Dynamics of HIV-1 Replication In Vivo

David D. Ho
Aaron Diamond AIDS Research Center, The Rockefeller University, New York 10016

The human immunodeficiency virus type 1 (HIV-1) was first identified in 1983 and shown in 1984 to be the causative agent of the acquired immunodeficiency syndrome (AIDS). Early studies found insufficient virus in infected persons to account for the marked CD4 lymphocyte depletion, thus prompting some investigators to propose autoimmune mechanisms of HIV-1 pathogenesis. Beginning in 1989, however, studies began to report substantially higher levels of infectious virus in the plasma and peripheral blood mononuclear cells of patients (1), thereby showing HIV-1 infection to be a persistently active process in vivo. Similarly, with the advent of polymerase chain reaction, viral DNA was also detected in increased amounts in infected individuals. Subsequent studies convincingly demonstrated the presence of high levels of HIV-1 RNA in plasma (2) and lymph nodes (3, 4) of all patients throughout the course of infection. Collectively, these findings show that continuous viral replication plays a central role in HIV-1 pathogenesis.

An initial burst of HIV-1 replication occurs during primary infection (5). The viral load then decreases substantially in temporal association with the development of specific cellular immune responses (6). In many cases, the plateau concentration of plasma viremia after the primary syndrome is maintained for years. This steady state level or "setpoint" is predictive of the long-term clinical outcome (7, 8). However, measurement of viral load provides only a static view, without real insights into the kinetics of HIV-1 replication. Using potent antiretroviral agents to perturb the steady state between virus and host, followed by careful analyses of the subsequent changes in viral load, a new understanding of the dynamics of HIV-1 replication in vivo has emerged from the studies of Wei et al. (9) and our group (10–12).

In our initial study (10), infected patients were treated with a potent protease inhibitor, ritonavir. Every patient had a rapid and dramatic decline in plasma viremia over the first 2 wk of treatment. The initial decline was always exponential, demonstrated by a straight-line fit to the data on a log plot. The slope of this line, as defined by linear regression, permitted a minimum estimate of half-life ($t_{1/2}$) of viral decay: range of 1.3–3.3 d, with a mean of 2.1 d. The latter value indicated that on average at least half of the plasma virions turns over every 2 d. Factoring in each patient's estimated plasma and extracellular fluid volume based on body weight, we determined the minimum rate of HIV-1 production (which equals clearance at steady state) to be in the range of $0.05–2.07 \times 10^9$ virions/d, with a mean of 0.68×10^9/d. Although we knew that these values represented only minimum estimates, the initial findings nevertheless served as a definitive demonstration that HIV-1 replication in vivo must be continuous and highly productive. The paradigm of HIV-1 pathogenesis was conclusively changed from one of prolonged viral quiescence to one of constant and dynamic viral replication.

In the study of Wei et al. (9), patients were treated with ritonavir, indinavir, or nevirapine, and found to have a similar mean decay $t_{1/2}$ for plasma virus of $\sim$ 2.0 d. In addition, in patients who received nevirapine therapy, they also showed that the wild-type population of HIV-1 in plasma was quickly replaced by drug-resistant variants, thus independently verifying the rapid and constant turnover of plasma virus.

The rapid, exponential decay of plasma viremia after treatment observed in these studies reflects a composite of two separate effects: clearance of free virions from plasma and loss of virus-producing cells. To understand the kinetics of these two viral compartments more precisely, we conducted a second study (11). Again, viral loads in infected subjects were closely monitored after the administration of ritonavir. Each patient responded with a similar pattern of viral decay, with an initial lag of $\sim$ 1.5 d followed by an approximately exponential decline in plasma viral RNA. Using mathematical modeling and nonlinear regression to fit the measurements of plasma HIV-1 RNA for each patient, separate estimates for virion clearance and loss of virus-producing cells were determined. Clearance of free virions was found to occur with a mean $t_{1/2}$ of $\leq$ 6 h. Since the production rate of virus must equal its clearance rate at steady state, we obtained an estimate for the rate of viral production before ritonavir administration. The total daily viral production and clearance rates ranged from 0.4×10^9 to 32.1×10^9 virions/d, with a mean of 10.3×10^9 virions/d released into the extracellular fluid. Although this rate is 15-fold faster than our previous estimate (10), it is still only a minimum estimate. The actual rates are likely to be 10–30-fold higher (our unpublished results). The loss of virus-producing cells was slower than that of free virions, with a mean $t_{1/2}$ value of $\sim$ 1.6 d.

It is now apparent that the repetitive replication of HIV-1, shown on the left side of Fig. 1, accounts for most of the plasma viruses in infected individuals, as well as for the high destruction rate of CD4 lymphocytes. The demonstration of the highly dynamic nature of this cyclic process provides not only a detailed kinetic picture of the pathogenesis of HIV-1, but also theoretical principles to guide the development of treatment strategies. Based on our estimates of the viral dy-

Address correspondence to David D. Ho, Aaron Diamond AIDS Research Center, The Rockefeller University, 455 First Avenue, New York, NY 10016. Phone: 212-725-0018; FAX: 212-725-1126; E-mail: dho@adarc.org

Received for publication 29 April 1997.

