

RESEARCH ARTICLE

Simulating the entire natural course of HIV infection by extending the basic viral dynamics equations to include declining viral clearance

Janka Petravac* and David P. Wilson

Burnet Institute, 85 Commercial Rd, Melbourne, VIC 3004, Australia

*Corresponding author: Burnet Institute, 85 Commercial Rd, Melbourne, VIC 3004, Australia. Tel: +61 439586834; E-mail: janka.petravac@gmail.com**One sentence summary:** The authors describe a simple coupling of free virus clearance to viral load in the basic model of viral dynamics that allows for the simulation of the whole course of untreated HIV infection.

Editor: Olivier Restif

ABSTRACT

The basic model of viral dynamics is a relatively simple set of equations describing the most essential features of the host–pathogen interactions. Coupled with data, it has been used extensively and successfully to reproduce and explain the features of the early acute phase of HIV infection and the effects of antiretroviral treatment, as well as to estimate the lifespan of infected cells, viral growth and clearance rates and predict early outcomes under different circumstances. However, it cannot reproduce the entire natural course of untreated HIV infection consistently with constant parameters. Here we show that it is possible to qualitatively reproduce the whole course of untreated HIV infection within the general framework of the basic model by assuming progressively declining viral clearance coupled with viral load. We discuss the interpretation of this model as proof-of-concept that may inspire further research into the role of viral clearance in HIV infection.

Keywords: HIV infection; mathematical models; free virus clearance

INTRODUCTION

Approximately 37 million people were living with human immunodeficiency virus (HIV) infection in 2017 (UNAIDS Global HIV and AIDS Statistics 2018), and it remains a major cause of morbidity and mortality worldwide. In the last 35 years, a huge amount of basic and applied research has been conducted with the aim of understanding the progression of the disease within an infected individual, how to control and reverse disease progression, and understanding the spread and control of the epidemic at the population level. Significant progress has been made in development of successful antiretroviral therapies (ART) to control the virus, allowing people living with HIV to reach a near-normal life expectancy and prevent onward transmission to others. However, drug treatment needs to be lifelong

and the best outcome of treatment is a chronic infection requiring regular clinical management to maintain successful virus suppression.

Much of the success in controlling disease due to HIV and its transmission has been due to the unprecedented amount of data collected from a large number of experimental and clinical studies, from the molecular level to host pathogenesis and immune response and transmission in the population. Mathematical modelling has played an essential role in understanding and interpreting these data. The analyses of the decay of viral load under ART provided the first insights into viral dynamics (Ho *et al.* 1995; Wei *et al.* 1995), such as the extent of viral production, estimates of the turnover of infected cells, rates of cell infection and free virus clearance. Later studies addressed HIV mutation

Received: 3 November 2018; Accepted: 8 August 2019

© FEMS 2019. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

and explained mechanisms of viral evolution and immune evasion (Althaus and de Boer 2008; Davenport et al. 2008), as well as explained the main mechanisms driving immunological escape (Balamurali et al. 2010; Elemans, Seich Al Basatena and Asquith 2012). More recent modelling work has estimated the dynamics of latently infected cells and provided insights around the action of drugs proposed as latency-reversing agents (Hill et al. 2014; Petracic et al. 2017).

Despite the empirical research advances, supported with quantitative models, and the undeniably world-changing impact of translational clinical successes, there remains much to be understood about the nature and pathogenesis of HIV infection. One of the dynamic features of untreated infection that is still not well understood is the CD4 + T cell decay in the chronic phase accompanied by a slow increase in viral load, the main cause of which is still under debate. Opinions are mostly divided among the ‘tap and drain’ (Ho et al. 1995) hypothesis, the immune activation hypothesis (Grossman et al. 2002; Yates et al. 2007), and hypotheses that assume a waning immune response associated with CD4 + T cell decay, or blame viral evolution (Ribeiro et al. 2006). Another unsolved puzzle that has evaded a plausible explanation is associated with the final stages of the AIDS phase, when CD4 + T cells (both uninfected and infected cells that can produce virus) are scarce, but viral load often exceeds the peak it reached in the acute phase, when there were large numbers of host cells in which to generate more virus (Pantaleo, Graziosi and Fauci 1993). Mathematical models have demonstrated that activation theories can partially explain the decay in total CD4 + T cells, but they cannot reproduce the final sharp increase in viral load at very low CD4 + cell count. The only notable exception, to our knowledge, is a viral evolution model that includes a ‘switch’ between the strain of virus that can infect only a subset of CD4 + T cells to the strain that can infect the whole CD4 + T cell population (Ribeiro et al. 2006), an effect which is not observed generally. The basic models of viral dynamics have been extremely useful in providing insights into the early acute HIV infection, yet they have not been able to reproduce the entire natural history of untreated HIV disease.

In this paper we investigate how to make minimal modifications to the basic model that could qualitatively reproduce the whole course of untreated HIV infection. Our particular interest is in being able to reproduce the signature of the final AIDS stage of disease: dramatic rise of viral load with very low frequency of infected cells and cells susceptible to virus. While the possibility of changes of different factors in the disease dynamics during the course of infection have been discussed previously without achieving this goal, the changes in viral clearance have not yet been considered. Here we present a proof-of-concept model based on a hypothesis that viral clearance may progressively decline during the course of untreated HIV infection due to the coupling with free virus, and show that such a mathematical model can qualitatively account for the whole course of HIV infection. We discuss what could cause such changes in virus clearance and how they could in principle be detected experimentally.

