[62] these measures were [illegible]

Transfusion measures taken in other countries are essentially based [illegible] have stayed in an "endemic" area. For example, the Canadian authorities [illegible] measures to exclude from donation individuals who became at risk [illegible] vCJD epidemic: in 1999, all people who had spent a cumulative period [illegible] excluded from donation; in 2000, it was the same criteria for [illegible] France or the UK was reduced to 3 months; and that of a stay abroad [illegible] period of 5 years.[65,66]

After a case of vCJD in an individual who visited the UK for less than [illegible] 2001 an illness that led to his death in 2004, Japan also took precautionary [illegible] considering that the patient had become infected in the UK, even though [illegible] have been identified with BSE. Having already excluded donors who had stayed [illegible] Japanese health authorities took the decision to exclude all individuals who had [illegible] country between 1980 and 1996. One can see that prion infection and [illegible] two common points: they cross all frontiers and spread in an unforeseen manner.

THE VARIABLES OF RISK OF TRANSFUSION TRANSMISSION OF [illegible] COMPONENTS

At this stage of medical knowledge, it is clear that all the elements of [illegible] are not clarified. Certain elements are however identifiable:

1. The number of labile blood components received by the patient and [illegible] to the dates of the epidemic and to the application of precautionary [illegible] leukoreduction, etc.).
2. The prevalence of infection in blood donors: a great uncertainty [illegible] upon that in the general population who were exposed throughout [illegible] taking into account the higher end of the estimate of between [illegible] French population and imagining these 300 cases among the [illegible] be blood donors (18-65 years) and eligible to be donors, and [illegible] the whole incubation period, results in a prevalence of 1 in 1,2[illegible] individuals per 1 million donors, which is close to 1 infected donor [illegible] In a recent reevaluation, the number of expected vCJD cases in France [illegible] 100 cases instead of 300), leading to an estimate of 1 donor infected [illegible] calculation in the UK gives a prevalence of 1 in 10,000 donors, [illegible] available in the UK with results from the National Anonymous Survey [illegible] progress.
3. Infectivity of a labile blood component with regard to prions is still [illegible] an "infectious dose," defined as the minimal dose capable of transmitting [illegible] the mode of contamination given. At present, the infection of a unit of [illegible]
   - The stage of infection in the donor: the level of circulating prion and [illegible] increases with the duration of the incubation period.[68] This might [illegible]

the infected subject becomes infective for the recipient of the blood: an infected donor, donating during the early part of the incubation period, may not be infectious to a recipient. According to animal studies, blood infectivity can be demonstrated at least at the start of the second half of the incubation period and perhaps also earlier (the infectivity of blood precedes the presence of pathological prion in the brain and the organs).[68] Even though experiments suggest that infectivity will be absent or minimal during the first third of the incubation period, caution dictates, in the current state of knowledge, that a labile blood component originating from a donor in the incubation period contains at least one infectious dose.[69] As many years have passed since the peak of the dietary epidemic, infected individuals are no longer in the initial stages of infection. The paradox could be that even though the number of infections is no longer increasing, the number of infectious subjects could still increase over time.

- Second, the efficacy of leukoreduction for cellular components and for plasma: leukoreduction of hamster blood contaminated with a scrapie prion removed only a little less than half (42%) of the infectivity present, because the infectivity divides almost equally between the WBCs and the plasma.[57,70,71] Leukoreduction may therefore be less effective than originally calculated. As demonstrated in studies based on experimentally infected rodent blood, total blood infectivity will be, during the asymptomatic phase, from 20-30 IU per mL,[35] and the distribution in the compartments of blood is in the order of 30 percent in the buffy coat and 50 percent in the plasma.[71] The presence of RBC and PLT infectivity has not been established in a formal manner: it seems at any rate to be little or none.[72,73] Thus, after the implementation of leukoreduction of labile blood components (which must have a residual WBC count of $<1 \times 10^6$/unit), the infectivity of RBC or PLT components is dependent on the amount of residual plasma. Use of optimal additive solutions for cellular components helps to reduce the quantity of plasma and therefore the infectious dose in the case of an infected blood component.

