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Viral persistence, latent reservoir, and blips: a review on HIV-1 dynamics and modeling during HAART and related treatment implications

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Abstract

HIV-1 eradication from infected individuals has not been achieved with the use of highly active antiretroviral therapy (HAART) for a prolonged period of time. The cellular reservoir for HIV-1 in resting memory $CD4^+$ T cells remains a major obstacle to viral elimination. The reservoir does not decay significantly over long periods of time as is able to release replication competent HIV-1 upon cell activation. Residual ongoing viral replication may likely occur in many patients because low levels of virus can be detected in plasma by sensitive assays and transient episodes of viremia, or HIV-1 blips, are often observed in patients even with successful viral suppression for many years. Here we review our current knowledge of the factors contributing to viral persistence, the latent reservoir, and blips, and mathematical models developed to explore them and their relationships. We show how mathematical modeling can help improve our understanding of HIV-1 dynamics in patients on HAART and the quantitative events underlying HIV-1 latency, reservoir stability, low-level viremic persistence, and emergence of intermittent viral blips. We also discuss treatment implications related to these studies.

Key words. HIV-1, HAART, low-level viremia, latency, viral reservoirs, blips, mathematical models

1 Introduction

Eradication of HIV-1 from infected individuals is the ultimate goal of antiretroviral therapeutic interventions. However, this possibility seems unlikely at present despite a great deal of progress that has been made in developing potent antiretroviral drugs and understanding the molecular biology of HIV-1 replication (see reviews in [8, 77, 155, 176, 184]). Although highly active antiretroviral therapy (HAART) based on administration of at least three different drugs from two or more classes has proved extremely effective in reducing the viral load in HIV-infected patients to below 50 RNA copies/mL [32, 183], the detection limit of current standard assays, a low level of viremia can be detected in plasma by more sensitive assays even after years of treatment [43, 139, 140]. Moreover, a number of patients experience transient episodes of detectable viremia, or blips, even when the viral load had been suppressed to below the limit of detection for many years [56, 70, 126, 177]. These phenomena indicate that residual active viral replication is very likely to continue in many patients on HAART.

HIV-1 can establish a state of latent infection in resting memory $CD4^+$ T cells [18, 24]. These latently infected cells are capable of escaping from viral cytopathic effects and host immune mechanisms due to very low levels of HIV-1 messenger RNA (mRNA) and proteins they express [73, 105]. Because of the nature of memory $CD4^+$ T cells [180], they remain in the resting state in the presence of potent

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combination therapy for a very long period of time [28, 47, 196]. However, they can produce new virus when stimulated by relevant antigen [21]. Thus, a viral rebound seems inevitable when therapy is withdrawn.

Determining the decay rate of the latent reservoir remains an important issue since it is directly related to the possibility and the time needed for the antiretroviral regimens currently in use to cure the infection. Estimates of the half-life of the latent reservoir are quite divergent, ranging from about 6 months [25, 157, 201] to 44 months [46, 172]. Therefore, combination treatment over as long as 73 years might be required for eradication of the latent reservoir [46]. Considering drug toxicities and medical tolerance, emergence of drug resistance, and treatment cost, such a lifetime therapy may not be reasonable for HIV-infected patients. Devising efficient strategies to accelerate the decay of the latent reservoir is a prerequisite for viral eradication.

In this paper we review our current understanding of the factors that contribute to the viral and latent reservoir persistence and viral blips. We start with a review of a few basic models used to study viral infection and estimate parameters that govern viral production and clearance. We then discuss recent developments of models proposed to study virus dynamics in patients on potent combination treatment and explore possible mechanisms underlying low-level viral persistence, stability of the latent reservoir, and occurrence of intermittent viral blips. These models offer a quantitative investigation of the influence of ongoing viral replication on the viral load dynamics and the latent reservoir decay characteristics observed in HAART-treated patients. Finally, we discuss related treatment implications for clinical practice. Due to limited space, we did not include models with immune responses, and the reader is referred to [138, 145, 195] and references cited therein.

2 Multiphasic viral decay

Quantitative analysis of HIV-1 replication *in vivo* has made significant contributions to our understanding of AIDS pathogenesis and antiretroviral treatment (reviewed in [48, 145]). After a few months of HIV-1 infection, the plasma virus usually attains a set-point, i.e., a relatively constant viral level ranging from 10^2 to 10^7 copies/mL [154] in different patients, that persists for years [76]. At the set-point viral production and clearance in infected individuals must be in equilibrium. Even though quantitative methods like RT-PCR can quantitate viral load in HIV-1 infection, they cannot determine whether virus is produced quickly or slowly. Work by Ho et al. [78] and Wei et al. [193] revealed that drug-induced perturbations of the steady-state viral loads would provide information on HIV-1 production and clearance and on $CD4^+$ T cell turnover. Combined with mathematical models developed by Perelson et al. [146, 148] and others [10, 11, 36, 109, 127, 133, 137, 153, 159, 168, 169, 181], significant progress has been made in understanding important features of HIV-1 dynamics and their clinical implications for antiretroviral treatment.

Analysis of the viral decay following initiation of potent combination drug therapy has suggested that the plasma viral load declines in multiple distinct phases (Fig. 1, see reviews in [48, 95, 145, 176]).

2.1 The first phase

The first studies that provide kinetic information on virus and $CD4^+$ lymphocyte turnover *in vivo* were carried out by perturbing the viral set-point in patients during the asymptomatic phase of HIV infection with antiretroviral drugs, such as the protease inhibitors ritonavir [78] or saquinavir [193], as well as the reverse transcriptase (RT) inhibitor nevirapine [193]. If viral replication is completely blocked by inhibitors, then the viral decay reveals the clearance rate of free virus. If it is not completely blocked,

then the rate of viral decay will also depend on the death rate of productively infected cells and the effectiveness of the drug [145]. Administration of these different agents resulted in a rapid decline (~ 2 log) in plasma virus levels in the first two weeks [78, 193], showing that the decline was not dependent on a particular drug but rather was the effect of blocking HIV replication in any manner. Further, this observation suggested that to maintain a set-point viral load there must be rapid production of the plasma virus and infection of $CD4^+$ T cells. Thus, the replication of HIV-1 *in vivo* is a dynamic process involving continuous rounds of *de novo* virus infection and reproduction.

Perelson et al. [148] and Wei et al. [193] developed mathematical models to further analyze the HIV-1 viral load data collected from patients after the administration of a protease inhibitor. They studied two separate processes that contribute to the viral decay observed after drug administration: the clearance of free virus from plasma and the loss of productively infected cells. The model for viral dynamics is described by the following equations [148]:

$$\begin{aligned} \frac{d}{dt}T(t) &= \lambda - dT - kVT, \\ \frac{d}{dt}T^*(t) &= kVT - \delta T^*, \\ \frac{d}{dt}V(t) &= N\delta T^* - cV, \end{aligned} \tag{1}$$

where $T(t), T^*(t)$ and $V(t)$ denote the concentration of uninfected $CD4^+$ T cells, productively infected cells, and free virus at time t, respectively. λ represents the recruitment rate of uninfected T cells, d is the per capita death rate of uninfected cells, k is the rate constant at which uninfected cells are infected by free virus. Here the infection is modeled by a simple but commonly used method, i.e., a 'mass action' term kVT. δ is the per capita death rate of infected cells, N (burst size) is the total number of virus particles produced by a productively infected cell during its lifetime and c is the clearance rate of virus. Therefore, $N\delta$, which is N divided by the cell life-span, $1/\delta$, gives the per capita viral production rate.

RT inhibitors can effectively block RT's enzymatic function and prevent completion of synthesis of the viral DNA from HIV RNA. Thus, the infection rate k is reduced by a quantity, $(1 - \epsilon_{RT})$, where ϵ_{RT} is the efficacy of RT inhibitors and $0 \leq \epsilon_{RT} \leq 1$. Protease inhibitors prevent HIV protease from cleaving the HIV polyprotein into functional units, causing infected cells to produce immature virus particles that are noninfectious. Thus, only a part, $(1 - \epsilon_{PI})$, of newly produced virus is infectious, where ϵ_{PI} is the protease inhibitor efficacy and similarly, $0 \leq \epsilon_{PI} \leq 1$.

Considering the effect of RT and protease inhibitors, model (1) can be modified to:

$$\begin{aligned} \frac{d}{dt}T(t) &= \lambda - dT - (1 - \epsilon_{RT})kV_IT, \\ \frac{d}{dt}T^*(t) &= (1 - \epsilon_{RT})kV_IT - \delta T^*, \\ \frac{d}{dt}V_I(t) &= (1 - \epsilon_{PI})N\delta T^* - cV_I, \\ \frac{d}{dt}V_{NI}(t) &= \epsilon_{PI}N\delta T^* - cV_{NI}, \end{aligned} \tag{2}$$

where V_I and V_{NI} are the concentration of infectious and non-infectious virus, respectively. $V = V_I + V_{NI}$ is the total amount of virus.

If one assumes that only a 100% effective protease inhibitor ($\epsilon_{PI} = 1$, $\epsilon_{RT} = 0$) is administered to an infected individual at quasi steady state with initial viral load, V_0, and that the uninfected target cells remain approximately at a constant level, T_0, over the time period of interest, then the viral load

at time t can be solved from Eq. (2) [148]:

$$V(t) = V_0 e^{-ct} + \frac{cV_0}{c-\delta}\left[\frac{c}{c-\delta}(e^{-\delta t} - e^{-ct}) - \delta t e^{-ct}\right]. \tag{3}$$

Using nonlinear regression analysis, the parameters, c and δ, could be estimated by fitting Eq. (3) to the plasma HIV-1 RNA data [148]. The estimates of c, with a mean of 3 day^{-1}, were similar for different patients, suggesting that the plasma virus has an intrinsic constant decay rate. The corresponding half-life ($t_{1/2} = \ln 2/c$) of plasma virus is very short, with a mean of ~ 6 hours. Estimates of δ have a mean of 0.5 day^{-1}, with a mean half-life of 1.6 days. These estimates give upper bounds for the half-lives of the virus and productively infected cells (i.e., the estimated values of c and δ are minimal estimates) because therapy in reality is not 100% effective and additional viral clearance and/or loss of virus-producing cells is required to account for residual viral replication.

These results had enormous implications for HIV-1 pathogenesis and antiretroviral treatment. Using the estimate of c and the pretreatment viral concentration, V_0, it was estimated that more than 10^{10} virions were cleared daily. Therefore, at steady state the same number of virions, 10^{10}, were produced and released into the extracellular fluid in the average mid-stage HIV-1-infected untreated patient per day [148]. Production of a large amount of virus [16, 65, 80] and the short half-life of productively infected cells suggest that a large number of CD4$^+$ T cells are actively infected daily. Due to the highly error-prone reverse transcription from HIV-1 RNA genome to DNA, not only does every possible single mutation arise [30], but $\sim 1\%$ of all possible double mutations can be generated daily [147]. High replication and high mutability of HIV-1 have greatly advanced our understanding of the evolution of drug resistance mutations and viral escape from immune responses. When drugs are administered individually, virological failure (treatment fails to achieve successful viral suppression to below the limit of detection) seems inevitable because of the rapid emergence of drug resistant virus variants in patients. Recent reviews on HIV drug resistance can be found in [29, 161]. Mathematical models have been developed to study HIV resistance during treatment, see [12, 92, 96, 122, 130, 160, 162, 163, 178, 179, 192, 197] and references cited therein.

2.2 The second phase

Clinical studies showed that simultaneous administration with multiple antiretroviral drugs would overcome the rapid emergence of drug resistance when a single agent was used and lead to a significantly prolonged benefit [61, 68]. Perelson et al. [146] analyzed changes in viral loads of eight HIV-1-infected patients after initiation of treatment with combined antiretroviral agents and found that each patient responded with a similar pattern of viral decline: an initial rapid exponential decline followed by a slower second-phase viral decay with a half-life of $1 - 4$ weeks. The plasma HIV-1 RNA levels in all patients dropped below 1000 copies/mL by 8 weeks of treatment, and below 50 copies/mL at week $16 - 20$, demonstrating the potency of combination drug therapy and the lack of emergence of drug resistance during the study period.

The nature of the cellular or anatomical compartments that are responsible for the second phase viral decay remains obscure and several cell populations or sanctuary sites might contribute. One possibility is the existence of a long-lived population of productively infected cells [146], such as infected macrophages, which are less susceptible to viral cytopathic effects than are CD4$^+$ T cells [79]. Other sources include, but are not restricted to, infected CD4$^+$ T cells in a latent state [202], which can be activated to produce virus when encountering specific antigen, and release of the virus trapped in tissue reservoirs, for example, on the surface of follicular dendritic cells (FDCs) [74, 75]. The contribution of these sources

to the plasma virus in untreated patients is minor but becomes influential when combination therapy is administered and the infection of cells that produce most of the virions in plasma is largely blocked.

A new mathematical model was proposed to analyze the observed two-phase viral load decay [146]. The model incorporated into the basic model (1) additional sources that could contribute to the plasma virus, such as long-lived productively infected cells and activation of latently infected cells, to explain the second-phase viral decline. Let M^* be the population of long-lived infected cells and L latently infected cells, the model before therapy is described by the following equations:

$$\begin{aligned}
\frac{d}{dt}T^*(t) &= kVT + aL - \delta T^*, \\
\frac{d}{dt}L(t) &= fkVT - \mu_L L, \\
\frac{d}{dt}M^*(t) &= k_M VM - \mu_M M^*, \\
\frac{d}{dt}V(t) &= N\delta T^* + pM^* - cV,
\end{aligned} \tag{4}$$

where cells, M, which upon infection with a rate, k_M, become long-lived infected cells, which produce virus at a rate p and are lost with a rate μ_M. Latently infected cells, L, which are generated with a rate fk, smaller than k by a factor $f < 1$, die with a rate δ_L, and are activated into productively infected cells with a rate a, giving a total rate constant of loss $\mu_L = a + \delta_L$. When used to fit short-term data, the level of susceptible $CD4^+$ T cells was assumed to be constant over the study period [146].

