HIV-1 Dynamics in Vivo: Virion Clearance Rate, Infected Cell Life-Span, and Viral Generation Time

Alan S. Perelson, Avidan U. Neumann, Martin Markowitz, John M. Leonard, David D. Ho

A new mathematical model was used to analyze a detailed set of human immunodeficiency virus-type 1 (HIV-1) viral load data collected from five infected individuals after the administration of a potent inhibitor of HIV-1 protease. Productively infected cells were estimated to have, on average, a life-span of 2.2 days (half-life $t_{1/2} = 1.6$ days), and plasma viremia was estimated to have a mean life-span of 0.3 days ($t_{1/2} = 0.24$ days). The estimated average total HIV-1 production was $10.3 \times 10^9$ virions per day, which is substantially greater than previous minimum estimates. The results also suggest that the minimum duration of the HIV-1 life cycle in vivo is 1.2 days on average, and that the average HIV-1 generation time—defined as the time from release of a virion until it infects another cell and causes the release of a new generation of viral particles—is 2.6 days. These findings on viral dynamics provide not only a kinetic picture of HIV-1 pathogenesis, but also theoretical principles to guide the development of treatment strategies.

HIV-1 replication in vivo occurs continuously at high rates (1, 2). Ho et al. (1) found that when a protease inhibitor was administered to infected individuals, plasma concentrations of HIV-1 decreased exponentially, with a mean $t_{1/2}$ of 2.1 ± 0.4 days. Wei et al. (2) and Novak et al. (3) found essentially identical kinetics of viral decay after the use of inhibitors of HIV-1 protease or reverse transcriptase. The viral decay observed in these studies was a composite of two separate effects: the clearance of free virions from plasma and the loss of virus-producing cells. To understand the kinetics of these two viral compartments more precisely, we closely monitored the viral load in five HIV-1-infected patients after the administration of a potent protease inhibitor. Using a mathematical model for viral dynamics and nonlinear least squares fitting of the data, we obtained separate estimates of the viral clearance rate, the infected cell life-span, and the average viral generation time in vivo.

Ritonavir (4, 5) was administered orally (600 mg twice daily) to five infected patients, whose baseline characteristics are shown in Table 1. After treatment, we measured HIV-1 RNA concentrations in plasma at frequent intervals (every 2 hours until the sixth hour, every 6 hours until day 2, and every day until day 7) by means of an ultrasensitive modification (1, 5) of the branched DNA assay (6). Each patient responded with a similar pattern of viral decay: an initial lag followed by an approximately exponential decline in plasma viral RNA (see Fig. 1 for examples).

After ritonavir was administered, a delay in its antiviral effect was expected because of the time required for drug absorption, distribution, and penetration into the target cells. This pharmacokinetic delay could be estimated by the time elapsed before the first drop in the titer of infectious HIV-1 in plasma (Table 1 and Fig. 1B). However, even after the pharmacokinetic delay was accounted for, a lag of ~1.25 days was observed before the plasma viral RNA concentration fell (Fig. 1). This additional delay is consistent with the mechanism of action of protease inhibitors, which render newly produced viral virions infectious but do not inhibit either the production of virions from already infected cells or the infection of new cells by previously produced infectious virions (7). In our previous study (1), this additional delay was missed because measurements were less frequent (every 3 days), and the results were fitted to a single exponential, which was sufficient to provide minimum estimates of HIV-1 kinetics. In contrast, in the present study, we obtained 15 data points during the first 7 days, which allowed a careful analysis of the results by means of a new mathematical model for viral kinetics.

