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

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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 virions were 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 Nowak 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 following 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 $\sim 1.25$ 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 virions noninfectious 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 of viral kinetics.

We assumed that HIV-1 infects target cells (T) with a rate constant $k$ and that it becomes productively infected cells (T*). Before drug treatment, the dynamics of cell infection and virion production are represented by

\[ \frac{dT^*}{dt} = kVT - 8T^* \]  

\[ \frac{dv}{dt} = N \bar{T}^* - v \]
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 nonspecific 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,

\[
\frac{dT^*}{dt} = kV\delta T^* - \delta T^* \quad (3)
\]

\[
\frac{dV_i}{dt} = -cV_i \quad (4)
\]

\[
\frac{dV_{NI}}{dt} = N\delta T^* - cV_{NI} \quad (5)
\]

where \( V_i \) is the plasma concentration of virions in the infectious pool [produced before the drug effect; \( V_i(t = 0) = V_i \)], \( V_{NI} \) is the concentration of virions in the noninfected pool [produced after 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, \( T_{ss} \), for 1 week after drug administration \((1, 5)\), we find from Eqs. 3 through 5 that the total concentration of plasma virions, \( V = V_i + V_{NI} \), varies as

\[
V(t) = V_0 \exp(-c(t)) + \frac{cV_0}{c - \delta} \left[ \exp(-c(\delta t) - \exp(-(ct)) - \delta t \exp(-c(\delta t)) \right] \quad (6)
\]

which differs from the equation derived by Wei et al. \((2)\); see \((11)\). 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 \((1)\) (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 \( c \) ranged from 2.06 to 3.81 day\(^{-1}\), with a mean of 3.07 ± 0.64 day\(^{-1}\) \((Table 1\) (12)). The corresponding \( t_{1/2} \) values for free virions \((t_{1/2} = \ln 2/c) \) ranged from 0.18 to 0.34 days, with a mean of 0.24 ± 0.06 days (~6 hours). Confirmation of the virion clearance rate was obtained from an independent experiment that measured by quantitative cultures \((13)\) the rate of loss of viral infectivity in plasma for patient 105 (Fig. 1B). The loss of infectious virions occurred by first-order decay, with a rate constant of 3.0 day\(^{-1}\), which is within the 68% confidence interval of the estimated \( c \) value for that patient \((Table 1)\.

At steady state, the production rate of virus must equal its clearance rate, \( cV \). Using the estimate of \( c \) and the pretreatment viral concentration \( V_{ss} \), we obtained an estimate for the rate of virion production before ritonavir administration. Each patient’s plasma and extracellular fluid volumes were estimated on the basis of body weight. Total daily virion production and clearance rates ranged from 0.4 × 10\(^6\) to 32.1 × 10\(^6\) virions per day, with a mean of 10.3 × 10\(^6\) virions per day released into the extracellular fluid \((Table 1)\) (14). The rate of loss of virus-producing cells, as estimated from the fit of Eq. 6 to the HIV-1 RNA data, was slower than that of free virions. 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)\.

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, \( \tau \)

<table>
<thead>
<tr>
<th>Patient</th>
<th>CD4 cells (per mm(^3))</th>
<th>Plasma virions (10^9) per ml</th>
<th>Pharm. delay (hours)</th>
<th>CD4 cells (per mm(^3))</th>
<th>Plasma virions (10^9) per ml</th>
<th>Infected cell loss (day(^{-1}))</th>
<th>Total virion production (10(^6) per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>16</td>
<td>294</td>
<td>2</td>
<td>3.81</td>
<td>1.93</td>
<td>7.03</td>
<td>0.18</td>
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<tr>
<td>103</td>
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<td>12</td>
<td>6</td>
<td>2.73</td>
<td>2.04</td>
<td>3.70</td>
<td>0.25</td>
</tr>
<tr>
<td>104</td>
<td>2</td>
<td>52</td>
<td>2</td>
<td>3.88</td>
<td>2.53</td>
<td>6.19</td>
<td>0.19</td>
</tr>
<tr>
<td>105</td>
<td>11</td>
<td>643</td>
<td>6</td>
<td>2.06</td>
<td>1.42</td>
<td>3.76</td>
<td>0.34</td>
</tr>
<tr>
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<td>412</td>
<td>77</td>
<td>2</td>
<td>3.09</td>
<td>2.56</td>
<td>4.55</td>
<td>0.22</td>
</tr>
<tr>
<td>Mean</td>
<td>170</td>
<td>216</td>
<td>3.6</td>
<td>3.07</td>
<td>2.10</td>
<td>5.05</td>
<td>0.24</td>
</tr>
</tbody>
</table>
= 1/c + 1/8, can be shown formally (Table 2). The average value of \( \tau \) for the patients was 2.6 \pm 0.8 days (Table 2).