In the presence of RT and protease inhibitors, Eq. (4) can be modified as in Eq. (2). Assuming that both RT and protease inhibitors are 100% effective, and that the level of target cells remains constant at value T_0, the viral level after drug therapy can be solved [146]:

$$V(t) = V_0\Big[Ae^{-\delta t} + Be^{-\mu_L t} + Ce^{-\mu_M t} + (1 - A - B - C)e^{-ct}\Big], \tag{5}$$

where

$$A = \frac{NkT_0}{c-\delta}(1 - \frac{af}{\delta - \mu_L}), B = \frac{af\delta NkT_0}{\mu_L(\delta - \mu_L)(c - \mu_L)}, C = \frac{c - NkT_0(1 + \frac{af}{\mu_L})}{c - \mu_M},$$

and the level of infected cells in blood is given by

$$I(t) = T^*(t) + L(t) = \frac{kV_0T_0}{\delta}\Big[(1 - \frac{af}{\delta - \mu_L})e^{-\delta t} + \frac{f\delta}{\mu_L}(1 + \frac{a}{\delta - \mu_L})e^{-\mu_L t}\Big]. \tag{6}$$

Simultaneously fitting $V(t)$ and $I(t)$ to the plasma virus and peripheral blood mononuclear cells (PBMC) infectivity data showed that the loss of long-lived infected cells ($t_{1/2}$ of $1 - 4$ weeks) was a major contributor to the second phase of viral decay, whereas the activation of latently infected cells was only a minor source [146].

The above-mentioned models were extremely useful and popular, but not unique, in explaining the multiple viral decay observed in patients after initiation of potent combination treatment. Grossman et al. [57, 58] and Bucy [13] developed different models to study viral dynamics, although there are technical problems with the analysis of the Grossman model [133]. The underlying mechanisms for these models are reviewed in [155].

2.3 The third phase: eradication?

After several months of HAART, most patients, particularly those who have not taken any antiretroviral medicines previously, achieve viral loads that are below the detection limit of current standard assays

[61, 68, 146]. These observations raised the hope that prolonged potent antiretroviral treatment in those patients who have suppressed their viral loads to very low levels would eradicate HIV-1 from both short and long-lived compartments. Perelson and his colleagues [146] were the first to make such predictions based on the second-phase viral decay observed in the eight patients who took combination antiretroviral drugs. They predicted that, even if the initial number of chronically infected cells was as large as 10^{12} cells, which is larger than the estimated number of $CD4^+$ T cells ($\sim 2 \times 10^{11}$ cells [203]), the virus could be completely eliminated from these two compartments after $\sim$3 years of treatment. However, this prediction was made with the assumption that antiretroviral regimen is 100% effective and more importantly, no additional viral compartments or sanctuary sites exist. Now it is clear that other long-lived viral reservoirs exist (see below), rendering eradication of HIV-1 unattainable at present. An excellent review of the cell types and anatomical sites that may serve as potential reservoirs for HIV-1 is given in [8].

3 Low-level viremic persistence

Although potent combination therapy can suppress the viral loads in many patients to below the detection limit of present assays, 50 RNA copies/mL, this does not imply that virus production has been completely stopped by the therapy. On the contrary, in many patients with suppressed plasma viral levels for a prolonged time, a low level of viremia can still be detected by more sensitive assays that quantify HIV-1 RNA down to one or a few copies/mL [43, 139, 140]. This phase with plasma viral levels below 50 copies/mL is usually referred to as the third phase [48]. The dynamics and sources of residual viremia in the third phase are of great interest because they are related to the issue of whether viral eradication can be achieved.

The factors contributing to this low-level viremic persistence have not been well characterized. It is possible that antiretroviral therapy is not completely suppressive and virus replication is still occurring [8, 167], albeit at low levels. Another possibility is that even if therapy is fully suppressive, HIV-1 establishes a state of latent infection in resting memory $CD4^+$ T cells (see Section 4) [18, 24, 28, 47, 196], and a small number of virus particles are continuously released from the reservoir by activation of these latently infected cells [21]. A more reasonable scenario is that both contribute to viral persistence — ongoing viral replication replenishes the latent reservoir while the latent reservoir releases virus that fuels ongoing active viral replication. Further understanding of these factors and their relative contributions is critical for the goal of eventual viral elimination.

3.1 Ongoing viral replication

A number of studies have suggested that residual viral replication continues in patients under potent combination therapy even when the viral loads have been suppressed to below the limit of detection for a long time [17, 26, 27, 52, 101, 143]. This low-level ongoing virus replication involves generation of new infected cells through *de novo* infection from the virus released from other infected cells. The evidence for ongoing replication during HAART comes from the isolation of replication-competent virus [28, 47, 196] from both PBMC and seminal cells [43, 200], the detection of unintegrated proviral DNA, both linear [28] and circularized [170, 171], and cell-associated HIV RNA [53, 132, 143, 201, 204]. However, care must be taken in comparing studies, as newer drugs may be more potent than older ones, patient compliance with therapy may vary, and the existence of drug resistance mutations may be different for different drug regimens and patient's prior drug exposure.

Another line of evidence for residual viral replication is that treatment intensification can lead

to better viral suppression and acceleration of the HIV-1 decay rate [71, 158]. In one study, the decay rate of the latent reservoir increased and the frequency of intermittent viremia decreased in 5 patients who underwent treatment intensification compared with 5 patients with comparable baseline characteristics who remained on standard combination therapy [158]. The result suggested that ongoing virus replication during standard antiretroviral therapy is at least partially due to the inadequate antiviral potency of some regimens. In another cohort of 14 patients whose HIV RNA levels had been sustained at < 50 copies/mL for more than 5 years, Havlir et al. [71] added the RT inhibitor abacavir to the regimen and found HIV plasma RNA levels declined rapidly and reached a new lower steady state that persisted over 5 years, suggesting that productive infection contributes to residual ongoing viremia and can be further inhibited with treatment intensification.

Other evidence for ongoing replication includes the occurrence of occasional viral measurements above the limit of detection of conventional assays [70, 158, 187], and changes in HIV-1 proviral sequences in PBMCs [201]. Ongoing replication is also found in other non-plasma compartments during HAART (reviewed in [97]), such as the central nervous system (CNS) [104].

Ongoing virus replication during therapy should inevitably lead to selection of drug resistant mutants. However, studies on viral evolution by analysis of clonal sequences have demonstrated mixed results. Some studies have detected drug resistance mutations in individuals on highly suppressed therapy [52, 62, 118, 120, 142], whereas other studies have not found resistance mutations [6, 63, 93, 141]. The discrepancies between these studies may be due to sampling differences [110] but some other factors, such as local drug concentration [92] and the level of target T cells, may also affect the development of drug resistance in patients on HAART [97].

Mathematical models have been developed to describe sustained, low-level production of virus under potent antiretroviral drugs. However, many models, as surveyed by Callaway and Perelson [14], cannot robustly produce a steady state viral load below the limits of detectability. Before we address the shortcoming of the basic model (2) and its variations in describing low-level viremia, we note one can reduce model (2) to a simpler form using the fact that the ratio of infectious to noninfectious virus is nearly a constant after a short time following initiation of treatment [164]:

$$\begin{aligned}
\frac{d}{dt}T(t) &= \lambda - dT - (1-\epsilon)kVT, \\
\frac{d}{dt}T^*(t) &= (1-\epsilon)kVT - \delta T^*, \\
\frac{d}{dt}V(t) &= N\delta T^* - cV,
\end{aligned} \tag{7}$$

where $V = V_I + V_{NI}$ is the total viral load and ϵ is the overall drug efficacy, which is defined as $\epsilon = 1 - (1-\epsilon_{RT})(1-\epsilon_{PI})$.

There is only one possible positive steady state of the viral load:

$$\bar{V} = \frac{\lambda N}{c} - \frac{d}{k(1-\epsilon)}. \tag{8}$$

It is clear that $\bar{V} > 0$ if and only if the overall drug efficacy ϵ is less than a 'critical drug efficacy', ϵ_c, above which virus is predicted to be eradicated. ϵ_c is given by

$$\epsilon_c = 1 - \frac{dc}{kN\lambda}. \tag{9}$$

The derivative of $\bar{V}$ with respect to ϵ is $-\frac{d}{k(1-\epsilon)^2}$, whose absolute value is very large when ϵ increases to approach ϵ_c [10, 14]. This implies that the steady state viral load is very sensitive to small changes

of the drug efficacy ϵ around ϵ_c. Thus, if the model reflects reality, then many patients should have cleared the virus, contrary to what has been observed in the clinic.

Two classes of models were shown not to exhibit extreme sensitivity of the steady state viral load to changes in drug efficacy [14]. In the first model, the death rate of productively infected cells, δ, is assumed to be dependent on the infected cell density. This form is motivated by the notion that infected cells could be cleared by the immune response at a rate proportional to the density of infected cells. Thus, if the death rate of infected cells, δ, is a function of the density of immune effector cells, E, and E is a function of the density of infected cells, T^*, then as suggested by Holte et al. [81], a simple way to achieve a density-dependent infected cell death rate is via a power-law function, for example, $\delta(T^*) = \delta' T^{*\omega}$, where ω governs the size of the immune effect on the death rate. Holte et al. [81] further showed that using $\omega > 0$ gave a significant better fit to viral load decay obtained from children on HAART than the standard model with $\omega = 0$. With this power-law function, the basic model (Eq. (7)) can be modified to

$$\begin{aligned}
\frac{d}{dt}T(t) &= \lambda - dT - (1-\epsilon)kVT, \\
\frac{d}{dt}T^*(t) &= (1-\epsilon)kVT - \delta' T^{*\omega} T^*, \\
\frac{d}{dt}V(t) &= p_v T^* - cV.
\end{aligned} \tag{10}$$

Since the model includes a density-dependent infected cell death rate, the viral production rate is decoupled from the cell death rate and virus is assumed to be produced at a constant rate, p_v.

There is only one possible positive equilibrium. The steady state viral load is given by $\bar{V} = p_v \bar{T}^*/c$, where $\bar{T}^*$ is determined by the equation

$$\delta' \bar{T}^{*\omega+1} + \frac{dc\delta'}{(1-\epsilon)kp_v} \bar{T}^{*\omega} - \lambda = 0.$$

Callaway and Perelson [14] noted that while the steady state viral load in the basic model is very sensitive to changes in drug efficacy, particularly when drug efficacy approaches ϵ_c, in the model with density-dependent infected cell death (Eq. (10)) this does not occur (Fig. 2).

Another class of models in which different populations of target cells respond differentially to antiretroviral drugs are also able to model low-level steady state viral loads robustly [14]. The model considers the infection process to occur in two distinct compartments, with one regarded as a drug sanctuary such as the brain or testes, and can be described by the following equations:

$$\begin{aligned}
\frac{d}{dt}T_1(t) &= \lambda - dT_1 - (1-\epsilon)kV_1T_1, \\
\frac{d}{dt}T_2(t) &= \lambda - dT_2 - (1-f\epsilon)kV_2T_2, \\
\frac{d}{dt}T_1^*(t) &= (1-\varphi)(1-\epsilon)kV_1T_1 - \delta T_1^*, \\
\frac{d}{dt}T_2^*(t) &= (1-\varphi)(1-f\epsilon)kV_2T_2 - \delta T_2^*, \\
\frac{d}{dt}C_1^*(t) &= \varphi(1-\epsilon)kV_1T_1 - \mu C_1^*, \\
\frac{d}{dt}C_2^*(t) &= \varphi(1-f\epsilon)kV_2T_2 - \mu C_2^*, \\
\frac{d}{dt}V_1(t) &= N_T\delta T_1^* + N_C\mu C_1^* - cV_1 + D_1(V_2 - V_1), \\
\frac{d}{dt}V_2(t) &= N_T\delta T_2^* + N_C\mu C_2^* - cV_2 + D_2(V_1 - V_2),
\end{aligned} \tag{11}$$

where T_i, T_i^*, C_i^*, and V_i represent the concentration of target cells, short-lived infected cells, long-lived chronically infected cells, and free virus, respectively, where $i = 1$ in the main compartment and $i = 2$ in the drug sanctuary. The drug efficacy in the sanctuary is reduced by a factor f. The two compartments are coupled by allowing transport of virus between them, which is governed by two rate constants D_1 and D_2. A subpopulation of chronically infected cells are included in the model because otherwise the viral load may become unreasonably low before it approaches the steady state. A fraction φ of infection events result in long-lived or chronically infected cells. These chronically infected cells die with a rate constant, μ, and each produces N_C virus particles during its lifetime.

There is only one nontrivial equilibrium point of Eq. (11). The steady state viral load is plotted as a function of drug efficacy in Fig. 3. The dashed line describes the two-compartment model and the solid line describes the corresponding one-compartment (the main compartment) model. Because of transport from the sanctuary site to the main compartment, the virus in the main compartment cannot be eradicated even with 100% drug efficacy there. In fact, the dashed curve becomes concave up from concave down near the point of the critical drug efficacy ($\sim$0.8) in the one-compartment model, suggesting that the steady state viral load in the two-compartment model is not sensitive to small changes in drug efficacy.

