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REVIEW

Blood infectivity, processing and screening tests in transmissible spongiform encephalopathy

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Surprising advances have been made in the areas of blood infectivity, infectivity removal and, especially, blood screening tests for transmissible spongiform encephalopathy (TSE) in the past few years. In fact, if anyone as recently as last year had suggested that a screening test for preclinical human infection might be available before the end of 2005, the statement would have been met with smiling disbelief. Nevertheless, it can be confidently predicted that the diagnostic misfolded 'prion' protein (PrP^{TSE}) will soon be reliably detectable in blood during the symptomatic phase of disease, and it is highly probable that it will also be detectable in blood from at least a proportion of infected individuals during the preclinical phase of disease.

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Blood infectivity in transmissible spongiform encephalopathy

Investigation of blood infectivity in transmissible spongiform encephalopathy (TSE) goes back nearly a half century, to the early 1960s (Table 1), with results that until very recently led to the conclusion that although low levels of infectivity could often be detected in the blood of rodents with experimentally induced disease, no infectivity could be demonstrated in the blood of animals with naturally acquired disease. The situation in humans was complicated by the fact that each of the few reported successes could be questioned on the basis of technical issues or non-reproducibility [1]. Within the past few years, however, this conclusion has been discarded with respect to both animals and humans (Table 2): blood or blood component transfusions from sheep with natural scrapie have yielded a transmission rate of ≈ 40%, and packed red cell donations from two young adults who, 18–36 months later, developed variant CJD (vCJD) almost certainly were responsible for infecting two older adults [2–4].

What level of infectivity is responsible for these transmissions? We know from experimentally infected rodents that the concentration of infectivity in buffy coat can range from 10 to 20 infectious doses (ID) per ml during the preclinical phase of disease, and increases to 50–110 ID/ml at the terminal stage of illness. The corresponding levels in plasma range from undetectable to 5 ID/ml during the preclinical period, and 20–30 ID/ml at terminal illness. We also know that blood components from TSE-inoculated chimpanzees inoculated into susceptible squirrel monkeys resulted in only a single transmission, from purified leucocytes (P. Brown *et al.*, unpublished data). Thus, it can be stated with confidence that infectivity is predominantly associated with the leucocyte fraction of circulating blood. From the viewpoint of therapy, however, it is equally important to note that because of the large difference in component volumes, the total amount of infectivity in blood is almost equal between the leucocyte fraction and the plasma fraction.

It is also important to recognize that these levels of infectivity are most unlikely to be present in the blood of humans with sporadic CJD. Even 5 ID/ml, multiplied by 200 ml to yield a total 1000 ID/ml in a unit of fresh frozen plasma, would have long since caused at least some cases of disease in recipients of contaminated whole blood or plasma, whereas no cases were identified in several older statistical and 'look-back' studies [1], or in a still-ongoing systematic study of hundreds of recipients of potentially contaminated blood, of whom 116 are either still living or have died from non-CJD illnesses, at least 5 years after receiving the donation (http://www.nbdrc.org/research/cjd.htm).

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Table 1 20th Century attempts to transmit transmissible spongiform encephalopathy (TSE) from blood or blood components

