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QUANTITATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 IN THE BLOOD OF INFECTED PERSONS

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Abstract We used end-point-dilution cultures to measure the level of infectious human immunodeficiency virus type 1 (HIV-1) in peripheral-blood mononuclear cells (PBMC) and plasma of 54 infected patients who were not receiving antiviral chemotherapy.

HIV-1 was recovered from the plasma and PBMC of every seropositive patient, but from none of 22 seronegative control subjects. The mean titers in plasma were 30, 3500, and 3200 tissue-culture-infective doses (TCID) per milliliter for patients with asymptomatic infection, the acquired immunodeficiency syndrome (AIDS), and the AIDS-related complex, respectively. In PBMC, the mean titers were significantly higher for symptomatic patients (AIDS, 2200, and AIDS-related complex, 2700 TCID per 10^6 PBMC) than asymptomatic patients (20 TCID per 10^6 PBMC). The values for the symptomatic

patients were considered to indicate that at least 1 in 400 circulating mononuclear cells harbored HIV-1.

The HIV-1 titers of seven patients with AIDS or AIDS-related complex treated with zidovudine for four weeks decreased significantly in plasma but not in PBMC. In addition, the mean titer in the plasma of 20 patients receiving long-term zidovudine treatment (130 TCID per milliliter) was 25-fold lower than the mean for comparable untreated patients with AIDS or AIDS-related complex.

We conclude that the levels of HIV-1 in plasma and PBMC are much higher than previous estimates. This high degree of HIV-1 viremia raises the possibility that the direct cytopathic effect of this retrovirus alone may be sufficient to explain much of the pathogenesis of AIDS. (N Engl J Med 1989; 321:1621-5.)

HUMAN immunodeficiency virus type 1 (HIV-1) was initially identified as the etiologic agent of the acquired immunodeficiency syndrome (AIDS) in 1983.^{1,2} Much has been learned subsequently about the structure and function of this virus. In addition, HIV-1 has been shown to be harbored by T helper lymphocytes³ and monocyte-macrophages,^{4,5} and it is detectable in plasma,⁶ genital secretions,^{7,9} and central nervous system tissue.¹⁰ However, no studies have been reported in which HIV-1 has been systematically quantified in vivo. A study using in situ hybridization has shown that only 1 in 10^5 peripheral-blood mononuclear cells (PBMC — i.e., lymphocytes and monocytes) of patients with AIDS expresses HIV-1 messenger RNA.¹¹ This observation and that of an earlier study showing that the rate of isolation of HIV-1 from infected persons is low¹² have contributed to the general notion that the level of HIV-1 expression in vivo is low. In fact, this belief has led some to suggest that

HIV-1 may not be the causative agent of AIDS.¹³ We report findings from a systematic, quantitative study to define the level of infectious HIV-1 in the PBMC and plasma of seropositive subjects during different stages of the disease. The amount of infectious HIV-1 detected was higher by orders of magnitude than previously estimated. This high degree of viremia has important implications for the pathogenesis and transmission of HIV-1.

METHODS

Patients

Blood samples were obtained from 54 HIV-1-seropositive adults (53 men and 1 woman) who were not receiving antiviral treatment; 16 patients had asymptomatic infection, 18 had AIDS-related complex, and 20 had AIDS. The mean CD4+ lymphocyte counts in these three subgroups were 462, 147, and 88 per cubic millimeter, respectively. Eighteen patients had taken zidovudine previously, but not for a minimum of four weeks. Twenty-two seronegative homosexual men served as controls.

Four untreated patients with AIDS-related complex donated blood for sequential analyses over a period of 12 to 20 weeks. Their clinical courses during this period were stable.

Twenty patients with AIDS or AIDS-related complex (mean CD4+ lymphocyte count, 112 per cubic millimeter), who had been treated with zidovudine (400 to 1200 mg per day) for at least four weeks, also provided blood for quantitative analyses. In addition, seven other patients with AIDS or AIDS-related complex were

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studied before drug treatment, as well as after four weeks of treatment (800 to 1200 mg of zidovudine per day).

