

Detection of Infectivity in Blood of Persons with Variant and Sporadic Creutzfeldt-Jakob Disease

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We report the presence of infectivity in erythrocytes, leukocytes, and plasma of 1 person with variant Creutzfeldt-Jakob disease and in the plasma of 2 in 4 persons whose tests were positive for sporadic Creutzfeldt-Jakob disease. The measured infectivity levels were comparable to those reported in various animals with transmissible spongiform encephalopathies.

Among humans, Creutzfeldt-Jakob disease (CJD) is a low incidence disease (≈ 1 case per million per year) that occurs as either a sporadic (sCJD) or a familial/genetic (fCJD) form. Whereas familial disease forms are linked to a mutation in the prion protein gene (*Prnp*), no clear epidemiologic risk factors have been identified for sporadic disease forms. sCJD is not a uniform disorder in terms of clinical and neuropathological phenotype. sCJD cases are classified as type 1 or 2 according to the polymorphism at codon 129 of the protease-resistant prion protein (PrP) sequence (methionine/valine) and to the electromobility of the proteinase K-resistant core of the abnormal PrP (PrP^{res}) (1). Type 1 and type 2 isoforms in sCJD are believed to correspond to different transmissible spongiform encephalopathy (TSE) agents

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Despite their relative rarity, several hundred iatrogenically transmitted CJD cases were identified during the past 60 years (2). Some data supporting the presence of infectivity in the blood of sCJD-affected patients were reported following the intracerebral inoculation of blood fractions from affected patients into rodents. These observations remain ambiguous because other studies did not confirm them (3,4).

In 1996, a new form of CJD, named variant CJD (vCJD), was identified in humans. Variant CJD was demonstrated to be caused by the agent that causes bovine spongiform encephalopathy in cattle (5). In the United Kingdom, 4 vCJD transmissions (3 clinical cases and 1 asymptomatic infection) were probably caused by the transfusion of non-leuco-depleted erythrocyte concentrates prepared from donors who later had positive test results for vCJD (6). More recently, a presumed additional case of vCJD infection was reported in the United Kingdom in a hemophilic patient who had received fractionated plasma products, including some units linked to a donor who had vCJD diagnosed (7). Despite the epidemiologic evidence of bloodborne transmission in vCJD, bioassays performed on conventional rodent models failed to demonstrate the presence of infectivity in the blood (8). The lack of TSE transmission in conventional rodent models could be a consequence of a low infectivity level in blood from vCJD- and sCJD-affected patients (as described in sheep and rodent TSE models) (9) or of the existence of the species barrier phenomenon that limits the transmission of human prions to these animal models. The development during the last decade of transgenic mice models expressing PrP from other species that abrogate the species barrier now offers the potential to detect low level of infectivity (10).

In this study, we used 2 transgenic mouse models that displayed a high sensitivity to the vCJD or sCJD TSE agents to estimate the infectious titer in certain blood fractions from vCJD- and sCJD-affected patients. According to legislation of the United Kingdom, Germany, and France, the experimental protocol, including the use of human samples, was approved by UK National CJD Research & Surveillance Unit tissue bank: REC reference number 2000/4/157-German TSE reference center: Ref Nr 11/11/93, PHRC ref 2004-D50-353 for patient from France.

The Study

Previous studies reported a high sensitivity in transgenic mice overexpressing bovine PrP (tgBov) for the detection of the bovine spongiform encephalopathy agent. To demonstrate that tgBov also displays a high sensitivity to vCJD infection, we titrated to endpoint a vCJD isolate (10% brain homogenate) by intracerebral inoculation in this model (Tg110) (11). Considering the potential diversity

Table 1. Titration of sCJD and vCJD isolates in transgenic mice expressing the human or bovine prion protein*†