J. Clin. Invest.

0021-9738/97/06/2565/03 $2.00
Volume 99, Number 11, June 1997, 2565–2567

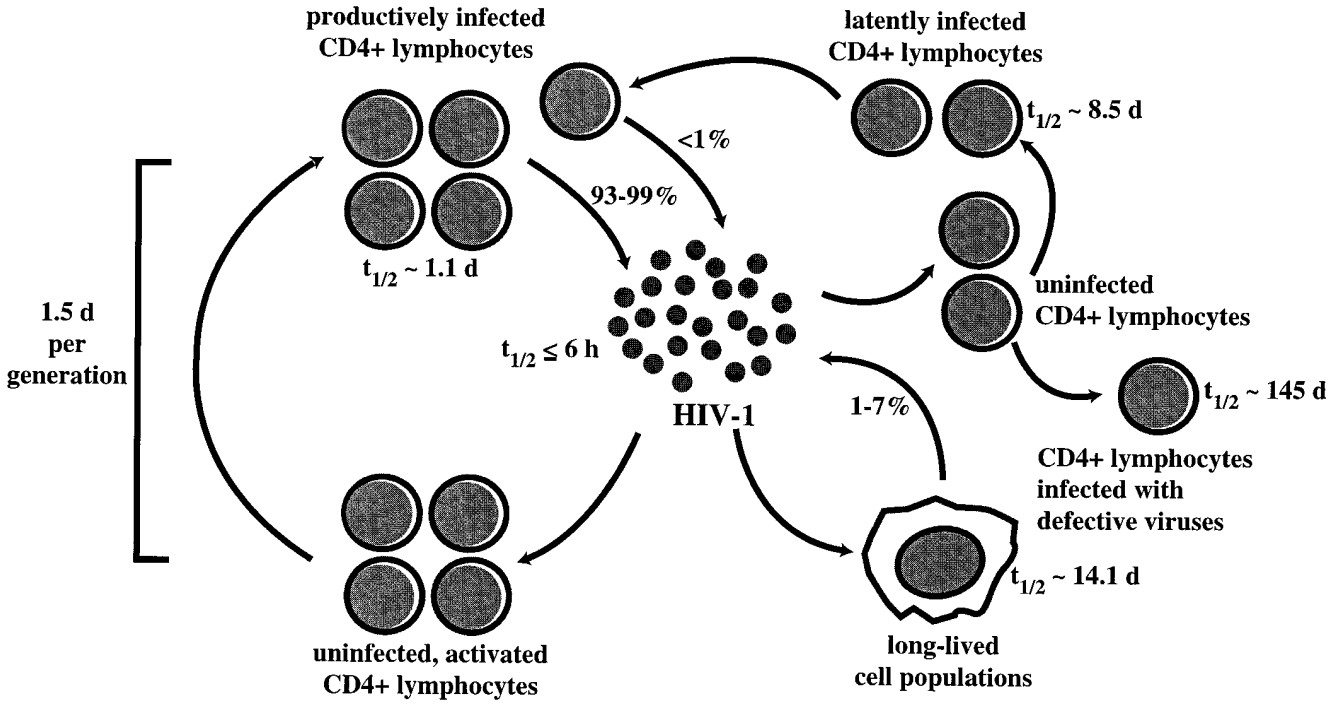

Figure 1. Schematic summary of the kinetics of known HIV-1 compartments in vivo (modified from reference 11). The life spans of the indicated cell populations are estimates derived from our latest study (12).

namics along with the mutation rate of HIV-1 (3.4×10^{-5}/bp per replication cycle) (13) and the genome size (10^4 bp), it can be argued that on average every mutation at every position in the genome occurs numerous times each day. Therefore, the failure of the current generation of antiviral agents, when used as monotherapy, is the inevitable consequence of the dynamics of HIV-1 replication. Moreover, that the process of producing mutant viruses is repeated for 240 generations each year argues strongly for aggressive therapeutic intervention early if a dramatic clinical impact is to be achieved (14).

Apparently, the "raging fire" of active HIV-1 replication depicted by the left side of Fig. 1 could be put out by potent antiretroviral agents in ~ 2 wk (9–12). However, the dynamics of other viral compartments remain unknown. Although they contribute relatively little to the plasma virus, each viral compartment depicted on the right side of Fig. 1 could serve as the "ember" to re-ignite high-level viral replication when the therapeutic regimen is withdrawn. Therefore, we were interested in determining the decay rate of long-lived, virus-producing populations of cells such as tissue macrophages, as well as the activation rate of cells latently carrying infectious proviruses. This objective was successfully accomplished in our third study on HIV-1 dynamics (12).