A similar model that accounts for heterogeneous drug responsiveness in two types of target cells cocirculating in a single compartment was also shown capable of simulating the persistence of residual HIV replication in the face of potent drug treatment [14]. This points in the direction of improving drug efficacy in all cell types and physiological compartments in order to improve the chance of ultimately eradicating virus from infected individuals.

3.2 Virus released from latent reservoirs

Release of virus by activation of latently infected cells in latent reservoirs may also contribute to low-level persistent viremia. Direct evidence of proviral latency came from the isolation of resting memory $CD4^+$ T cells with stably integrated HIV-1 DNA [24]. In these latently infected cells virus production is extinguished because of the absence of active forms of the host transcriptional activators that are believed to be necessary for HIV-1 gene expression [105]. Hence, latently infected cells are almost indistinguishable from uninfected $CD4^+$ T cells. The virus persists in the resting cells as integrated DNA, unaffected by antiretroviral drugs. When the cells encounter their recall antigen or are activated by cytokines or other stimuli in the future, upregulation of viral gene expression results in active viral production. The establishment, dynamics and nature of the latent reservoir that consists of latently infected $CD4^+$ T cells in the setting of HAART will be discussed in detail in Section 4. Here we review the evidence that low-level persistent viremia may originate from the latent reservoir and discuss developments of mathematical models that describe low-level viremic persistence by considering latent cell activation.

In the majority of patients who received uninterrupted antiviral therapy and remained aviremic for up to 9.1 years, Chun et al. [27] detected substantially higher levels of HIV proviral DNA in activated $CD4^+$ T cells than resting $CD4^+$ T cells. Considering the short half-life of activated $CD4^+$ T cells and phylogenetic analysis of viral sequences isolated from resting and activated $CD4^+$ T cells, they suggested that virions released during activation of latently infected resting $CD4^+$ T cells might spread to and infect neighboring activated as well as resting $CD4^+$ T cells. In this way, activation of latently infected resting $CD4^+$ T cells, most likely as a consequence of normal immunologic responses to various relevant antigens or routine vaccination [182] or induction of cytokines [21], may offer a plausible mechanism to explain HIV-1 persistence in infected individuals receiving effective therapy.

Eighteen HIV-infected patients, including both children and adults, were enrolled in another longitu-

dinal study of HIV-1 reservoirs [72]. Many of them achieved viral loads below 50 RNA copies/mL after treatment. Among the patients with prior nonsuppressive therapy, resistance mutations were found but consistent with pre-HAART treatment. These resistance profiles suggest that low-level viremia does not lead to development of new resistance mutations and may result from archival, pre-HAART virus that has been deposited in the latent reservoir [72]. Genetic analyses of low-level plasma HIV-1 in children were further performed to fully characterize the persistent viremia and the evolution of resistance mutations in the protease gene [150]. Despite the frequent use of nelfinavir, a drug that has a low mutational barrier to resistance, over a long observation period, accumulation of mutations in protease was not observed. Extensive commingling of protease sequences from plasma virus and replication-competent HIV-1 recovered from resting $CD4^+$ T cells indicated that the low-level plasma virus likely originated from the latent reservoir [150].

Study of rebounding virus upon cessation of suppressive drug therapy may also provide a useful way to characterize the low-level persistent viremia in suppressed individuals because continuously produced virus is most likely to rebound when treatment is stopped. HIV-1 quasispecies that rebound following a single interruption of HAART were found to be similar to the viral quasispecies present before initiation of therapy, suggesting that rebounding plasma viremia might originate from archived virus present in the pool of resting $CD4^+$ T cells [84]. This is supported by the observation of rapid reappearance of wild-type viral variants in a number of patients with drug resistant variants after interruption of treatment [37, 38, 125, 191]. The replacement of the resistant by the wild-type virus was abrupt and fast, implying that it was a consequence of reappearance of archived wild-type virus from the latent reservoir rather than the reversal of mutations from drug resistant viral variants [136]. Zhang et al. [199] reported on the genetic characterization of rebounding virus in eight patients with plasma virus below the limit of detection for about three years. The rebound virus in five patients who did not show viral replication was genetically identical to sequences isolated from the latent reservoir. In two patients with some degree of residual viral replication, the rebound virus was genetically different from the latent reservoir virus, corresponding instead to minor viral variants detected during the course of treatment in lymphoid tissues. This supports the idea that in cases with apparently complete HIV-1 suppression by HAART, low-level viremia persistence may originate from activation of virus from the latent reservoir. Whereas in patients with incomplete suppression, the low-level virus is likely triggered by low-level ongoing replication [135].

Mathematical models have been developed to consider activation of latently infected cells [146] and to examine whether this activation is responsible for the observed low-level persistent viremia. A basic model that includes latent cell activation is

$$\begin{aligned}
\frac{d}{dt}T(t) &= \lambda - d_T T - (1-\epsilon)kVT, \\
\frac{d}{dt}L(t) &= \eta(1-\epsilon)kVT - d_L L - a_L L, \\
\frac{d}{dt}T^*(t) &= (1-\eta)(1-\epsilon)kVT - \delta T^* + a_L L, \\
\frac{d}{dt}V(t) &= N\delta T^* - cV,
\end{aligned} \tag{12}$$

where η is the fraction of infections that result in latency rather than the active production of HIV particles. a_L is the rate at which latently infected cells become activated. d_T and d_L represents the death rate of susceptible target cells and latently infected cells, respectively.

There is a single nontrivial positive equilibrium of model (12):

$$\bar{V} = \frac{N\lambda}{c}(1 - \frac{d_L}{a_L + d_L}\eta) - \frac{d_T}{(1-\epsilon)k}. \tag{13}$$

Similarly, $\bar{V} > 0$ if and only if the efficacy ϵ is less than a critical efficacy, ϵ_c, which is given by

$$\epsilon_c = 1 - \frac{cd_T}{kN\lambda(1 - \frac{d_L}{a_L+d_L}\eta)}. \tag{14}$$

By the same arguments as in Section 3.1 we can establish that the steady state viral load is very sensitive to small changes in drug efficacy and thus it is difficult to obtain robust low-level viremic persistence during therapy with Eq. (12).

Rong and Perelson have recently extended this model by considering asymmetric cell division [164] or programmed expansion and contraction [165] of latently infected cells upon exposure to antigen, and suggested that the low-level viral load during HAART could be maintained solely by latent cell activation. These models will be discussed in detail in Section 5.

Taken together, several factors, including residual viral replication in the presence of HAART, virus released from the reservoir of latently infected $CD4^+$ cells and other possible fluid, anatomical and cellular compartments that have reduced drug penetrance may all contribute to low-level viremic persistence. Quantitative understanding of these sources and their relative contributions would provide valuable information that potentially allows the design of more efficient treatment strategies.

4 Latent reservoir

Because latently infected $CD4^+$ cells serve as a potential source of inducing productive viral infection when treatment is withdrawn and they have a very slow decay rate in the majority of patients on HAART [47, 157, 172, 201], the latent reservoir has been considered as a major obstacle to viral eradication (see reviews in [8, 23]).

4.1 Establishment during primary infection

Why an HIV-infected $CD4^+$ T cell becomes latent remains largely obscure. A few molecular mechanisms have been proposed to explain the latency (see a review in [105]), for example, the inaccessibility of the integrated provirus to the transcriptional machinery [89], the absence of host transcriptional factors [44, 131] and of HIV-1 Tat [91], an important protein that upregulates HIV-1 gene expression. Since latent viral genomes have been shown to reside within the introns of actively transcribed genes [69], the first mechanism seems less likely [105]. Weinberger et al. [194] presented an integrated experimental and computational study of an HIV-1 model vector and showed that Tat fluctuations can generate distinct phenotypes analogous to productive and latent viral infection.

Another critical issue related to HIV-1 latency is how early the latent reservoir is established during the course of HIV-1 infection. Chun et al. [20] demonstrated that initiation of HAART in patients as early as 10 days after the onset of symptoms of primary infection did not prevent generation of a latent reservoir. There was no significant correlation between the frequency of resting $CD4^+$ T cells harboring integrated HIV-1 DNA and the time of initiation of treatment, suggesting that once plasma viremia is present and virus is disseminated to lymphoid organs, the latent reservoir is already established.

4.2 Decay characteristics of the latent reservoir

The decay characteristics of the latent reservoir remain controversial and estimates of its half-life have been quite divergent. Finzi et al. [46] estimated the mean half-life of the reservoir in chronically infected patients to be $\sim$44 months. Thus, even with a conservative estimate that the latent reservoir consists of only 10^5 cells, more than 60 years of treatment would be required to eradicate this compartment in

most patients. In fact, the mean slope of the decay in some patients was not statistically different from zero, implying that the reservoir in these patients may not decay in the setting of HAART.

A more rapid decay rate was reported by Zhang et al. [201] in a subset of acute seroconvertors who started combination therapy within 90 days of infection. Eight men were selected from over 100 subjects enrolled in the clinical trial because they had been fully compliant with the prescribed antiretroviral therapy and plasma viremia was suppressed to below 50 copies /mL after 5 months of treatment. Using two independent approaches, they estimated that the half-life of the latent, replication-competent virus in resting $CD4^+$ cells was approximately 6 months. In a recent study, Chun et al. [25] obtained an even shorter half-life of ~4.6 months in patients who initiated antiretroviral therapy early in infection. The result suggested that it would take up to 7.7 years of therapy to completely eradicate this reservoir.

The apparent discrepancy between half-life estimates was addressed in the study of Ramratnam et al. [157]. With a quantitative micro-culture assay, they demonstrated that the latent reservoir decayed with a mean half-life of about 6 months in patients who consistently maintained plasma virus of less than 50 copies/mL, whereas in those patients who experienced intermittent episodes of viremia the decay was much slower. Thus, the latent reservoir persistence is likely to some extent due to the continual replenishment by active viral replication. In addition, many other factors, such as unintentional selection of patients with rapid decay of the reservoir, the duration of follow-up studies, and differences in the assay sensitivities, may also strongly impact the estimate of the decay rate of the reservoir [172].

The above estimates have assumed that long-lived infected cells belong to a homogeneous population exhibiting exponential decay characteristics. However, Strain et al. [186] suggested that the decay rate of latently infected cells decelerates during treatment. They analyzed the decay kinetics of HIV-1 DNA and of cell-associated infectivity, and estimated that the latent reservoir had a median half-life of 20 weeks during the first year of therapy, whereas the decay slowed significantly during the subsequent 3 years with a median half-life of 70 weeks. Furthermore, it seems that the deceleration is continuing. The decelerating decay of the latent reservoir can be explained by the heterogeneity in the activation rate of latently infected cells [94, 129, 185]. Cells specific for frequently encountered antigens may be preferentially activated and quickly removed from the reservoir during the first year, whereas cells specific for rarely encountered antigens may persist without activation or be activated slowly in subsequent years.

A simple mathematical model was employed by Muller et al. [129] to study the decay characteristics of viral load by considering the heterogeneity of latent cell activation after prolonged fully suppressive therapy. Let $L(t)$ be latently infected cells, which die at rate δ_L and are activated at rate a into the productively infected class, T^*. The model reads:

$$\begin{aligned} \frac{d}{dt}L(t) &= -(a+\delta_L)L, \\ \frac{d}{dt}T^*(t) &= aL - \delta T^*, \\ \frac{d}{dt}V(t) &= pT^* - cV. \end{aligned} \tag{15}$$

Since the dynamics of latently infected cells are much slower than those of productively infected cells, Muller et al. [129] assumed the latter to be a quasi-steady state. Setting the second equation to zero, one has $T^*(t) = (a/\delta)L(t)$. Similarly, as virus turns over much faster than productively infected cells [148], the viral load will follow the latter as a constant ratio: $V(t) = (p/c)T^*(t)$. Therefore, the viral load maintained by activation of latently infected cells is

$$V_L(t) = \frac{pa}{c\delta}L_0 e^{-(a+\delta_L)t}.$$

Considering the heterogeneity in the activation from the latent to productive infection, they assumed that the latently infected cell pool had an initial distribution of activation rates. Using a continuous range of activation rates, instead of a single fixed value, the above viral load equation changes to

$$V_L(t) = \frac{pe^{-\delta_L t}}{c\delta} \int_0^{a_{max}} aL_0(a)e^{-at}da,$$

where $L_0(a)$ represents the initial distribution of latently infected cells with respect to the activation rate, which has the maximum value, a_{max}.

To obtain the total viral load from initiation of therapy, they also incorporated other virus-producing cell populations into the model. From the results given in [146], they obtained the total viral load

$$V(t) = V_L(t) + \frac{p}{c}T_0^* e^{-\delta t} + \frac{p_M}{c}M_0 e^{-\delta_M t}, \tag{16}$$

where T_0^* and M_0 represent the initial population of productively infected cells and long-lived infected cells, respectively. p_M and δ_M are the virus production rate and death rate, respectively, of the latter cells.

The graph of $V(t)$ according to (16) is a triphasic decline in which the third phase reflects the long tail of the decelerating production by activated latently infected cells [129]. This suggests that the population of latently infected cells gradually shift toward cells with a small probability of activation, i.e., cells specific for increasingly rare antigens. In this regard, the progressively decelerating clearance of latently infected cells can explain the observed persistence and long-term dynamics of HIV-1 during effective therapy.