THE NATURAL COURSE OF HIV INFECTION

HIV disease involves infection and progressive loss of the memory CD4 + ‘helper’ T lymphocytes preferentially expressing the CCR5 co-receptor of activated effector-memory phenotype, and preferentially during division (Douek, Picker and Koup 2003). Further preference seems to be for HIV-specific CD4 + T cells (Douek et al. 2002), but this may be because they are the most

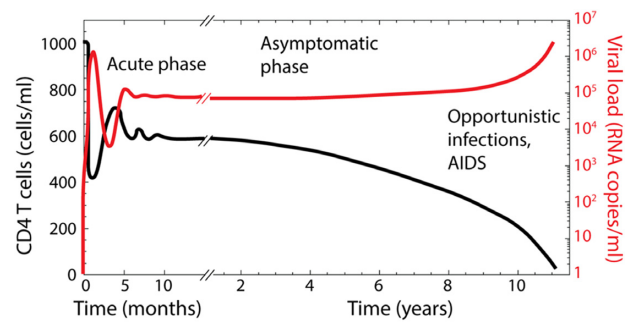


Figure 1. A typical course of untreated HIV infection.

activated. In the initial acute phase of infection, virus spreads through the CD4 + T cell population, causing their depletion in blood and mucosal tissues (Lim et al. 1993), especially gut (Brenchley et al. 2004), and a high peak viral load (Fig. 1). After the peak, occurring a few weeks after the initial inoculation, viral load decreases to a ‘set point’ and CD4 + T cells partially recover, resulting in a chronic, mostly asymptomatic phase lasting around a decade on average. The chronic phase is characterised by a slow gradual loss of CD4 + T cells and a slow increase in viral load. The almost constant levels of CD4 + T cells and viral load in the chronic phase characterise the transitory ‘set point’ of HIV infection. Infected cell frequency stays low throughout the untreated infection course. Because CD4 + T cells are essential for protection against many pathogens, their loss eventually leads to immunodeficiency. In untreated infection, when the CD4 + T cell frequency in blood drops below approximately 200 cells/ μ l, various opportunistic infections tend to arise, there is a faster decay of CD4 + T cells, and an accelerated rise in viral load. This defines the final AIDS stage of infection and causes high rates of mortality.

Adaptive immune responses to HIV arise during the acute phase. The most well-studied adaptive immune defence against HIV is mediated by CD8 + T cells (Borrow et al. 1994; Schmitz et al. 1999), which can kill infected cells (McCune 2001) or limit viral replication via soluble factors (Tomaras et al. 2000; Neil et al. 2007). On the other hand, HIV-specific neutralising antibodies are thought to have only a limited effect in controlling HIV infection (Overbaugh and Morris 2012), either contributing to free virus clearance or to infected cell death through antibody-dependent cell-mediated cytotoxicity (ADCC) (Bruel et al. 2016). Based on data from experiments with simian immunodeficiency virus (SIV) infection of rhesus macaques, it is assumed that most of the free virus clearance occurs in organs like the liver, spleen, lymph nodes and kidneys, with the liver playing a major role (Zhang et al. 2002).

HIV has a high mutation rate of approximately one mutation per replication (Nowak 1990), which drives the high diversity of the viral population and allows the virus to temporarily evade CD8 + T cell responses. However, escape mutations usually carry a fitness cost that reduces their replicative capacity, although the cost can be avoided by other compensatory mutations. The importance of escape mutants in an evolving viral population is determined by the balance between fitness cost and the lower immune pressure. High mutation rate is behind the constant race between viral evolution and immune response adaptation (Korthals Altes, Ribeiro and de Boer 2003).

The acute phase is followed by chronic inflammation, possibly partially caused by extensive depletion of CD4 + T cells in

the gut and microbial translocation from the gut to the bloodstream (Brenchley *et al.* 2006). This is accompanied by generalised immune activation, which is thought to accelerate replication and differentiation of CD4 + T cells (Ribeiro *et al.* 2006) and may shorten their lifespan (Sousa *et al.* 2002). Increased activation may also contribute to T cell exhaustion and senescence (Galvani 2005), decreasing CD8 + T cell immune effectiveness and CD4 + T cell capacity for help. In HIV infection, immune activation is the strongest correlate and the best predictor of progress to AIDS (Giorgi *et al.* 1999).

The above overview of the features of the dynamics of untreated HIV infection is by no means exhaustive and does not encompass all the rich research in this area. We have only mentioned the features of the untreated infection that the simple model that will be used will attempt to reproduce, that is, how the uninfected and infected CD4 + T cells and viral load behave in different stages of HIV infection, and we have mentioned the immune responses that may modulate the model parameters.

One of the important features of HIV infection is the existence of a pool of latently infected cells, that are a small fraction of all infected cells, do not produce virus and do not contribute to CD4 + T cell loss. They do not play an important role in the course of untreated infection, but in treated individuals latently infected cells form a large reservoir of virus in the body that is resistant to ART (Chun and Fauci 1999). They can reactivate virus production after prolonged time periods, causing re-emergence of acute viral replication shortly after treatment interruption. This makes ART a life-long treatment and prevents HIV cure.

THE BASIC MODEL OF VIRAL INFECTION

Mathematical models attempt to describe the system of interest with sufficient complexity to capture the most important components while also being as simple as possible, eliminating details that obscure analysis and understanding. The goal is for the quantitative framework to provide phenomenological structure around available data to elucidate key mechanisms, understand if and when various subcomponents are important drivers of observations, estimate rates and provide useful predictions and insightful implications. The basic mathematical model of viral infection (Nowak and May 2000) accomplishes this aim. This model framework is well-established and is the simplest model of a viral infection. It considers the interactions of only the three most important variables: frequencies of target cells for the virus (T), infected cells (I) and the free virus (V) (Fig. 2A). The rate of change in time *t* of these variables is described by the equations:

$$dT/dt = \lambda - d_T T - \beta VT, \tag{1}$$

$$dI/dt = \beta VT - \delta I, \tag{2}$$

$$dV/dt = pI - cV. \tag{3}$$

Here, uninfected target cells are supplied from a source at the rate λ , and naturally die at the rate d_T . In the absence of infection ($V = 0$), the target cell frequency is $T_0 = \lambda/d_T$. Target cells are infected by free virus at a rate assumed to be proportional to the