We assumed that HIV-1 infects target cells (7) with a rate constant $k$ and causes them to become productively infected cells (7*). Before drug treatment, the dynamics of cell infection and viral production are represented by

$$\frac{dI}{dt} = kVT - 8T$$

(1)

$$\frac{dT}{dt} = N8T^{*} - cV$$

(2)

where $I$ = number of infected cells, $T$ = number of virions in the plasma, $V$ = number of virions in the infected cells, $k$ = rate constant of infection, $c$ = rate constant of production of new virions, and $N$ = number of virions produced per infected cell. The differential equations are solved with initial conditions $I(0) = 0$, $T(0) = 0$, and $V(0) = 0$. The parameters are estimated by nonlinear least squares fitting of the model to the data, where the model predictions are compared to the observed plasma viral RNA concentrations (Fig. 1). The parameters are estimated by nonlinear least squares fitting of the model to the data, where the model predictions are compared to the observed plasma viral RNA concentrations (Fig. 1).
where \( V \) is the concentration of viral particles in plasma, \( \delta \) is the rate of loss of virus-producing cells, \( N \) is the number of new virions produced per infected cell during its lifetime, and \( c \) is the rate constant for virion clearance (8). The loss of infected cells could be the result of viral cytopathicity, immune elimination, or other processes such as apoptosis. Virion clearance could be the result of binding and entry into cells, immune elimination, or non-specific removal by the reticuloendothelial system.

We assumed that ritonavir does not affect the survival or rate of virion production of infected cells, and that after the pharmacological delay, all newly produced virions are noninfectious. However, infectious virions produced before the drug effect are still present until they are cleared. Therefore, after treatment with ritonavir,

\[
\frac{dT}{dt} = kV_{t}T - \delta T
\]

\[
\frac{dV_{t}}{dt} = -cV_{t}
\]

\[
\frac{dV_{NI}}{dt} = N\delta T - cV_{NI}
\]

where \( V_{t} \) is the plasma concentration of virions in the infectious pool, produced before the drug effect; \( V_{t}(t = 0) = V_{0} \), \( V_{NI} \) is the concentration of virions in the noninfectious pool, produced before the drug effect; \( V_{NI}(t = 0) = 0 \), and \( t = 0 \) is the time of onset of the drug effect. In our analyses, we assumed that viral inhibition by ritonavir is 100%, although the model can be generalized for nonperfect drugs (9).

Assuming that the system is at quasi steady state before drug treatment (10) and that the uninfected cell concentration \( T \) remains at approximately its steady-state value, \( T_{0} \), for 1 week after drug administration (1, 5), we find from Eqs. 3 through 5 that the total concentration of plasma virions, \( V = V_{t} + V_{NI} \), varies as

\[
V(t) = V_{0} \exp(-\delta t) + \frac{cV_{0}}{c - \delta} \left( \exp(-\delta t) - \exp(-\delta t) \right) - \delta \exp(-\delta t)
\]

which differs from the equation derived by Wei et al. (2); see (1)). Allowing \( T \) to increase necessitates the use of numerical methods to predict \( V(t) \) but does not substantially alter the outcomes of the analyses given below (12).

Using nonlinear regression analysis (Fig. 1), we estimated \( c \) and \( \delta \) for each of the patients by fitting Eq. 6 to the plasma HIV-1 RNA measurements (Table 1) (12). The theoretical curves generated from Eq. 6, using the best-fit values of \( c \) and \( \delta \), gave an excellent fit to the data for all patients (see Fig. 1 for examples). Clearance of free virions is the more rapid process, occurring on a time scale of hours. Values of \( \delta \) ranged from 0.26 to 0.68 day\(^{-1} \), with a mean of 0.49 ± 0.13 day\(^{-1} \); the corresponding \( t_{1/2} \) values were 1.02 to 2.67 days, with a mean of 1.55 ± 0.57 days (Table 1). A prediction of the kinetics of virus-producing cells can be obtained by solving Eq. 3 (15).

Several features of the replication cycle of HIV-1 in vivo could be discerned from our analysis. Given that \( c \) and \( \delta \) represent the decay rate constants for plasma virions and productively infected cells, respectively, then \( 1/c \) and \( 1/\delta \) are the corresponding average life-spans of these two compartments. Thus, the average life-span of a virion in the extracellular phase is 0.3 ± 0.1 days, whereas the average life-span of a productively infected cell is 2.2 ± 0.8 days (Table 2). The average viral generation time \( \tau \) is defined as the time from the release of a virion until it infects another cell and causes the release of a new generation of viral particles; hence, \( \tau \) should equal the sum of the average life-span of a free virion and the average life-span of a productively infected cell. This relation, \( \tau \)

\[
\tau = \frac{1}{c} + \frac{1}{\delta}
\]