Kim and Perelson [94] developed another model that studies the decay characteristics of the latent reservoir. Their model includes two novel features. First, as suggested by the observations in [186], the model assumes that the rate of latent cell activation decreases with time during antiretroviral therapy, leaving behind cells that are specific for increasingly rare antigens and less frequently activated. Second, the model includes bystander proliferation of latently infected cells without transitioning them into active viral production, which contributes to the maintenance of the latent reservoir. Bystander proliferation is proliferation that is not driven by the cell engaging its antigen-specific receptor, and hence could be due to cytokines in the environment for example. The model is described as follows:

$$\begin{aligned}
&\frac{d}{dt}a(t) = -\omega(a - a_{min}),\\
&\frac{d}{dt}L(t) = \eta(1-\epsilon)kT_0V + rL - aL,\\
&\frac{d}{dt}T^*(t) = (1-\eta)(1-\epsilon)kT_0V + aL - \delta T^*,\\
&\frac{d}{dt}V(t) = N\delta T^* - cV,
\end{aligned} \tag{17}$$

where $a(t)$ is the activation rate that exponentially decays with the deceleration rate ω, from the initial value, a_0, to a minimum value, a_{min}. r represents the net effect of bystander proliferation and death, i.e., $r = p_{bs} - d_L$, where p_{bs} is the bystander proliferation rate and d_L is the death rate of latently infected cells.

From the $L(t)$ equation in (17), T cell bystander proliferation, latent cell activation, and ongoing viral replication (determined by drug efficacy) all impact the decay of the latent reservoir. In the case of perfect treatment ($\epsilon = 1$), the final size of the latent reservoir is fully determined by the relationship between the net proliferation rate, r, and the minimum activation rate, a_{min}. If a_{min} is assumed to be

zero, then the pool size of the latent reservoir keeps increasing when $r > 0$, stabilizes to a steady state when $r = 0$, and is always decreasing when $r < 0$. However, even if the proliferation rate r is slightly greater than zero, the model still cannot maintain a low-level viremia (Fig. 4). This can be explained analytically from the T^* equation in (17). As $\epsilon = 1$ and $a(t) \to a_{min} = 0$, the asymptotic behavior of productively infected cells is determined by the equation: $dT^*/dt = -\delta T^*$, which yields $T^* \to 0$ and thereby $V \to 0$. In order to simulate the persistence of both low-level viremia and the latent reservoir, the minimum activation rate, a_{min}, has to be greater than zero (Fig. 5). In the scenario of $a_{min} > 0$, the size of the latent reservoir keeps on increasing when $r > a_{min}$, approaches a steady state when $r = a_{min}$ (Fig. 5), and decreases when $r < a_{min}$. The viral load can be maintained at a low level as long as $a_{min}L = \delta T^*$.

When treatment is not 100% effective ($\epsilon < 1$), the analysis becomes complicated. In the case of $a_{min} = 0$, there exists a critical drug efficacy, $\epsilon_c = 1 - c/((1-f)NkT_0)$. For $\epsilon > \epsilon_c$, both the virus and the latent reservoir decay toward zero. The contribution of ongoing viral replication to the level of latently infected cells remains insignificant. When $\epsilon = \epsilon_c$, the viral load approaches a nonzero steady state, whereas the latent cell population does not stabilize unless $r < 0$. When ϵ is slightly less than ϵ_c, the viral load keeps increasing and the latent reservoir eventually blows up after an initial decline.

These results suggest that ongoing viral replication together with bystander proliferation of latently infected cells is able to describe the extremely slow decay or stability of the latent reservoir and the persistence of low-level viremia during potent treatment. However, the model is still sensitive to the net proliferation rate, r, and the drug efficacy, ϵ. In Section 5, recent developments of models that simulate viral blips will be discussed. Some models are shown to be robust in maintaining the latent reservoir as well as low-level persistent viremia. More interestingly, they demonstrate the potential to explain the differences between the divergent estimates of the half-life of the latent reservoir in the literature.

4.3 Mechanisms of the reservoir stability

Although latently infected cells are rare in plasma, with a frequency on the order of 1 in 10^6 resting $CD4^+$ T cells [18], they are of considerable concern because they decay extremely slowly and can be activated to produce virus, which can rekindle infection when treatment is interrupted. The factors that contribute to the remarkable stability of the latent reservoir have not been fully elucidated. As discussed above, a low level of ongoing active viral replication may reseed the latent reservoir in the presence of HAART [27, 157, 185]. Another mechanism for the latent reservoir persistence stems from the intrinsic stability of latently infected cells. The latent reservoir consists at least of a number of resting memory $CD4^+$ T cells [46, 172, 174, 185], whose intermitotic half-life is around 6 months [121, 124]. As memory T cells can be maintained by occasional proliferation in some murine systems [180, 188], latently infected $CD4^+$ cells might reseed the reservoir if they can also undergo such occasional proliferation upon activation that does not fully turn on HIV-1 gene expression [155]. In this situation, the latent reservoir may decay with a much longer half-life than 6 months. This idea is supported by the model of Kim and Perelson [94] discussed above (Section 4.2).

Recent evidence from genotypic analysis seems to favor the hypothesis that the reservoir stability comes principally from the intrinsic stability of latently infected cells. Strain et al. [185] exploited a predictable drug resistance mutation in the viral reverse transcriptase active site to track cells infected during suppressive therapy and identify cells within the latent reservoir replenished by ongoing viral replication. This allowed them to quantify both the intrinsic turnover rate of the reservoir and the extent of its replenishment by residual replication. The result showed that even though ongoing replication can replenish the reservoir, it cannot explain the observation that cells that were recently infected and contained viral DNA bearing the mutation were cleared more rapidly than cells that were infected

earlier and contained the wild-type codon. Further analysis suggested that the latent reservoir has a heterogeneous and dynamic composition, and that the reservoir stability reflects decreasing decay rates of subpopulations within the latent pool [185]. In the study of Ruff et al. [166], archival wild-type virus was found to persist in resting $CD4^+$ T cells despite selective pressure that favors drug resistant mutants in a cohort of children for a prolonged period of time. In addition, viral sequences isolated from latently infected cells at later time points did not show greater divergence than from earlier time points, indicating that HIV-1 persists in a latent form in long-lived resting memory $CD4^+$ T cells. In some patients failing HAART, Monie et al. [128] found that resting $CD4^+$ T cells harbored a diverse array of wild-type and archival drug resistant viruses. However, their *pol* sequences were genetically distinct from contemporaneous plasma virus that consisted of a relatively homogeneous population of drug resistant viruses. These results all support the idea that the reservoir stability results primarily from the intrinsic stability of resting memory $CD4^+$ T cells. The influence of ongoing viral replication on the decay of the latent reservoir remains insignificant, particularly in patients who have suppressed plasma viral loads for long periods of time. However, the precise extent to which residual viral replication reseeds the latent reservoir during HAART has not been fully delineated.

Mathematical modeling has the potential to shed light on the quantitative contribution from ongoing viral replication. Siliciano and his colleagues detected a predominant plasma clone (PPC) of HIV-1 that dominates the residual viremia in some patients on HAART [3]. If the latent reservoir is replenished by viral replication, then this unique sequence would be incorporated into the latent reservoir and be readily detected at later time points since its genotype is different from sequences in resting $CD4^+$ T cells. In this regard, the PPC sequence can serve as a functional label for measuring the rate of replenishment for the latent reservoir in the setting of HAART. They developed a model for the dynamics of the latent reservoir and estimated the maximum daily flow of cells into the latent reservoir [169]. The model includes two variables, latently infected cells containing PPC proviruses (L_1) and latently infected cells containing all other proviruses (L_2), and is described by the following equations:

$$\begin{aligned} \frac{d}{dt}L_1 &= k_{in}f - k_{out}L_1, \\ \frac{d}{dt}L_2 &= k_{in}(1-f) - k_{out}L_2, \end{aligned} \tag{18}$$

where k_{in} is the rate at which free viruses enter the latent reservoir, k_{out} is the decay rate of latently infected cells, and f represents the fraction of plasma virus that consists of the PPC.

Introducing $K = k_{in}/k_{out}$, the above system of equations can be solved explicitly to give

$$\begin{aligned} L_1(t) &= [L_1(0) - Kf]e^{-k_{out}t} + Kf, \\ L_2(t) &= [L_2(0) - K(1-f)]e^{-k_{out}t} + K(1-f). \end{aligned} \tag{19}$$

Assuming that the PPC permanently disappears from the plasma after a period of time t_e, the two subpopulations of latently infected cells at time $t > t_e$ are governed by the equations:

$$\begin{aligned} \frac{d}{dt}L_1 &= -k_{out}L_1, \\ \frac{d}{dt}L_2 &= k_{in} - k_{out}L_2. \end{aligned} \tag{20}$$

The corresponding solution for $t > t_e$ is given by

$$\begin{aligned} L_1(t) &= L_1(t_e)e^{-k_{out}(t-t_e)}, \\ L_2(t) &= L_2(t_e)e^{-k_{out}(t-t_e)} + K(1 - e^{-k_{out}(t-t_e)}), \end{aligned} \tag{21}$$

where $L_1(t_e)$ and $L_2(t_e)$ are solutions (19) evaluated at t_e. Thus, the fraction of latently infected cells containing the PPC at time $t > t_e$, Φ_{PPC}, can be calculated from equations (21) as

$$\Phi_{PPC} = \frac{L_1}{L_1 + L_2} = \frac{L_1(0) + Kf(e^{k_{out}t_e} - 1)}{L_1(0) + L_2(0) + K(e^{k_{out}t} - 1)},$$

where $K = k_{in}/k_{out}$. The probability that there are m PPC sequences out of n total sequences can be approximated by a binomial distribution:

$$P(k) = \begin{pmatrix} n \\ m \end{pmatrix} \Phi_{PPC}^m (1 - \Phi_{PPC})^{n-m}.$$

Using this probability, a maximum likelihood approach was employed to estimate the value of k_{in} from patient data [169]. The magnitude of the maximum daily flow of cells into the latent reservoir was very small compared with the overall latent reservoir size, suggesting that ongoing viral replication is unlikely to significantly influence the decay of the latent reservoir.

5 Intermittent viral blips

Although many infected individuals exhibit sustained low-level viremia on HAART, a number of them have occasional viral load measurements above the detection limit. Such transient episodes of detectable viremia are called 'blips' (Fig. 1). Since viral blips are relatively rare events, neither their occurrence timing, frequency, duration and amplitude nor their etiology is well known. With more extensive sampling viral blips are more likely to be identified in the majority of patients at some time.

5.1 Characteristics of viral blips

There are three main features characterizing intermittent viral blips: blip frequency, viral amplitude, and duration of occurrence. Di Mascio et al. [39] examined viral blip time series obtained from 123 HIV-1 infected patients and found that the mean blip frequency was 0.09±0.11/sample, and the mean blip amplitude was 158±132 RNA copies/mL. Although the frequency and amplitude of blips did not increase with longer periods of observation, the frequency was inversely correlated with the $CD4^+$ T cell count at the start of therapy [39]. By comparing the dynamics of blips in two treatment subgroups, they concluded that blip frequency was roughly two-fold higher in patients treated during chronic infection than in patients treated during acute infection [40]. They also suggested that blips were not isolated random events, but rather extended transient episodes of viremia with a duration of roughly 3 weeks [41]. The amplitudes of blips observed in these patients do not share a common probability distribution, and thus blips cannot be interpreted solely by assay variations [144]. They further showed that the blip amplitudes were consistent with random sampling from a profile in which the viral load rises rapidly to a peak, followed by a slower biphasic decay [42].

5.2 Origin of blips

A number of mechanisms have been proposed to explain the occurrence of viral blips. By comparing the proportion of episodes occurring in patients who received or did not received triple-drug therapy, it has been suggested that intermittent viremia might occur because of higher levels of viral replication [70], including the selection of drug resistant variants [31]. Thus, there was concern that viral blips could represent viral evolution [62] through active virus replication and, thereby, signal imminent virological failure [56]. However, in other studies viral blips were not associated with virological failure [70, 119,

126, 177], even though in some cases they were associated with the emergence of new drug resistant HIV variants [31, 114]. By comparing viral sequences derived from transient viremia with sequences from PBMC collected before and during HAART, Tobin et al. [187] showed that viral blips could result from production of virus following immune activation and clonal expansion of latently infected cells. Other mechanisms for the generation of viral blips include antigen-driven target cell activation [51, 52] due to vaccination [64, 86] or opportunistic infections [87, 88]. However, viral blips can also be the result of laboratory error or statistical variation rather than genuine viral rebound especially when of low amplitude [107, 134]. Nettles et al. [134] examined the characteristics of viral blips by intensive sampling of 10 patients over a period of 90 days. In their sample, blips were common (9 of 10 patients), brief in duration (median, less than 3 days), low in magnitude (median, 79 copies/mL), and poorly reproducible on independent testing. Moreover, blip frequency was not related to illness, vaccination, or drug concentrations. These observations indicate that random biological or statistical variation around a mean viral load less than 50 copies/mL might be responsible for the aberrant viral load measurements they observed. The nature and clinical significance of viral blips are not well known and require more clinical and experimental studies.

5.3 Models that simulate blips

Recently a few mathematical models have been developed to describe intermittent viral blips, as well as the extremely slow decay of the latent reservoir. These models mainly consider two types of cell activation: target T cell activation and latently infected cell activation.

