REVIEW

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Dynamic models of viral replication and latency

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Purpose of review
HIV targets primary CD4$^+$ T cells. The virus depends on the physiological state of its target cells for efficient replication, and, in turn, viral infection perturbs the cellular state significantly. Identifying the virus–host interactions that drive these dynamic changes is important for a better understanding of viral pathogenesis and persistence. The present review focuses on experimental and computational approaches to study the dynamics of viral replication and latency.

Recent findings
It was recently shown that only a fraction of the inducible latently infected reservoirs are successfully induced upon stimulation in ex-vivo models while additional rounds of stimulation make allowance for reactivation of more latently infected cells. This highlights the potential role of treatment duration and timing as important factors for successful reactivation of latently infected cells. The dynamics of HIV productive infection and latency have been investigated using transcriptome and proteome data. The cellular activation state has shown to be a major determinant of viral reactivation success. Mathematical models of latency have been used to explore the dynamics of the latent viral reservoir decay.

Summary
Timing is an important component of biological interactions. Temporal analyses covering aspects of viral life cycle are essential for gathering a comprehensive picture of HIV interaction with the host cell and untangling the complexity of latency. Understanding the dynamic changes tipping the balance between success and failure of HIV particle production might be key to eradicate the viral reservoir.

Keywords
cure, dynamic model, HIV, latency, transcriptome dynamics

INTRODUCTION

Current antiretroviral therapy (ART) is successful in inhibiting viral replication (defined as undetectable plasma viremia using standard assays, i.e., below 20–50 copies/ml) and transmission, but fails to completely eliminate HIV. Indeed, the presence of continuous low-level viremia (detectable using ultrasensitive assays) or occasional viral blips under ART and the observation that viremia rebounds rapidly upon ART cessation indicate the existence of viral reservoirs [1]. Viral reservoirs are established early, during the first 3 days upon viral exposure [2▪,3,4]. Their exact nature has yet to be completely understood, but includes anatomical sanctuaries and cellular reservoirs. Although still controversial, anatomical sanctuaries are described as sites with incomplete ART penetration, where infected cells may reside with continuous low levels of viral replication and may include lymph nodes, gut-associated lymphoid tissue, and the central nervous system [5–8]. Cellular reservoirs are described as being latently infected cells, i.e. infected cells that do not produce replication-competent virions and include mostly long-lived resting memory CD4$^+$ T cells [8–10]. The current dogma is that the major HIV reservoir originates from activated CD4$^+$ T cells that have been infected and survive while reverting to a resting state, thereby becoming a memory cell. Because of the intrinsic physiology of resting memory cells, no infectious virions are produced, hence the concept of latent infection. Bursts of viral production, however, may occur from time to time as manifested by viral blips. Although the cellular mechanisms contributing to sudden induction of viral blips in otherwise aviremic individuals are yet

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KEY POINTS

- Viral processes (replication, latency, and reactivation from latency) are dependent on the cellular composition – and thus on the cell type/subset, cell state (resting or dividing), and nature of latency (transcriptional or posttranscriptional block) – and on the extracellular environment (type of stimuli and exposure time) (Fig. 1).
- Time series experiments are useful in resolving the causes and effects of virus–host interactions.
- Dynamic models give insight into time-dependent changes that take place in the host cell environment and thus into viral reproductive success.

poorly defined, viral production and viral latency appear to be intertwined and closely linked to cell physiology and activation (Fig. 1).

Viral infection and replication are successful in activated $CD4^+$ T cells, while poorly efficient in resting $CD4^+$ T cells (Fig. 1a). The timing of the infection event might impact on the type of post-integration block in the latent cell (Fig. 1a and b). Therefore, viral reactivation from latency might need different types of stimuli to reverse several types of latency [11▪,12▪▪]. Furthermore, different types of latent cells may need to be exposed for different time lengths to successfully trigger a productive infection (Fig. 1c). Finally, the efficiency of viral production or the type of latency can impact the kinetics of cell death and thus the elimination of the reservoir of latently infected cells (Fig. 1d). An additional layer of complexity may reside in the possibility of having multiple blocks within the same cells, which would require multiple stimuli with specific time schedules of administration to be reactivated. In conclusion, the dynamics of establishment of a successful infection and production, including the type of cell stimulation, the time required for stimulation, the stimulated subset of cells, and the type of block, might be key for understanding viral latency and for the development of appropriate strategies aiming at purging viral reservoirs and eradicating HIV.