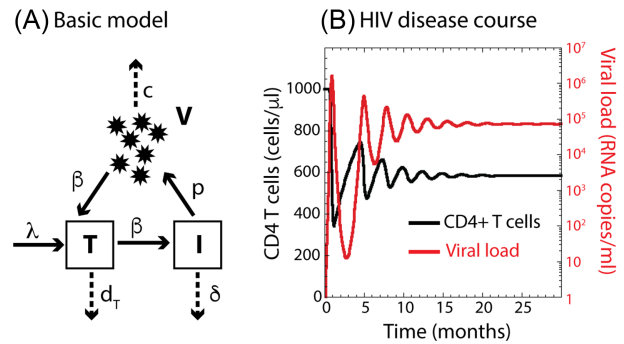


Figure 2. The basic model of viral dynamics. (A) Target cells susceptible to virus (T) are replaced at the rate λ and die at the rate d_T . They get infected by the virus (V) with probability proportional to infectivity β , to become infected cells (I). Infected cells produce virus at the rate p and die at the rate δ . (B) The solution of the basic model qualitatively reproduces the acute phase of HIV infection to the set point. Here the parameter values were chosen as $\lambda = 10$ cells/ μ l/day, $d_T = 0.01$ day⁻¹, $\beta = 10^{-7}$ ml/RNA copies/day, $\delta = 1$ day⁻¹, $p = 600$ RNA copies/cell/day and $c = 35$ day⁻¹.

amount of virus (mass action law), with the coefficient of proportionality β called the infectivity parameter (equation 1). As cells become infected with virus, they leave the target cell pool and join the infected cell population, dying at the rate δ , faster than the death rate of uninfected targets (equation 2). Infected cells also produce free virions at the rate p , that are cleared at a clearance rate c (equation 3).

For any infection model, the average number of cells infected by one typical infected cell during its lifetime at the start of infection is called the basic reproductive ratio R_0 . If the basic reproductive ratio is greater than unity, the infection will ‘take off’, otherwise it will ‘die out’. For the basic model, the basic reproductive ratio is

$$R_0 = T_0/T^* = \lambda\beta p / (d_T\delta c). \tag{4}$$

Provided that the basic reproductive ratio is greater than unity, the basic model will generate qualitatively similar dynamics: an initial exponential viral growth to a peak in viral load, a nadir in target cell levels, after which free virus and CD4 + T cells will both settle to their steady-state levels, which can be interpreted as the infection set point. Mathematically, the steady-state levels of target cells and virus depend only on the equation parameters and are respectively:

$$T^* = \delta c / (\beta p), \tag{5}$$

$$V^* = \lambda p / (\delta c) - d_T / \beta. \tag{6}$$

Viral load is related to the frequency of infected cells:

$$V^* = (p/c) I^*. \tag{7}$$

The two variables V^* and I^* are proportional as long as p and c are constant.

Differences in the basic model parameters result only in changes to the timing and the values at virus peak, target cell nadir and the steady-state values of the variables (Petravic *et al.* 2008; Petravic and Davenport 2011). The standard basic model

predicts the qualitative features of the HIV infection up to the set point, although all features cannot be consistently fitted with the same set of parameters. These dynamics are just what occurs during the first days, weeks and months of HIV infection (Fig. 2B).

Since the model does not contain any immunity effects that would build over time, the decay of virus after the peak in Fig. 2 is due to the virus not having enough cells to infect, which is called target cell limitation and is an important feature of the basic model. In the case of HIV, target cells are mostly a subset of CD4 + T cells expressing the effector-memory coreceptor CCR5, the frequency of which cannot be easily determined. In this simple model, target cells are often approximated to all CD4 + T cells, which may cause problems in modelling the effects of immune activation. Despite these shortcomings, the model can qualitatively reproduce the main features of HIV and SIV (the HIV-like virus in monkeys) dynamics up to the set point. The initial exponential rise of viral load has been used to estimate the basic reproductive ratio (Ribeiro et al. 2010), and the steep exponential decay after the peak can serve as an estimate for the death rate of infected cells (used for example in (Balamurali et al. 2010)).

The model can be best fitted to the viral load decay under ART, when target cell dependence is strongly limited by the effectiveness of the drugs. Fitting the multi-phase exponential decay of viral load under ART to the basic model has allowed estimation of the initial fast death rate of infected cells (Markowitz et al. 2003) and revealed the existence of infected cell populations with vastly different lifespans (Perelson et al. 1997; Palmer et al. 2008), some of which (latently infected cells) are so long-lived that they prevent HIV eradication. Fits for different ART drug types and combinations helped estimate the death rate of infected cells (Markowitz et al. 2003), suggesting $\delta \approx 1 \text{ day}^{-1}$ for the shortest-lived main infected CD4 + T cell population. Fitting the model to data in the initial hours after ART initiation has helped estimate virus infectivity, production and clearance (Ho et al. 1995). Independent experiments have provided direct estimates of clearance and production to validate the modelled estimates. Plasma apheresis in HIV patients in the chronic phase provided the only direct estimates of virus clearance in humans *in vivo* (Ramratnam et al. 1999), ranging between 9.1 and 36 day^{-1} . This is around 10-fold slower than clearance rates found in the primary phase of infection in rhesus macaques (Zhang et al. 1999). Most experiments used clearance rate estimates to determine viral production with the range of values between a few thousand and fifty thousand per infected cell (Chen et al. 2007; Reilly et al. 2007), with the exception of a single-cell experiment by Hockett et al. (Hockett et al. 1999) that measured a similar production rate independently of clearance and found production rates of 3162–5011 virions/cell irrespective of patient's viral load and stage of infection.