5.3.1 Target CD4$^+$ T cell activation

Motivated by the observations that vaccination [64, 86] or opportunistic infection [123] is associated with enhanced viral replication, and the modeling studies of Ferguson et al. [45] and Fraser et al. [50, 51] that antigen-driven T cell proliferation can result in a burst of virus production, Jones and Perelson [87] proposed a mathematical model for immune cell expansion in the presence of pathogen. The model is an extension of the two-compartment model (11), in which two co-circulating populations of target cells have different drug penetration and hence one population has reduced drug efficacy. Let A be a pathogen or some other growing antigen that stimulates an immune response. The model that considers antigen-driven target cell activation is given by

$$\begin{aligned}
\frac{d}{dt}T_1(t) &= \lambda_1 + f(A)T_1 - (1-\epsilon)k_1VT_1 - d_TT_1,\\
\frac{d}{dt}T_2(t) &= \lambda_2 + f(A)T_2 - (1-f\epsilon)k_2VT_2 - d_TT_2,\\
\frac{d}{dt}T_1^*(t) &= (1-\varphi)(1-\epsilon)k_1VT_1 - \delta T_1^*,\\
\frac{d}{dt}T_2^*(t) &= (1-\varphi)(1-f\epsilon)k_2VT_2 - \delta T_2^*,\\
\frac{d}{dt}C_1^*(t) &= \varphi(1-\epsilon)k_1VT_1 - \mu C_1^*,\\
\frac{d}{dt}C_2^*(t) &= \varphi(1-f\epsilon)k_2VT_2 - \mu C_2^*,\\
\frac{d}{dt}V(t) &= N_T\delta(T_1^*+T_2^*) + N_C\mu(C_1^*+C_2^*) - cV,
\end{aligned} \tag{22}$$

where T_1 and T_2 represent two co-circulating CD4$^+$ T cells. In one population (T_1), drug efficacy is ϵ, and in the second population (T_2), drug efficacy is reduced by a factor $f < 1$. As in Eq. (11), the model

also includes productively infected cells, T_1^* and T_2^*, and chronically infected cells, C_1^* and C_2^*.

$f(A)$ is an activation function, which depends on the antigen concentration and can be approximated by a type-II functional response:

$$f(A) = a\frac{A}{A + K_4},$$

where a is a maximum T-cell activation rate and K_4 is a half-saturation concentration of antigen that stimulates a $CD4^+$ cell response.

On exposure to antigen, naive $CD8^+$ T cells experience a burst of proliferation, undergoing a programmed cascade of divisions that culminate in the production of mature activated effector cells [90]. Let N_0 be the initial naive $CD8^+$ T cells, and N_i the $CD8^+$ cells that have completed i divisions. The following equation describes such T-cell proliferation under antigenic stimulation [87]:

$$\begin{aligned}
\text{Antigen:} \quad & \frac{d}{dt}A(t) = r_0 A(1 - \frac{A}{A_{max}}) - \gamma AE, \\
& \frac{d}{dt}N_0(t) = -(p_0(A) + d_0)N_0, \\
\text{CD8 response:} \quad & \frac{d}{dt}N_1(t) = 2p_0(A)N_0 - (p + d)N_1, \\
& \frac{d}{dt}N_i(t) = \begin{cases} 2pN_{i-1} - (p + d)N_i & \text{i=2, 3, 4,} \\ 2pN_{i-1} - (p + d_E)N_i, & \text{i=5, 6, ... , k-1,} \end{cases} \\
& \frac{d}{dt}N_k(t) = 2pN_{k-1} - d_E N_k.
\end{aligned} \tag{23}$$

Here pathogen undergoes density-dependent growth governed by a logistic term with a maximum growth rate, r_0, and the carrying capacity, A_{max}. γ is the clearance rate constant for the pathogen due to effector cells, E. Cells are assumed to become effectors after a number of divisions, say, four, and stop proliferating after more divisions, say, eight. Thus, $E = \sum_{i=5}^{8} N_i$. p is the constant proliferation rate for all divisions after the first; d_0, d, and d_E ($d_0 < d < d_E$) are the mortality rate of naive cells, non-effector proliferative cells, effector cells, respectively.

The rate of the first T cell division is antigen-dependent and is described by the function [87]

$$p_0(A) = p_0\frac{A^n}{A^n + K_8^n}, \tag{24}$$

where p_0 is the maximum proliferation rate, K_8 is antigen half-saturation for stimulating $CD8^+$ T cells, and n is the Hill coefficient that determines the steepness of the response.

Following exposure to antigen described by Eq. (23), target cell activation (Eq. (22)) is shown capable of generating a transient increase of the viral load (Fig. 6). Thus, these episodes of viremia can be explained by occasional activation of the immune system by opportunistic infection [87].

5.3.2 Latently infected cell activation: asymmetric division

Two classes of models have recently been developed that study activation of latently infected cells upon stochastic antigenic stimulation in HIV-infected patients on HAART [164, 165]. In the first model [164], latently infected T cells are hypothesized to undergo asymmetric divisions like stem cells [67, 83, 85] or T cells [15] on exposure to their relevant antigen. When a latently infected cell divides, one daughter cell is activated that can produce new virus, whereas the other daughter cell remains in the resting state

with varied probability, providing the potential to renew the latent reservoir. To study the influence of asymmetric division on the latent reservoir and viral load, the following model was considered:

$$\begin{aligned}
\frac{d}{dt}T(t) &= \lambda - d_T T - (1-\epsilon)kVT, \\
\frac{d}{dt}L(t) &= \eta(1-\epsilon)kVT - d_0 L + f(t)(-a_L L + 2a_L p_L L), \\
\frac{d}{dt}T^*(t) &= (1-\eta)(1-\epsilon)kVT - \delta' T^{*\omega} T^* + 2f(t)(1-p_L)a_L L, \\
\frac{d}{dt}V(t) &= p_v T^* - cV,
\end{aligned} \tag{25}$$

where p_L represents the probability that a daughter cell remains in the latent state when a latently infected cell divides upon antigen stimulation. After each division, $2p_L$ latently infected cells and $2(1-p_L)$ activated infected cells are generated. Similar to (10), the model includes density-dependent infected cell death and virus is produced at a constant rate, p_v, from each infected cell.

The dynamics of antigen were not modeled explicitly as in [87]. Instead, a basic 'on-off' model that has successfully describe the immune response to lymphocytic choriomeningitis virus (LCMV) infection [34, 35] was adopted to approximate antigenic stimulation of latently infected cells. The activation function $f(t)$ takes on the value 0 when there is no activation and 1 when there is full activation [34], i.e.,

$$f(t) = \begin{cases} 0 & \text{if } t < T_{on}, \\ 1 & \text{if } T_{on} \le t < T_{off}, \\ 0 & \text{if } t \ge T_{off}, \end{cases} \tag{26}$$

where T_{on} is the time at which activation starts and T_{off} is the time when the activation ends. $\Delta t = T_{off} - T_{on}$ is the duration of the activation.

Figure 7 shows the results of stochastic simulations of Eq. (25). The timing, frequency and duration of the blips are determined by when, how often and for how long the activation occurs. Several factors, including the probability that a daughter cell remains in the latent state, the level of latently infected cells, and the drug efficacy, all impact the amplitude of viral blips in the simulation. An interesting result is that occasional replenishment of the latent reservoir induced by asymmetric division of latently infected cells upon intermittent antigenic stimulation is able to explain the differences between the divergent estimates of the half-life of the reservoir. With different distributions of the probability p_L, Fig. 7 exhibits three distinct decay profiles of the latent reservoir. In column (a), a statistically significant decay of the reservoir was not seen. This is consistent with the clinical observations in some patients [46]. In column (b), although occasional activation replenishes the latently infected cell pool, the size of the reservoir diminishes gradually. However, the decay is extremely slow due to the occasional replenishment. This scenario might correspond to a long half-life of ~44 months estimated in references [46, 172]. Column (c) is with a small probability p_L, which implies that the renewal ability of the reservoir is poor. Occasional encounters with antigen activate latently infected cells to produce virus and consequently consume the latent reservoir more quickly than in previous scenarios. This case might explain the short half-life (for example, ~6 months) estimated in other references [25, 157, 201].

5.3.3 Latently infected cell activation: programmed expansion and contraction

Both $CD4^+$ and $CD8^+$ T cell responses to infectious agents, e.g., LCMV [82], consist of three distinct phases: initial antigen-driven expansion and differentiation into effector cells, followed by rapid contraction of activated cells and formation of a small number of memory cells [2]. By developing mathematical

models that include these phases, De Boer et al. [34, 35] studied $CD4^+$ and $CD8^+$ T cell responses to LCMV. Using a model that accounts for the programmed cascade of divisions during expansion of the $CD8^+$ T cell response (Eq. (23)), Jones and Perelson [88] showed that latent cell activation is able to explain transient episodes of viremia observed in well-suppressed patients on effective treatment. They developed the following model:

$$\begin{aligned}
&\frac{d}{dt}T(t) = \lambda - d_T T - (1-\epsilon)kVT,\\
&\frac{d}{dt}L_0(t) = \eta(1-\epsilon)kVT - (p_0(A)+d_0)L_0 + \rho\sum_{i=1}^{k} Li,\\
&\frac{d}{dt}L_1(t) = 2p_0(A)L_0 - (\rho+p+d_{LA}+a_L)L_1,\\
&\frac{d}{dt}L_i(t) = 2pL_{i-1} - (\rho+p+d_{LA}+a_L)L_i \quad \text{i=2, ..., k-1},\\
&\frac{d}{dt}L_k(t) = 2pL_{k-1} - (\rho+d_{LA}+a_L)L_k,\\
&\frac{d}{dt}T^*(t) = (1-\eta)(1-\epsilon)kVT + a_L\sum_{i=1}^{k} L_i - \delta(T^*)T^*,\\
&\frac{d}{dt}V(t) = p_v T^* - cV,
\end{aligned} \tag{27}$$

where L_0 denotes resting latently infected cells that have not divided and L_i denotes cells that have been stimulated by antigen and have completed i divisions. $p_0(A)$ is the proliferation rate of a cell in its first division, which can be defined as in (24) but with K_8 now denoting the half-saturation of antigen that stimulates a latent cell into division. Activated cells proliferate at rate p, die at rate d_{LA}, transition into the productively infected class at rate a_L, and revert to resting at rate ρ. Antigen dynamics are described in (23). The model also uses a density-dependent death rate of productively infected cells as in Eq. (10).

Simulations of Eq. (27) together with (23) demonstrate that latent cell activation caused by sporadic immune activation can generate viral blips [88], as shown in Fig. 6.

Based on (27), Rong and Perelson [165] developed a new model in which latently infected cells are hypothesized to experience a programmed expansion and contraction in the latently infected $CD4^+$ T cell response to their specific antigen. With the assumption that a small portion of the activated cells revert back to a resting state by the process that normally generates memory $CD4^+$ T cells, the latently infected cell pool can be replenished by intercurrent activations. Let L_0 represent resting latently infected $CD4^+$ T cells, which encounter their relevant antigen and are activated to enter L_a, the class of activated cells. The model describing the programmed expansion and contraction of latently infected cells upon antigen encounter is as follows:

$$\begin{aligned}
&\frac{d}{dt}T(t) = \lambda - d_T T - (1-\epsilon)kVT,\\
&\frac{d}{dt}L_0(t) = \eta(1-\epsilon)kVT - d_0 L_0 - f(t)aL_0 + (1-f(t))\rho L_a,\\
&\frac{d}{dt}L_a(t) = f(t)(aL_0 + pL_a) - (1-f(t))(\sigma+\rho)L_a - a_L L_a,\\
&\frac{d}{dt}T^*(t) = (1-\eta)(1-\epsilon)kVT - \delta(T^*)T^* + a_L L_a,\\
&\frac{d}{dt}V(t) = p_v T^* - cV.
\end{aligned} \tag{28}$$

where the activation function $f(t)$ is given by (26). When the antigen is present, resting latently infected cells, L_0, can be activated into the L_a class with a constant rate a. Activated cells proliferate at rate p. Following the expansion phase, there is a contraction phase in which activated cells have death rate σ, and can revert to the resting state at rate ρ. In addition, activated cells transition into productively infected cells at rate a_L. A density-dependent death rate of productively infected cells is employed again as in (10) to maintain the low steady state viral load.

Simulations of Eq. (28) show that it is able to generate viral blips with amplitude and duration consistent with the findings of Di Mascio et al. [39] (Fig. 8). The antigen induces temporary substantial proliferation of activated cells, which then reseed the latent reservoir. The extent to which the latent cell pool is replenished depends on the proliferative ability of activated cells. In Fig. 8(a), the proliferation rate is chosen to be $p = 1.4$ day^{-1}, which implies that cell divisions occur $8 - 12$ times over an interval of $4 - 6$ days. In this case, the activation induces a high level of activated cells, and hence the amplitude of viral blips remains relatively high. Accordingly, there are many activated cells going back to the resting state. Thus, no statistically significant decay of the latent reservoir was observed, suggesting that the viral reservoir can be extremely stable. Figure 8(b) shows an example with a slightly smaller proliferation rate, $p = 1.35$ day^{-1}. Although activated cells and the viral load increase to high levels, there is still a decay of the latent reservoir. However, the decay is very slow, with a half-life of approximately 44 months. In Fig. 8(c), the proliferation rate is chosen to be $p = 1$ day^{-1}, which means cells divide $6 - 8$ times during $4 - 6$ days. In this situation, fewer activated cells are produced, and the reservoir replenishment is limited. Consequently, the latent reservoir decays relatively quickly because cell activation consumes latently infected cells. Figure 8(c) shows a realization of Eq. (28) in which the half-life of the reservoir decay is about 6 months.

