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The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy

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BACKGROUND: The administration of blood components from donors who subsequently develop Creutzfeldt-Jakob disease has raised the issue of blood as a possible vehicle for iatrogenic disease.

STUDY DESIGN AND METHODS: We examined infectivity in blood components and Cohn plasma fractions in normal human blood that had been "spiked" with trypsinized cells from a scrapie-infected hamster brain, and in blood of clinically ill mice that had been inoculated with a mouse-adapted strain of human transmissible spongiform encephalopathy. Infectivity was assayed by intracerebral inoculation of the blood specimens into healthy animals.

RESULTS: Most of the infectivity in spiked human blood was associated with cellular blood components; the smaller amount present in plasma, when fractionated, was found mainly in cryoprecipitate (the source of factor VIII) and fraction I+II+III (the source of fibrinogen and immunoglobulin); almost none was recovered in fraction IV (the source of vitamin-K-dependent proteins) and fraction V (the source of albumin). Mice infected with the human strain of spongiform encephalopathy had very low levels of endogenous infectivity in buffy coat, plasma, cryoprecipitate, and fraction I+II+III, and no detectable infectivity in fractions IV or V.

CONCLUSION: Convergent results from exogenous spiking and endogenous infectivity experiments, in which decreasing levels of infectivity occurred in cellular blood components, plasma, and plasma fractions, suggest a potential but minimal risk of acquiring Creutzfeldt-Jakob disease from the administration of human plasma protein concentrates.

Concern has mounted in recent years about the possibility of transmitting Creutzfeldt-Jakob disease (CJD) through blood or blood components because a proportion of patients dying of CJD have been regular blood donors, and because the blood of experimentally infected animals, and humans with CJD, may sometimes contain low levels of the infectious agent.[1]

See also: Dodd RY, Sullivan MT. Creutzfeldt-Jakob disease and transfusion safety: tilting at icebergs? (editorial). Transfusion 1998;38:221-3.

The purposes of this study were, first, to determine the distribution of infectivity among the various components and plasma fractions of normal human blood that had been "spiked" with a high concentration of infectious agent, to obtain information about agent clearance during the process of blood separation and fractionation; and, second, to determine the distribution of infectivity (if present) in the same components and fractions in an experimental model of transmissible spongiform encephalopathy (TSE) characterized by a low blood level of endogenous circulating pathogen, analogous to the probable situationin humans with CJD.[2]

ABBREVIATIONS: CJD = Creutzfeldt-Jakob disease; GSSD = Gerstmann-Sträussler-Scheinker disease; LD_{50} = mean lethal dose; PBS = prosphate-buffered saline; PrP = protein-resistant protein; TSE = transmissible spongiform encephalopathy.

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MATERIALS AND METHODS

High input infectivity ("spiking") experiment

Preparation of material used in spiking experiment. One half of each brain from two terminally ill golden Syrian hamsters that had been infected with the 263K strain of scrapie agent were combined (total 1.0 g wet tissue) and minced into very fine fragments. The fragments were then suspended in 9 mL of phosphate-buffered saline (PBS) at pH 7.0 containing 0.025-percent trypsin and 0.05-percent EDTA, and incubated with constant stirring at 37°C for 30 minutes to disperse cells. Residual fragments were resuspended and similarly incubated in fresh trypsin-EDTA solution. No fragments remained after the second trypsinization, and the pooled pellets from each specimen (following centrifugation at 600 × *g* for 15 min) were washed two times in 50 mL of PBS. The final washed pellet contained 1.6×10^9 neuronal and glial cells, of which 99 percent were viably intact as evidenced by failure to stain with trypan blue, and contained 9.1 mean lethal dose ($\log_{10}/LD_{50}$) infectious units as determined by endpoint dilution assay in hamsters. The pellet was resuspended in 46.8 mL of normal whole human blood containing CPD (United States Pharmacopoeia) at an anticoagulant-to-blood ratio of 1:9.