Although the basic viral dynamics model does not contain any immune response explicitly, one can think of it as being included in the constant infection parameters as a constant average rate. The death rate of infected cells would include cytolysis by CD8 + T cells and ADCC, infectivity and virus production could be modified by noncytolytic CD8 + T cell factors and virus clearance would be the compound effect of organ clearance and antibody activity. Because of this, the basic model parameters could in fact be made to be time-dependent, as was done for example in Vaidya et al. 2010, where time-dependent infectivity was used to better explain early SIV dynamics. Many have extended the basic model to explicitly include immune factors (for example Perelson 1989; Nowak and May 2000; Schwartz et al.

2016; Conway and Ribeiro 2018); this has involved numerous additional equations and parameters with greater data needs and explicit assumptions, introducing more of the inherent mechanisms into the viral dynamics, but without necessarily providing more insight into the onset of AIDS.

Although in the basic model there is a steady state established over the scale of months, which can be interpreted as corresponding to the infection set point, this model does not capture the slow changes in level of virus and CD4 + T cells during the asymptomatic phase, nor the very dynamic end stage of infection (Fig. 2B), over a timespan of years. The basic model has not needed to reproduce these later-stage infection dynamics to remain extremely insightful in unravelling the key phenomena, calculating rates, and making predictions associated with various conditions. Much greater complexity could be included in a quantitative framework or computer simulation, but this defeats the objective of models to be as simple as possible so as not to end up being 'black boxes'.

We sought to extend the basic model through the introduction of simple, biologically plausible, time-dependent rates. We first compared the results of parameter change with the infection in the steady state. The decrease in viral clearance showed the changes in the steady-state values that were the most similar to the natural infection course. We then constructed a self-contained extension of the basic model with monotonic waning of clearance caused by persistent viral load, which can, with a suitable choice of parameters, lead to a time course qualitatively similar to the natural HIV course.

DEPENDENCE OF BASIC MODEL STEADY STATES ON INFECTION PARAMETERS

Let us assume that the infection parameters change slowly, over the course of years, reflecting a slow weakening of the host's immunity. We consider the expectant changes in the target cells and viral load dynamics after the set point caused by such slow monotonic parameter changes, as predicted by the basic model.

The basic model parameters for HIV infection are generally chosen so that the steady state is achieved within less than 1 year, and a slow parameter change over the next 10 years or so would cause target cells, infected cells and viral load to continuously adjust to the values predicted by the steady-state equations (5–7). The changes in the steady-state variables with the changes in each of the infection parameters are shown in Fig. 3. We first consider the effect of changes in infectivity and the death rate of infected cells, which do not change the proportionality of viral load and the frequency of infected cells (equation 7), and then we analyse the effect of varying viral clearance and viral production rate, which remove this proportionality.

Increasing infectivity and declining death rate of infected cells

Increasing infectivity in the basic model (Fig. 3A) has a limited impact on viral load and infected cells in the chronic phase (red and green lines respectively), as can also be seen from the functional form in equation (6). As infectivity increases, the second term on the right-hand side of equation (6) (the term responsible for increase in viral load) becomes less and less important, and viral load asymptotically approaches $\lambda p / (\delta c)$. The reason for this is that, as infectivity increases, more and more cells get infected, but as they live only for a short time, the target cell pool gets