4. Recipient methionine homozygosity at codon 129 has an impact on the risk of developing illness, with perhaps a hierarchy of risk, moving in descending order from MM homozygotes to MV heterozygotes to VV homozygotes. Furthermore, nonhomozygosity for MM does not appear to confer absolute protection from infection, as indicated by the second UK recipient case (an MV heterozygote, nonetheless infected through the transfusion route) and in experimental animals.[13] What is certain is that the clinical outcome of transfusion transmission appears to be greatest for MM homozygotes, since they alone of the "exposed" population at risk have developed the disease.
5. Finally, the length of the incubation period, an essential factor and of which much is currently unknown and to which must be added two important parameters: the age of the recipient and the posttransfusion survival, which is heavily influenced by deaths due to the underlying illness in the initial years after the blood transfusion.

PRION FILTERS

Specific prion reduction filters applicable for certain labile blood components have been undergoing validation. The first donations processed with these prion filters demonstrated their capacity to reduce spiked infectivity of blood by three logs, which would without a doubt make a significant contribution to reducing transfusion risk.[74] These filters have been produced by two companies with a view to use for RBC preparations: application to PLT preparations and to plasma await further work. The validation work has been carried out on the Pall leukotrap affinity prion reduction filter, integrated in the filter CompoSafe Pr Fresenius,[75-77] and the TSE affinity ligand of the pathogen removal and diagnostic technologies, integrated in the P-Capt MC (MC for Macopharma) filter.[78] Changes were made to the Pall filter after the initial validation, which affected performance and led to its withdrawal. A new combined leukoreduction and prion removal filter from the same manufacturer is now under development. These affinity filters are assumed to remove all detectable traces of infection in a contaminated unit and to reduce infectivity by transfusion. This capacity has been demonstrated by a study based on inoculation, in hamsters, of leukoreduced whole blood taken from animals infected by a TSE. When the blood was treated with passage over a filter, no hamster became infected. When the blood was not filtered, some hamsters developed illness associated with the presence of prions in tissues.[79] Nevertheless, although the potential of these filters has been demonstrated by experimental infectivity transmissions in animal models, their efficacy in the prevention of human transfusion transmission remains to be validated.[80] [illegible] prion circulating in different human blood components may differ from that [illegible] manner, in particular from brain extracts. These artificial situations [illegible] quantitative characteristics of the human prionemia. The most infectious aggregates [illegible] are those that are formed of 14 to 28 molecules,[81] but the size of circulating [illegible] unknown. Furthermore, the consequences of using prion reduction filters on blood [illegible] maintenance of PLT function) and on plasma proteins is totally unknown.[82] [illegible] the risk of neoantigenicity and induction of inhibitors.

A MUCH-AWAITED DIAGNOSTIC TEST

Lacking nucleic acid and not provoking any immune response by the infected host, the [illegible] detected by molecular or serologic methods usually used in viral diagnosis. [illegible] markers has, up to now, reached a dead end.[83-85]

In asymptomatic or symptomatic infection, the most useful diagnostic test [illegible] pathologic prion in the blood. However, the form that the prion takes in the blood [illegible] central nervous system. PrP^{sc} has an aggregated form in the brain and a [illegible] that difference could influence the effectiveness of diagnostic tests, the majority [illegible] capacity to detect the cerebral form. Furthermore, the pathologic form only represents [illegible] prions, but it is this pathologic form that the test must detect. Most of the diagnostic [illegible] depend on the physicochemical differences in the two forms of prion, the normal and the [illegible] on the resistance of the pathological form to proteinase K.[11,36-45]

A large number of unknowns relating to the transmissibility, epidemiology [illegible] no doubt be resolved when one or several diagnostic tests, having the [illegible] specificity, and reproducibility, become available and usable on a large scale. [illegible] being made to develop such tools, which could be used in the screening of blood donations [illegible] reduce even further the risk of transfusion transmission of prions. These tests [illegible] criteria:[93,94]

1. A very high sensitivity, to detect an infectious load that may be very [illegible] a low level of PrP^{sc} in circulating blood is likely to be infectious for [illegible]
2. High specificity is essential, since the normal protein is present in the [illegible] results could have disastrous consequences, in terms of notifying individuals whose [illegible] concluded "positive," not to mention the unjustified deferral of a large number of donors [illegible] infections, every reactive result obtained through blood donation screening tests must [illegible] confirmatory test to separate true-positive results from false-positive results. [illegible] true confirmatory test will be available, whether the solution for prions will [illegible] simultaneously, or if one will be used for "confirmation" of a positive result [illegible] specificity, it has been calculated that, if a diagnostic test having a [illegible] specificity was applied to the screening of blood donations in a population [illegible] in 10,000 (which is the estimate for donors in the UK), 99 individuals would [illegible] phase of the infection and would correspond to "true positives" for 1 million tested [illegible] donors would give a false-positive result. On the other hand, there would [illegible] 1 million tests.[96] In France, where an estimate of prevalence [illegible] carriers of the variant would be detected, but the number of false-positive [illegible] the UK: 10,000 per 1 million donors, who would not be allowed to give [illegible] informed of their biologic status.
3. Finally, these tests will have to be reproducible, usable [illegible] time scale that is compatible with the shelf life of PLT concentrates [illegible] obtained) for nucleic acid testing in transfusion.