The study was approved by the local institutional review board, and all study subjects gave written, informed consent.

Assays for HIV-1 Antibody and p24 Antigen

HIV-1 antibodies were measured with an enzyme-linked immunosorbent assay and a radioimmunoprecipitation assay.

Serum samples and culture supernatant fluids were assayed for HIV-1 p24 core antigen with a commercial enzyme immunoassay (Abbott Laboratories, North Chicago).

End-Point-Dilution Cultures of HIV-1

PBMC

Blood samples were processed within 60 minutes after venipuncture. Decreasing numbers of PBMC (2×10^6 , 2×10^5 , 2×10^4 , 2×10^3 , 2×10^2 , and 1×10^2) isolated by Ficoll-Hypaque gradient centrifugation were cocultured with 2×10^6 phytohemagglutinin-activated PBMC from healthy blood-bank donors in 1.5 ml of RPMI-1640 medium with 15 percent fetal-calf serum and 10 percent (vol/vol) interleukin-2 (Cellular Products, Buffalo, N.Y.). Twenty-four hours later, all cultures were washed four times with medium. Cocultures were subsequently monitored for the presence of p24 antigen in supernatant fluid, twice weekly for up to 28 days. During this period, PBMC were not added to or removed from the cultures, and 50 percent medium changes were performed twice weekly. A culture was considered positive if the concentration of p24 antigen in the supernatant exceeded 1000 pg per milliliter (typical cutoff value, approximately 30 pg per milliliter) on a single determination or ≥ 200 pg per milliliter on two or more determinations. The lowest number of PBMC required to produce a positive culture was taken as the end point, and the titers of infectious HIV-1 were then expressed as 0.5, 5, 50, 500, 5000, or 10,000 tissue-culture-infective doses (TCID) per 10^6 PBMC.

Plasma

Plasma was obtained by centrifugation of blood at $3000 \times g$ for 15 minutes, which is sufficient to remove all cells. Decreasing volumes of plasma (1000, 200, 40, 10, and 2 μ l; and in selected patients, 0.2 and 0.02 μ l) were cultured with 2×10^6 phytohemagglutinin-activated PBMC from healthy blood-bank donors as described above. Again, the PBMC in cultures were washed four times 24 hours later and subsequently monitored for p24-antigen expression as outlined above. The smallest volume of plasma required to produce a positive culture was taken as the end point, and HIV-1 titers were then expressed as 1, 5, 25, 100, 500, 5000, or 50,000 TCID per milliliter of plasma.

RESULTS

The results of quantitative cultures of plasma and PBMC are summarized in Figure 1. All seronegative control subjects had negative cultures for HIV-1 in both plasma and PBMC, demonstrating that the method employed had a specificity of 100 percent and that laboratory contamination did not occur. In contrast, in every seropositive patient, HIV-1 was recovered from both plasma and PBMC (100 percent sensitivity). The titers of virus in plasma were higher in the patients with more advanced disease. Although there was a wide range of HIV-1 titers for patients in each disease category, the mean titers in plasma were 30, 3200, and 3500 TCID per milliliter for those with asymptomatic infection, AIDS-related complex, and AIDS, respectively (Fig. 1A). Using these mean values, we calculated that the plasma of patients with

symptoms contained approximately 1 TCID per 0.3 μ l and that the plasma of patients without symptoms had a level 100-fold lower.

The viral titers in PBMC were also higher in patients with advanced disease (Fig. 1B). Patients with AIDS-related complex and those with AIDS had mean HIV-1 titers of 2700 and 2200 TCID per 10^6 PBMC — approximately 1 TCID per 400 PBMC. In other words, at least 1 in 400 mononuclear cells harbored HIV-1. Fifteen of the 38 symptomatic patients (39 percent) had titers of ≥ 5000 TCID per 10^6 cells (Fig. 1B), or ≥ 1 TCID per 200 cells. The mean titer in PBMC of the 16 asymptomatic seropositive patients was 20 TCID per 10^6 cells, about 125 times lower than the mean titer of the symptomatic patients.