Separation of blood into its components. A scaled-down version of the "three-bag" protocol used by the American Red Cross was used for component separation. Anticoagulated whole blood was centrifuged (Sorvall SS-34 rotor, DuPont Medical Products Clinical Diagnostics, Wilmington, DE) at 4300 rpm (2280 × *g*) for 4 minutes at ambient temperature. The supernatant plasma was carefully withdrawn by pipette down to the edge of the buffy coat overlying the red cell sediment, transferred to a new 50 mL tube, and centrifuged at 5800 rpm (4200 × *g*) for 8 minutes at ambient temperature. The supernatant plasma was pipetted into a new tube, leaving behind a very small sedimented pellet. Without disturbing their contents, all specimens were frozen intact at –70°C. While frozen, the buffy coat layer overlying the red cell sediment was sliced apart and combined with the pellet from the plasma centrifugation step to yield a single white cell and platelet specimen for assay.

Cohn fractionation of plasma component. Fractionation was carried out in a scaled-down version of a protocol in wide commercial use,[3] and yielded a protein profile similar to that of the production-scale process. Approximately 10 mL of plasma was transferred from –70°C to –20°C for overnight "tempering," then exposed to a final 30-minute thaw inside a 50-mL jacketed reaction beaker connected to a refrigerated circulating bath set at 1 to 2°C. The thawed plasma was transferred to a weighed, cold, 15-mL centrifuge tube and centrifuged at 6800 rpm (5600 × *g*) for 15 minutes at 1 to 2°C. The pellet was weighed and then frozen at –70°C (cryoprecipitate).

The supernatant was again placed into the reaction beaker-circulating bath apparatus set at 1 to 2°C, and the pH was adjusted to 6.65 to 6.70 with acetate buffer, pH 4.0. (10.9 g sodium Acetate, 24 g glacial acetic acid, 71 mL water). Slowly, over a period of 1 hour, repeated small amounts of cold 95-percent ethanol were added to achieve a final ethanol concentration of 20 percent. After addition of one half of the ethanol, the pH was verified to be in range of 6.80 to 7.00, and the circulating bath temperature was lowered from 1 to 2°C to –5°C. The plasma-ethanol mixture was transferred to a weighed, cold centrifuge tube and centrifuged at 6800 rpm (5600 × *g*) for 15 minutes at –5°C. The pellet was weighed and frozen at –70°C (fraction I+II+III).

The supernatant was again placed into the reaction beaker-circulating bath apparatus set at –5°C. The pH was adjusted to 5.16 to 5.22 with acetate buffer in 20-percent ethanol, pH 4.0, and then further adjusted to a final pH of 5.75 with 1 M $NaHCO_3$. Slowly, over a period of 1 hour, small quantities of cold 95-percent ethanol were added to achieve a final ethanol concentration of 40 percent and a final pH of 5.92 to 5.98. The plasma-ethanol mixture was transferred to a weighed, cold centrifuge tube and centrifuged at 6800 rpm (5600 × *g*) for 15 minutes at –5°C. The pellet was weighed and frozen at –70°C (fraction IV_1/IV_4).

The supernatant was placed into a tube containing 2 mg of filter aid per mL of supernatant, mixed, and filtered through a 20-mL syringe containing a filter (CPX70, Cuno, Meriden, CT). The filtrate was placed into the reaction beaker-circulating bath apparatus set at –5°C. The pH was adjusted to 4.78 to 4.82 by slowly adding acetate buffer in 40-percent ethanol, pH 4.0. The plasma mixture was placed into a weighed, cold centrifuge tube and centrifuged at 6800 rpm for 15 minutes at –5°C. The pellet was weighed and frozen at –70°C (fraction V). The supernatant was also frozen at –70°C (fraction V supernatant).

Infectivity bioassays. On the day of the test, specimens (inoculum, whole blood, blood components, and Cohn fractions) were thawed, serial 1-in-10 dilutions were made in PBS (pH 7.4), and specimens were inoculated intracerebrally in volumes of either 30 µL (for components) or 50 µL (for fractions) to groups of 4 to 8 female weanling hamsters per dilution. Two cages of uninoculated hamsters served as "sentinels" to monitor laboratory cross-contamination. Animals were observed for 8 months, and the brains from a random sampling of clinically positive animals in all higher dilution groups were examined to verify the presence of spongiform neuropathology. None of the uninoculated sentinel animals showed clinical or neuropathological signs of scrapie.