Species					
Donor	**Assay**	**Inoculum**	**Route of inoculation**	**Positive/total donors**	**Reference[a]**
Scrapie (natural)					
Goat	Mouse	Blood clot/serum	i.c.	0/3	Hadlow 1980
Sheep	Mouse	Blood clot/serum	i.c.	0/18	Hadlow 1982
BSE (natural)					
Cow	Mouse	Blood clot/serum/	i.c. + i.p.	0/2	Fraser 1994, cited
		Buffy coat	i.c. + i.p.	0/2	in Bradley 1999
Scrapie (experimental)					
Goat	Goat	Whole blood	i.c.	0/14	Pattison 1962
Sheep	Mouse	Serum	i.c.	1/1	Gibbs 1965
Mouse	Mouse	Whole blood	i.c.	0/39	Eklund 1967
Mouse	Mouse	Serum	i.c.	1/1 (pool)	Clarke 1967
Rat	Rat	Serum	i.c.	1/1 (pool)	Clarke 1967
Mouse	Mouse	Whole blood	i.c.	3/13	Dickinson 1969
Goat	Mouse	Blood clot	i.c. or s.c.	0/20	Hadlow 1974
Hamster	Hamster	Whole blood	i.c.	0/9	Diringer 1984
Hamster	Hamster	Blood extract	i.c.	5/5 (pools)	Diringer 1984
Hamster	Hamster	Blood extract	i.c.	10/11 (pools)	Casaccia 1989
Mink encephalopathy (experimental)					
Mink	Mink	Serum	i.c.	0/1	Marsh 1969
Mink	Mink	Whole blood, or components	i.c.	0/8 (pools)	Marsh 1973
CJD (experimental)					
Guinea pig	Guinea pig	Buffy coat	i.c., s.c., i.m., i.p.	10/28	Manuelidis 1978
GSS (experimental)					
Mouse	Mouse	Buffy coat	i.p.	4/7 (pools)	Kuroda 1983
Mouse	Mouse	Buffy coat/plasma	i.c.	5/5 (pools)	Brown 1999
		Buffy coat/plasma	i.v.	2/2 (pools)	Brown 1999

[a]Citations for most of the studies can be found in the reference lists of articles [1] and [5].

BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; GSS, Gerstmann-Sträussler-Scheinker syndrome; i.c., intracerebral; i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous.

The situation with respect to vCJD is less sanguine. The first presumptive case of transfusion-associated disease occurred in a 69-year-old man who became ill 7 years after having received packed red cells from a young adult who did not begin to show signs of vCJD until 3 years after making the donation [3]. A second transmission occurred in a 77-year-old recipient who died of an unrelated illness 5 years after having received packed red cells from another young adult who did not become ill until 18 months after donating the blood [4]. This transmission was only identified by the postmortem discovery of PrP^{TSE} in a lymph node and spleen (the brain was normal), and it will never be known whether the infection would have persisted in a silent carrier state, or evolved into clinical disease. These two cases of transfusion-associated infection have occurred within a group of 50 recipients of whole blood or labile blood components donated by individuals later dying of vCJD; of these 50 recipients 20 lived, or are still alive, at least 5 years afterwards. An additional 25 individuals were recipients of blood from three vCJD patients in France, of whom five are still alive, or died of non-CJD illnesses at least 5 years afterwards (Dr J. P. H. Trouvin, personal communication).

Genetic susceptibility may affect the outcome of transfusion exposure to vCJD, as it does to primary oral exposure to bovine spongiform encephalopathy (BSE). All 133 tested cases of orally transmitted vCJD were found to be homozygous for methionine at the polymorphic codon 129 of the PRNP gene that encodes the 'prion' protein. Homozygosity at this site apparently predisposes to all forms of human TSE, including sporadic, familial and iatrogenic CJD, as ≈ 80% of patients are homozygous for either methionine or valine, compared to a 50% frequency in the general population [6].

Table 2 21st Century attempts to transmit transmissible spongiform encephalopathy (TSE) from blood or blood components