By a heuristic procedure, we found minimal estimates for the average duration of the HIV-1 life cycle and of its intracellular or eclipse phase (from virion binding to the release of the first progeny). The duration of the HIV-1 life cycle, \( S \), is defined as the time from the release of a virion until the release of its first progeny virus; we estimated \( S \) by the lag in the decay of HIV-1 RNA in plasma (Fig. 1) after the pharmacologic delay (Table 1) is subtracted. The shoulder in the RNA decay curve is explained by the fact that virions produced before the pharmacologic effect of ritonavir are still infectious and capable of producing, for a single cycle, viral particles that would be detected by the RNA assay. Thus, the drop in RNA concentration should begin when target cells interact with drug-affected virions and do not produce new virions. These “missing virions” would first have been produced at a time equal to the minimum time for infection plus the minimum time for production of new progeny. The estimated values for \( S \) were quite consistent for the five patients, with a mean duration of 1.2 \pm 0.1 days (Table 2). In steady state, \( 1/c = 1/\left( NKT_0 \right) \) is the average time for infection (Table 2, legend); if this average time is assumed to be greater than the minimal time for infection, then a minimal estimate of the average duration of the intracellular phase of the HIV-1 life cycle is given by \( S - (1/c) = 0.9 \) days (16).

Previous studies that used potent antiretroviral agents to perturb the quasi steady state in vivo provided a crude estimate of the \( T_{1/2} \) of viral decay in which the life-span of productively infected cells could not be separated from that of plasma virions (1, 2). Our results show that the average life-span of a productively infected cell (presumably an activated CD4 lymphocyte) is 2.2 days; thus, such cells are lost with an average \( T_{1/2} \) of \( \sim 1.6 \) days (Fig. 2). The life-spans of productively infected cells were not markedly different from the five patients (Table 2), even though individuals with low CD4 lymphocyte counts generally have decreased numbers of virus-specific, major histocompatibility complex class I-restricted, cytotoxic T lymphocytes (17).

The average life-span of a virion in blood was calculated to be 0.3 days. Therefore, a population of plasma virions is cleared with a \( T_{1/2} \) of 0.24 days; that is, on average, half of the population of plasma virions turns over approximately every 6 hours (Fig. 2). Because our analysis assumed that the antiviral effect of ritonavir was complete and that target cells did not recover during treatment, our estimates of the virion clearance rate and infected cell loss rate are minimal estimates (12, 16). Consequently, the true virion \( T_{1/2} \) 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 \( \sim 30 \) min. Thus, the total number of virions produced and released into the extracellular fluid is at least \( 10^3 \times 10^9 \) particles per day (14); this rate is about 15 times our previous minimum estimate (1). At least 99% of this large pool of virus is produced by recently infected cells (1, 2) (Fig. 2). At quasi steady state, the virion clearance rate \( \lambda V \) equals the virion production rate \( NB_T \). Because \( c \) has similar values for all patients studied (Table 1), the degree of plasma viremia is a reflection of the total virion production, which in turn is proportional to the number of productively infected cells \( T \) and their viral burst size \( N \). The average generation time of HIV-1 was determined to be 2.6 days, which suggests that \( \sim 140 \) viral replication cycles occur each year, about half the number estimated by Coffin (19).

It is now apparent that the repetitive replication of HIV-1 (left side of Fig. 2) accounts for \( \approx 99% \) 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 previous estimates of the viral dynamics (1, 2) and data on the mutation rate of HIV-1 (3.4 \times 10^{-3} per base pair per replication cycle) (20) and the genome size (10^6 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,
when used as monotherapy, is the inevitable consequence of the dynamics of HIV-1 replication. Effective treatment must, instead, force the virus to mutate simultaneously at multiple positions in one viral genome by means of a combination of multiple, potent antiretroviral agents. Moreover, because the process of producing mutant viruses is repeated for ∼140 generations each year, early and aggressive 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 latentely 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 "burn out."

**REFERENCES AND NOTES**

2. X. Wei et al., ibid., p. 117.
9. The effect of a nonperfect drug can be modeled by simply adding the term (1 − νdWt) to Eq. 4 and multiplying the first term in Eq. 5 by the factor that represents the drug activity (for example, D = Dc + Dc(1 − νdWt)).
10. In quiescent state, dT = 0 and dV = 0. Thus, υdW = 0 and ∂E = 0, where the subscript 0 indicates a quiescent state. Combining these equations yields dN = c = 0.
11. The difference between T and N represents the drug clearance at rate C.

![Diagram](https://example.com/diagram.png)

**Fig. 2.** Schematic summary of the dynamics of HIV-1 infection in vivo. Shown in the center is the cell-free virus population that is sampled when the viral load in plasma is measured.
Opposite Modulation of Cocaine-Seeking Behavior by D₁- 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 D₂-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-press 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 discriminate the D₁- from the D₂-like class of receptors, they do not adequately distinguish the various subtypes within each class in vivo. The priming ability of these dopamine receptor agonists was assessed by their ability to reinstate nonreinforced lever-pressing for saline infusions at the lever that 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 2A). Quinpirole also induced selective responding at the drug-paired lever and with higher potency but with less efficacy and dose-dependency than responding induced by 7-OH-DPAT. These differences cannot be explained by the relative selectivity or affinity of the two agonists at D₁ or D₂ receptor subtypes (12) and therefore probably reflect different pharmacokinetic properties of the drugs. The possibility of a generalized rate-increasing effect of the D₂-like agonists 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 (15, 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, the