Using the method of Reed and Muench,[4] $\log_{10} LD_{50}$ infectivity titers were calculated except for the plasma specimen, for which infectivity was estimated comparing its incubation period curve to that of whole blood at dilutions 10^{-1} through 10^{-4} (the highest dilution of plasma that was

inoculated). This estimate makes use of the inverse relationship between the amount of infectivity and the length of the incubation period (the greater the infectivity, the shorter the interval between inoculation and disease)—a type of "dose-response" curve. Although not as precise as an endpoint dilution titration, it is reassuring that the whole blood, red cell, and buffy coat specimens, which had nearly identical endpoint dilution titers, also had nearly superimposable incubation period curves, and that the plasma curve was parallel to the whole blood curve at a 1.2 $\log_{10}$ unit lower level.

Endogenous infectivity experiment

Experimental model. Weanling Swiss-Webster mice (Charles River Laboratories, Wilmington, MA) were inoculated intracerebrally with a 10-percent clarified homogenate of a mouse-adapted Fukuoka-1 strain of human P102L Gerstmann-Sträussler-Scheinker disease (GSD).[5,6] When mice began to show symptoms of disease (approx. 4 months after inoculation), they were lightly anesthetized and bled by open chest direct cardiac puncture into CPD containing 5 units of heparin per mL blood to counteract the unusually strong clotting tendency of mouse blood. At the time of exsanguination, brains and spleens were also removed from each animal; tissue pools of each organ were made into separate 10-percent tissue suspensions in PBS for infectivity titrations performed at the same time as those for the blood specimens.

Collection and processing of blood specimens. A total of 75 mice yielded a pooled sample volume of 52 mL (45 mL of blood and 7 mL of citrate containing 225 units of heparin). The blood was immediately separated into its red cell, white cell-platelet, and plasma components, frozen at −70°C. A portion of the plasma was later thawed and processed into Cohn fractions, as described in the spiking experiment. The only difference was that, in this experiment, we did not combine the buffy coat layer of the red cell sediment with the centrifuged plasma pellet, choosing instead to assay the two specimens separately.

Infectivity bioassays. All specimens were inoculated intracerebrally in 30-µL volumes into groups of weanling Swiss-Webster mice, and two cages of uninoculated sentinel animals were included as cross-contamination controls. Because of anticipated low or undetectable infectivity levels in most specimens, this experiment was conducted in a facility that had never been used for TSE experiments, and specimens were inoculated into groups of up to 130 mice. Undiluted inocula proved to be highly toxic, causing nearly instantaneous death that was probably due to a combination of high osmolarity, anticoagulant, and (in the case of Cohn fractions) residual alcohol; dilutions of 1-in-4 to 1-in-5 were well tolerated and were therefore used for most inoculations. Serial 1-in-10 dilutions were inoculated for specimens expected to have higher infectivity titers, such as brain, spleen, and the white cell-platelet component of blood.

Animals were observed for clinical signs of disease for a period of 13 months, at which time all surviving mice were sacrificed. The brains from most animals that died during this observation period, as well as the brains of all animals sacrificed at the conclusion of the experiment, were removed and stored at −70°C for Western blot detection of proteinase-resistant protein (PrP).

Of a total of 537 mice that survived the immediate postinoculation period, 21 mice inoculated with various blood specimens died during the next 6 months, when the earliest verified deaths from spongiform encephalopathy began to occur in animals used for parallel titration of brain infectivity. Brains from 10 of these 21 mice were examined and found by Western blot to have been PrP-negative. We therefore presumed that all 21 deaths were due to intercurrent non-CJD-related illness and excluded them from our analysis.