A density-dependent infected cell death rate has been exploited in the above model to generate a low steady state viral load in the absence of antigen. Equation (28) can be modified to show that sporadic activation of latently infected cells is able to generate low-level persistent viremia as well as intermittent viral blips during HAART [165]. Without assuming density-dependent cell death, the viral load in last model decreases very quickly in the absence of activation because activated cells decline rapidly to an extremely low level after the contraction phase, with not enough cells entering the productively infected state. If activated cells are maintained at a low level rather than decreasing to zero quickly during the contraction phase, then low steady state viral loads are possible. In fact, in the study of Chun et al. [27], high levels of HIV proviral DNA were found in activated CD4$^+$ T cells in maximally suppressed patients. Although a fraction of these cells could harbor defective provirus, the evidence that spontaneous release of virus during overnight culture without any activating stimuli would argue for the persistence of infectious virus in activated CD4$^+$ T cells.

Motivated by the observation of a multiphasic contraction phase in the CD4$^+$ T cell response during acute LCMV infection [34, 82], a model with a biphasic contraction phase in the latent CD4$^+$ T cell response was developed as follows [165]:

$$\begin{aligned}
\frac{d}{dt}T(t) &= \lambda - d_T T - (1-\epsilon)kVT, \\
\frac{d}{dt}L_0(t) &= \eta(1-\epsilon)kVT - d_0 L_0 - f(t)aL_0 + (1-f(t))\rho L_a, \\
\frac{d}{dt}L_a(t) &= f(t)(aL_0 + pL_a) - (1-f(t))(\delta_a + \rho)L_a - g(t)\sigma L_a - a_L L_a, \\
\frac{d}{dt}T^*(t) &= (1-\eta)(1-\epsilon)kVT - \delta T^* + a_L L_a, \\
\frac{d}{dt}V(t) &= N\delta T^* - cV,
\end{aligned} \tag{29}$$

where $f(t)$ is defined by Eq. (26). Following the expansion phase, there is a biphasic contraction: a rapid contraction phase of length Δt days, during which activated cells die rapidly by apoptosis or activation-induced cell death, at a rate σ, and a slower phase where activated cells die at their base mortality rate δ_a. $g(t)$ denotes the contraction function. During the rapid contraction phase, $g(t) = 1$, otherwise, $g(t) = 0$.

With different proliferation rates chosen to characterize the different potentials of activated cells to proliferate during expansion, simulations of Eq. (29) (Fig. 9) exhibit three distinct decay profiles of the latent reservoir as shown in Fig. 8: (a) there is almost no decay; (b) the latent reservoir decays at a very slow rate; (c) the reservoir decays at a faster rate. The viral load does not decline to an unreasonably low level in the absence of antigenic stimulation because it is maintained by a small number of activated cells that transition into the productive stage after the rapid contraction phase. Relative contributions of ongoing viral replication to the viral and latent reservoir persistence were also examined quantitatively with this model [165]. The result showed that residual ongoing replication during HAART is only a minor factor, consistent with the conclusions in recent studies [115, 169].

To summarize, occasional activation of latently infected cells by antigen can produce a large number of activated cells temporarily, and thereby generate intermittent viral blips. Different potentials of activated cells to proliferate during the initial clonal expansion phase are able to explain the different decay characteristics of the latent reservoir observed in different studies. The levels of persistent viremia and latently infected cells are not correlated with treatment potency, suggesting that the stability of the latent reservoir may not arise from ongoing residual replication during HAART.

6 Treatment implications

Low-level persistent viremia, a stable latent reservoir, and intermittent viral blips observed in infected individuals on potent combination treatment all suggest that current HAART regimens are unable to eradicate HIV-1 from patients. Studies of the dynamics, mechanisms and relationships between them could have important treatment implications.

6.1 Treatment intensification

Given that the latent reservoir has been identified as a major barrier to virus eradication, elimination of the reservoir by novel therapeutic approaches is necessary before the ultimate goal of viral eradication can be achieved. Although treatment intensification can decrease the viral load to a lower level even in patients with years of highly suppressive therapy [71], and in some cases accelerate the decay of the latent reservoir [158], its long-term effects have not been clearly documented [60, 115]. Experimental evidence and modeling have both suggested that the remarkable stability of the latent reservoir is most likely due to the intrinsic stability of resting memory $CD4^+$ T cells and/or occasional replenishment by antigenic stimulation. If this is the case, then simply intensifying current HAART regimens is unlikely to have an influential impact on the long-term decay of the latent reservoir.

6.2 Early therapy

The best time to initiate antiretroviral therapy remains controversial. In order to prevent progressive immune damage, treatment ideally should be started as early as possible following infection. The impetus for early therapy also comes from the initial prediction that prolonged potent combination therapy would be able to eradicate the virus from infected individuals. However, the attractiveness of early treatment has to be balanced against the drawbacks of prolonged overall duration of therapy. The

discovery that integrated virus can hide in latently infected cells and persist in the presence of immune responses and potent therapy also tempers the enthusiasm for the initial early and aggressive approach. Once the pool of latently infected cells is established, it becomes very difficult to eradicate.

Chun et al. [20] and Finzi et al. [47] showed that initiation of HAART as early as 10 days after the onset of symptoms of primary infection could not prevent generation of latently infected $CD4^+$ T cells even though the plasma viremia could be successfully controlled shortly after the adminstration of HAART. However, in other studies, early treatment was shown to some extent to reduce the frequency of latently infected cells, confine the size of the latent reservoir [7, 112], limit the evolution of HIV-1 in viral reservoirs [149], and lower viral blip frequency [40]. Strain et al. [186] reassessed the impact of initiating early treatment on the latent reservoir size in 27 patients who initiated therapy before or < 6 months after seroconversion and whose viremia was successfully suppressed to below 50 copies/mL. Total cell-associated infectivity measuring the population size of cellular reservoirs could not be detected in most patients after one year of treatment. In contrast, replication-competent virus could be recovered from all 17 control patients who initiated treatment during chronic infection. These results show that early therapy may reduce the size and/or accelerate the decay of the latent reservoir. However, failure to recover replication-competent virus does not necessarily imply that latently infected cells are completely eradicated as the frequency of latently infected cells may simply fall below the detection limit of the assay employed [9]. Furthermore, partial reduction in the latent reservoir size may not be of significant clinical benefit [9, 66] because theoretically one infectious virion released from latently infected cells could reignite infection quickly. This notion is supported by the rapid HIV rebound to the pretreatment viral level when treatment is interrupted in patients with sustained viral suppression [84, 117, 199].

Recently, the shortest half-life of latently infected $CD4^+$ T cells (~ 4.6 months) has been reported by Chun et al. in patients who initiated antiviral therapy early after infection [25]. This raises the hope that < 10 years of continuous therapy would possibly eliminate latently infected cells in infected individuals who undergo early treatment. However, a close examination of the data shows that in at least 4 out of 7 patients the reservoir decay appears to exhibit a second, slower phase [116], which is consistent with the result that the latent pool has a heterogeneous and dynamic composition [185]. The possibility that HIV may persist in multiple cellular or anatomical reservoirs makes viral eradication even more difficult. Considering the treatment-associated side-effects and cost, whether initiating antiretroviral therapy early has clinical and immunological benefits awaits more long-term follow-up studies.

6.3 Treatment interruptions

Currently, lifelong treatment may be required for most HIV patients. However, lifelong antiretroviral regimens may be largely limited by toxicities, emergence of drug resistance, and cost. In an attempt to reduce drug exposure without compromising efficacy, investigators have proposed structured treatment interruptions (STI) (see reviews in [5, 59, 111]). In patients who initiate treatment during acute infection, STI that allows viral rebound is hypothesized to preserve or boost cellular immune responses. In patients who receive therapy in chronic infection with controlled viremia, STI may enhance immune responses as in the scenario of acute infection, and minimize drug-related adverse effects and cost. In patients with chronic treated infection but with virologic failure because of multi-drug resistance, STI has been proposed as a way of generating a reversion from resistant to wild-type virus, improving virological responses to subsequent (salvage) therapy. Mathematical models have also been developed to assess possible therapeutic outcomes of STI [1, 4, 99].

However, the available evidence does not support that STI is associated with better immunologic, virologic or clinical outcomes than continuous antiretroviral therapy, although short-term STI may have the potential to allow patients drug holidays in carefully controlled clinical trials [5]. Interruptions of

antiretroviral drugs have often been accompanied by a decline in $CD4^+$ T cell counts and an exponential growth of plasma HIV-1 RNA levels [49, 199], which is likely to repopulate HIV cellular reservoirs and sometimes can result in severe AIDS-related clinical events, particularly when treatment interruption and resumption cycles are not adequately controlled [113]. The intention that STI would restore drug sensitivity and thereby preserve subsequent treatment options in those patients who developed multi-drug resistance has been difficult to realize because drug resistant viral quasispecies can be archived in the latent reservoir and confer lifelong resistance [106, 151, 166]. In this regard, even if there is a shift from multiply-resistant to wild-type virus in some cohort studies during treatment interruptions [37, 125], drug resistant variants can reemerge when therapy is reinitiated. Therefore, failure to respond better to salvage therapy for those patients who initiated STI than patients who directly switched to salvage therapy is not surprising [106, 152]. Due to these interruption-associated risks, the use of STI has not been recommended outside the setting of rigorously controlled clinical trials with frequent monitoring and close follow-up.

6.4 Activation strategies

In an attempt to flush out the latent reservoir, immune stimulation with activating agents have been proposed. It was expected that reactivated infected cells would be vulnerable to eradication by viral cytopathic effects and host immune responses, and that *de novo* infection could be prevented by potent antiretroviral therapy. However, such activation strategies to date have shown only limited success. For example, the combination of proinflammatory cytokines interleukin-2 (IL-2), IL-6 and TNF-α was shown to increase the proliferation of resting $CD4^+$ T cells [189] and HIV-1 replication in latently infected $CD4^+$ cells [21]. Intermittent administration of only IL-2 with continuous HAART resulted in a greater reduction in the pool of resting $CD4^+$ T cells containing replication-competent HIV-1 compared with patients who received HAART alone [22]. However, when therapy was interrupted, high levels of viral RNA were observed in most patients [19, 33], suggesting that long-term suppressive HAART in combination with IL-2 did not eliminate HIV-1 infection. Besides the reservoir of latently infected $CD4^+$ T cells, viral rebound was believed to come from other unidentified reservoirs, such as tissue-bound macrophages [55].

The monocyte/macrophage activator IFN-γ along with IL-2 administered to HAART-treated patients during early infection was associated with a proviral DNA decrease and immune reconstitution but was still unable to induce HIV-1 remission [102]. When a broad-spectrum T cell stimulator OKT3 and IL-2 were added to the immune activation therapy, patients failed to achieve measurable purging of the cellular HIV reservoir despite apparent T cell activation and proliferation [156]. Furthermore, side-effects of high drug doses were serious and antibodies against OKT3 developed rapidly in all patients [156].

Some other activation strategies, such as HAART supplemented with a histone deacetylase (HDAC) inhibitor valproic acid [108] or the nontumorigenic phorbol ester prostratin [98, 100], have been proposed to purge the latent virus out of the reservoir. Although valproic acid induced latent viral expression from resting $CD4^+$ T cells in vitro [198] and a small clinical trial suggested a possible effect of valproic acid in accelerating clearance of HIV from resting $CD4^+$ T cells [108], its clinical effect on the decay of the latent reservoir was doubted by a later study [173]. Prostratin might be a promising activating agent that induces latent viral expression *in vivo* with minimum activation effects on the immune system [98, 100], but its effects await future clinical studies.

Several issues need to be kept in mind when developing activation strategies. First, global T cell activation has to be avoided since it would significantly increase the number of susceptible T cells and induce viral replication beyond the threshold that can be contained by current antiretroviral treatment.

Second, if latently infected cells can proliferate then activation of these cells may also induce the renewal of the latent reservoir, as suggested by models in Section 5.3. Third, partial reduction of the size of the latent reservoir might not be of clinical significance if activating agents in combination with potent antiretroviral drugs cannot lead to a complete eradication of all cellular/anatomical reservoirs for HIV-1.

6.5 Resistance testing

Despite great advances in the development of antiretroviral drugs and simultaneous administration of these agents, most patients develop some degree of resistance. The problem of resistance has been exacerbated by the fact that the latent reservoir can serve as a lifelong archive for all forms of viral strains that ever evolved and replicated [136, 175]. Recent studies have suggested that both the wild-type virus that circulated before the onset of HAART and different strains of drug-resistant variants that evolved during non-suppressive periods of therapy remain detectable even after a long period of successful HAART [103, 166, 190]. All of the virions in the latent reservoir have the potential to reemerge when therapy is stopped or selective drug pressure is exerted.

The detection of HIV drug resistance has been an important management tool recommended for newly diagnosed patients with acute or recent infection and patients who need to change therapy in case of treatment failure [54]. There are currently two basic types of resistance tests: genotypic and phenotypic tests. Genotypic resistance assays identify specific mutations in the virus that are associated with resistance to specific drugs, whereas phenotypic assays measure the ability of clinical isolates to reproduce in the presence of selected drugs relative to a wild-type reference virus. All currently available forms of resistance testing require a minimum viral load (about 500 to 1000 copies/mL) and they cannot detect either low-level minority populations of virus in a mixture (e.g., less than 20% to 30% of quasispecies) or resistant viral variants archived in viral reservoirs. Therefore, resistance testing only complements patient-specific factors such as drug access, safety and adherence, longitudinal viral load values, immunologic status and treatment history whenever a decision needs to be made about initiating a new drug or drug combination.