Species					
Donor	**Assay**	**Inoculum**	**Route of inoculation**	**Positive/total donors**	**Reference[a]**
Scrapie (natural)					
Sheep	Sheep	Whole blood	i.v.	4/10	Houston 2005
		Buffy coat	i.v.	4/11	
Scrapie (experimental)					
Hamster	Hamster	All components	i.c.	1/1 (pool)	Rohwer 2000
		Whole blood	i.c.	31/124	(unpublished)
		Whole blood	i.v.	3/108	
BSE (experimental)					
Cow	Mouse	Buffy coat	i.c. + i.p.	0/11 (pools)	Wells 2000
	Cow	Buffy coat	i.c.	0/4 (pools)	
Mouse	Mouse	Plasma	i.c.	4/48	Taylor 2000
Microcebe	Microcebe	Buffy coat	i.c.	1/1	Bons 2002
Sheep	Sheep[b]	Whole blood	i.v.	5/11	Houston 2005
		Buffy coat	i.v.	1/7	
Monkey	Monkey	Buffy coat			Lasmezas 2005
		Plasma			
GSS (experimental)					
Chimp	Monkey	Leucocytes	i.c. + i.v.	1/1	Brown 2005
		Plasma	i.c. + i.v.	0/1	(unpublished)
		Platelets	i.c. + i.v.	0/1	
sCJD (experimental)					
Monkey	Monkey[b]	Whole blood	i.v.	0/4	Brown 2005
Chimp	Monkey	Leucocytes	i.c. + i.v.	0/2	(unpublished)
		Plasma	i.c. + i.v.	0/2	
		Platelets	i.c. + i.v.	0/2	
Human	Monkey	Buffy coat	i.c. + i.v.	0/2 (pools)	
		Plasma	i.c. + i.v.	0/2 (pools)	
Human	Chimp	Buffy coat	i.c.	0/3	
vCJD (experimental)					
Mouse	Mouse	Buffy coat	i.c.	2/2 (pools)	Cervenakova 2003
			i.v.	2/2 (pools)	
		Plasma	i.c.	2/2 (pools)	
			i.v.	2/2 (pools)	
Monkey	Monkey[b]	Whole blood	i.v.	0/4	Brown 2005
Human	Monkey	Buffy coat	i.c. + i.v.	0/3 (pool)	(unpublished)
		Plasma	i.c. + i.v.	0/3 (pool)	

[a]Citations for most of the studies can be found in the reference lists of articles [1] and [5].

[b]Ongoing experiment.

BSE, bovine spongiform encephalopathy; GSS, Gerstmann-Sträussler-Scheinker syndrome; i.c., intracerebral; i.p., intraperitoneal; i.v., intravenous; sCJD, sporadic Creutzfeldt–Jakob disease; vCJD, variant Creutzfeldt–Jakob disease.

As expected, the first transfusion-related case of vCJD was also homozygous for methionine, but the second (asymptomatic) case was a heterozygote. It has been suggested that this genotype could have prolonged the preclinical phase of disease, but it should be borne in mind that the original (homozygous) patient did not begin to show symptoms until 7 years after his transfusion, an interval 2 years longer than that between transfusion and death of the second patient from unrelated illness.

One other piece of information bears on the issue of case number prediction: random screening for PrP^{TSE} in tonsils and appendices removed from adolescents and young

adults during the period since ≈ 1990 have revealed three positives among a tested population of 12 674 young adult individuals, leading to an age-adjusted estimate of as many as 3800 infected, but presymptomatic, apparently healthy, individuals in the UK [7]. Without some type of screening test to identify any such presymptomatic individuals there is no way to prevent them from donating blood and thus exposing an unsuspecting recipient population to the risk of iatrogenic transfusion-transmitted vCJD.

Finally, the unexpected similarity of blood study results in experimental models of vCJD and sporadic CJD (sCJD) raises a question as to whether blood from patients with sCJD might not, after all, pose a risk despite the absence of recognized transfusion-associated cases. The problem may simply be recognition, which will probably require the reverse conditions of the vCJD cases, that is, an older adult with typical sCJD donating blood to an adolescent or young adult, both of whom subsequently proceed to develop neuropathologically verified sCJD. Even then, the occasional occurrence of sporadic disease in people under the age of 30 years would make the association less convincing than was the case for vCJD.

Blood processing and validation issues

There exist, at present, only two strategies to minimize the risk of exposure to contaminated blood: eliminating infected blood donors; and eliminating the infectious agent during blood processing. Eliminating the source of infectivity by donor-deferral policies can never be perfect, depending as it does on information volunteered by the donor, but the deferral of various categories of donors at higher-than-normal risk of harbouring infection can certainly remove a major source of potentially infectious blood. Policies vary from country to country, but generally limit donations from individuals who have resided for stipulated periods of time in countries in which BSE occurs, and in some cases deferrals have been extended to include individuals in the UK or France who had received blood donations, or who had been vaccinated during the past 20 years. However, even the most stringent deferral policy cannot prevent donations from apparently healthy individuals in the preclinical phase of vCJD (we already know that blood can be infectious for at least 3 years prior to the onset of symptoms), and so a second line of defence is clearly desirable.