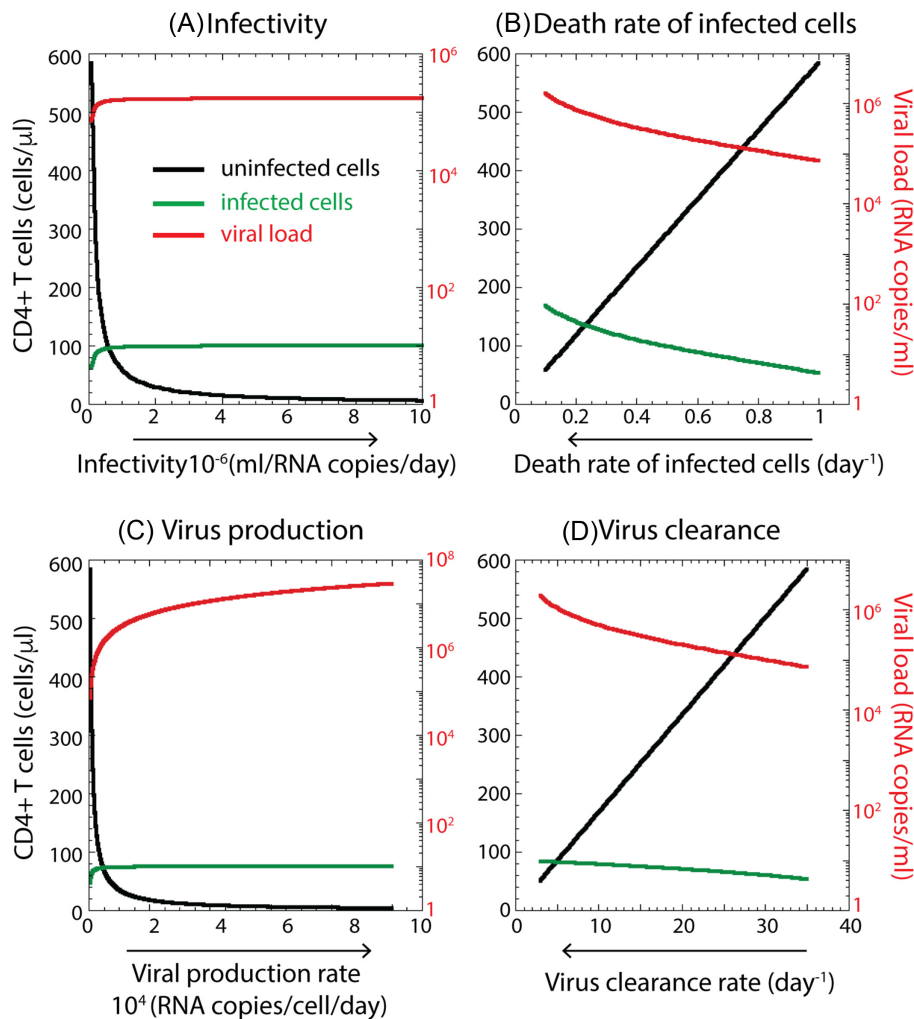


Figure 3. Dependence of steady-state values of target cells, infected cells and viral load on infection parameters. Infected cells (in cells/ μl) are shown on the same log scale (on the right-hand side) as viral load. (A) Change of steady-state values depending on infectivity; (B) change of steady-state values depending on death rate of infected cells; (C) change of steady-state values depending on virus production rate; (D) change of steady-state values depending on free virus clearance. For the relevant changes to the steady states, one should consider parameter changes (increase or decrease) in the direction of the arrows. Constant parameters are as in Fig. 2B.

depleted fast. Consequently, when infectivity is at the highest, there are not many target cells available to infect.

If infectivity were to increase monotonically (in the direction of the arrow below Fig. 3A) after the set point in an untreated infection (irrespective of the functional form of this increase), the prediction of the basic model is that viral load and infected cells would asymptotically approach a fixed limit.

The productive lifespan of HIV-infected cells is limited by viral cytotoxicity and the immune response, mediated mostly by cytotoxic T-lymphocyte killing and, to a lesser degree, by ADCC. Both of these responses depend on CD4 + T cell help and may in principle weaken as CD4 + T cells decline. The effect of a decreasing death rate of infected cells (in the direction of the arrow below Fig. 3B) is an accelerating increase in viral load (red line) and a steady decrease in uninfected target cells (black line).

This is also what the basic model would predict if we assumed that the death rate of infected cells slowly decreased over time from the set point, and what we would expect in an untreated HIV infection. However, the increase in viral load is accompanied by a proportional increase in infected cells (green line in Fig. 3B). Consequently, the total (uninfected and infected)

CD4 + T cell count would possibly stall or even increase as the death rate of infected cells decreased. Moreover, in the final stages the total CD4 + T cell count would be dominated by infected cells. Another consequence of too low a death rate of infected cells is that burst size (total viral production over the lifespan of infected cell) grows to become implausibly high.

In contrast, in a natural untreated HIV infection infected cells always remain a very low fraction of the total CD4 + T cell count during the whole natural course of HIV. In addition, there is no significant variation in the viral decay rate under ART, irrespective of the timing of the start of ART, meaning that the death rate of infected cells does not change much during HIV infection.

Another parameter whose increase would cause the increase in steady-state viral load in the basic model, while preserving the proportionality between free virus and infected cells, is target cell replacement λ . While such an increase in the basic model does not seem directly relevant for the modelling of HIV infection, it has applications in understanding the outcomes of the more sophisticated activation models. Activation models introduce the additional complexity of explicitly considering the transition of CD4 + T cells from the unsusceptible (naïve) to

the susceptible, activated subset (memory) in varying detail (for example Ribeiro *et al.* 2006; Yates *et al.* 2007; Chan *et al.* 2010). The common feature of these models is that they contain an infection module that is in fact a basic model. In this module, the target cells are cells susceptible to virus (for example memory cells) with time-varying replacement due to activation of the unsusceptible (naïve) pool. Activation rate may be a function of total CD4 + T cell loss (homeostatic mechanisms (Hazenberg *et al.* 2000)) or immune activation caused by virus. Activation models are important because they can reproduce the slow loss of CD4 + T cells in the chronic phase and explain why unsusceptible CD4 + T cells are also depleted in the course of HIV infection (Grossman *et al.* 2006). However, contrary to observations, susceptible cells reach a steady state fast, at a rate dictated by the death rate of infected cells. In addition, such models cannot successfully describe the approach to AIDS because of the intrinsic obstacle of proportionality between viral load and infected cells. Indeed, in Yates *et al.* 2007 there is no mention of viral load, neither in the figures nor in the equations, since this proportionality is implicit at all times. Target cell replacement and activation models are discussed in more detail in Supplementary Material S1.

A general conclusion from this section is that any extension of the basic model aiming to reproduce the AIDS stage of HIV infection (rapid increase in viral load with scarce infected cells) by allowing a change in infectivity, death rate of infected cells or target cell replacement must also include some modification of equation (3), such that it includes additional terms containing these parameters.

Increasing viral production and declining virus clearance

Increasing viral production rate (Fig. 3C) or decreasing viral clearance (Fig. 3D) in the basic model has a fundamentally different effect on steady-state values, because there are additional causes of increasing viral load: progressively more virus produced by each infected cell in the case of increasing virus production rate, or accumulation of uncleared virus in the case of declining clearance. Mathematically, the factor p/c in the relationship between viral load and infected cells in equation (7) is no longer constant, and the proportionality of the two variables breaks down. While the steady-state viral load increases as production and clearance change in the direction of the arrows below Fig. 3C and Fig. 3D respectively, the growth of infected cells is limited as they asymptotically approach the constant value λ/p . This type of behaviour is what we would also see if we allowed production or clearance to change slowly and monotonically in the direction of the arrows below the respective figures. Changes in viral production and clearance in the basic model would therefore result in the broad features present in untreated HIV infection.