The lack of a test with the above-mentioned characteristics has major consequences [illegible] from blood donation all those who are carriers of vCJD and the necessity of basing [illegible] blood donations on nonspecific or partially effective measures such as exclusion [illegible] leukoreduction of blood donations, and so forth; the impossibility of detecting [illegible] risk recipients; the difficulty in collecting data about the mean duration of incubation [illegible]

and, further to all this, the impossibility of rehabilitating donors excluded because of a stay in the UK during the affected years (all the more because, among the cases of vCJD identified in France, such a history has been found only once and it becomes a paradox to exclude donors on the pretext of a visit to the UK when almost all the French patients who had the illness were infected in their own country). It would be necessary, furthermore, to take care that the positive effect of such a "rehabilitation" for some donors was not offset by a negative effect, by announcing, in the media, the use of a specific transfusion screening test. This could raise concern in the donor population, of learning, through going blood, that they carry the infectious agent of an illness for which there is no preventive or curative treatment.

While awaiting validation, and predicting their potential use in detecting donors who are infected by vCJD, the first tests could be usefully applied in studies of sample repositories, to determine the spread of the epidemic in the overall population and in the transfused population. Assessment of the prevalence of vCJD in donors and recipients of blood, as well as its transmissibility through plasma products, could be carried out via anonymous plasma samples of infected donors and recipients. This is one of the possibilities provided by the repository presently undertaken on a European scale, called "BOTTIA" (Blood and Organ Transmissible Infectious Agents).[97] Indeed, for obvious ethical reasons, one cannot use imperfectly validated tests on nonanonymous samples.

Meanwhile, the absence of a diagnostic test and strong uncertainties about a transfusion epidemic of vCJD requires maintenance of the preventive measures established by the UK, France, and other countries. If a specific test is used in transfusion in the future, there will be the opportunity to consider relaxation of these measures.

UNCERTAINTIES

An illness whose pathogenicity is not well known, with an uncertain prevalence of the infectious agent in at-risk groups and in the general population, the absence of a screening test, infectiousness and duration of incubation poorly defined, and the absence of any therapy, make up the elements that influence the transfusion risk of vCJD and handicap its prevention. Many questions have no answers, and the order in which we enumerate them probably does not correspond to the sequence in which solutions will be found:

1. After the end of the UK dietary epidemic and after the peak of the vCJD epidemic in 1999, will there be a second peak of transfusion origin? Up to now, the epidemic has remained relatively limited: approximately 600 cases worldwide, of which three-quarters have been in the UK. The initial pessimistic hypotheses on future number of cases have been revised downward. Furthermore, the peaks that followed the initial peak of CJD cases linked to injection of contaminated growth hormone were smaller and smaller, as if patients of other genotypes were less susceptible to infection and/or to the development of clinical illness. It is not known if the same will happen with vCJD, but the hypothesis of a secondary transfusion epidemic, with amplification of the phenomenon through asymptomatic carriers of the prion, cannot be excluded. Nevertheless, it is now 14 years since the first cases of vCJD occurred, and no evidence of clinical cases in heterozygotes has appeared, in contrast to observations in the growth hormone epidemic. Finally, intraspecies transmission of prions induces, compared to interspecies transmission, a shorter incubation period and increased effectiveness of transmission. This could cause a larger outbreak of infection through transfusion than through contamination by food.
2. What is the prevalence of infection in the general population of the UK and in France, and how many potentially infected donors are there? The results of a retrospective British study on the prevalence of vCJD in surgical tissues from appendectomies and tonsillectomies pointed in the direction of a much higher prevalence of asymptomatic carriers than was implied by the known number of symptomatic cases. Furthermore, since, in the British MV transfused recipient carrying the variant, PrP^{res} was only detectable in the spleen and the cervical lymph nodes, and not in the appendix or the tonsils, this retrospective epidemiologic study based on detection of the pathologic prion in the appendix could have underestimated the size of the epidemic in the general population.
3. What are the kinetics of the appearance of circulating prion during the incubation phase? For estimation of the transfusion risk, the working hypothesis is that of blood infectivity and thus potential transmissibility throughout this phase, but the prion level in circulating blood may be too low, in the first months or first years of infection, to transmit infection by transfusion.
4. What is the effect of the current precautionary measures in transfusion, especially leukoreduction? The margin of safety that this measure gives is unknown. Has a reduction in infectivity prevented, or will it prevent, some transmissions by blood components? Up to now, the most feared contradiction would be the appearance of vCJD in a recipient transfused solely with leukoreduced components. Such a finding has not yet been reported.