This additional precaution resides in blood processing, which could, in principle, involve either inactivation or simple removal. However, the resistance of TSE agents to all but the harshest methods of inactivation (e.g. exposure to bleach, lye, or autoclaving) makes this approach impossible. Removal, on the other hand, can be accomplished in a variety of ways. The three most commonly used processing steps that have been shown to remove agent infectivity are precipitation, depth filtration and chromatography. All act in a non-specific manner by aggregate precipitation or adsorption, and have been shown to reduce infectivity by 2–6 $\log_{10}$ mean lethal doses (LD_{50}) per ml, with an average of ≈ 3 $\log_{10}$ LD_{50}/ml [8,9]. Recently, a filter incorporating a ligand with specific affinity for PrP has been reported to reduce infectivity in blood spiked with scrapie-infected hamster brain by nearly 4 $\log_{10}$ LD_{50}/ml [10].

Table 3 Considerations about the choice of infectious 'spikes' for validation studies

Categories	Alternatives
TSE strain	Scrapie
	BSE
	CJD
	vCJD
Animal model	Mice
	Hamsters
	Guinea pigs
	Sheep
	Bovines
	Primates
Tissue	Brain
	Spleen
	Lymph nodes
Preparation	Tissue homogenate
	Trypsinized cells
	Microsomal fractions
	Caveolar-like domains
	Purified PrP^{TSE}
Assay	Western blot of PrP^{TSE}
	Animal bioassay
	Cell culture bioassay

BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; PrP^{TSE}, misfolded 'prion' protein; TSE, transmissible spongiform encephalopathy; vCJD, variant Creutzfeldt–Jakob disease.

The ideal model for validating risk reduction in blood-processing protocols would employ tissue containing a high level of infectivity in a form known to circulate in human blood. The problem is that such a model does not exist, and thus a compromise is necessary between the need for high-infectivity material and relevance to the circulating blood of a human donor.

Several aspects of the compromise require consideration (Table 3). With respect to endogenous infection there is the choice of TSE strains and animal species, which could be scrapie, BSE, or human TSE strains in rodents, sheep, cows, or primates. Most work, to date, has been performed in mice and hamsters, using rodent-adapted strains of scrapie or human TSE, and although it is always problematic to what extent

such models reflect the human situation, reported data show a good parallelism in processing reductions using a variety of TSE strains [9]. The major disadvantage of rodents is the need to use a large number of animals to obtain sufficient blood to study; the major disadvantage of ruminants is their cumbersome size; and the major disadvantage of primates is their expense and limited availability. To date, only rodent blood has been titrated to determine levels of infectivity associated with experimentally induced endogenous infections, and those levels are never greater than 1–2 $\log_{10}$ LD_{50}/ml, many orders of magnitude lower than those of brain tissue from the same animals.

The choice of TSE strains and animal species applies equally to validation studies using exogenous (spiked) material, and the *raison d'être* of spiking experiments – a high level of input infectivity – mandates the use of brain tissue, because peripheral tissues such as spleen or spleen fractions have infectivity levels not much higher than those of circulating leucocytes [11]. An additional consideration with respect to spikes is the choice of tissue preparations: published data comparing several different protocols indicate that purified PrP^{TSE} provides a less stringent challenge than crude brain homogenate [12]. It seems improbable, however, that the form of infectious agent in brain tissue is exactly the same as that circulating in blood, and so its relevance to blood infectivity studies is moot, especially in regard to removal strategies that depend on the minimum size of an infectious particle (which is unknown and may well be heterogeneous). The observation that a spike consisting of trypsinized brain cells showed a partitioning of infectivity in blood similar to that found in endogenous infections [13] deserves further exploration as a way to minimize the 'artificiality' of a crude brain homogenate and still have a high level of infectivity.