THE OBSTACLE OF THE INDUCIBLE RESERVOIR

Initial studies measuring the size of the latent viral reservoir aimed at quantifying the number of circulating latently infected cells in the plasma. For this purpose, the gold standard is the viral outgrowth assay: resting $CD4^+$ T cells are collected from patients, serially diluted, and activated with phytohemagglutin/interleukin 2 (IL-2) and 10× excess of irradiated peripheral blood mononuclear cells. $CD4^+$ lymphoblasts (from a healthy HIV donor) are added the next day (day 2) and at days 7 and 14, and viral production is measured by p24 ELISA at day 21 [13▪,14]. Using this assay, 1 of 10^6 cells was reported to produce infectious particles and thus considered to be latently infected [13▪]. Interestingly, using PCR to detect proviral DNA, 1000 of 10^6 cells were shown to be infected by HIV [13▪]. Recent findings [15] showed that 88.3% of infected cells carry defective copies of HIV DNA and are thus not able to produce infectious and replication-competent viral particles. The remaining infected cells (11.7%) carry intact viral DNA and are potentially able to be induced (hence referred to as the inducible viral reservoir) and produce infectious particles, suggesting that the size of the viral reservoir is on the order of 60 latently infected per 1 million cells. Only a fraction of these inducible cells, however, is efficiently stimulated *in vitro* using the standard viral outgrowth assay. An additional round of cell stimulation allows inducing viral production from additional cells, but not all, suggesting that activation conditions, time, and stochasticity play a role in successful induction of viral production from latently infected cells [15]. Similar observations were reported upon stimulation of resting $CD4^+$ T cells using CD3/CD28 antibodies for 7 days in the presence of efavirenz to block viral replication and measuring cell-associated and supernatant viral RNA by reverse transcription followed by quantitative polymerase chain reaction (RT-qPCR) [16▪▪,17▪▪]. Under these conditions, cell-associated viral RNA was detected in 7.5% of latently infected cells, while only 1.5% of cells were able to be induced and produce viral particles (as detected by viral RNA in the supernatant), confirming previous observations [15]. Of note, spontaneous virion production in the absence of activation was detected in 0.041% of latently infected cells.

These recent findings highlight the current gap between the experimentally induced reservoir and the total inducible reservoir, and demonstrate that the type and time of stimulation affect the size of the induced reservoir. This underlines the need for new experiments that investigate the stimulation dynamics leading to maximal viral reactivation, so that the total inducible reservoir is *de facto* induced.

DYNAMIC MODELS

Numerous modeling approaches have been applied to facilitate understanding of various aspects of HIV biology [18–20]. Here, we consider two major categories of modeling approaches applied to the

