Effect of Platelet-Associated Virus on Assays of HIV-1 in Plasma

In their recent report M. Piatak *et al.* found high concentrations of RNA from human immunodeficiency virus–type 1 (HIV-1) (up to 22 million RNA genomes per milliliter) in the plasma of each of the 66 seropositive patients they evaluated (*1*). This concentration, as determined by their "quantitative competitive polymerase chain reaction" (QC-PCR) assay (*1*), was highly correlated with $CD4^+$ T cell counts and with the progression of the AIDS disease; concentration declined with antiretroviral therapy. These results led Piatak *et al.* to recommend clinical use of quantitative determination of HIV-1 RNA in plasma for monitoring the health of seropositive persons.

As Piatak *et al.* note, the concentrations they measured greatly exceeded those observed by other investigators who used viral culture or alternative reverse transcriptase–polymerase chain reaction (RT-PCR) methods. Piatak *et al.* attribute this difference to the optimized design of their QC-PCR assay, to their use of ultracentrifugation, and to other steps taken to maximize recovery of viral RNA. On the basis of recent experiments conducted in our laboratories, we suggest that this difference can be explained, at least in part, by the fact that their QC-PCR assay does not account for contribution to viremia of HIV-1 associated with platelets.

As part of a project to investigate the feasibility of inducing viral depletion or inactivation in blood for transfusion, we have studied the compartmentalization of HIV-1 in blood. We found that platelets subjected to filtration (to remove leukocytes) and to serial washes (to remove plasma) retained high concentrations of tightly associated HIV-1 RNA; presumably this reflected bound viral particles. The concentrations of virus associated with platelets frequently exceeded those free in plasma by a factor of ten or more.

When we used the protocol of Piatak *et al.* to process 15 blood samples (centrifugation at 200*g* for 15 min; 1000*g* for 15 min), we found that plasma specimens (which they would designate "cell-free") in fact contained an average of 7×10^6 platelets per milliliter (range, 3 to 14×10^6 platelets per milliliter). Six of these samples (from four HIV-1 seropositive subjects) were further processed (in a step not taken by Piatak *et al.*) by centrifugation at 10,000*g* for 15 min to yield platelet pellets and plasma samples that were truly platelet-free (Fig. 1). We also prepared purified platelets from these patients by centrifugation of whole blood at 250*g* for 5 min, leukodepletion of the platelet-rich plasma (PL-F1 filters, Pall, Glen Cove, New York), pelleting of platelets at 10,000*g* for 15 min, and five washes of the pellet to remove plasma. The platelet-free plasma and the two platelet pellets from each sample were then assayed in parallel to detect HIV-1 RNA with the use of a sensitive RT-PCR assay (*2*). To recover virions from the cell-free plasma, we used an immunocapture technique with anti-gp-120/41–coated latex microparticles (*2*).