The concentrations of p24 antigen in serum were determined in 53 patients, and the results were correlated with the HIV-1 titers in plasma ($r = 0.689$, $P < 0.001$; Fig. 2). Nevertheless, although p24 antigen was not detected in the serum of 14 patients, infectious HIV-1 was recovered from their plasma; the plasma titers ranged from 5 to 500 TCID per milliliter (Fig. 2). Thus, plasma culture was more sensitive than serum p24 antigen measurement in detecting the presence of cell-free HIV-1 in blood.

Serial HIV-1 titers in plasma and PBMC were determined in four patients with AIDS-related complex who were in clinically stable condition and who were not receiving antiviral chemotherapy. Table 1 shows that the titers in both components of blood were relatively stable for up to 20 weeks. In contrast, the plasma titers of seven patients with AIDS or AIDS-related complex treated with zidovudine for four weeks (800 to 1200 mg per day) decreased from a mean of 1700 TCID per milliliter to 100 TCID per milliliter (Fig. 3), representing a 94 percent reduction in the load of cell-free virus. Although this decrease was paralleled by a similar decrease in the level of p24 antigen, the PBMC titers did not change consistently (Fig. 3). That zidovudine had an *in vivo* effect on plasma HIV-1 titers was supported by the results of studies of plasma obtained from 20 patients who were receiving long-term zidovudine therapy (> 4 weeks; 400 to 1200 mg per day). Their plasma titers ranged from 0 to 500 TCID per milliliter, with a mean of 130 TCID per milliliter (Fig. 1A) — a value 25-fold lower than the mean plasma titer in the untreated patients with AIDS or AIDS-related complex. Indeed, during the course of this study, only four seropositive patients had no infectious HIV-1 in plasma, and all were receiving zidovudine.

DISCUSSION

We found that in infected but asymptomatic patients with HIV-1, 1 in 50,000 PBMC (20 TCID per 10^6 cells) harbored the virus. When such a patient's condition progressed to AIDS-related complex or AIDS, the viral titer increased significantly, to approximately 1 in 400 PBMC (2200 to 2700 TCID per