5. Do non-MM subjects (that is, 60% of the general population) [illegible] might they develop it after a longer period of incubation? This latter hypothesis would [illegible] the epidemic, which might, furthermore, be partially masked by other causes of [illegible] situations, infected asymptomatic subjects would not be [illegible] recipients could become symptomatic if they have the MM status. Such a situation [illegible] epidemiologically disastrous: an MV or VV infected donor [illegible] and not become ill him- or herself, but the recipients would develop illness [illegible] circumstances have been observed in viral transfusion transmissions, notably [illegible] infected recipient could develop symptomatic illness several years before [illegible] donors who are carriers of vCJD but do not develop the illness [illegible] 129 would not be identifiable without a specific diagnostic test, except by [illegible] their common donor status in two (or more) recipients infected by [illegible] because of a nonprotecting genotype. Such studies would [illegible] regular, infected donor and of interrupting a chain of transmission [illegible] extent the deferral of transfused patients from giving blood [illegible] "contamination cycle" between the donor population and [illegible] this precaution has probably avoided several transfusion [illegible] based on a mathematical model, has concluded that the effect [illegible] majority of donors were infected from dietary sources and [illegible] excluded from blood donation.[99]
6. How many donors and recipients will develop vCJD during the next few years [illegible] transfusion lookbacks and investigations? In the TMGR study, among recipients [illegible] infected donors, the proportion of recipients who developed the [illegible] period of time (less than a decade), taking into account that the [illegible] genetic status of codon 129.
7. Will the threat of transfusion transmission of prions be limited solely to the [illegible] in other countries such as Spain and Saudi Arabia, of cases of vCJD [illegible] that the problem has now taken on an international dimension, [illegible] transfusion safety.

CONCLUSIONS

The possibility of a blood component recipient developing vCJD [illegible] regular donor developing it after the same amount of time, and that [illegible] with the help of a transfusion traceability almost as prolonged as the [illegible] dietary epidemic is now a problem of chronic asymptomatic carriers [illegible] infection via medical devices used in surgery or in [illegible] of epidemic.

An essential notion is that of protection provided by [illegible] scenario, where cases of vCJD would show up in recipients [illegible] blood components and thus infected by the residual [illegible] the screening of blood donations, would be to have recourse [illegible] and harmlessness is resolved, or to only use washed [illegible] concerns[101]—even if a partial reduction of prion level [illegible] infected recipients and/or to induce a longer incubation period [illegible] proportional to the original contaminating inoculum).[102]

Many professionals in the field of transfusion infectious [illegible] acknowledging that demonstration of their clinical efficacy remains difficult for [illegible] and which are dominated by the absence of a diagnostic test usable on a large [illegible] these filters is problematic and leads to as many questions as not using them. [illegible] test that would be applicable for blood donations will raise a no less difficult question: [illegible] test?

Procedures for the inactivation of infectious agents in labile blood components [illegible] since they are aimed at the nucleic acids of these agents, they will not be effective [illegible]

the prion could become the ultimate transfusion-transmissible agent, while the risk connected to viruses, bacteria and parasites, known or emerging, would be controlled by pathogen inactivation.

If transfusion transmission of vCJD is a certainty from now on, benefits of transfusion obviously remain immeasurable compared to this risk. One must put in perspective the number of lives saved every day by transfusion and the number of cases of transfused vCJD counted on a worldwide scale. One also must compare this risk, which mainly concerns two European countries, with the infectious risks faced by transfused patients in parts of the globe where the means are so limited that safety is not always assured even for major blood-borne agents.

Never before have so many measures been taken in transfusion to counteract a risk that is numerically so low, some taken even before the first case of vCJD by blood transfusion had been reported. The precautionary principle has not just gone into the law: it has also penetrated the senses.

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