7 Conclusion

Both residual ongoing viral replication and virus released from viral reservoirs may contribute to the persistence of low-level viremia in patients during suppressive HAART. Viral evolution seems to be largely halted in patients who have optimal adherence and viral loads below the detection limit. Long-term control of viral replication is possible in these patients if drug toxicities can be overcome and patients adhere to the therapy. The remarkable stability of the latent reservoir appears to be maintained by the intrinsic stability of latently infected resting $CD4^+$ T cells and/or renewal due to factors such as residual ongoing replication, homeostatic proliferation and latent cell activation. Treatment intensification in combination with activating agents at the current time cannot eradicate the latent reservoir. Intermittent viral blips may represent enhanced viral replication or increased viral release from the latent reservoir due to occasional activation of target $CD4^+$ T cells or latently infected cells, or when of low amplitude just stand for normal biologic or statistic variations around the detection limit of current assays.

At present, many new antiviral drugs have been developed. Some of them may have an improved penetration ability and a higher barrier to drug resistance. Further knowledge of the persistence of all possible cellular and anatomical reservoirs is crucial in the management of HIV-1 infection. Greater efforts are being put on new activation strategies that purge latent virus from HIV-1 reservoirs. Math-

ematical models, in conjunction with experimental data, have helped evaluate the effectiveness of new drugs (and drug combinations), and as shown here, test the possible mechanisms of viral persistence, HIV-1 latency, reservoir stability and viral blips. They have also been used to explore why the immune system cannot induce long-term viral control, and provided insights into the slow depletion of $CD4^+$ T cells, viral evolution and disease progression. We believe that mathematical modeling would provide more information for future research of HIV-1 treatment.

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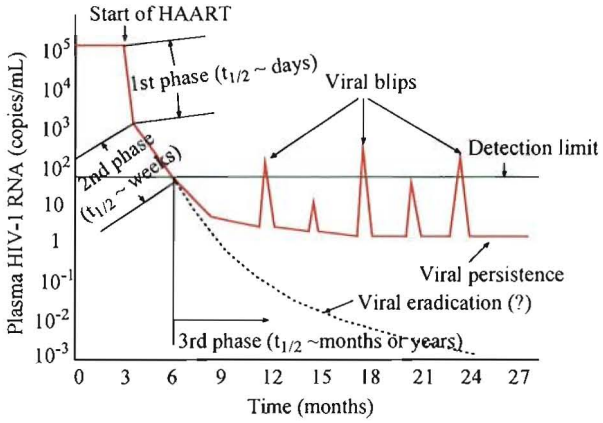

Figure 1: The plasma viral load remains at a relatively constant level during chronic infection before initiation of HAART. Following treatment the plasma RNA level undergoes a multiphasic decay and declines to below 50 copies/mL after $3-6$ months. However, virus cannot be eradicated with current antiretroviral therapy. A low level of viremia persists in patients with apparently suppressive treatment for many years. A number of patients experience intermittent viral blips, with transient HIV-1 RNA levels above the detection limit.

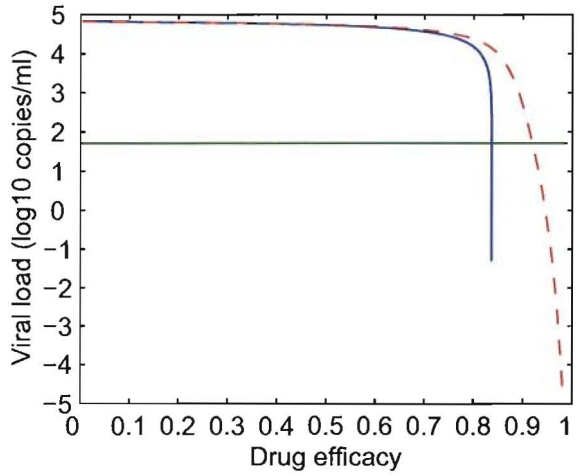

Figure 2: The steady state viral load vs drug efficacy for both the basic model (Eq. (7), solid line) and the model with density-dependent infected cell death (Eq. (10), dashed line). The values of parameters are [14]: $\lambda = 10^4$ mL^{-1} day^{-1}, $k = 8 \times 10^{-7}$ mL day^{-1}, $c = 13$ day^{-1}, $\delta = 0.7$ day^{-1}, $N = 100$, $d = 0.01$ day^{-1}, $\omega = 0.1$, $p_v = 70$ day^{-1}. δ' is chosen to be 0.274 day^{-1}(mL/cell)$^{\omega}$ such that both models have the same initial viral load when $\epsilon = 0$. The basic model is much more sensitive to changes in drug efficacy, particularly when ϵ is close to the critical drug threshold (~ 0.85). The green horizontal line represents the detection limit.

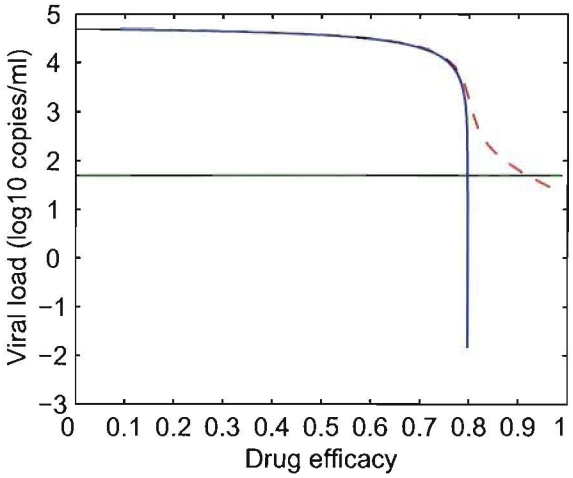

Figure 3: The steady state viral load vs drug efficacy for the two-compartment model (Eq. (11), dashed line) and its corresponding one-compartment model (solid line). The values of parameters are [14]: $f = 0.45$, $\varphi = 0.195$, $\mu = 0.07$ day^{-1}, $N_T = 100$, $N_C = 4.11$, $D_1 = 0.1048$ day^{-1}, $D_2 = 19.66$ day^{-1}. The other parameter values are the same as in Fig. 2. The two-compartment model is less sensitive to changes in drug efficacy than the one-compartment model. The green horizontal line represents the detection limit.

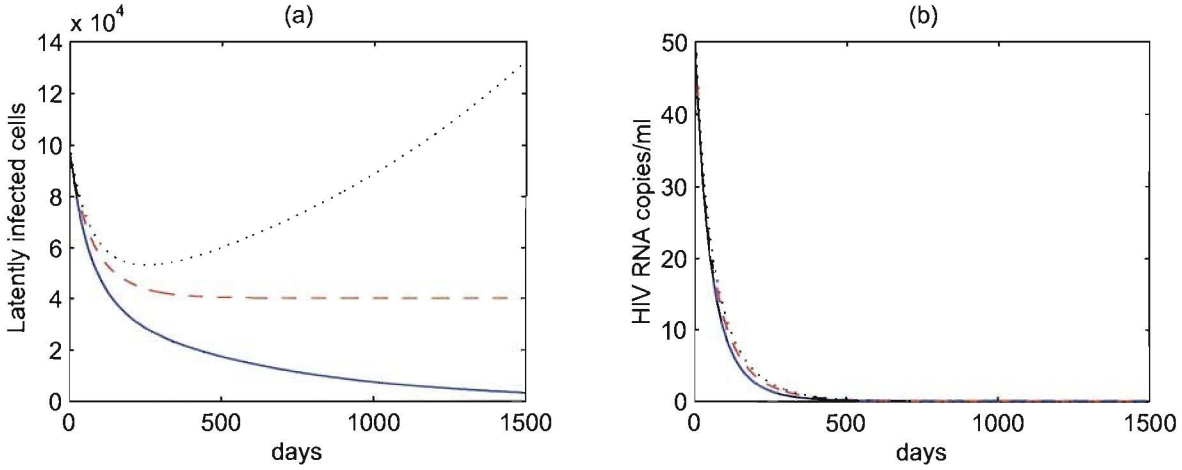

Figure 4: Simulations of Eq. (17) with $a_{min} = 0$ and $\epsilon = 1$. The net proliferation rate r varies: $r = -0.00171$ day^{-1} (blue solid line); $r = 0$ (red dashed line); $r = 0.0008$ day^{-1} (black dotted line). The other parameter values are [94]: $\omega = 9.39 \times 10^{-3}$ day^{-1}, $\eta = 3 \times 10^{-6}$, $k = 2.4 \times 10^{-8}$ mL day^{-1}, $T_0 = 595$ cells/μl, $N = 2 \times 10^4$, $\delta = 1$ day^{-1}, $c = 23$ day^{-1}. (a) The pool size of latently infected cells increases as $r > 0$, decreases as $r < 0$, and stabilizes to a steady state as $r = 0$. (b) In each case, the viral load decreases to zero. Thus, low-level viremic persistence cannot be generated by Eq. (17) with $a_{min} = 0$ and $\epsilon = 1$.

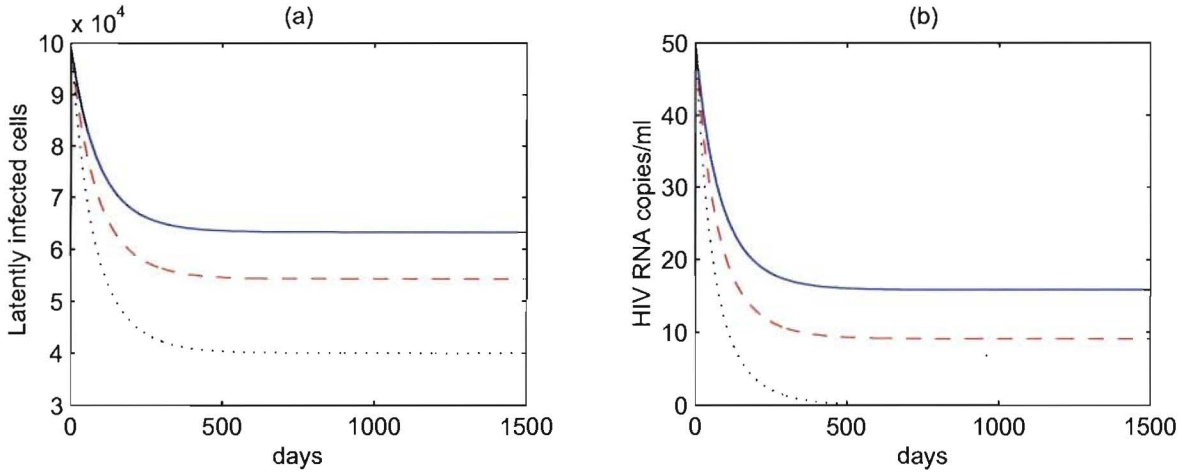

Figure 5: Simulations of Eq. (17) with $a_{min} \geq 0$ and $\epsilon = 1$. The value of r varies: $r = a_{min} = a_0/2$ (blue solid line); $r = a_{min} = a_0/3$ (red dashed line); $r = a_{min} = 0$ (black dotted line), where $a_0 = 8.625 \times 10^{-3}$ day^{-1} is the initial activation rate of latently infected cells. The other parameter values are the same as in Fig. 4. Both the viral and latent reservoir persistence can be obtained when $r = a_{min} > 0$. The larger the value of r, the higher the levels of latently infected cells and viral load.

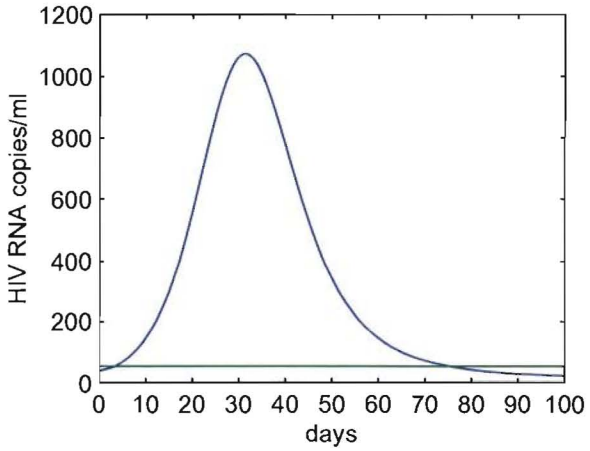

Figure 6: Simulation of the target cell activation model (Eq. (22)). The model can generate a transient viral load increase. The parameter values are [87]: $\lambda_1 = 10^4$ mL^{-1} day^{-1}, $\lambda_2 = 56$ mL^{-1} day^{-1}, $k_1 = 8 \times 10^{-7}$ mL day^{-1}, $k_2 = 10^{-4}$ mL day^{-1}, $\epsilon = 0.9$, $d_T = 0.01$ day^{-1}, $f = 0.6$, $\varphi = 0.195$, $\delta = 0.7$ day^{-1}, $\mu = 0.07$ day^{-1}, $N_T = 100$, $N_C = 4.11$, $c = 23$ day^{-1}, $a = 0.2$ day^{-1}, $K_4 = 1000$ mL^{-1}, $r_0 = 2$ day^{-1}, $A_{max} = 10^8$ mL^{-1}, $\gamma = 10^{-3}$ day^{-1}, $d_0 = 0.01$ day^{-1}, $p = 2.92$ day^{-1}, $d = 0.1$ day^{-1}, $d_E = 0.325$ day^{-1}, $p_0 = 1$ day^{-1}, $n = 1$, $K_8 = 1000$ mL^{-1}. The green horizontal line represents the detection limit.