Dilution	sCJD MM1 in tgHu		vCJD in tgBov	
	Positive transmission in mice	Incubation period, d	Positive transmission in mice	Incubation period, d
Not diluted	6/6	186 ± 10	6/6	249 ± 2
10 ⁻¹	6/6	213 ± 15	6/6	283 ± 15
10 ⁻²	6/6	240 ± 13	6/6	316 ± 21
10 ⁻³	6/6	263 ± 24	6/6	342 ± 10
10 ⁻⁴	6/6	296 ± 26	6/6	453 ± 66
10 ⁻⁵	6/6	323 ± 29	4/6	499 ± 17
10 ⁻⁶	1/6	316	1/6	502
10 ⁻⁷	0/6	>650	0/6	>700
Infectious titer, ID ₅₀ /g of brain (95% CI)	10 ^{6.67} (10 ^{6.33} –10 ^{6.97})		10 ^{6.33} (10 ^{5.84} –10 ^{6.82})	

*sCJD, sporadic Creutzfeldt-Jakob Disease; tgHu, human PrP gene; PrP, protease-resistant prion protein; vCJD, variant CJD; tgBov transgenic mice overexpressing bovine PrP, ID, infectious dose.

†Successive 1/10 dilutions of 10% brain homogenate (frontal cortex) from patients affected by vCJD and sCJD were injected intracerebrally to tgHu (n = 6) and tgBov (n = 6) mice, respectively. Those 2 patients were different from the 1 whose blood was tested in bioassay (Table 2). Mice were euthanized when they showed clinical signs of infection or after 650 days postinfection. Mice were considered infected when abnormal prion protein deposition was detected in the brain by western blot by using Sha31 monoclonal antibody, which recognizes amino acids 145–152 (YEDRYRE) of the sheep prion protein. Infectious titers were estimated by the Spearman-Kärber method (14).

of TSE agents that may cause sCJD, we decided to focus only on type 1 homozygous for methionine at codon 129 of the PRP gene (MM1) sCJD cases. An endpoint titration of a MM1 sCJD 10% brain homogenate was performed in a mouse model that express the methionine 129 variant of the human PrP gene (tgHu:Tg340) (12). This enabled confirmation of the capacity of the tgBov and tgHu models to detect the vCJD and sCJD MM1 agent, respectively, up to a 10⁻⁶

dilution of the reference brain homogenates (Table 1; 13). This value was within the range of the brain/blood relative infectivity reported in various TSE animal models (9,14).

In the next step of our experiment, blood fractions (erythrocytes, plasma, and leukocytes) from 1 vCJD-confirmed patient were injected intracerebrally in tgBov mice. Similarly, plasma samples from 4 sCJD MM1 patients were inoculated with tgHu (Table 2). The blood fraction

Table 2. Intracerebral inoculation of blood components collected from 1 vCJD and 4 sCJD cases (MM1) in transgenic mice expressing the bovine or human prion protein gene*†

Mouse model	Donor	Specimen	Inoculated mice	Positive mice	Incubation period, d	ID/mL (95%CI)‡	
tgBov	vCJD	Leukocyte	24	3	476, 567, 576	2.23 (0–4.87)	
		Plasma	24	1	453	2.12 (0–6.52)	
		Erythrocyte	24	1	433	2.12 (0–6.52)	
tgHu	sCJD case 1	Plasma	14§	1	338	3.70 (0–11.65)	
		Brain	6	6	216 ± 2	NA	
	sCJD case 2	Plasma	24	0	>700	0 (0–6.24)	
		brain	6	6	217 ± 5	NA	
	sCJD case 3	Plasma	24	1	233	2.12 (0–6.52)	
		Brain	6	6	205 ± 5	NA	
	sCJD case 4	Plasma	24	0	>700	0 (0–6.24)	
		Brain	6	6	207 ± 3	NA	
	tgHu	Control human	Plasma	12	0	>650	NA
	tgBov	Control human	Plasma	12	0	>650	NA
	tgHu	Control human	PBS	12	0	>700	NA
	tgBov	Control human	PBS	12	0	>700	NA
tgHu	Control human	Brain	24	0	>700	NA	
tgBov	Control human	Brain	24	0	>700	NA	
tgHu	Control human	None	24	0	>750	NA	
tgBov	Control human	None	24	0	>750	NA	

*vCJD, variant Creutzfeldt-Jakob disease; sCJD, sporadic Creutzfeldt-Jakob disease; dpi, days postinfection; ID, infectious dose; tgBov, bovine prion protein; tgHu, human prion protein; PBS, phosphate-buffered saline.