Of the remaining 516 animals, 264 later died while under observation, and 252 remained well until the conclusion of the experiment. Only one of these 252 mice was PrP-positive, so that the "lethal" and "infectious" doses were essentially identical. Our final analysis was based upon 461 animals in these two groups whose brains were examined for the presence of PrP (brains from 55 mice in various groups that died during the course of the experiment, and that were found dead more than 24 hours postmortem, were considered unsatisfactory for Western blot examination). None of the uninoculated sentinel animals became ill or had PrP in their brains.

For Western blots, 100 µL of a 10-percent brain homogenate of each brain was digested at 37°C in the presence of 100 µg per mL of Proteinase K (Boehringer-Mannheim, Indianapolis, IN) and 2-percent sodium dodecyl sulfate (Gibco, Gaithersburg, MD). A second 100-µL aliquot was incubated with a 0.0125 M solution of the protease inhibitor phenylmethylsulfonylfluoride (PMSF, Gibco). After 20 minutes, PMSF was added to the sample containing Proteinase K, and both samples were autoclaved at 121°C for 30 minutes to denature the proteins and inactivate infectivity. The samples were electrophoresed in adjacent lanes on precast 14-percent tris-glycine acrylamide gels (Novex, San Diego, CA), and electroblotted to membranes (Immobilon, PVDF, Millipore, Burlington, MA). PrP was detected using chemiluminescence (ECL + Western Blot Detection System, Amersham, Arlington Heights, IL) with a mouse PrP antibody (#78295) kindly provided by Dr. Richard Rubenstein (Institute for Basic Research, Staten Island, NY). Control specimens of scrapie-infected and uninfected mouse brain were included with every digestion and gel.

RESULTS

Scrapie-infected hamster brain cell spike of normal human blood

Infectivity was found at comparable concentrations in whole blood, red cells, and buffy coat (values of ±0.5 $\log_{10}$ are not

TABLE 1. Distribution of infectivity among blood components and Cohn plasma fractions in normal human blood "spiked" with $10^{9.4}$ LD_{50} of scrapie infectivity contained in a trypsinized suspension of viable brain cells from hamsters infected with the 263K strain of scrapie agent*

Specimen	Specimen vol (or wt)	Infectivity concentration ($log_{10}LD_{50}$/mL or g)	Total infectivity ($log_{10}LD_{50}$)†	Fractional recovery of infectivity(%)†
Whole blood	46.8 mL	8.3	9.3×10^9	100
Red cells	20.0 mL	8.0	2.0×10^9	22
White cells/platelets‡	2.0 mL	8.5	6.3×10^8	7
Plasma§	24.0 mL	7.1	3.0×10^8	3
Fractionated plasma (11mL)				
Plasma§	11.0 mL	7.1	1.4×10^9	100
Cryoprecipitate	0.26 g	6.6	1.0×10^5	0.71
Fraction I+II+III	0.93 g	6.1	1.2×10^6	0.86
Fraction IV_1+IV_4	0.87 g	4.0	8.7×10^3	0.006
Fraction V	1.66 g	2.5	0.5×10^3	0.0004
Fraction V supernatant	11.5 mL	ND¶		

* Specimens were assayed by intracerebral inoculation of healthy weanling hamsters.
† For components, the amount of infectivity in the component compared to the amount of infectivity in whole blood; for fractions, the amount of infectivity in the fraction compared to amount of infectivity in the plasma sample used for fractionation. Note that because differences of less than ±0.5 log infectivity concentration between any two specimens are not necessarily significant, fractional recovery percentages could be correspondingly higher or lower in a repeat experiment.
‡ Recovered from centrifuged plasma (4200 × g for 8 min).
§ Infectivity estimated from comparison of incubation period time curve to that of whole blood (see Methods section).
¶ ND = none detected (no disease transmissions in groups of four hamsters inoculated with undiluted through 10^{-6} dilutions).

appearance" could have been due to the imprecision of the bioassay (±0.5 log_{10} variability of LD_{50} titers), and some could have resulted from adherence of infective particles to containers used for experimental manipulations. It is also possible that some infectivity was lost as a result of Cohn fractionation, although low pH and ethyl alcohol by themselves have previously been shown not to inactivate the agents of TSE.[7,8]