**Table 1. Summary of HIV-1 clearance rate, infected cell loss rate, and virion production rate for the five patients. Base-line values are average of measurements taken at days -7, -4, -1, and 0; each virion contains two RNA copies. Pharamacologic delay was estimated from the first drop in plasma infectivity for patients 102, 106, and 107. Delay was estimated by best fit of viral load to Eq. 6 for patients 103 and 104. Lower and upper 68% confidence intervals were calculated by a bootstrap method (22) in which each experiment was simulated 100 times. Total virion production was calculated from plasma and extracellular fluid volumes estimated from body weights, assuming that plasma and extracellular fluid are in equilibrium.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Base-line values</th>
<th>Pharmacologic delay (hours)</th>
<th>Virion clearance</th>
<th>Infected cell loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4 cells (per mm(^2))</td>
<td>Plasma virions (10(^6) per ml)</td>
<td>c (day(^{-1}))</td>
<td>Lower</td>
</tr>
<tr>
<td>102</td>
<td>16</td>
<td>294</td>
<td>3.81</td>
<td>1.93</td>
</tr>
<tr>
<td>104</td>
<td>408</td>
<td>12</td>
<td>2.73</td>
<td>2.04</td>
</tr>
<tr>
<td>106</td>
<td>2</td>
<td>52</td>
<td>3.68</td>
<td>2.53</td>
</tr>
<tr>
<td>107</td>
<td>11</td>
<td>643</td>
<td>2.06</td>
<td>1.42</td>
</tr>
<tr>
<td>Mean</td>
<td>170</td>
<td>216</td>
<td>3.09</td>
<td>2.56</td>
</tr>
<tr>
<td>( \pm SD )</td>
<td>196</td>
<td>235</td>
<td>2.0</td>
<td>0.44</td>
</tr>
</tbody>
</table>
rate are minimal estimates (12, 16). Consequently, the true virus t1/2 may be shorter than 6 hours. For example, Nathanson and Harrington (18) found that monkeys clear the Langat virus from their circulation on a time scale of ~30 min. Thus, the total number of virions produced and released into the extracellular fluid is at least 10^3.3 

It is now apparent that the repetitive replication of HIV-1 (left side of Fig 2) accounts for ~99% of the plasma viruses in infected individuals (1, 2, 19) as well as for the high destruction rate of CD4 lymphocytes. The demonstration of the highly dynamic nature of this cyclic process provides several theoretical principles to guide the development of treatment strategies.

1) An effective antiviral agent should detectably lower the viral load in plasma after only a few days of treatment. 

2) On the basis of the previous estimates of the viral dynamics (1, 2) and data on the mutation rate of HIV-1 (3.4 x 10^-5 per base pair per replication cycle) (20) and the genome size (10^4 base pairs), Coffin has cogently argued that, on average, every mutation at every position in the genome would occur numerous times each day (19). The larger turnover rate of HIV-1 described in our study makes this type of consideration even more applicable. Therefore, the failure of the current generation of antiviral agents,
repetition, when used as monotherapy, is the inevitable consequence of the dynamics of HIV-1 replication. Effective treatment must, instead, decrease the virus to mutate simultaneously at multiple positions in one viral genome by means of a combination of multiple, potent, and specific antiretroviral agents. Moreover, because the process of producing mutant viruses is repeated for ~140 generations each year, each and every therapeutic intervention is necessary if a marked clinical impact is to be achieved (21).

3) From our study and previous reports (1, 2, 5), it is now clear that the "raging fire" of active HIV-1 replication (left side of Fig. 2) could be put out by potent antiretroviral agents in 2 to 3 weeks. However, the dynamics of other viral compartments must also be understood. Although they contribute <1% of the plasma virus, each viral compartment (right side of Fig. 2) could serve as the "ember" to reignite a high rate of viral replication when the therapeutic regimen is withdrawn. In particular, we must determine 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 information, someday, will enable the design of a treatment regimen to block de novo HIV-1 replication for a time sufficient to permit each viral compartment to be "burned out."