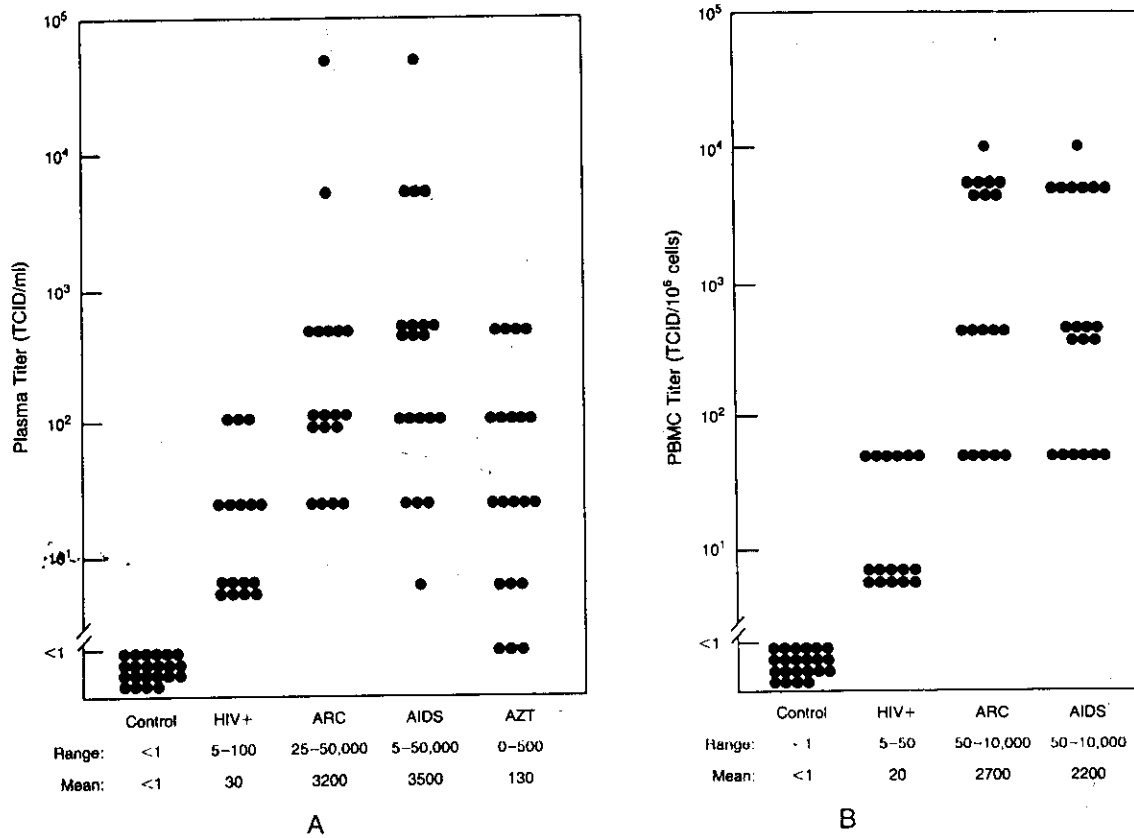


Figure 1. Titers of Infectious HIV-1 in Plasma and PBMC of 22 Control Subjects and 54 Patients in Different Stages of HIV-1 Infection. HIV+ denotes asymptomatic seropositive patients; ARC, patients with the AIDS-related complex; AZT, patients receiving long-term zidovudine treatment; and PBMC, peripheral-blood mononuclear cells.

10^6 cells), as shown in Figure 1B. It is unclear from this study, however, what percentage of the infected cells carry HIV-1 latently and what percentage of the cells express the virus actively. If 1 in 10^5 PBMC from a patient with AIDS expresses viral messenger RNA, as shown by Harper et al.¹¹ using in situ hybridization, then 99.6 percent of the infected mononuclear cells harbor the virus latently and the remaining 0.4 percent express it actively. Nevertheless, the total burden of HIV-1 that we found in PBMC was much higher than previous estimates.^{11,13,14} Furthermore, if one assumes that 10 percent of the mononuclear cells of a patient with AIDS are CD4+, 1 in 40 CD4+ PBMC harbors the virus.

HIV-1 was detected in the plasma of every seropositive patient who was not receiving antiviral treatment, even though some did not have detectable levels of p24 antigen. This finding suggests that in the absence of chemotherapeutic intervention, HIV-1 replication in the host is not completely latent. The level of the virus in plasma increased from approximately one infective dose per 30 μ l in asymptomatic patients to three infective doses per 1 μ l in symptomatic patients (Fig. 1A). The finding of high titers of infectious virus in plasma clearly shows that circulating antibodies are insufficient to neutralize HIV-1 in vivo.

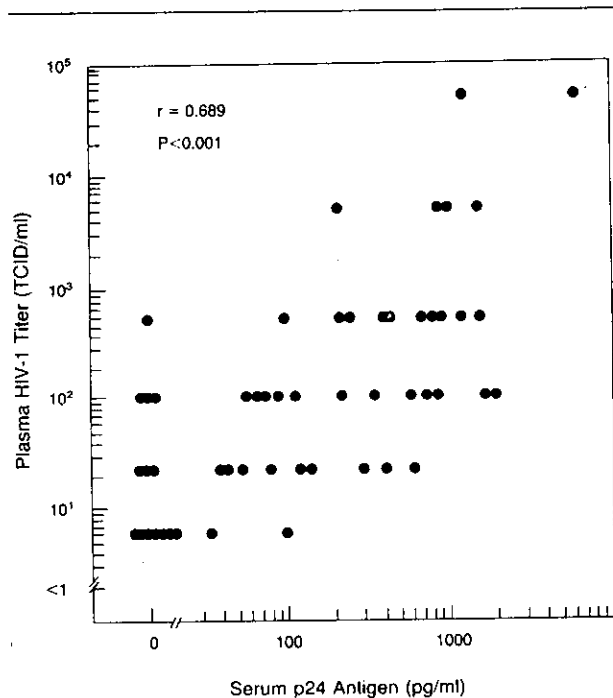


Figure 2. Serum p24 Antigen Concentrations in Relation to Plasma HIV-1 Titers in 53 Patients with HIV-1 Infection.