Endogenous blood infectivity in TSE mouse model

From clinically ill mice that had 4 months earlier been inoculated intracerebrally with a mouse-adapted strain of human TSE, specimens of buffy coat, plasma, cryoprecipitate, and Cohn fraction I+II+III transmitted disease to a few animals, but no transmissions occurred from whole blood, red cells, or Cohn fractions IV and V (Table 2).

considered to be significant in single-assay comparisons); somewhat lower levels in plasma and the first two plasma fractions; and substantially lower levels (4-6 log_{10} reduction) in the last two fractions (Table 1). The absence of transmissions in the small group of animals inoculated with the final fraction V supernatant is consistent with a range of infectivity (using a Poisson distribution calculation) from zero to 1.4 log_{10}, that is, less than the demonstrated infectivity in fraction V.

Considering the total amount of infectivity (rather than its concentration) in these same components and fractions, about equal amounts (3-7%) of infectivity were recovered in buffy coat and plasma, of which a very small amount of plasma infectivity found its way to the cryoprecipitate and fraction I+II+III, and virtually none (<0.01%) to the last two fractions.

It may be remarked that a significant proportion of input spike infectivity was not recovered, either in the blood components or in the plasma fractions. Some of this apparent "dis-

TABLE 2. Infectivity in blood components and plasma fractions processed from the pooled blood of 75 mice experimentally infected 4 months earlier with a mouse-adapted strain (Fukuoka-1) of Gerstmann-Sträussler-Scheinker disease*

Specimen	Specimen vol (or wt)	Proportion of specimen inoculated (%)†	Specimen dilution	Positive animals‡	Negative animals‡
Whole blood	45.0 mL	0.15	1-in-5	0	11
Red cells	18.0 mL	0.22	1-in-5	0	7
Buffy coat§	3.5 mL	2.3	1-in-5	2	10
			1-in-50	0	6
Plasma pellet¶	0.2 mL	60	1-in-6	4	19
			1-in-60	0	10
Plasma‖	22.6 mL	3.5	1-in-5	8	124
			1-in-50	0	10
Fractionated plasma (11.3 mL)					
Cryoprecipitate	0.15 g	29	1-in-4	5	6
			1-in-40	1	3
Fraction I+II+III	0.40 g	37	1-in-4.5	6	37
Fraction IV_1+IV_4	0.86 g	38	1-in-4	0	86
Fraction V	1.22 g	30	1-in-4	0	94

* Specimens were assayed by intracerebral inoculation of healthy weanling mice.
† Amount of inoculated specimen divided by the amount contained in the 45-mL volume of whole blood (taking into account the volume and dilution of each inoculated specimen; dilution of anticoagulant; and for fractions, the fractionated plasma volume).
‡ Confirmed by Western blot tests for PrP in brain extracts. Sixteen animals inoculated with higher dilutions of the plasma pellet, fraction IV, and fraction V, tested negative.
§ Sliced from top 5 mm of red cell sediment frozen after centrifugation of whole blood. The amount of infectivity may be greater than shown, as several more animals that died at about the same time as the positive animals were not tested for PrP and were thus excluded from the table.
¶ Pellet after plasma centrifugation for 8 minutes at 4200 × g (see Methods).
‖ Supernatant after plasma centrifugation for 8 minutes at 4200 × g (see Methods).

The presence of infectivity in the separately assayed specimens of buffy coat and the centrifuged plasma pellet probably reflects the presence of white cells in both specimens, but raises the possibility that platelets as well as white cells might contain the infectious agent. It should also be noted that the absence of transmissions from the whole blood and red cell specimens does not imply the absence of infectivity (which would be unreasonable in view of its presence in buffy coat and plasma), because only very small proportions of these specimens were assayed, due to the necessity of using diluted inocula. The separate pools of brains and spleens collected from the same 75 animals had infectivity titers of approximately 10^5 LD_{50} per g and 10^2 LD_{50} per g, respectively, similar to titers observed in an earlier experiment using the same mouse model.[2]