Infected patients naive to antiretroviral agents were treated with a protease inhibitor, nelfinavir, and two reverse transcriptase inhibitors, zidovudine and lamivudine. Each patient responded with a similar pattern of viral decay: an initial rapid exponential decline of nearly 2-logs (first phase), followed by a slower exponential decline (second phase). The second phase in the decay profile is probably due to sources of HIV-1 not included in previous analyses, such as infected tissue macrophages or dendritic cells, activation of latently infected lymphocytes, or released of trapped virions. If such secondary sources are initially responsible for, say, 1% of the virions in plasma, then the rapid decline in viremia would slow when productively infected CD4+ T cells had decayed to ~ 1% of their initial level. Further decay of productively infected CD4+ T cells at their fast $t_{1/2}$ would then leave the secondary sources as the major producers of virions. The slower decay of the major secondary source would then be reflected in the slope of the second-phase decline. In this manner, by careful mathematical analysis of plasma viremia data as well as sequential measurements of HIV-1 infectivity titers in peripheral blood mononuclear cells, we were able to determine the decay $t_{1/2}$ of long-lived, infected tissue macrophages to be ~ 1–4 wk, with a mean value of ~ 14 d. Similarly, the decay $t_{1/2}$ of latently infected CD4 lymphocytes was estimated to be ~ 0.5–2 wk, with an average of ~ 8.5 d. The $t_{1/2}$ for productively infected CD4 lymphocytes was adjusted to a mean value of ~ 1.1 d (Fig. 1).

The above parameter estimates allowed us to compute the contribution of each cellular compartment to the viral load as measured in plasma. As shown in Fig. 1, 93–99% of the plasma viremia are sustained by productively infected CD4 lymphocytes. Infected tissue macrophages and other long-lived cells contribute ~ 1–7%, whereas the contribution by activation of latently infected T cells is < 1%.

These results have direct implications for the possibility of eradicating HIV-1 from an infected person. If de novo infection is completely inhibited by antiretroviral drugs, the treatment duration necessary to permit all viral compartments to burn out would depend on the decay $t_{1/2}$ and pool size of each element. Given their rapid kinetics, cell-free virions and productively infected CD4 lymphocytes would be eliminated in < 2 mo. Above estimates for the decay of infected tissue macrophages and latently infected lymphocytes suggest that these compartments of virus could be eliminated after ~ 2–3 yr of

treatment with a completely inhibitory antiretroviral regimen (12). However, to eradicate HIV-1 completely from an infected person, treatment may need to be administered for a longer period, because of the possible existence of small, unforeseen viral compartments that decay more slowly, or sanctuary sites (such as the brain) which are impenetrable by some of the drugs.

In summary, by using antiretroviral agents to perturb the equilibrium between the host and virus, we have been able to dissect out important kinetic features of HIV-1 replication in vivo. The continuous high-level replication of the virus is the principal engine driving the pathogenesis of this viral infection. This new paradigm has in turn allowed us to formulate therapeutic strategies to effectively control HIV-1 in infected persons.

References

1. Ho, D.D., T. Moudgil, and M. Alam. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N. Engl. J. Med.* 321: 1621–1625.

2. Piatak, M., M.S. Saag, L.C. Yang, S.J. Clark, J.C. Kappes, K.-C. Luk, B.H. Hahn, G.M. Shaw, and J.D. Lifson. 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science (Wash. DC).* 259:1749–1754.

3. Embretson, J., M. Zupancic, J.L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A.T. Haase. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature (Lond.).* 362:359–362.

4. Pantaleo, G., C. Graziosi, J.F. Demarest, L. Butini, M. Montroni, C.H. Fox, J.M. Orenstein, D.P. Kotler, and A.S. Fauci. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature (Lond.).* 362:355–358.

5. Daar, E.S., T. Moudgil, R.D. Meyer, and D.D. Ho. 1991. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N. Engl. J. Med.* 324:961–964.

6. Koup, R.A., J.T. Safrit, Y. Cao, C.A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D.D. Ho. 1994. The initial control of viremia during primary HIV-1 syndrome is temporally associated with the presence of cellular immune responses. *J. Virol.* 68:4650–4655.

7. Mellors, J.W., C.R. Rinaldo, P. Gupta, R.M. White, J.A. Todd, and L.A. Kingsley. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science (Wash. DC).* 272:1167–1170.

8. Ho, D.D. 1996. Viral counts count in HIV infection. *Science (Wash. DC).* 272:1124–1125.

9. Wei, X., S.K. Ghosh, M.E. Taylor, V.A. Johnson, E.A. Emini, P. Deutsch, J.D. Lifson, S. Bonhoeffer, M.A. Nowak, B.H. Hahn, et al. 1995. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature (Lond.).* 373:117–122.

10. Ho, D.D., A.U. Neumann, A.S. Perelson, W. Chen, J.M. Leonard, and M. Markowitz. 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature (Lond.).* 373:123–126.

11. Perelson, A.S., A.U. Neumann, M. Markowitz, J.M. Leonard, and D.D. Ho. 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science (Wash. DC).* 271:1582–1586.

12. Perelson, A.S., P. Essunger, Y. Cao, M. Vesanen, A. Hurley, K. Saksela, M. Markowitz, and D.D. Ho. 1997. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature (Lond.).* 387:188–192.

13. Mansky, L.M., and H.M. Temin. 1995. Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *J. Virol.* 60:5087–5094.

14. Ho, D.D. 1995. Time to hit HIV, early and hard. *N. Engl. J. Med.* 333: 450–451.