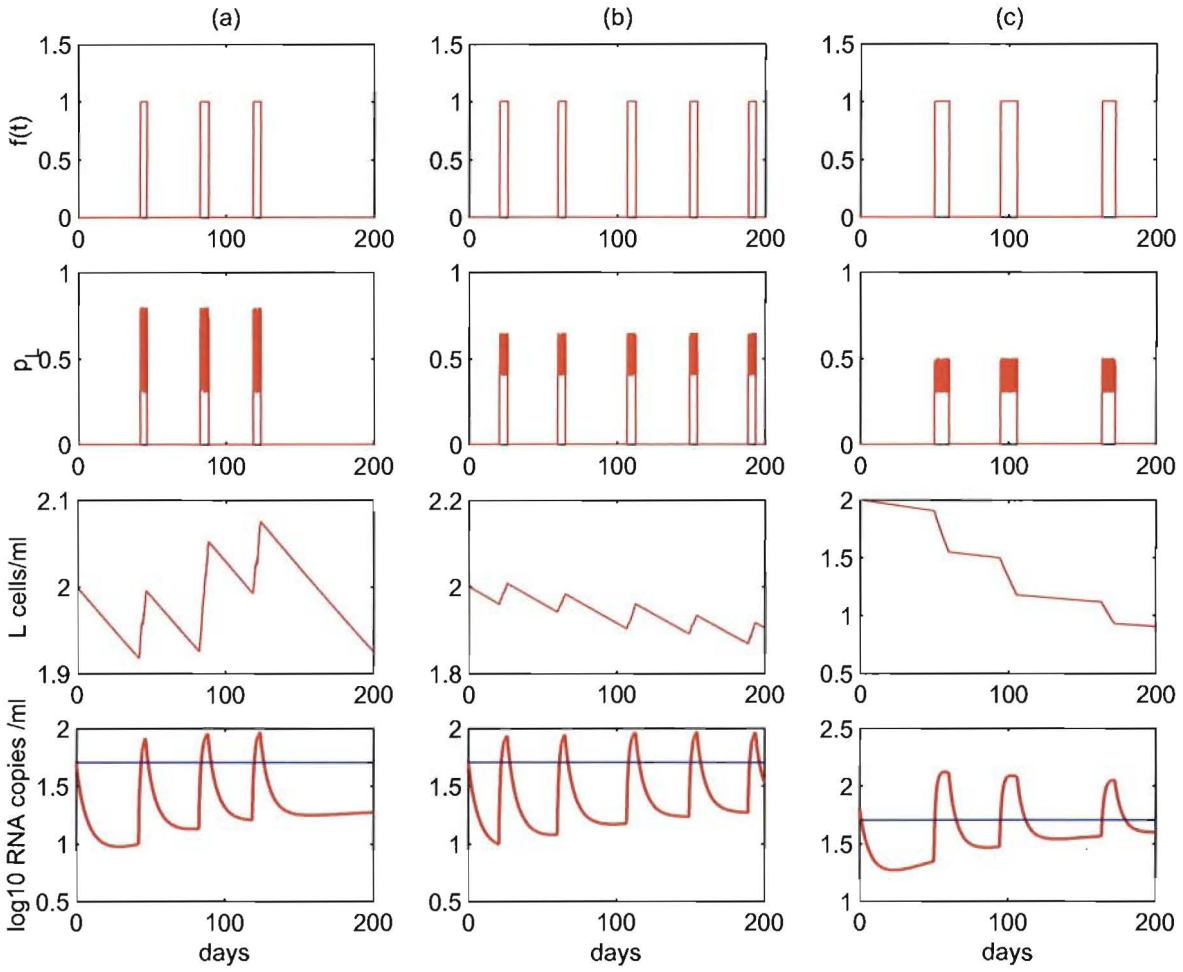

Figure 7: Simulations of Eq. (25) show that it is able to generate intermittent viral blips. (a) The interval between two consecutive activations, ΔT, obeys a normal distribution $N(50, 10)$. The duration each activation lasts, Δt, obeys a uniform distribution $U(4, 6)$. The probability p_L obeys a uniform distribution $U(0.3, 0.8)$. The decay rate of the latent reservoir is not significantly different from zero. (b) $\Delta T \sim N(40, 10)$, $\Delta t \sim U(4, 6)$, $p_L \sim U(0.4, 0.65)$. The latent cell pool size decreases, but the decay rate is very small, representing a very long half-life, for example, 44 months. (c) $\Delta T \sim N(50, 10)$, $\Delta t \sim U(7, 14)$, $p_L \sim U(0.3, 0.5)$. The latent reservoir shows a quicker decay than in the scenario (b), corresponding to a shorter half-life, such as 6 months. The other parameter values used in the simulations are [164]: $\lambda = 10^4$ mL^{-1} day^{-1}, $d_T = 0.01$ day^{-1}, $\epsilon = 0.85$, $k = 2.4 \times 10^{-8}$ mL day^{-1}, $\eta = 0.001$, $d_0 = 0.001$ day^{-1}, $a_L = 0.1$ day^{-1}, $\delta' = 0.7863$ day^{-1}(mL/cell)$^{\omega}$, $\omega = 0.44$, $p_v = 2000$ day^{-1}, $c = 23$ day^{-1}.

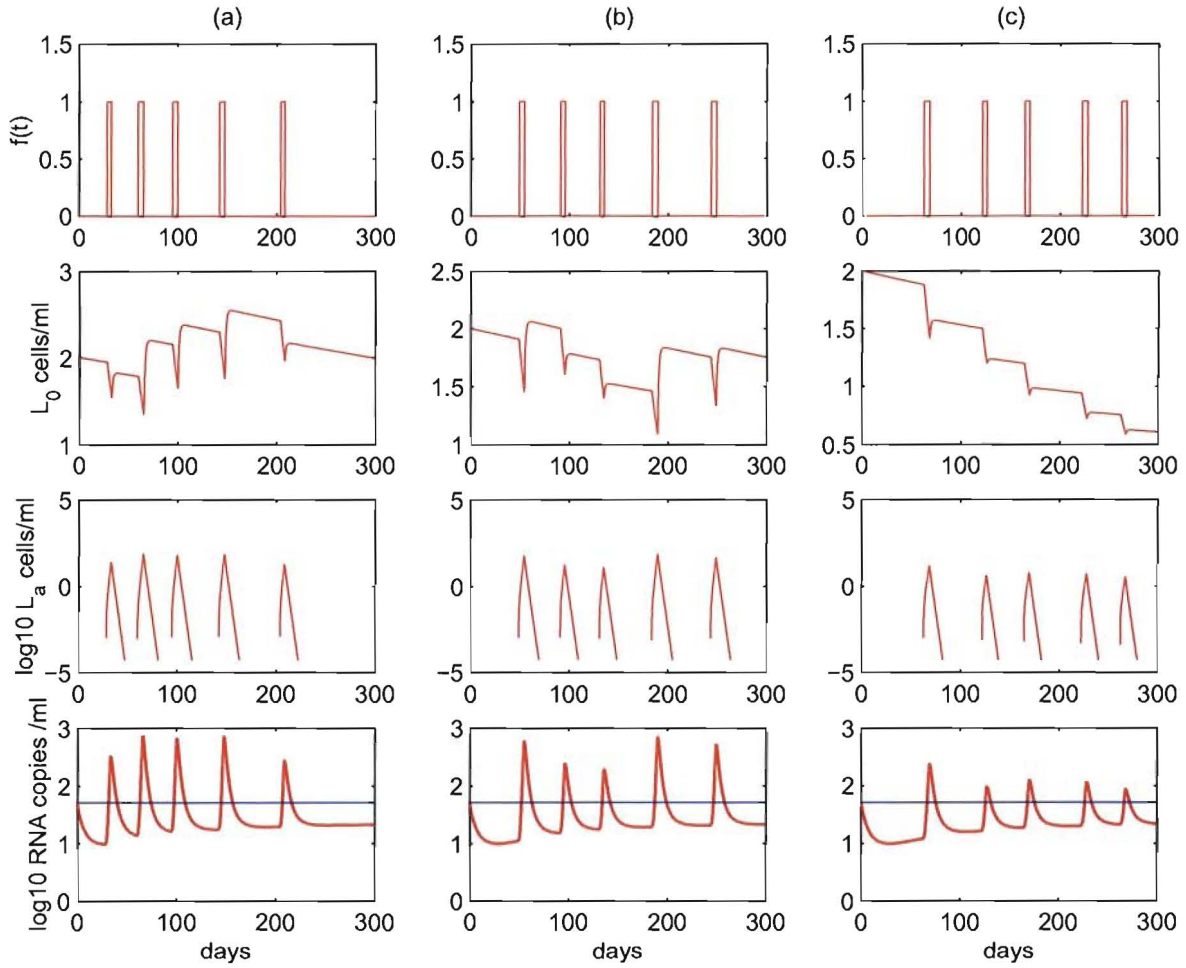

Figure 8: Simulations of Eq. (28) show that it can robustly generate viral blips. The decay of the latent reservoir is primarily determined by p, which represents the potential of resulting activated cells to proliferate during the latently infected cell response. (a) $p = 1.4$ day^{-1}. No statistically significant decay of latently infected cells was observed. (b) $p = 1.35$ day^{-1}. The latent reservoir decays very slowly. This realization shows a half-life of ~ 44 months. (c) $p = 1$ day^{-1}. The latent reservoir decays more quickly than in (b), representing a half-life of about 6 months. The parameter values used are [165]: $\Delta T \sim$ N(50, 10), $\Delta t \sim$ U(4, 6), $a = 0.05$ day^{-1}, $\sigma = 0.8$ day^{-1}, $\rho = 0.01$ day^{-1}. The other parameter values are the same as in Fig. 7.

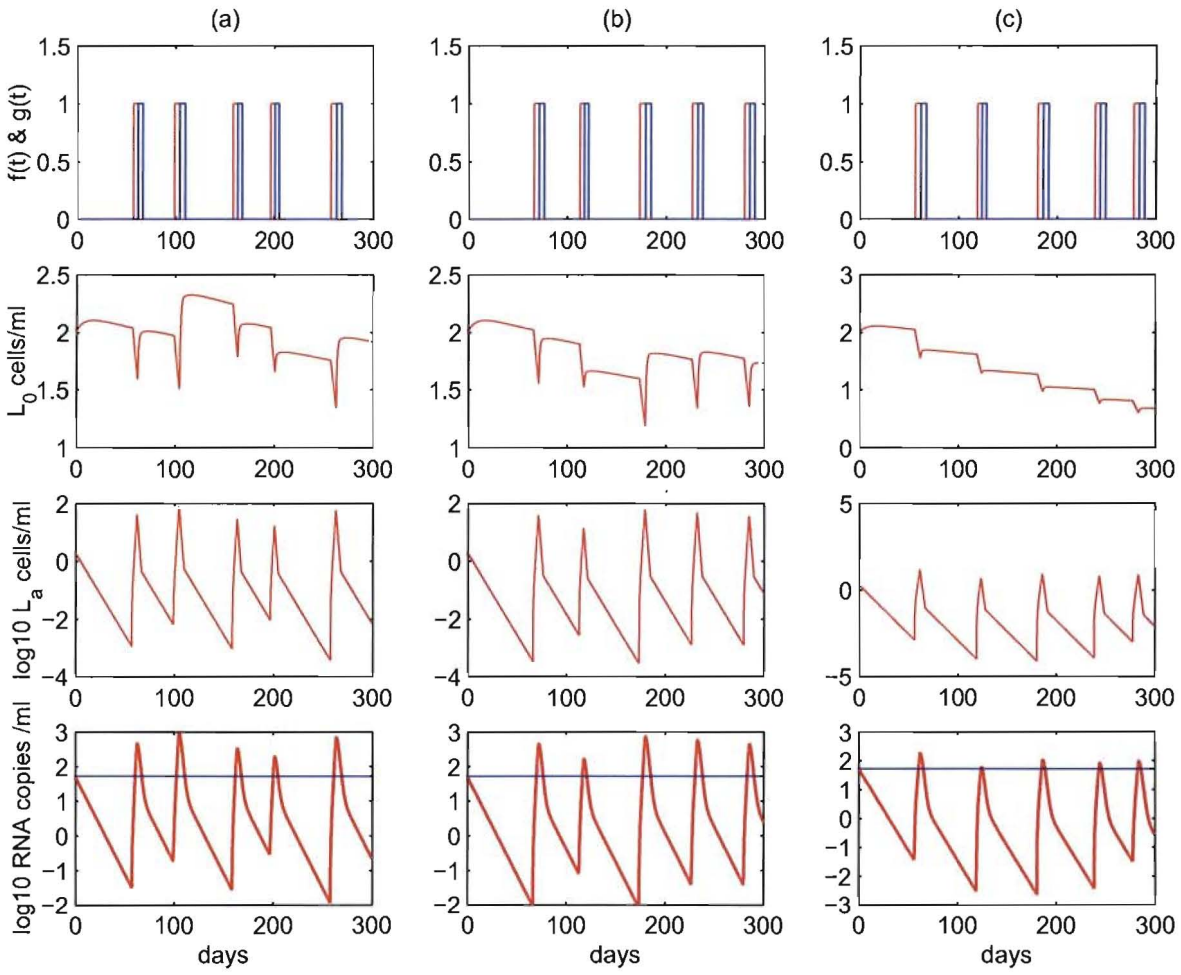

Figure 9: Simulations of Eq. (29) show that it can generate both intermittent viral blips and low-level persistent viremia. As in Fig. 8, different proliferation rates, i.e., (a) $p = 1.4$ day^{-1}, (b) $p = 1.35$ day^{-1}, and (c) $p = 1$ day^{-1}, result in different decay characteristics of the latent reservoir. The death rate δ_a is 0.02 day^{-1}. The rapid contraction phase has the same duration as the expansion phase. The other parameter values are the same as in Fig. 8.