Scrapie Infectivity in Hamster Blood Is Not Associated with Platelets

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The infectivity of hamster scrapie strain 263K was measured in platelets isolated from blood pooled from six hamsters with clinical scrapie. The total number of infectious doses present in the blood pool was 220, out of which only 3.5 infectious doses were associated with platelets. A larger proportion of the total infectivity was recovered from the mononuclear leukocyte fraction. This result indicates that platelets are not the source of blood-borne infectivity in transmissible spongiform encephalopathy-infected hamsters.

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases of both humans and animals (5). TSE infectivity is present in the blood of experimentally infected rodents showing symptoms of the disease, albeit at very low levels (3, 4, 9). This infectivity partitions to both cellular and plasma fractions during component separation in a manner suggesting an association with blood platelets, which frequently contaminate other blood fractions (3, 4; Rohwer laboratory, ongoing studies). Moreover, human platelets contain a significant concentration of the cellular prion protein, PrPc (7, 10). Much lower concentrations are present in rodent platelets (8).

To measure the concentration of TSE infectivity in platelets, we purified platelets from the pooled blood of six hamsters in the terminal stages of scrapie after intracerebral inoculation with a dilution of hamster scrapie strain 263K brain homogenate containing approximately 10^2 infectious doses (ID) per inoculum. Low-titer inoculation was utilized to prevent any chance of reisolating the inoculum itself in the blood collected 144 days later during clinical disease. The hamsters were anesthetized, their chest cavities were opened, and blood was collected into 3.8% sodium citrate (9:1) by cardiac puncture, with great care being taken not to expose the needle to any other tissue. A total of 22 ml of blood in 2-ml aliquots was collected. Platelets were prepared by a method previously optimized for hamster blood (8). The blood of individual hamsters was diluted with an equal volume of physiological saline (PS) and centrifuged through Ficoll. The cellular band containing platelets and mononuclear leukocytes was collected from the top of each gradient, and the pooled fraction was diluted with 30 ml of washing buffer (12.9 mM sodium citrate, 30 mM glucose, 120 mM NaCl, 5 mM EDTA [pH 6.5]). The mononuclear cells were pelleted from the suspension by centrifugation at 250 \times g for 5 min. The supernatant was collected, and residual mononuclear cells were removed by a second centrifugation at 250 \times g for 5 min. The platelets in the supernatant from the second

spin were then pelleted by centrifugation at 1,200 \times g for 15 min. The platelet pellet was washed twice to remove residual plasma and Ficoll. The concentration of Ficoll or plasma in the final platelet fraction was less than 0.01%. The washed platelets were resuspended in 1 ml of PS and counted with a hemocytometer. The final preparation contained 5.2 \times 10^9 platelets in 1 ml of PS, which represents a recovery of approximately 50% from whole blood at a count 10 times greater than in whole blood. The number of leukocytes contaminating the platelet fraction was less than or equal to 1 cell/μl as determined with a Nageotte counting chamber. This was more than 1,000-fold fewer than the number in the original whole blood.

The white blood cells pelleted from the platelet suspensions were combined, washed once with buffer, and resuspended in 1 ml of PS. Microscopic evaluation confirmed that this mononuclear cell fraction contained mainly mononuclear leukocytes, with some platelets and a few red blood cells. The residual concentration of Ficoll or plasma in this fraction was estimated to be 0.6%.

Aliquots of the pooled whole blood and of the isolated platelets and mononuclear cells were frozen and stored at −80°C. Immediately before inoculation, all the fractions were thawed, sonicated, and diluted 1:1 and 1:4 with PS. Scrapie infectivity was measured by intracerebral inoculation of 50-μl aliquots into weanling male golden Syrian hamsters (HsdHan: AURA; Harlan Sprague Dawley, Inc., Indianapolis, Ind.). The animals were housed four per cage and observed for 14 months for the presence of clinical signs of scrapie. The animal incubations were terminated at 418 days postinoculation. Western blot detection of proteinase K-resistant prion protein (PrPres) in brain samples from each animal confirmed all positive clinical scores and revealed three additional subclinical infections.

The results of the infectivity study are shown in Table 1. Six of 24 animals inoculated with the twofold dilution of whole blood developed scrapie (incubation, 250 to 382 days). Eleven out of 20 and 3 out of 12 hamsters in the groups inoculated with mononuclear cells diluted 1:1 and 1:4, respectively (incubation, 143 to 418 days), developed scrapie. In contrast, there was only one infection among the animals inoculated with either dilution of the platelet preparation (incubation, 318 days). All of the transmissions in this study had incubation...
times in excess of 140 days, which is indicative of infections initiated by single infectious doses (R. G. Rohwer, unpublished data). In the group inoculated with the 1:1 dilution of platelets, two hamsters died from undetermined causes prior to any possibility of clinical disease (at 51 and 73 days after inoculation) and were not included in the analysis even though their brains tested negative for the presence of PrPSc.

Total scrapie infectivity in the inoculated blood fractions was calculated as shown in Table 1. The original 22 ml of whole blood contained approximately 220 ID or 10 ID/ml, which is in good agreement with the results of ongoing studies in the Rohwer laboratory. Only 1.75 ID were recovered in the platelet fraction, and as the recovery of platelets was approximately 50%, a total of 3.5 ID were associated with platelets in the original blood sample. The mononuclear leukocyte fraction isolated with the platelets contained 22.6 ID, consistent with the higher levels of infection detected in the blood of scrapie-infected mice and hamsters (3, 4). Since the method for isolation of platelets was optimized at the expense of the other cellular components, we are not comfortable attempting to draw quantitative conclusions from the results obtained with the mononuclear cell fraction. However, we suspect that the percentage of mononuclear cells recovered was significantly greater than the 10% of total infectivity recovered with this fraction, recovered indicating that mononuclear cells are not the only source of TSE infectivity in hamster blood.

In previous work, flow cytometry was unable to detect PrPSc on hamster platelets and PrPSc was only barely detectable on hamster blood leukocytes (8). In contrast, PrPSc was readily observed on human platelets and mononuclear leukocytes (1, 6, 7). However, even higher concentrations of PrPSc have been found in association with many tissues containing little, if any, infectivity (2). Thus, it is not clear that blood-associated PrPSc has any role in the acquisition and transport of TSE infectivity in blood.

This study demonstrates that platelets are not a significant source of TSE infectivity in the blood of scrapie-infected hamsters. The single transmission observed in the platelet group is most likely the result of residual contamination by plasma, leukocytes, or some other entity bearing infectivity. Since the level of this contamination will vary with purification method and since therapeutic platelet concentrates are never produced by the methods applied here, the levels of residual infectivity in platelet concentrates may be different.

The views of the present authors represent scientific opinion and should not be construed as opinion or policy of the U.S. Food and Drug Administration.

**REFERENCES**