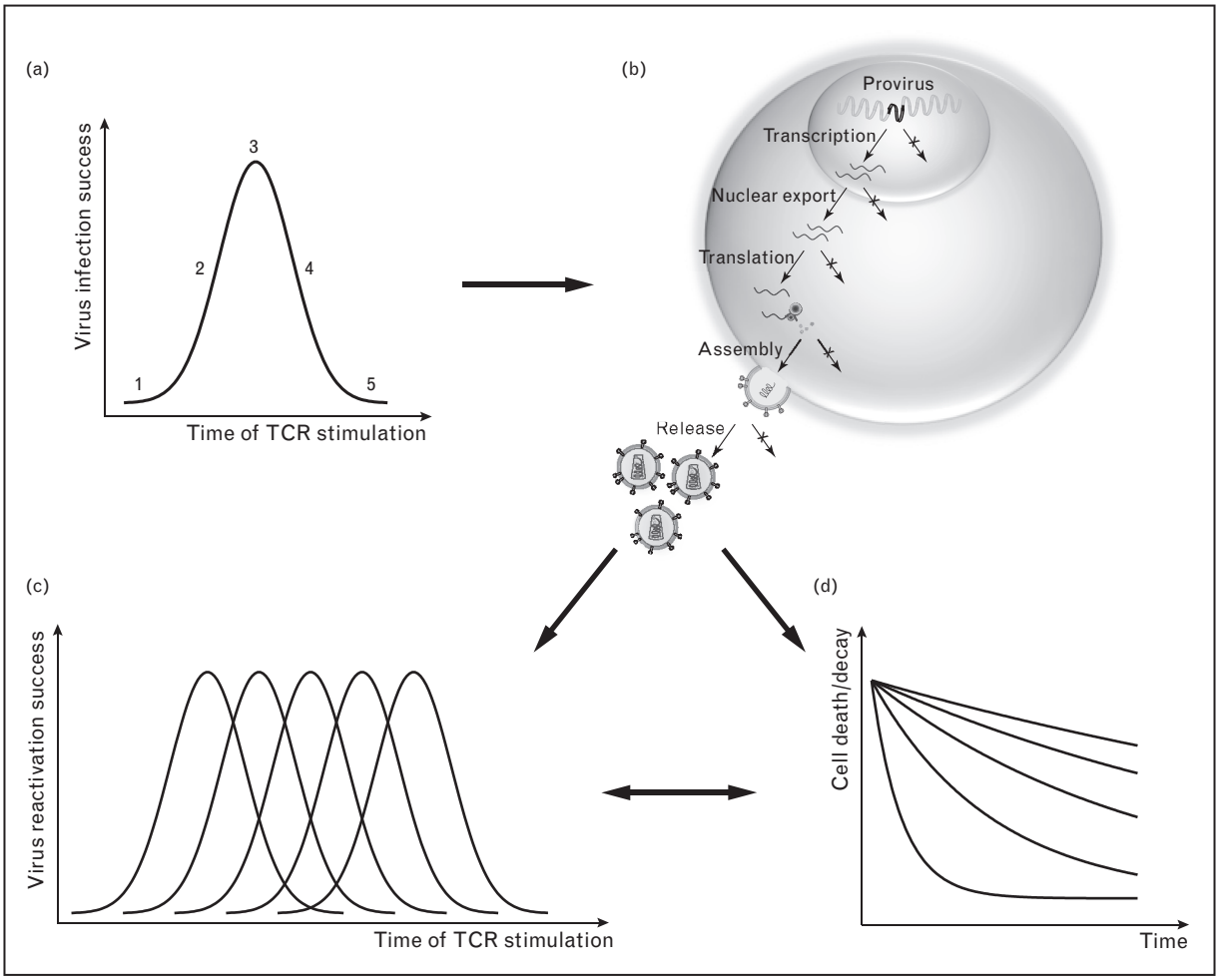

FIGURE 1. The possible relationship between cell physiology varying over time, viral production (infection success, induction from latency), and cell death. (a) Viral production success upon infection of the host cell *in vitro* depends on the time after T-cell receptor (TCR) stimulation. Numbering 1–5 reflects the continuum of different physiological states of the cell after TCR stimulation, which associate with different infection successes, i.e. a highly successful peak of infection (phase 3) or inefficient infection (phase 1 and 5). (b) Viral latency is characterized by the lack of successful viral production. Latency, however, is multifactorial, including many possible blocks (crossed arrows), such as transcription, nuclear export, translation, assembly, and release. (c) Each specific latency block may associate with one specific reactivation kinetic. Hence, exposure to TCR stimulation (or other types of stimuli) may induce viral production (i.e., reactivate cell production from latently infected cells) with different kinetics depending on the nature of latency. (d) Success of viral production and viral reactivation impact the kinetics of cell death.

study of the dynamics of viral replication and latency: mechanistic models that incorporate detailed biological knowledge into systems of differential equations in order to explain the dynamic behavior of biological systems, and descriptive statistical models, on the basis of large datasets obtained from genome-wide transcriptomic and proteomic measurements that explore patterns of similarity.

Mechanistic models

Mathematical analysis of HIV infection has traditionally been performed using mechanistic models. Early pioneering mechanistic models provided insight into the pathogenesis and treatment of HIV infection. These models present a bottom-up approach to mechanistically describe complex kinetic patterns observed in HIV infection. Prominent examples include the complex dynamics of viremia during the course of untreated HIV infection and the multiphasic decline of viral load under effective treatment [18]. Early models of HIV infection estimated the average half-life of productively HIV-infected cells to be approximately 1 day. A recent study by Petravic *et al.* suggests that this average life span poorly represents reality as individual infected cells may die within a few hours to a few days. The authors [21] observed that the rate of infected cell

death decreased over time and was not correlated with viral protein production. This observation may impact the so-called 'shock and kill' strategy that aims at reactivating viral protein expression in latently infected cells, thereby mediating virus-induced cytotoxicity to kill the infected cells [22–25].

Mechanistic models have previously been developed to provide insight into the long-term dynamics of viral latency, including degradation of the virus, viral blips in virologically suppressed patients, reactivation of quiescent infection, and time needed to eliminate HIV under ART [19]. Immonen and Leitner used a joint phylogenetic and differential equation approach to model the evolutionary divergence of the virus taken from plasma and the latently infected cells from 26 patients. They [26] observed an overdispersion of evolutionary divergence relative to the molecular clock model, suggesting that a major fraction of infected cells have experienced periods of latency at some point in the past. The stability of the latent reservoir and the emergence of viral blips could be explained by stochastic expression of HIV [27,28▪]. Hill *et al.* [28▪] used a stochastic model to describe viral rebound after ART interruption and to define a quantitative goal for latency reactivation approaches. The authors conclude that a 10 000-fold decrease in the latent reservoir may be necessary to reach permanent viral remission in half of all individuals. Petravic *et al.* [29] devised a mathematical model to study the efficacy of antilatency drugs under different strategies and suggested that antilatency treatment should be administered early upon initiation of ART to achieve optimal outcomes. A recent study by Althaus *et al.* used longitudinal data from five chronically infected HIV patients to model the dynamics of different types of HIV-infected cells defined by the splicing patterns of the viral transcripts. In line with the study of Petravic *et al.*, their model suggests that the reservoir is smaller during acute infection; thus, eradication strategies should be started early on in combination with ART [30].