**REFERENCES AND NOTES**

7. The rate of virus production is expressed as the product Nk to convey either of two possibilities: HIV-1 is produced continuously at an average rate given by the total production of virus-Nk divided by HIV-1, 1/c or (N) N viruses are produced in lytic cells occurring at the cell death rate.
8. The effect of a nonperfect drug can be modeled by simply doubling the term (1 - e^{-k}) to Eq. 4 and multiplying the first term in Eq. 5 by the factor n, where n represents the drug's inhibitory activity (for example, n = nD/N = 0, in which D is the plasma concentration of drug at the time of infection).
9. In quasi steady state, 0 = c + 0 and 0 = c, where NkD = 157 and NkD = 157, whereas the subscripts 0 indicates a quasi-steady state. Combining these equations yields NkD, c. Each virus infects cells at rate NkD, with each infection leading to an exponential increase of Nk77. At steady state, the production of new virus at rate NkD must balance the virus clearance rate at c.
10. Equation 5 differs only in the expression for the effect of drug treatment on viral load. Their analysis was based on Eqs. 1 and 2 and the assumption that no new infections occur after drug treatment (k = 0 after treatment). Equation 5 is a new model appropriate for protease inhibitors, which do not prevent infections arising from preexisting mature infectious viruses. Because of the symmetry between c and h in the Weit et al. equation, the viral clearance rate cannot be distinguished from the infected cell death rate by data fitting.
11. Because our parameter estimates are based on the assumption of complete inhibition of the production of new infectious viruses and no increase in target cells, we expect our parameter estimates to be minimal estimates. Generating a model to relax these assumptions, we can show that 0 is always a minimal estimate (17) and that, with target cell growth, c is typically a minimal estimate. We tested how the estimates of c and 0 depend on the assumption that inhibition is 100% effective as follows: We generated viral load data assuming different drug effects in different drugs, 10, 0.90, 0.80, we estimated c = 3,000, 3,000, 3,015, and 3,023, respectively, and 0.5, 0.50, 0.494, 0.470, and 0.461, respectively. Thus, our estimate of c remains essentially unchanged, whereas that of 0 is a slight underestimate (for example, for 0 = 0.47 rather than the true 0.5). Consequently, if
drug is not completely effective, cell life spans may be somewhat less than we estimate. If the target cells were allowed to increase by the maximum factor observed in the five patients (that is, fivefold), we find that the derived values of \( c \) and \( b \) are minimal estimates. Thus, for example, for data generated with \( \eta = \), with \( c = 3.0 \) and \( b = 0.50 \), we find that our fitting procedure yields estimates of \( c = 2.76 \) and \( b = 0.499 \).


14. Virions that are not released into the extracellular fluid are not included in this estimate. Thus, the total production in the body is even larger.

15. The solution to Eq. 3 is

\[
T_N(t) = \frac{T_0}{c - b} \left[ \exp(-bt) - \exp(-ct) \right]
\]

(7)

If cellular RNA data were obtained, this equation could be fitted to these data, and the parameter estimates for \( c \) and \( b \) could be verified for consistency with the viral kinetics.

16. In principle, more accurate estimates of the duration of the intracellular or eclipse phase of the viral life cycle can be obtained with a model that explicitly includes a delay from time of infection until the time of viral release. For example, Eq. 2 can be replaced by

\[
v(t) = N_0 \int_0^t T_N(t') dt' - cv
\]

where \( w(t) \) is the probability that a cell infected at time \( t = 0 \) produces virus at time \( t \). Explicit solutions to our model, with \( w(t) \) given by a gamma distribution, will be presented elsewhere (A. S. Perelson et al., in preparation). Alternatively, if virtually all infected cells \( T_N \) are destroyed, Eq. 1 can be replaced by

\[
\frac{dT_N}{dt} = kI \int_0^t T_N(t') w(t-t') dt' - bT_N
\]

Models of this type can also be solved explicitly when \( w(t) \) is given by a gamma function. M. Nowak and A. Heit (personal communication) have solved this model for the case where \( w(t) \) is a delta function, in which case the delay simply adds to the pharmacokinetic delay and Eq. 6 is regained after this combined delay. Analysis of current data by non-linear least squares estimation has so far not allowed accurate simultaneous estimation of \( c, b, \) and the intracellular delay. However, the qualitative effect of including the delay in the model is to increase the estimate of \( c \) which is close to \( c \) for comparable choices of \( c \) and \( b \) in Table 1 are minimal estimates. Higher values of \( c \) (hence lower values of \( 1/c \)) will lead to increased estimates of the intracellular delay, \( \lambda \) (1/c). Thus, our estimate of the duration of the intracellular phase, as derived above and given in Table 2, is still a minimal estimate.