†The leukocyte(s) from a single vCJD case corresponding to a starting volume of 3 mL of blood were suspended in 1 mL of 5% glucose solution. The leukocyte suspension and the crude erythrocytes were homogenized by using a high speed cell disrupter. The leukocyte and erythrocyte homogenates (vCJD case) and crude plasma (vCJD and sCJD cases) were intracerebrally injected into mice (20 µL per mouse). For the 4 sCJD MM1 cases, brain homogenate (10%, temporal cortex) were also inoculated in tgHu. Mice were euthanized when they showed clinical signs of infection or after 650 or 750 dpi. Mice were considered infected when abnormal protease-resistant prion protein deposition was detected in brain tissue by using Western blot analysis with Sha31 monoclonal antibody: epitope amino acids 145–152 (YEDRYRE) of the sheep PrP sequence. For samples showing 100% attack rate, incubation periods are reported as mean (± SD). For other samples, individual incubation period of CJD-positive mice are presented; their infectious titers were estimated by using limiting dilution titration method (application of Poisson model) described by Brown et al (13).

‡Leukocyte titer is expressed as ID/mL of the starting whole blood. Plasma and erythrocyte titers are expressed as ID/mL of inoculum.

§24 mice were inoculated; 10 died because of the acute toxicity of the sample.

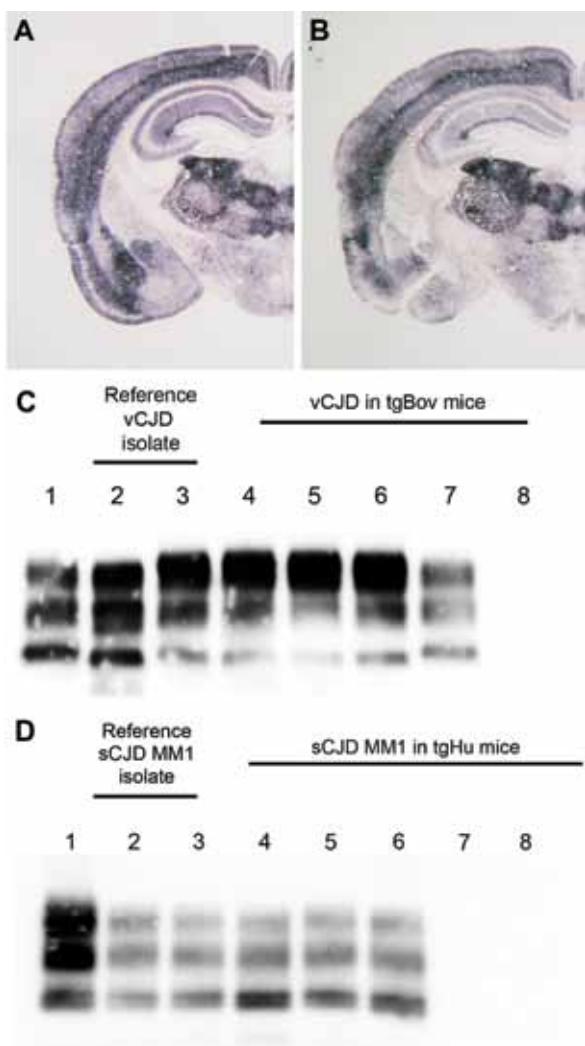


Figure. Abnormal prion protein (PrP^{res}) detection by using Western blot (WB) and paraffin-embedded tissue (PET) blot in the brain of transgenic mice expressing the methionine 129 variant of the human prion protein (PrP) (tgHu) or bovine PrP (tgBov). A, B) PET blot PrP^{res} distribution in coronal section (thalamus level) of tgHu mice inoculated with sporadic Creutzfeldt-Jakob disease (sCJD) MM1 isolates (10% brain homogenate): A) reference isolate used for the endpoint titration in Table 1; B) sCJD case 1 (Table 2). C) PrP^{res} WB of variant Creutzfeldt-Jakob disease (vCJD) reference isolate (used for endpoint titration in Table 1) and tgBov mice inoculated with the same vCJD reference isolate or vCJD blood fractions. Lane 1, WB-positive control; lanes 2 and 3, reference vCJD isolate; lane 4, leukocytes; lane 5, erythrocytes; lane 6, plasma; lane 7, WB-positive control; lane 8, healthy human plasma in tgBov. D) (PrP^{res} Western blot of the sCJD reference isolate (used for endpoint titration in Table 1) and tgHu mice inoculated with the same sCJD reference isolate and plasma from sCJD cases. A proteinase K-digested classical scrapie isolate in sheep was used as positive control for the blots in panels C and D. (PrP^{res} immunodetection in PET and Western blots was performed by using Sha31 monoclonal antibody (epitope: 145YEDRYRE152 of the human PrP). Lane 1, WB-positive control; lanes 2 and 3, reference sCJD MM1 isolate; lane 4, brain tissue from case 1; lane 5, plasma from case 1; lane 6, plasma from case 3; lane 7, plasma from case 2; lane 8, plasma from case 4.