Table 1. Serial HIV-1 Titers in Plasma and PBMC of Four Untreated Patients with AIDS-Related Complex.

WEEK	HIV-1 TITER	
	PLASMA	PBMC
	TCID/ml	TCID/10 ⁶ cells
Patient 1		
0	500	5000
4	500	500
5	100	500
8	500	5000
10	500	5000
20	500	5000
Patient 2		
0	500	500
5	500	50
7	500	5000
20	500	5000
Patient 3		
0	500	5000
2	500	500
6	500	5000
10	500	5000
12	500	5000
Patient 4		
0	100	500
8	500	500
11	500	5000
13	500	500
15	500	5000
17	500	5000

The total titer of HIV-1 in whole blood from asymptomatic patients and symptomatic patients (those with AIDS and AIDS-related complex) can be determined with the use of the mean values for plasma and PBMC levels shown in Figure 1. If 1 ml of blood is assumed to contain an average of 0.65 ml of plasma and 2×10^6 PBMC, the blood of asymptomatic patients contains approximately 60 TCID per milliliter

and that of patients with AIDS or AIDS-related complex contains about 7000 TCID per milliliter (total viral titer per volume of blood; Fig. 4). The presence of high levels of HIV-1 viremia raises the possibility that the direct cytopathic effect of this retrovirus alone may be sufficient for AIDS to develop, without necessarily involving indirect autoimmune mechanisms.¹⁵ In addition, this information on the quantitation of HIV-1 should reduce residual doubts about whether HIV-1 is the true etiologic agent of AIDS.¹³

The model shown in Figure 4 has important practical implications. For example, one 250-ml unit of contaminated blood could contain 1.5×10^4 TCID of HIV-1 if it was obtained from an asymptomatic person or 1.75×10^6 TCID if obtained from a symptomatic patient. These values clearly show why the transfusion of contaminated blood results in an extremely high rate of HIV-1 infection among recipients.¹⁶ Our quantitative data could also be applied in hospital laboratories, where technicians typically handle 1 to 10 ml of blood, often without special containment facilities. If a blood sample is obtained from a patient with AIDS, the total infective dose would be approximately 7×10^3 to 7×10^4 TCID. These numbers serve to emphasize the importance of the use of strict guidelines and proper techniques and an appropriate level of caution among laboratory workers. When intravenous drug users share needles and syringes, perhaps 10 to 100 μ l of blood may be transmitted; according to the model in Figure 4, such an exposure would result in an infective dose of 0.6 to 6 TCID of HIV-1 if the shared apparatus had been used by an asymptomatic seropositive person. However, the risk would be markedly higher (70 to 700 TCID) if the apparatus had been shared with a patient with AIDS or AIDS-related complex. Finally, during needle-stick accidents in