DISCUSSION

Several earlier studies of TSE have tested one or another component of whole blood specimens for the presence of infectivity, with conflicting results: most of the successes were from buffy coat, but in a few studies, whole or extracted blood, and serum or concentrated serum were found to be infectious; and no infectivity was detectable in nearly half of such studies (including assays on the blood of sheep naturally infected with scrapie, and assays in primates inoculated with blood from humans with CJD).[1,9-11] None of these studies examined the distribution of infectivity in different blood components of a single specimen, and none examined infectivity in the Cohn fractions that represent an intermediate stage between crude plasma and therapeutic plasma protein concentrates.

Experimental design considerations

The primary goals of these experiments were to determine the effect of a standard protocol for blood separation and plasma fractionation in blood containing a high enough level of infectivity to permit an estimate of the degree to which processing caused a reduction in infectivity (agent clearance) and provide an idea of the distribution of the much lower levels of endogenous infectivity that would be expected to occur in the blood of experimentally infected animals.

No single experimental design can answer both questions. For clearance studies, a much higher level of infectivity is needed than occurs in the blood of experimentally infected animals to measure serial infectivity reductions in successive processing steps. Scrapie-infected hamster brain satisfies this condition of high-input infectivity. The choice of trypsinized and washed intact brain cells was based on evidence that blood infectivity is most likely cell-associated,[1] and thus, insofar as could be predicted, infected cells represent a more appropriate infectious vehicle than either infectious tissue homogenates or purified, cell-free PrP. We could not know in advance whether trypsinized infected brain cells would partition during separation in exactly the same manner as blood cells; in the event, they turned out to be a more accurate predictor of endogenous infectivity distribution than we imagined possible.

Relevance of experiments to human disease

The choice of mice inoculated with the Fukuoka-1 strain of human GSSD to investigate endogenous infectivity was based on its standing as a well-studied model in which, beginning about half-way through the incubation period, low but rising levels of infectivity were detected in the buffy coat.[2] It may be objected that this GSSD strain might not be an appropriate choice to investigate the behavior of CJD. However, most human TSE strains produce similar clinical signs and neuropathology when inoculated into rodents, and induce the same molecular species of host PrP in the infected animal; thus, there is every reason to suppose that the biology of blood infectivity can be considered as a generic phenomenon of TSE (the only potential exception would be "new variant" CJD, which has shown some degree of biologic distinctiveness).

Similarly, although experimental modeling of medical problems may or may not yield results that can be transposed to the human condition, rodents have provided a vast amount of information about the pathogenesis of TSE that appears to be widely applicable across many agent strains and host species, and there is thus reason to suppose that rodent models can also provide useful (even if not definitive) information on the question of blood infectivity and disease transmission. The ideal experiment, in which chimpanzees would be inoculated with human strains of CJD, would be almost forbiddingly expensive, and require many years to obtain meaningful results (nearly 2 years for the inoculated animals to become sick, and another several years to bioassay the collected blood specimens in healthy squirrel monkeys).[12]

Both the exogenous spike and endogenous infectivity experiments gave consistent results: infectivity was present in plasma as well as white cells, and when the plasma was subjected to Cohn fractionation, infectivity distributed almost entirely into the first two precipitates. Thus, although infectivity in the brain cell spike was added in a necessarily unnatural cellular element, the similarity of its distribution to that observed in the infected mouse model indicates that it accurately reflected the endogenous situation (the only major difference was the predictably lower level of infectivity in the endogenous model). The presence of infectivity in plasma raises a question as to its origin from either white cell (or white cell fragment) contamination of adjacent components during the comparatively low speed centrifugation separation, or as distinct cell-associated and cell-free forms of infectivity. The finding of infectivity in the plasma component of normal human blood that had been spiked with intact infected brain cells argues the case of a

cell-associated rather than cell-free origin, but further work needs to be done to resolve the issue.