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Opposite Modulation of Cocaine-Seeking Behavior by D1-like and D2-like Dopamine Receptor Agonists

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Activation of the mesolimbic dopamine system is known to trigger relapse in animal models of cocaine-seeking behavior. We found that this “priming” effect was selectively induced by D2-like, and not by D1-like, dopamine receptor agonists in rats. Moreover, D1-like receptor agonists prevented cocaine-seeking behavior induced by cocaine itself, whereas D2-like receptor agonists enhanced this behavior. These results demonstrate an important dissociation between D1- and D2-like receptor processes in cocaine-seeking behavior and support further evaluation of D1-like receptor agonists as a possible pharmacotherapy for cocaine addiction.

Relapse of cocaine use in cocaine-dependent people is often precipitated by episodes of intense drug craving even after prolonged abstinence. Cocaine craving has been described subjectively as resembling the positive or “high”-like qualities of the drug itself (1). In this sense, cocaine craving may differ from cravings for opiates or ethanol, which are sometimes described as a desire to alleviate the negative, withdrawal-associated symptoms of drug dependence (1). Both cocaine craving in humans and relapse in animal models of cocaine-seeking behavior are triggered by environmental stimuli associated with the drug experience (2, 3) and by low doses of cocaine itself (3, 4).

The priming effects of such cues in animal models of cocaine-seeking behavior can be mimicked by activation of the mesolimbic dopamine system (5), which is a major neural substrate of cocaine reinforcement (6). Dopamine acts at two general classes of dopamine receptors, termed D1-like and D2-like, that are distinguishable by their structural homology (7), opposite modulation of adenylate cyclase activity (8), and differential localization within the brain (9).

We tested the ability of full D1- or D2-like dopamine receptor agonists to induce relapse in an animal model of cocaine-seeking behavior. Male Sprague-Dawley rats were trained to press a lever to self-administer intravenous cocaine (10, 11). A daily 4-hour reinstatement procedure was followed in which rats self-administered cocaine for 2 hours, after which saline was substituted for the cocaine during the final 2 hours. During the time that saline was substituted, the rats’ “nonreinforced” lever pressing responses progressively diminished, a behavioral phenomenon known as extinction. After responding had diminished (11), the rats were given intraperitoneal priming injections of either the D2-like-selective receptor agonist 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) (12) or quinpirole (13), or the D1-like-selective receptor agonist SKF 82958 (14). Although these dopamine agonists can selectively differentiate the D1- from the D2-like class of receptors, they do not adequately distinguish the various subtypes within each class (15).

The priming ability of these dopamine receptor agonists was assessed by the ability to reinstate nonreinforced lever-pressing for saline infusions at the lever to which previously delivered cocaine infusions (drug-paired lever) during the cocaine phase of the test session (Fig. 1).

The D2-like agonist 7-OH-DPAT induced large dose-related increases in nonreinforced responding at the drug-paired lever as compared with very low levels of responding induced both by the drug vehicle and at an inactive lever (Figs. 1 and 2). Quinpirole also induced selective responding at the drug-paired lever and with high potency but with less efficacy and dose dependency than responding induced by 7-OH-DPAT. These differences cannot be explained by the relative selectivity of the two agonists at D2 or D1 receptor subtypes (12) and therefore probably reflect different pharmacokinetic properties of the drugs. The possibility of a general rate-increasing effect of the D2-like agonist is eliminated by the lack of significant responding at the inactive lever and by previous studies in which D2-like agonists produced decreases rather than increases in responding when animals were treated during cocaine self-administration tests (16). Thus, we conclude that the D2-like agonists initiate neural processes that trigger relapse in an animal model of cocaine-seeking behavior.

In contrast to the D2-like agonists,