The last issue in a consideration of validation studies concerns the choice of an assay that is used to monitor the effectiveness of agent clearance. Measurement of the misfolded protein, PrP^{TSE}, is easy, rapid and comparatively inexpensive, and for purposes of validation tests using high-infectivity spikes, a very good surrogate for the much more time-consuming and expensive infectivity bioassay [14]. It should not, however, be considered as a substitute for the bioassay, but rather as a method that can provide a good approximation of the reduction inherent in the various steps of a processing protocol.

Although dilution end-point bioassay determinations remain the final arbiter of validation, there is a good correlation between the 'dose' of infectious agent and the length of the incubation period 'response' to the dose: the greater the dose, the shorter the incubation period. This correlation permits a good estimation of the infectivity end-point from the length of the incubation period, requiring only a single untreated control titration against which to compare any number of single-dilution inoculations of test samples. However, it is vulnerable to inaccuracy at the low blood infectivity levels of endogenous infections. TSE bioassays have historically been performed in animals, but two recently developed tissue culture bioassays have been shown to have sensitivities comparable to those of animal bioassays, and could greatly reduce the time, space and financial constraints of bioassay testing [15] (Dr Benöit Flan, presentation at Cambridge HealthTech Institute Conference on Transmissible Spongiform Encephalopathies, McLean, VA, February 14–15, 2005).

All things considered, perhaps the most rigorous and realistic protocol would use both exogenous spikes and endogenous infections: high-input infectivity spikes would allow evaluation of the broadest possible range of agent clearance, including reduction for the process as a whole, as well as for individually spiked processing steps, whereas low-input endogenous infectivity would provide at least some reassurance that the process is applicable to the naturally occurring situation. Spikes of individual process steps could be assessed by Western blot tests, leaving only one or two critical steps and the final product for infectivity bioassays. Endogenous infections would necessarily require bioassays because the levels of PrP^{TSE} are beneath the threshold of detection unless the protein can be amplified by one of the methodologies under current development for use in screening tests.

Screening tests

The holy grail of risk reduction is the development of a reliable, practical test for preclinical TSE infection, and enormous progress towards this goal has been made during the past 2 years. We will look at the chronology of this progress, briefly noting earlier methodologies that have run into difficulties, and then describe, in some detail, three tests that are currently verging towards success (Table 4). All are based on detection of the pathologically misfolded protein.

It became clear, from the very first attempts, that Western blot assays and enzyme-linked immunosorbent assays (ELISAs) would not be sufficiently sensitive to detect the low levels of PrP^{TSE} found in the blood, even in the clinical phase of disease. The first test to show promise employed a combination of competitive antibody capture and capillary electrophoresis (CE). However, despite reports of success in identifying normal vs. scrapie-infected sheep [16,17], the method failed to discriminate between normal and CJD-infected chimpanzees or humans, or saline controls, and thus remains in limbo with respect to a human screening test methodology [18].

The next test that seemed to have a bright future was based on conformational changes in the protein during denaturation, and was named the conformation-dependent immunoassay (CDI) [19]. Interesting results have been presented at scientific meetings, showing high sensitivity for recombinant

Table 4 Candidate tests for detecting PrP^{TSE} in blood from infected animals or humans

			Animal blood		Human blood	
Test method	Recombinant PrP	Brain PrP^{TSE}	Clinical	Preclinical	Clinical	Preclinical
Capillary electrophoresis (CE)	+	+	(+)	(+)	-	NT
Screening for intensely fluorescent targets (SIFT)	NA	+	NT	NT	NT	NT
Conformation-dependent-immunoassay (CDI)	+	+	(+)	NT	NT	NT
Immuno-PCR	+	+	NT	NT	NT	NT
Protein misfolding cyclic amplification (PMCA)	NA	+	(+)	NT	NT	NT
Polyanion ligand (Seprion)	NA	+	(+)	(+)	(+)	NT
Polypeptide ligand (Adlyfe)	NA	+	+	(+)	(+)	NT