Statistical models

New transcriptomics and proteomics technologies have enabled the collection of large-scale snapshots of the cellular state that can provide a holistic view of the cellular changes occurring upon viral infection at the cell population level. Repeated high-throughput measurements carried out in longitudinal experiments make allowance for a top-down analysis of the dynamics of the cell. The information provided by such time series experiments facilitates distinguishing causes and consequences observed upon viral infection, viral latency, and reactivation from latency. Permissive T-cell lines have been used to investigate transcriptional reprogramming of the host cell [31–34]. Mohammadi *et al.* [34] analyzed the joint virus–host transcriptome upon HIV infection with high temporal resolution over a 24 h period. A total of 73% of the expressed genes in the host cells were found to be regulated in concordance with the major viral replication steps, namely, reverse transcription, integration, and a late phase that spans from transcription to the release of new viral particles. The longitudinal design of the experiment and the mathematical analysis of the paired measurements of the viral life cycle intermediates and host transcriptome showed that the early regulated genes were likely due to response to the incoming virus. Mohammadi *et al.* observed a massive early downregulation probably reflecting a host cell response to viral presence. In contrast, late regulated genes are more likely to be regulated by newly produced viral proteins. These findings are consistent with a recent study that investigated phosphorylation changes upon HIV exposure [35▪]. Using infection with X4-tropic virus in primary resting $CD4^+$ T cells, the authors identified rapid changes of 239 phosphorylation sites from 175 genes, some as early as 1 min after exposure that may prepare the cell for successful viral replication. This finding is also in agreement with another proteomic study that found host-induced early posttranslational modifications of histones in response to the HIV infection occurring as early as 4 h after exposure [36]. Similar patterns of early regulation were observed in microRNAs (miRNAs) [32]. Peng *et al.* [37] performed a joint analysis of transcriptome by mRNA sequencing and total RNA sequencing at 12 and 24 h after infection in a T-cell model. Their results suggest that a total RNA sequencing assay, quantifying also nascent and nonmature transcripts, may detect transcriptome changes earlier than mRNA sequencing.

Mohammadi *et al.* [12▪▪] investigated the transcriptome of the infected cells establishing and exiting latency. Resting $CD4^+$ T cells were activated, infected with an HIV-based vector, and allowed to revert to a resting cellular state during 10 weeks using a feeder cell layer. Cells were then stimulated with various latency reactivating agents or with CD3/CD28/IL-2 for 8 or 24 h. This study demonstrated stable persistence of viral transcripts in latently infected cells over time, suggesting that in this system, the latently infected cells failed to produce viral particles because of posttranscriptional rather than transcriptional blocks. The analysis highlighted the biological state of the host cell, i.e. resting versus activated, as a major driver of differences between latent and productive infection [12▪▪,38▪]. Additional time series analyses focusing on the reactivation of cells will help to identify the

key determinants driving the cascade of regulatory events leading to successful induction of viral production in latently infected cells.

CONCLUSION

Mathematical models have proven beneficial to make long-term predictions about the viral and cellular behaviors. They help defining strategies aiming at viral eradication. These predictions include estimation of the ART duration required to eliminate the viral reservoir and the critical size of the viral reservoir for sustained viral remission.

Large-scale temporal analyses have been used to describe HIV replication at the cell population level. In the future, integrative analyses involving collections of paired transcriptomic and proteomic datasets should produce a more comprehensive picture of the sequence of events occurring upon cell exposure to the virus, latency establishment, and reactivation from latency. These analyses may benefit from recent technological developments, such as single-cell technologies to measure the state of multiple individual cells. Single-cell approaches should allow a better assessment of stochastic expression of latent HIV, the impact of latency reactivating agents, and the optimal conditions (treatment type and exposure time) required to induce viral production from different types of latently infected cells. Repeated analysis of the transcriptome or proteome of a single cell over time is currently not feasible. Combination of population and single-cell analysis over time, however, might help identifying and dissecting the multiple types of latent cells.

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Conflicts of interest

There are no conflicts of interest.

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