Infectivity estimates and risk assessment

What might be the likely limits of infectivity in the plasma of a patient with CJD? For this speculative calculation, we can reason as follows: if each of the assay mouse transmissions resulted from a single infectious unit, which seems likely in view of the small proportion of positive to inoculated animals in the 1-in-5 dilution and the absence of transmissions in the 1-in-50 dilution, then the number of observed transmissions (8) multiplied by the reciprocal of the percentage of plasma inoculated (100/3.5) predicts the number of infectious units (230) that would have been observed if all 22.6 mL of plasma had been inoculated. Thus, the mouse plasma contained approximately 10 infectious units per mL. Similar calculations yield infectivity estimates per mL of processed plasma of about 5 infectious units in cryoprecipitate, and one infectious unit in fraction I+II+III.

If the 10 infectious units per mL of plasma are considered as a concentration of infectivity applicable to both humans and mice, then a standard 450-mL blood donation (containing approx. 250 mL of plasma) would contain about 2500 infectious units. Even if an intravenously inoculated plasma specimen were only 1-in-100th as likely to produce infection as the intracerebral inoculation assay used in this experiment,[13] the consequent estimate of 25 infectious units still seems far too high in view of the fact that no case of CJD has yet been linked to the administration of blood or blood products.[14-17] It is possible that peripheral routes of infection are even less efficient than supposed, or that dilution of this comparatively low number of infectious units in large donor pools comes into play in further reducing the risk of disease transmission.

A question of immediate practical importance is the issue of which plasma products deserve the most attention as possible vehicles for the transmission of CJD. Our results suggest that the potential for transmission would be comparatively higher for cryoprecipitate and fraction I+II+III than for fractions IV and V. Albumin, made from fraction V, is an especially important product because it is used as an excipient and stabilizer in other plasma protein concentrates, as well as in various non-plasma-derived biologicals, including products as varied as vaccines, injectable diagnostic radiology dyes, and embryonic cultures for in-vitro fertilization procedures. Judging from the nearly 5 $\log_{10}$ reduction in infectivity in fraction V as compared to plasma in the spiking experiment, and the absence of fraction V infectivity in the TSE mouse model, the risk of contracting CJD from exposure to albumin must be extremely low.

CONCLUSIONS

The distribution of blood infectivity in two different experimental models of TSE—one using an infectious cellular spike of normal blood and the other using blood from experimentally infected mice—confirmed the previously demonstrated association of infectivity with buffy coat. An unexpected finding was the presence of infectivity in plasma, which may have resulted from the imperfect separation of cells and plasma in the course of a standard centrifugation separation protocol. Cohn fractionation of the infectious plasma further reduced its infectivity to very low or undetectable levels.

The levels of infectivity demonstrated in these model studies may not be fully representative of the actual risk of disease transmission from human blood components because: 1) blood from a CJD patient included in a donor pool will contribute only a minute proportion of plasma to the pool, which is usually made up from as few as 6000 to more than 100,000 donors[18]; 2) many therapeutic protein concentrates are derived from plasma fractions processed through chromatography columns that are known to adsorb (although not inactivate) TSE infectivity[19,20]; and 3) plasma products are administered via intravenous and parenteral injections, which have been shown to be comparatively inefficient routes of TSE disease transmission.[13]

Our results represent only the beginning of a rational approach to an assessment of the risk, if any, of acquiring CJD from the administration of blood components or plasma products. Among urgently needed additional pieces of information are answers to the following questions: 1) is there a similar amount and distribution of blood infectivity in the preclinical stage of disease (when humans would usually be donating blood)?; 2) is the infectivity present in plasma the result of contamination by white cells or white cell debris (special interest in white cells comes from the demonstration that B cells are important for neuroinvasion and clinical infection[21])?; 3) can the low levels of endogenous blood infectivity detected by intracerebral inoculation of assay animals also be detected by intravenous or intramuscular inoculation (the routes by which most therapeutic blood products are administered)?; 4) will such infectivity, if present in Cohn fractions, be carried through the additional processing steps used to produce therapeutic end products?; and finally, 5) does "new variant" CJD have the same biological characteristics with respect to blood infectivity as other types of TSE?

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