preparation was performed by using laboratory scale hematologic protocols (online Technical Appendix, wwwnc.cdc.gov/EID/article/20/1/13-0353-Techapp1.pdf), not by following the procedure applied by blood banking services. This method implies that the leucodepletion that is applied to blood labile products in most countries to reduce the vCJD bloodborne transmission risk was not performed. Brain tissue samples from each of the 4 sCJD cases were also inoculated with tgHu. On the basis of the incubation period (Table 2) and PrP^{res} distribution pattern in the brain as assessed by using paraffin-embedded tissue blot, the TSE agents in those isolates were indistinguishable from those in the MM1 sCJD case that was used for endpoint titration (Figure, panel A).

No TSE clinical signs or PrP^{res} accumulation were observed in the tgBov or tgHu mice inoculated with phosphate-buffered saline or brain and plasma from healthy human controls. The 3 blood fractions from the vCJD-affected patient caused a positive result but low attack rate among tgBov mice (Table 2). On the basis of these results, infectivity in erythrocytes and plasma was estimated to be 2.12 infectious dose (ID)/mL of inoculum. In leukocytes, the infectious titer was estimated to be 2.23 ID/mL of whole blood. According to these values and the hematocrit of the sample (online Technical Appendix), the global infectious titer whole blood in the tested patient would be ≈ 4.45 ID/mL. Such infectious level is approximately equivalent to 1.4 μg of the reference vCJD brain sample that was endpoint-titrated (Table 1).

In tgHu mice, positive transmission was observed among mice inoculated with 2 of 4 plasma samples (Table 2). The infectious titers in both positive plasma samples were estimated to be 2.12 and 3.7 ID/mL of plasma, which is equivalent to 0.3–0.5 μg of the reference sCJD MM1 brain sample that was endpoint titrated (Table 1). However, because of the limited number of mice inoculated ($n = 24$) and the overall sensitivity of the assay (upper CI limit 6.24 ID/mL), the absence of transmission in mice inoculated with the 2 other plasma samples cannot be interpreted conclusively.

In tgBov inoculated with vCJD and tgHu inoculated with sCJD, the PrP^{res} banding patterns observed by Western blot in animals challenged with brain homogenate and blood components were identical (Figure, panels C, D). These results support the contention that the TSE agent propagated in tgBov mice and tgHu were vCJD and sCJD agents, respectively.

Conclusions

The data reported here confirm the presence of infectivity in erythrocytes, leukocytes, and plasma from vCJD-affected patients and demonstrate unambiguously the presence of infectivity in the plasma of some, but not all, sCJD-affected patients. The infectivity levels that we

measured in the tested vCJD and sCJD blood components were comparable to those reported in various TSE animal models. The number of cases included in our study was limited; a new experiment that would include a larger number of cases and different blood fractions from sCJD cases will be necessary to refine the data. However, these results represent a substantial input for assessing the risk for interindividual bloodborne transmission of sCJD and vCJD.

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Technical Appendix

Biochemical Typing and PrP ORF Sequencing of Sporadic and Variant Creutzfeldt-Jakob Disease Genes

Confirmation of the disease diagnosis, PrP^{res} WB typing and *PrnP* gene sequencing in the patients were performed by the national CJD reference center of the country of origin of each patient. All patients were Methionine/Methionine at codon 129 and no other mutation was observed. sCJD cases were all originating from Germany. The vCJD case whose blood was tested by bioassay was originating from France. The vCJD case that was used in the endpoint titration experiment was provided by the UK CJD reference center in Edinburgh.