While it is possible that production of virions by infected cells increases as HIV infection progresses, possibly due to a gradual loss of noncytolytic functions of CD8 + T cells (for example, loss of interferon- α secretion (Neil *et al.* 2007)), the amount of this increase cannot be unlimited. One reason is that too high a virus production rate at constant lifespan would increase the burst size (Nelson *et al.* 2004) beyond accepted biological limits. The second reason is that virion assembly and production are governed by chemical reactions that are limited by cell resources and take a finite time to complete, a process that cannot be made arbitrarily fast. However, since not much is known about

the mechanisms of virus clearance, there are no known limits to how much it can decrease. These are the reasons why we chose to look into the effects of declining virus clearance and its effects on HIV dynamics more closely.

There are some experimental indications that virus clearance may be declining during the HIV infection course. Infected cells from lymph nodes of HIV-infected patients in different stages of disease before and during ART have been shown to contribute similar numbers of virions *ex vivo* (Hockett *et al.* 1999) despite vastly different viral loads. In the same study, the linear log-log dependence between viral load and the frequency of infected cells had a slope significantly greater than unity, suggesting that viral load increases faster than linearly with the increase in infected cells. In Hockett *et al.* 1999, the authors speculated that virus clearance rate diminished as viral load increased.

Mechanisms of free virion clearance are not very well understood, but it is accepted that, as in SIV-infected monkeys, it mostly takes place in internal organs, most notably in the liver (Zhang *et al.* 2002). One possible cause of declining clearance at higher viral loads could be liver damage, which is a common cause of morbidity in HIV patients, due to oxidative stress, systemic immune activation and HIV interactions with hepatocytes (Sherman, Peters and Thomas 2017). Another cause could be saturation of the amount of virus that can be cleared per day because of finite liver capacity. Interestingly, the HIV patient with the lowest measured clearance in Ramratnam *et al.* 1999 had hepatitis C virus coinfection and, presumably, liver damage.

A SELF-CONTAINED MODEL WITH DECREASING VIRUS CLEARANCE

Examination of the dependencies of the steady-state expressions of the basic model equations (5-7) revealed a major obstacle in modelling the AIDS phase of HIV infection by allowing infectivity, infected cell death or target cell replacement (or activation) in the basic model to change in time during the course of infection: proportionality of infected cells and viral load. The only basic model parameters whose change does not contain this proportionality are viral production rate and viral clearance. Here we chose to construct a disease course model in which viral clearance implicitly changes in time through its dependence on viral load.

In order to introduce a decreasing clearance rate as a self-contained extension of the basic model, we hypothesized that viral load causes some small damage to clearance mechanisms (for example, that virions temporarily saturate the liver or that they may be toxic to hepatocytes) that are being slowly but imperfectly repaired. The repair term is not necessary to reproduce the disease course, but we introduced it because there is no published evidence that the re-emergence of HIV disease after stopping long-term ART is more severe than the original infection course, as would be the case if the clearance decline were permanent. To this effect, we added an equation to the basic model equations (1-3):

$$dc/dt = -\alpha V + r(c_0 - c). \quad (8)$$

In equation (8), α is the weak coupling between clearance and viral load that describes the small cumulative damage to clearance mechanisms, and r is the slow recovery rate of these mechanisms. In order to have a monotonic decline in clearance, the

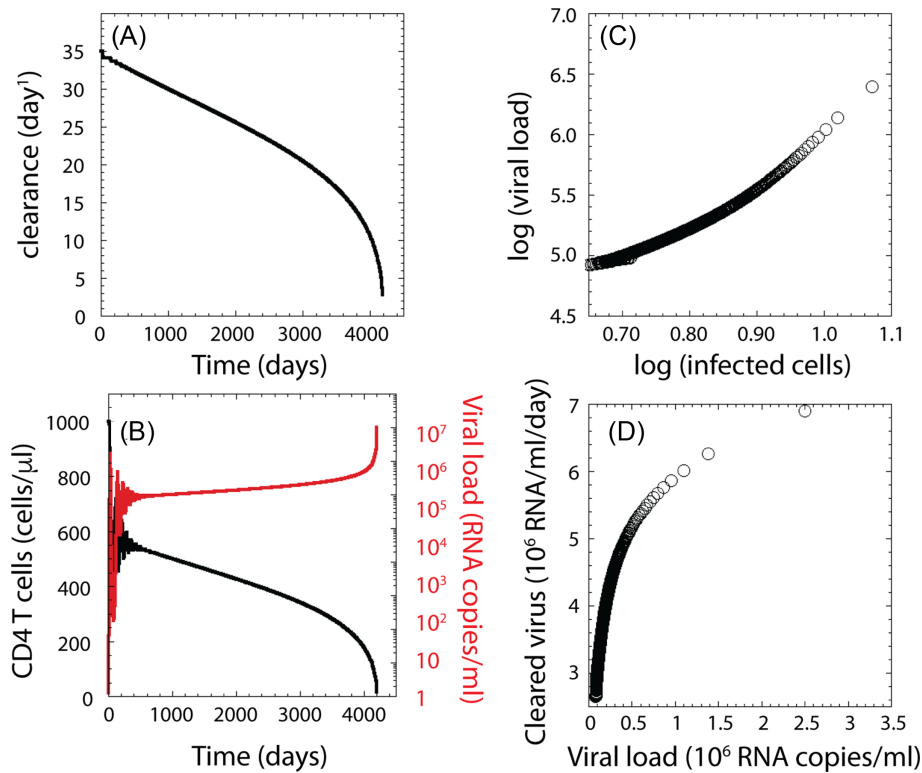


Figure 4. Effect of weak coupling of clearance and viral load. (A) Dependence of clearance on time. (B) Dynamics of CD4 + T cells and viral load predicted by the basic model and equation (8). Parameters used are as in Fig. 2B, with additional parameters $\alpha = 6.8 \times 10^{-8}$ ml/RNA copies/day², $r = 5 \times 10^{-4}$ day⁻². (C) Viral load grows faster than infected cells. (D) Amount of virus cleared per day $c(t)V(t)$ as a function of viral load after set point.

parameter values must be such that the right hand side of equation (8) is always negative (a conservative estimate is $\alpha V^* > r c_0$, where V^* is the steady state of the basic model, equation (6). In the absence of viral load, time to half-recovery would be $\ln 2/r$.