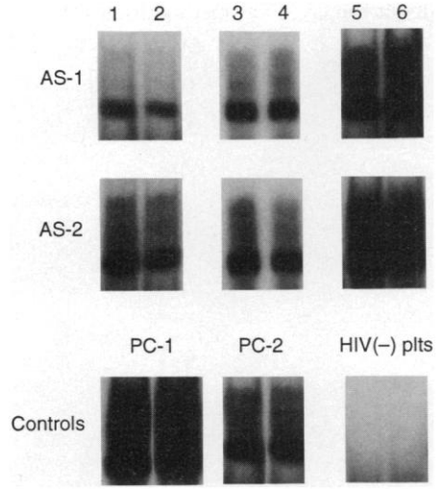

Fig. 1. Assays of relative concentrations of HIV-1 RNA associated with platelets and free in plasma. "Cell-free" plasma was prepared from two asymptomatic seropositive patients (AS-1 and AS-2) according to the protocol of Piatak *et al.* (*1*) (200*g* for 15 min; 1000*g* for 15 min). Residual platelets (14,000 and 10,000 per microliter, respectively) were pelleted (10,000*g* for 15 min) from 100 microliter of what Piatak *et al.* would designate "cell-free" (*1*) plasma. The cell-free supernatants, which had no detectable residual platelets, were subjected to immunocapture RT-PCR in duplicate (lanes 1 and 2) (*2*), and the platelet pellets were lysed and assayed in parallel by RT-PCR (lanes 3 and 4). Purified platelet pellets (lanes 5 and 6), prepared from 100 microliter of platelet-rich plasma (170,000 and 140,000 platelets per microliter, respectively) after leukocyte filtration and serial washes, were also assayed in parallel (lanes 5 and 6). Known HIV-1 viral particle standards [PC-1 (1000 copies per milliliter) and PC-2 (100 copies per milliliter)] were processed in parallel. As a control, a purified platelet pellet from 100 microliters of platelet-rich plasma from a seronegative person was tested in parallel under code HIV(−) plts.

Purified platelets (depleted of leukocytes and plasma), recovered from 100 μl of platelet-rich plasma, harbored much more virus than was present in platelet-free plasma (Fig. 1). These purified platelet preparations did not contain HIV-1 DNA as determined by a sensitive assay system (*3*), which showed that the HIV-1 RNA signal was not a result of low-level contamination by infected leukocytes.

Our findings are supported by other studies: megakaryocytes from seropositive subjects have been found to contain HIV-1 particles [by electron microscopy (*4*)] and viral RNA and antigen [by in situ hybridization and immunoblot and immunofluorescence analysis (*5*)]; HIV-1 RNA has been detected in platelet preparations by RT-PCR (*6*); and there is evidence that other viral agents are associated with platelets (*7*).

Tzong-Hae Lee
Irwin Memorial Blood Centers,
270 Masonic Avenue,
San Francisco, CA 94118
Robert R. Stromberg
American Red Cross,
Jerome Holland Laboratory,
Rockville, MD 20855
Denis Henrard
Abbott Diagnostics Division,
Abbott Park, IL 60064
Michael P. Busch
Irwin Memorial Blood Centers,
Laboratory Medicine,
University of California,
San Francisco, CA 94143

REFERENCES AND NOTES

1. M. Piatak *et al.*, *Science* **259**, 1749 (1993).
2. D. R. Henrard, W. F. Mehaffey, J.-P. Allain, *AIDS Res. Hum. Retrovir.* **8**, 47 (1992).
3. T.-H. Lee, F. J. Sunzeri, L. H. Tobler, B. G. Williams, M. P. Busch, *AIDS* **5**, 683 (1991).
4. D. Zucker-Franklin, S. Seremetis, Z. Y. Zheng, *Blood* **75**, 1920 (1990).
5. F. Louache *et al.*, *ibid.* **78**, 1697 (1991).
6. S. Bruisten *et al.*, *AIDS Res. Hum. Retrovir.* **9**, 259 (1993).
7. H. Terada, M. Baldini, S. Ebbe, M. A. Madoff, *Blood* **28**, 213 (1966); T. Bik, I. Sarov, A. Livne, *ibid.* **59**, 482 (1982); R. P. Larke and E. F. Wheelock, *J. Infect. Dis.* **122**, 523 (1970); D. Forghani and N. J. Schmidt, *Arch. Virol.* **76**, 269 (1983); D. Danon, Z. Jerushalmy, A. De Vries, *Virology* **9**, 719 (1959).

11 May 1993; accepted 7 October 1993

Response: Lee *et al.* suggest that viral RNA in platelet-associated virions could have contributed to the viral load that we found (*1*) in plasma from HIV-1–infected patients, as determined by QC-PCR. Our analyses to test this possibility with the use of QC-PCR do not agree with the data of Lee *et al.* In our studies we processed fresh, whole blood specimens (anticoagulated with acid citrate dextrose) from three

Table 1. Platelet and HIV-1 RNA content after sequential centrifugation preparation of plasma specimens for QC-PCR.