NA, not applicable; NT, not tested; PCR, polymerase chain reaction.
Parentheses indicate that results are based on a very limited number of specimens, or have not been reproducibly or independently verified.

protein (rPrP) and brain extracts of PrP^{TSE}, as well as an ability to distinguish between blood samples from normal and sick hamsters that had been infected with the 263K strain of scrapie (Dr Jiri Safar, presentation at Cambridge HealthTech Institute Conference on Transmissible Spongiform Encephalopathies, Washington, DC, February 12–13, 2003). Nothing further is known about the test, and no publications have appeared, so this method also remains in limbo.

A third test that showed early promise, but seems to have run into difficulties, is based on the fact that, in contrast to the normal cellular protein, its misfolded form tends to aggregate, thereby increasing the number of sites available to bind a labelled antibody. With appropriate gating of stronger signals from weaker signals, it seemed possible that screening for intensely fluorescent targets (SIFT) might provide a novel detection method for PrP^{TSE}. It was successfully used on rPrP as well as on PrP^{TSE} extracted from brain or spinal fluid [20], but has apparently not been used (or used successfully) to test blood or plasma.

The fourth promising methodology is an application of the polymerase chain reaction (PCR) to the amplification of proteins, using a ligand to bind the PrP^{TSE} to nucleic acid and then running a PCR protocol. This immuno-PCR test has been proven to have an exquisite sensitivity, detecting rPrP at attogram levels, and PrP^{TSE} in scrapie-infected hamster brain in dilutions as high as 10^{-8}, but has, as yet, not been attempted on infected blood [21].

Although this record of unrealized potential tends to dampen expectations about the introduction of promising new tests, the past year has witnessed three new methodologies that have been developed and refined to the point that it is now possible to hope that success may, after all, be at hand. One involves PrP^{TSE} amplification, another relies on an aggregate-specific ligand capture of PrP^{TSE}, and the third combines both ligand-based capture and amplification in a single step.

The PrP^{TSE} amplification method was first described in 2001 and extended the discovery, several years earlier, which found that when infected brain tissue containing the misfolded protein is mixed together with uninfected brain containing the normal protein, the misfolded protein acts as a template to convert a small amount of the normal protein into the misfolded form – a type of *in vitro* recuitment process [22]. It was reasoned that if these newly formed particles were dispersed by sonication, and then mixed with additional normal protein, and the process were repeated through many cycles, the misfolded protein particles would continue to convert more and more of the normal protein, and eventually produce a significant concentration of PrP^{TSE}. As originally described, the method achieved up to a 40-fold increase in the quantity of detectable PrP^{TSE} [23]. With refinement, it has recently been reported to produce at least a 6000-fold increase (as detected by Western blots) of end-point dilution series in pre- and post-treatment samples of 263K scrapie-infected hamster brain, and preliminary results indicate that this degree of amplification is sufficient to detect PrP^{TSE} in the blood of the same animals [24] (Dr C. Soto, personal communication).

The polyanion ligand-capture method is based on the affinity of sulphated polyanionic compounds, such as heparan sulphate and Congo Red, for β-sheet (amyloid) structured proteins, of which PrP^{TSE} is an example. The most avid of a series of synthesized molecules was selected for use as a ligand for screening tests. The method consists of coating magnetic beads with the (proprietary) ligand, exposing the beads

to the test specimen, eluting the captured material, and detecting PrP^{TSE} by using standard ELISA or Western blot procedures [25]. Results of blood testing have been presented, showing a clean discrimination of scrapie-infected from normal sheep, and both positive and negative tests were recorded for sheep that had been exposed to scrapie but were still healthy (correlation of these results with proof of the presence or absence of infection has not yet been carried out). In addition, the blood of one human patient with iatrogenic CJD was strongly positive, compared to negative results with blood from several uninfected controls (Dr Stuart Wilson, presentation at IBC International Conference on Transmissible Spongiform Encephalopathies, Reston, VA, December 6–7, 2004).