Provided a suitable choice of parameters is made, the virus clearance equation (8) introduces a new, initially slow-changing time scale into the system dynamics. Necessity for multiple time-scale dynamics has been extensively discussed in relation to the rapid approach to the steady state of target cells in the activation models (de Boer 2007; Yates et al. 2007). In equation (8), the adjustable parameters α and r control the slow changes of the model variables. It should be noted that setting $r = 0$ for small values of α is sufficient for obtaining the desired disease course, since $-\alpha V$ is always negative and clearance always decreases monotonically. In this case, α would control the variation of the changes to target cells, viral load and infected cells and the timing of AIDS. We discuss the stability of this extension to the basic model in Supplementary Material S2.

The results of this extended model with viral load dependence of clearance are shown in Fig. 4A–D. The values of the coupling and repair parameters were chosen so that the AIDS phase appears after >10 years. A change in the viral load coupling α or in the repair rate r would change the timing of AIDS. Clearance (Fig. 4A), which starts at 35 day⁻¹, decays very slowly in the chronic phase (to 20–30 day⁻¹), with a rapid decline around the point where CD4 + T cell frequency is close to 200 cells/μl.

The changes in viral load and CD4 + T cells are very slow and quasi-linear until the target cell threshold is reached, and then change rapidly (Fig. 4B), and the log-log dependence of viral load on infected cells is almost linear with a slope of 2.7 (Fig. 4C). This is greater than the average experimental slope of 1.6 (Hockett et al. 1999), but is a good qualitative resemblance for such a crude

model. The plot of the amount of virus cleared per day (equal to cV) depends linearly on viral load for low viral loads, but saturates at high viral loads (Fig. 4D), which can be interpreted as a transition from the first to zeroth order process, similar to what is seen in alcohol metabolism in the liver (Cederbaum 2012).

DISCUSSION

The standard, basic mathematical model of viral dynamics and its variants have been extraordinarily useful in describing the mechanisms and rates of early infection and implications of antiretroviral therapy. However, they have not been able to reproduce the entire natural history of HIV. Our goal in this paper was to investigate the possibility of a minimal modification of the basic model that would allow time-dependent changes in one of the parameters and would be able to qualitatively reproduce the whole course of HIV infection. We identified two important requirements for such an extension: (i) viral load and infected cells should not be proportional in the long run, with viral load growing much faster while the growth of infected cells is limited; (ii) the equation controlling the change of the chosen parameter should initially introduce a slow time-scale into the system to allow the description of the set point of infection.

We have shown that the two parameters in the basic model that can change in time to generate such a non-linear relationship are decreasing virus clearance or increasing virus production rate. The change in the other model parameters would predict CD4 + T cell and virus dynamics different from those observed, with almost all CD4 + T cells being infected when viral load soars in the AIDS stage. We have chosen to construct an

extension to the basic model with declining clearance because the increase in viral production would have biological limits.

We introduced the initial slow time-scale into the dynamics by an assumption that a cumulative decrease in clearance is weakly coupled to viral load (equation 8). The strength of the coupling controls the rate of decay of target cells and the rate of increase in viral load at the set point and the timing of the onset of AIDS. Similar dynamics is obtained with an addition of the very small 'repair term' that allows clearance to return to the disease-free level after ART. The coupling of the clearance rate with viral dynamics can be interpreted as the infection-mediated damage to clearance organs that is being slowly and incompletely repaired. Our model is at this stage pure speculation, but it is the first simple model that can successfully reproduce the conceptual dynamics of the entire HIV natural history time course.

Clearance mechanisms are not well understood. In the monkey SIV model, most clearance appears to take place in the liver and other organs (kidneys, lymph nodes, lungs). These are the organs that also show progressive damage in untreated HIV patients and may therefore be able to clear progressively less virus over time. Although free virus clearance rate has been measured cross-sectionally in HIV patients in the chronic stage, revealing a range between 9 and 36 per day, it is not known whether it declines longitudinally (Ramratnam *et al.* 1999). An *ex vivo* experiment on samples from HIV patients showed a viral load increasing faster than linearly with the increase in infected cell frequency at almost constant virus production, which seemed to indicate that clearance decreases with increasing viral load (Hockett *et al.* 1999). This experiment also had a cross-sectional approach, but shows that the idea of declining clearance rate corresponding to increased viral load in a single infection is not implausible.

The available evidence does not contradict our hypothesis that free HIV clearance rate decreases over time, since there are no longitudinal measures of clearance during the whole course of HIV/SIV infection. We now call on experimentalists to investigate the possibility of declining clearance rate. The first step towards testing this hypothesis would be to measure SIV clearance in rhesus macaques longitudinally all the way to the final stages of disease. If this hypothesis is validated, it may be that the research would also identify a different set of mechanisms and a different differential equation for clearance, but one that would also result in a monotonic decline during the disease course, possibly revealing another target mechanism for HIV control.

Mathematical modelling normally involves neglecting unnecessary complexities with the aim of arriving at a simple model that clearly reproduces and explains the considered behaviour. We have therefore only considered the assumptions under which the simplest of HIV models could describe the full HIV infection course, and in this we have only considered the changes in parameters coupled to variables. We have not considered changes in multiple parameters, dependencies between parameters or the infinity of possible changes to the existing equations by adding new terms (for example, including additional terms to equation (3) that could break the proportionality of viral load and infected cells). It is possible that models involving such modifications would point to entirely different mechanisms generating the dynamics of the later stages of HIV disease.