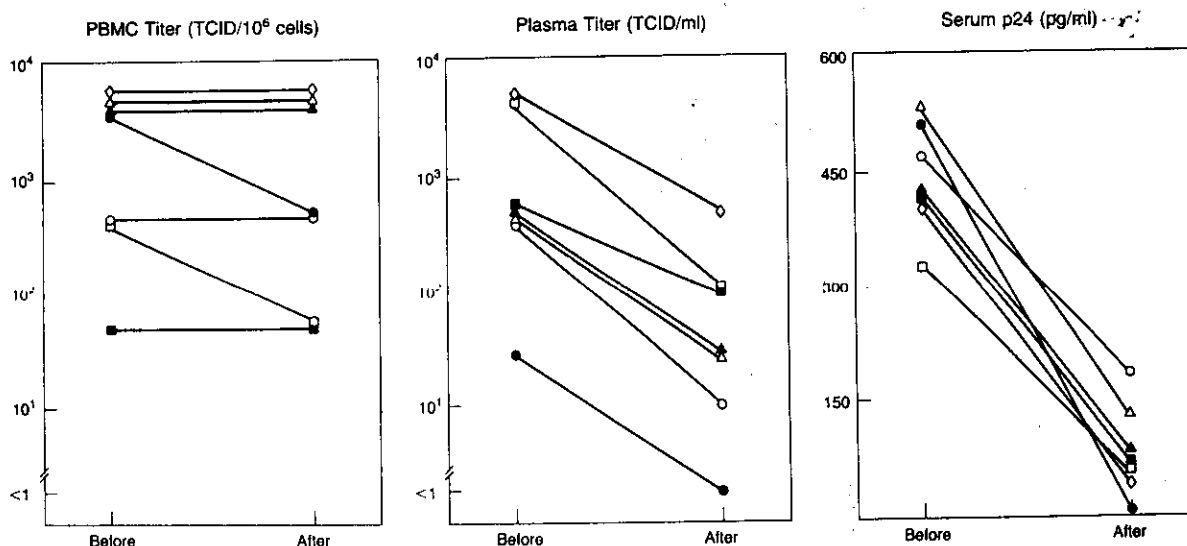


Figure 3. Changes in PBMC HIV-1 Titer, Plasma HIV-1 Titer, and Serum p24 Antigen Concentration in Seven Patients with AIDS-Related Complex or AIDS, before and after Four Weeks of Zidovudine Treatment.

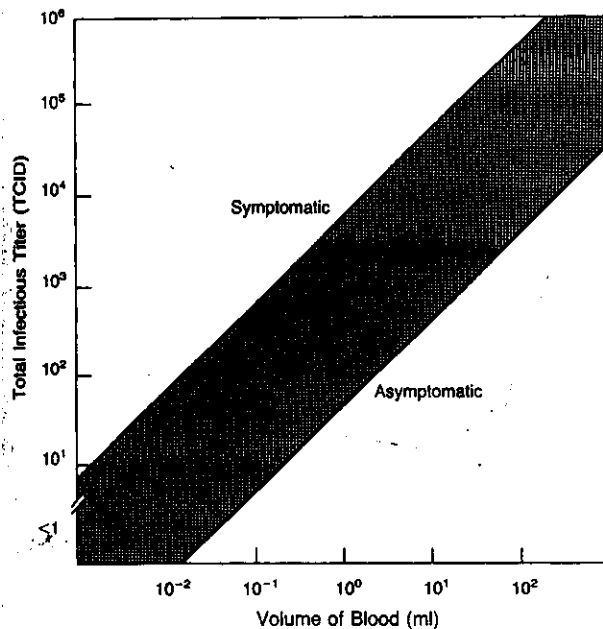


Figure 4. Total HIV-1 Titer in Relation to Volume of Blood from Asymptomatic Patients (Bottom Line) and Symptomatic Patients (Top Line).

The two lines were derived with the use of the data shown in Figure 1 and the assumptions given in the Discussion.

health care settings, the amount of blood involved is typically about 1 μ l, which would represent 0.06 TCID if the blood were that of an asymptomatic person or 7 TCID if that of a symptomatic patient. These values reflect relatively low titers and are consistent with the low (but not zero) rate of infection through such exposures.¹⁷ It will be important to conduct similar studies of genital secretions to quantify the amount of virus involved in the sexual transmission of HIV-1. Such information would be particularly useful in planning studies of active and passive immunization in animals and humans.

Our findings suggest that culturing plasma (200 μ l or more) is a sensitive and specific method of detecting HIV-1 in untreated patients. In addition, although laborious, this method can be made quantitative by using end-point-dilution cultures. We have shown that this method can detect the antiviral effect of zidovudine in treated patients, even in the absence of p24 antigen in the blood. It should be possible to use this

quantitative culture technique to monitor the anti-HIV-1 effect of other chemotherapeutic agents in vivo, as well as to assess clinical factors that can activate replication of the virus in vivo.

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