This ligand strategy would thus appear to hold bright prospects for use as a blood screening test in both animal and human TSE diseases, but requires study of experimentally induced infections and a much larger study of human specimens to determine whether it will succeed in detecting preclinical disease. Like the amplification method, the polyanion ligand protocol does not require a proteinase-digestion step, which could be advantageous in the event that partially misfolded, but still proteinase-sensitive, intermediates contribute to the population of circulating PrP^{TSE}.

The final method under consideration is also a ligand-based approach, but instead of a sulphated polyanion it uses a fluorescently labelled polypeptide sequence from the middle of the PrP molecule that is coupled to its mirror image (a palindromic polypeptide). When mixed with a test specimen containing PrP^{TSE}, the ligand folds into a β-sheet conformation, and in so doing induces more ligand molecules to adopt the same conformation (i.e. it acts as its own amplifier). The altered peptide conformational structure is detected by a change in the light-emission maxima of the fluorophore with which it is labelled [26].

Tests on plasma from symptomatic scrapie-infected sheep and normal sheep have achieved 100% sensitivity and specificity, and tests on plasma from BSE-infected and normal cattle have achieved 100% sensitivity and 95% specificity. Tests have also correctly identified a small group of clinical-phase sCJD patients and normal controls (Dr C. Orser, presentation at Cambridge Healthtech Institute Conference on Transmissible Spongiform Encephalopathies, McLean, VA, February 14–15, 2005, and Dr C. Orser, personal communication). More important from the standpoint of a screening test is that a scrapie hamster model began to yield positive signals in blood specimens taken about halfway through the incubation period.

Conclusion

It is a pleasure to be able to conclude that a preclinical blood screening test for vCJD may be imminent, rather than having always to say that it is on a (receding) horizon. Blood transmissions from preclinical vCJD donors imply that in at least this form of human disease, PrP^{TSE} should also be present before symptoms appear, and invites speculation that it may also be present in the blood of individuals incubating sporadic and familial forms of CJD. If present in a continuous rather than in an erratic manner, we have reason to expect that PrP^{TSE} will be detectable by one of the extremely sensitive methods currently in the advanced stages of development, and to hope that the holy grail of a preclinical screening test for TSE infection is finally within our grasp.

Would that the story could end on this optimistic note. However, the very likelihood of success has begun to evoke some questions that do not have ready answers. The first concerns the practical aspects of developing a test procedure with sufficient sample capacity, speed, and economy to allow its inclusion among the current panel of blood donor screening tests. Each of the three most promising tests is still in a prototype phase of development, but the transition to economical high-throughput protocols is likely to be accomplished by technological ingenuity.

The second question concerns the validation of a test based on the detection of PrP^{TSE}, but designed to monitor infectivity. The ideal choice would be to carry out PrP^{TSE} assays in parallel with bioassays on human blood samples, but no such human blood samples exist. It would thus be necessary to use experimental animal models, preferably primates, but in all likelihood, rodents, with the usual caveats about applicability of the results to human beings.

The third question is the most difficult: how do you interpret a positive test, and what do you tell the individual who tested positive? The current crop of tests appears to be heading towards a level of sensitivity that could be capable of detecting subinfectious quantities of PrP^{TSE} (this would have been unthinkable a few years ago, but is perfectly plausible, because it surely requires hundreds, if not thousands, of individual molecules to produce an infectious dose). This degree of sensitivity could be a methodological Achilles' heel, because it will be far more important to avoid false positives (specificity) than to miss a proportion of true positives (sensitivity).

In fact, the question actually divides into two quite separate considerations: first, does the presence of PrP^{TSE} imply that the blood is capable of transmitting disease? And, second, even if detectable at a subinfectious concentration, does its mere presence imply an eventual progression to symptomatic disease and death in the tested individual? In due time, both these questions will be answered, but it will take years of observation of both the donors and recipients to be sure of the outcome.

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