Specimen	Patient		
	BRDO 1565	HOWE 1483	GADA 0585
A. Platelet counts per milliliter of plasma ($\times 10^{-6}$)			
200*g* supernatant	188.4	105.5	114.0
1000*g* supernatant	11.5	5.8	3.6
10,000*g* supernatant	0.8	1.4	0.9
B. HIV-1 RNA copies per milliliter of starting plasma			
200*g* supernatant	1,238,000	1,404,000	8,778,000
1000*g* supernatant	1,239,000	1,547,000	12,999,000
10,000*g* supernatant	1,378,000	2,179,000	10,567,000
10,000*g* pellet	63,100	74,900	445,500

HIV-1–infected patients, according to our usual method (*1*), with sequential 15-min centrifugations at 200 and 1000*g*. After each centrifugation, aliquots of the plasma supernatant were removed and stored at −70°C for later QC-PCR analysis. The 1000*g* supernatant was subjected to an additional centrifugation (15 min, 10,000*g*) to pellet residual platelets. The resulting pellet was washed with phosphate-buffered saline supplemented with bovine serum albumin (1% w/v) and EDTA (4 mM) (PBE), pellets were prepared and resuspended in PBE, then frozen for later QC-PCR analysis. Platelet counts were made on unfrozen aliquots of supernatants from all three spins with the use of a Coulter Counter ZM (Coulter, Hialeah, Florida). Supernatants and the resuspended pellet were thawed, processed, and analyzed by QC-PCR to determine their content of virion-associated HIV-1 RNA copies, as described in our report (*1*).

The plasma supernatants obtained after the centrifugation at 1000*g* were largely, but not completely, depleted of platelets (Table 1, part A) and contained only a small fraction of the total platelets present in the platelet-rich supernatants from the 200*g* centrifugation. The centrifugation at 10,000*g* did indeed remove these residual platelets. However, the centrifugation at 1000*g*, which removed on the average more than 95% of the platelets, and the centrifugation at 10,000*g*, which removed essentially all remaining platelets, did not remove commensurate amounts of viral RNA. The amounts of viral RNA in the platelet-depleted 1,000 and 10,000 supernatants, as determined by QC-PCR, were comparable to amounts in the corresponding platelet-rich 200*g* supernatants. The amount of HIV-1 RNA associated with the 10,000*g* platelet pellets represented only a small fraction of the total viral RNA measured in the corresponding platelet-rich plasma supernatants (Table 1, part B). Thus, the contribution of platelet-associated virions to the total viral load we found in plasma specimens (*1*) was minimal, well within the range of the analytical variability of the QC-PCR method itself (*1*).

We used a single, well-described, quantitative, internally controlled PCR method (*1*) to measure viral RNA in plasma and platelet samples. Lee *et al.* used two different procedures, neither of which is rigorously quantitative or internally controlled, with and without an initial immunocapture step (*2*), to estimate the HIV-1 RNA content of different specimens. We suspect that these methodologic differences may contribute to our different results. These comparative analyses however, emphasize the point that, as more sensitive methods are developed for quantifying HIV-1 in human plasma, careful consideration should be given to specimen processing issues, including the potential contribution of platelet-associated virus to the determination of virus concentrations.

Michael Piatak Jr.
Division of HIV and Exploratory Research, Genelabs Technologies, Inc., Redwood City, CA 94063
George M. Shaw
Department of Medicine, University of Alabama, Birmingham AL 35294
Limei C. Yang
Division of HIV and Exploratory Research, Genelabs Technologies, Inc.
John C. Kappes
Michael S. Saag
Department of Medicine, University of Alabama
Jeffrey D. Lifson
Division of HIV and Exploratory Research, Genelabs Technologies, Inc.

REFERENCES

1. M. Piatak *et al.*, *Science* **259**, 1749 (1993).
2. D. R. Henrard, W. F. Mehaffey, J.-P. Allain, *AIDS Res. Hum. Retrovir* **8**, 47 (1992).

24 June 1993; accepted 7 October 1993