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25 July 1995; accepted 22 August 1995

Plasma Viral Load, CD4$^+$ Cell Counts, and HIV-1 Production by Cells

Recent papers have reported large amounts of human immunodeficiency virus (HIV) particles circulating in the blood of infected patients (*1–3*). In the total blood volume, the number of virions can equal 10^6 particles per milliliter, or an estimated 10^9 HIV particles per milliliter (*1*, *2*). In his article, John M. Coffin refers (*4*, p. 483) to an "extraordinarily large number of replication cycles that occur during [HIV] infection of a single individual." But retrovirus-infected cells, in general, produce large amounts of progeny virions, and most of these viruses are not infectious (*5*). Thus, the importance of the quantity of virus, both infectious and uninfectious, circulating in the blood should not be overemphasized. The most important question raised from these papers is how many cells in the body are needed to produce the billion virus particles observed in the symptomatic patients.

To address this issue, we cultivated for up to 3 days human T cells of the HUT78 line that were chronically infected with the HIV-1_{SF2} strain. This virus strain replicates with moderate kinetics in this T cell line. We monitored viable cell number with the use of trypan blue dye exclusion and determined the percent of productively infected cells with the use of indirect immunofluorescence assays (IFA) on fixed cells (*6*). Amounts of virus were measured by branched DNA with the use of a kit provided by Chiron Corporation (Emeryville, California).

The HUT78 T cells expressed HIV proteins as detected by IFA. After initial trypsinization to remove virions associated with the cell surface (*7*), we measured virus production by 2 million of these cells cultured in 2 ml of medium. Within 24 hours, we detected about 120 million viral RNA genome copies which means that virus production by 2×10^6 cells is about 30 virions per cell (that is, two RNA molecules per virion). After 48 hours the amount of virus production rose to 400 million RNA molecules, or about 100 particles per cell.

Because the initial studies on virus production used an established transformed T cell line (HUT78), we also examined virus production by infected peripheral blood mononuclear cells (PBMC), the major target of HIV. We acutely infected cultured PBMCs with either the highly cytopathic HIV-1_{SF33} strain or a molecular clone of the less cytopathic HIV-1_{SF2} strain. Cell culture fluids were changed daily. On the fifth day, when reverse transcriptase activity (~400,000 cpm/ml) in the cell culture fluids was high, the supernatants were collected, filtered, and assayed for viral RNA with the use of the branched DNA technique. The infected PBMCs were trypsinized to remove and inactivate cell-associated virus (*7*), monitored for cell viability and expression of viral proteins as detected by IFA, and plated as infectious centers on PHA-stimulated normal PBMCs. The dilutions involved duplicate cultures receiving 1000, 500, 100, 50, and 10 cells per well. At each dilution of cells, we performed the trypsinization procedure to eliminate any virus spread that might have occurred during the dilution.

We found that 10 to 15% of the PBMC showed HIV protein expression by both assays. Viral RNA production in 2 ml of culture fluid indicated the release of approximately 125 to 200 particles per cell in 24 hours (Table 1), which is similar to our result with the HUT78 cells. Studies by Dimitrov *et al.*, which used cultured HIV-1–infected CEM cells (*8*), showed that at the time of peak virus production 1000 particles were released by an infected cell. These data and ours demonstrate the large capacity for virus release by one infected cell and suggest that only about 10 million cells would be needed to maintain the billion virus particles observed in the plasma of some subjects (*1*, *2*). Possibly even fewer cells are required, given the limits of the in vitro studies conducted.

Table 1. HIV-1 replication in peripheral blood mononuclear cells. Data obtained from viral RNA measurements in 2 ml of fluid after culture of 2×10^6 infected PBMC for 24 hours. Number of cells releasing virus are estimated from percent viability, data from infectious center, and immunofluorescence assays. Computations do not include cells that may have died after virus release (for example, with HIV-1_{SF33}).

HIV-1 Isolate	Cells releasing HIV-1	Viral RNA genome copies per milliliter	Viral particles per cell
SF33	1.4×10^5	32×10^6	228
SF2	2.4×10^5	30×10^6	125

These findings on free virus production do not include the number of virons that remain cell-associated. Such viruses must be in much greater number than the free virus released, as they are easily detected as budding particles by electron microscopy, and as they can spread 100- to 1000-fold more effectively than can free virus (*8*). These observations underline the importance of combating the cellular source of HIV and not just de novo infection by its viral progeny (*9*).