Blood Collection and Fractionation

sCJD blood samples were collected by using S-Monovette® Coagulation Sodium Citrate 1 in 3 mL tubes according to manufacturer instruction (SARSTEDT AG & Co. · www.sarstedt.com) . Tubes were centrifuged for 20 minutes at 2000 rpm, plasma was then collected and cell-free fraction underwent another centrifugation step at 13000 rpm for 10 minutes. Supernatant was collected and stored frozen. The hematocrit values corresponding to the different samples were: sCJD case 1: 37.6%, sCJD case 2: 39.7%, sCJD case 3: 43%, sCJD case 4: 43.7%.

vCJD blood sample on EDTA and fractionated by a 10 minutes 3000 g centrifugation at 12°C . Plasma was collected and directly frozen stored. The buffy coat was collected and washed twice in NaCl 0.9% (2 min, RT) before being pelleted at 3000 g 10 min and frozen.

The sample was submitted to standard biochemical analyze and the blood formula was red cells $5.21 \cdot 10^{12}/L$, hemoglobin 149 g/L, hematocrit: 48%, total white cells $17.1 \cdot 10^9/L$, lymphocytes: 27.1%, monocytes 9.3%, neutrophils: 60%, eosinophil: 1.8%, Basophils: 1.8%, Platelets: $356 \cdot 10^9/L$.

Brain and Blood Samples Handling and Bioassay

Blood was collected during the diagnostic procedures when patients were evaluated for CJD diagnosis at notifying hospital. The time between blood sampling and patients' decease are reported in Technical Appendix Table 1.

For sCJD patients, blood was processed at the CSF reference laboratory of the National TSE Reference Center at the Department of Neurology Göttingen, Germany. Autopsy was performed by the Department of Pathology of the notifying hospital and reference material was sent to the Department of Neuropathology, Göttingen, Germany. Blood and brain samples were stored in separate department and handled by different staff in the Gottingen University hospital.

The vCJD blood sample was collected and fractionated in the Bron Hospital (France). In this hospital the department handling CSF and blood samples and the pathology department (post mortem sampling) are distinct. The vCJD reference brain sample was obtained from the UK CJD reference laboratory in Edinburgh.

All the samples were dispatched to the laboratory that performed the bioassays (UMR INRA ENVT 1225) in separated sealed containers. Samples were kept untouched and prepared only a few hours before their inoculation in mice.

The sCJD endpoint titration in tgHu mice was performed 1 year before the reception of sCJD plasma samples.

Plasma and Brain samples from the four sCJD affected patients were prepared and inoculated separately; Brain from the affected patients (text Table 2) were inoculated after the first positive transmission occurred in mice inoculated with sCJD plasma.

Similarly the vCJD endpoint titration experiment and the inoculation of the vCJD blood samples in tg Bov were performed at different dates (9 months interval).

Negative control (phosphate-buffered saline and healthy blood samples) were inoculated during the same inoculation session than the inoculation of the blood fractions from the vCJD and sCJD patients. Healthy brain controls (human and bovine) were inoculated during the same session than the endpoint titration of sCJD and vCJD brain material.

Technical Appendix Table. Clinico-pathological data and medical history in variant and sporadic Creutzfeld-Jakob Disease patients whose blood samples were tested in bioassay*

Patient	Sex	Age at onset	Disease duration	Blood collection†	CSF 14-3-3	MRI	EEG	Medical and surgical history
vCJD	M	46	12	12	-	+	-	None
sCJD 1	F	75	8	7	+	-	+	Gynecological
sCJD 2	F	75	3	2	+	+	+	Gynecological, orthopedic, hip fracture and replacement
sCJD 3	F	68	13	5	+	+	+	Cataract, hip replacement, tonsillectomy
sCJD 4	M	66	2	2	+	-	+	Hernia, appendectomy, tonsillectomy

*vCJD, variant Creutzfeld-Jakob Disease; sCJD, sporadic Creutzfeld-Jakob Disease; CSF, cerebrospinal fluid. EEG, electroencephalogram; MRI, Magnetic resonance imaging.

†Number of months after disease onset.