Finally, the immediate rise in the CD4$^+$ cell counts after antiretroviral therapy may reflect a response to the drug itself and not to a drug-induced protection from CD4$^+$ cell death, as hypothesized in other studies (*1*, *2*). Records from one individual provide valuable data. Within 30 minutes after a needle stick injury, this person was given 3′ azido-3 deoxythymidine (AZT) prophylaxis (1200 mg/day for 2 days, 1000 mg/day for 12 days, and then 500 mg/day for 2 weeks). He has been shown not to be infected. Total lymphocyte and CD4$^+$ and CD8$^+$ cell counts were conducted on a regular basis and examined in relationship to the pre- and post-drug therapy measures. Within 48 hours after AZT treatment, both lymphocyte and CD4$^+$ cell counts rose substantially from the baseline amount (Fig. 1). They increased by 30% by day 12 and remained at this high count throughout the course of

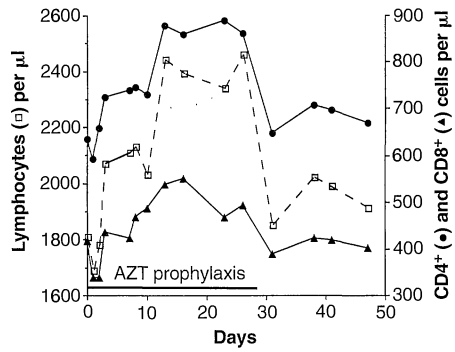

Fig. 1. Lymphocyte counts during and after prophylactic treatment of a healthy individual with AZT after an HIV needle stick injury. Zero represents time period just before initiation of therapy. The *y* axis on the left shows a total lymphocyte count; the *y* axis on the right shows a count for specific cell types.

treatment. $CD8^+$ T cells did not increase substantially, likely reflecting the stable distribution of CD4 to CD8 cells in this individual. When AZT therapy stopped, the lymphocyte and $CD4^+$ cell counts returned to the normal range. The source of the cells is not known. However, the rapid increase in $CD4^+$ cells and their memory subset (CD45RO) suggests a margination effect or redistribution of already formed cells. Because AZT treatment was not continued after 1 month, we do not know whether such therapy would have maintained such a high concentration of lymphocytes for a longer period. Most important, the cellular response that we observed to the treatment challenges current interpretations of the effect of AZT (and perhaps other antiviral drugs) on the number of lymphocytes in treated patients.

Our studies suggest experimental controls that should be considered when evaluating antiretroviral therapy in HIV-infected individuals. A 100-fold reduction in virus particles per milliliter observed during antiretroviral therapy (*1*, *2*) might represent the arrest of virus replication by only 10 million cells. More than 200 billion cells in the host are infected by the virus (*10*), so control of HIV infection would require much greater reduction in virus load. Moreover, measuring $CD4^+$ cell counts as an indication of antiretroviral efficacy (*11*) may not account for the cellular response of the host to the antiviral drug alone. Before advocating antiretroviral therapies, a clear assessment of their effect on parameters influencing the clinical course of the disease should be provided. A major target in therapy should be the source of the virus—the virus-infected cell—and not only its products (*9*), so that significant effects on viral load and clinical outcome might be achieved.

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23 October 1995; accepted 5 December 1995

Response: As shown by the experiments presented by Levy *et al.*, 10^9 virions is not very much virus (about a microgram, in fact). This much virus can easily be made in a day in a cell culture of modest proportion, and its production should hardly strain the resources of a 75-kilogram patient. The importance of the virus load is only as a relative measure of activity in an infected individual from one time point to the next. It is the number of infected calls and the kinetics of infection that are important. The actual amount of virus observed in blood is related to the balance of production, transit from the site of production, and decay or clearance. The final value could be anything measurable without changing important conclusions for understanding HIV pathogenesis.

Although, as concluded Levy *et al.*, the amount of virus detected *could* be made by relatively few cells, it cannot be concluded that it *is* made by a few cells. An infected patient is not a well-stirred system like a cell culture: The important sites of replication are the lymphoreticular tissues (in lymph nodes, spleen, tonsils, gut, skin, and elsewhere), which are not in free equilibrium with blood. Only a fraction of the virus made at these sites can get to the blood, and we have no idea what this fraction is and therefore have no way of using the virus load to directly calculate the number of infected cells. As I pointed out in my article, an important assumption in the use of virus load measurements for this purpose is that not that the fraction of virus released into blood be large or small, only that it be constant over the period of observation. While I believe this assumption to be correct, I also consider it of first importance that it be tested.

Although the case history regarding increased $CD4^+$ cell counts observed after AZT treatment is interesting, it does not bear on the kinetic studies (*1*) cited by Levy *et al.* because neither of these studies used AZT. As I have pointed out (*2*), a strength of the combined studies and those of some other groups (*3*) is that the use of multiple compounds makes the occurrence of artifacts resulting from unknown side effects unlikely. Unless Levy *et al.* were to postulate that nucleoside analogs, nonnucleoside reverse transcriptase inhibitors, and protease inhibitors all share the property of $CD4^+$ cell enhancement, or perhaps that all drugs have this property, this observation has no relevance to the situation.

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20 November 1995; accepted 5 December 1995