The simplicity with which we mathematically represent decreasing clearance while still predicting the whole course of

untreated HIV infection conceptually makes it compelling. However, it may be an instance of the proverbial 'simple and clear, but wrong solution to a complex problem'. The existence of different HIV strains, most notably the switch from a CCR5-tropic strain that infects only memory cells to the CXCR4-tropic strain that can infect all CD4 + T cells (Ribeiro *et al.* 2006) could be the most important contributing factor to the progression to AIDS. More complicated models, that consider different anatomical compartments (de Boer, Ribeiro and Perelson 2010) or full dynamics of immune response, may yet yield new insights and reveal different possible mechanisms at play.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSPD](https://academic.oup.com/femspd) online.

Conflict of interest. None declared.

REFERENCES

- Althaus CL, de Boer RJ. Dynamics of immune escape during HIV/SIV infection. *PLoS Comput Biol* 2008;**4**:e1000103.
- Balamurali M, Petravic J, Loh L *et al.* Does cytolysis by CD8+ T cells drive immune escape in HIV infection? *J Immunol* 2010;**185**:5093–101.
- Borrow P, Lewicki H, Hahn BH *et al.* Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol* 1994;**68**:6103–10.
- Brenchley JM, Schacker TW, Ruff LE *et al.* CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 2004;**200**:749–59.
- Brenchley JM, Price DA, Schacker TW *et al.* Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006;**12**:1365–71.
- Bruel T, Guivel-Benhassine F, Amraoui S *et al.* Elimination of HIV-1-infected cells by broadly neutralizing antibodies. *Nat Commun* 2016;**7**:10844.
- Cederbaum AI. Alcohol Metabolism. *Clin Liver Dis* 2012;**16**:667–85.
- Chan ML, Petravic J, Ortiz AM *et al.* Limited CD4+ T cell proliferation leads to preservation of CD4+ T cell counts in SIV-infected sooty mangabeys. *Proc R Soc Lond B Biol Sci* 2010;**277**:3773–81.
- Chen HY, Di Mascio M, Perelson AS *et al.* Determination of virus burst size in vivo using a single-cycle SIV in rhesus macaques. *Proc Natl Acad Sci USA* 2007;**104**:19079–84.
- Chun TW, Fauci AS. Latent reservoirs of HIV: obstacles to the eradication of virus. *Proc Natl Acad Sci USA* 1999;**96**:10958–61.
- Conway JM, Ribeiro RM. Modeling the immune response to HIV infection. *Curr Opin Syst Biol* 2018;**12**:61–69.
- Davenport MP, Loh L, Petravic J *et al.* Rates of HIV immune escape and reversion: implications for vaccination. *Trends Microbiol* 2008;**16**:561–6.
- de Boer RJ. Time Scales of CD4+ T Cell Depletion in HIV Infection. *PLoS Med* 2007;**4**:e193.
- de Boer RJ, Ribeiro RM, Perelson AS. Current Estimates for HIV-1 Production Imply Rapid Viral Clearance in Lymphoid Tissues. *PLoS Comput Biol* 2010;**6**:e1000906.
- Douek DC, Picker LJ, Koup RA. T cell dynamics in HIV-1 infection. *Annu Rev Immunol* 2003;**21**:265–304.
- Douek DC, Brenchley JM, Betts MR *et al.* HIV preferentially infects HIV-specific CD4+ T cells. *Nature* 2002;**417**:95–98.
- Elemans M, Seich Al Basatena NK, Asquith B. The efficiency of the human CD8+ T cell response: how should we quantify

- it, what determines it, and does it matter? *PLoS Comput Biol* 2012;**8**:e100381.
- Galvani AP. The role of mutation accumulation in HIV progression. *Proc Biol Sci* 2005;**272**:1851–8.
- Giorgi JV, Hultin LE, McKeating JA et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis* 1999;**179**:859–70.
- Grossman Z, Meier-Schellersheim M, Paul WE et al. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat Med* 2006;**12**:289–95.
- Grossman Z, Meier-Schellersheim M, Sousa AE et al. CD4+ T-cell depletion in HIV infection: Are we closer to understanding the cause? *Nat Med* 2002;**8**:319–23.
- Hazenberg MD, Otto SA, Cohen Stuart JWT et al. Increased cell division but not thymic dysfunction rapidly affects the T-cell receptor excision circle content of the naive T cell population in HIV-1 infection. *Nat Med* 2000;**6**:1036–42.
- Hill AL, Rosenbloom DIS, Fu F et al. Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. *Proc Natl Acad Sci USA* 2014;**111**:13475–80.
- Ho DD, Neumann AU, Perelson AS et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;**373**:123–6.
- Hockett RD, Kilby JM, Derdeyn CA et al. Constant mean viral copy number per infected cell in tissues regardless of high, low, or undetectable plasma HIV RNA. *J Exp Med* 1999;**189**:1545–54.
- Korthals Altes H, Ribeiro RM, de Boer RJ. The race between initial T-helper expansion and virus growth upon HIV infection influences polyclonality of the response and viral set-point. *Proc Biol Sci* 2003;**270**:1349–58.
- Lim SG, Condez A, Lee CA et al. Loss of mucosal CD4 lymphocytes is an early feature of HIV infection. *Clin Exp Immunol* 1993;**93**:448–54.
- Markowitz M, Louie M, Hurley A et al. A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo. *J Virol* 2003;**77**:5037–8.
- McCune JM. The dynamics of CD4+ T-cell depletion in HIV disease. *Nature* 2001;**410**:974–9.
- Neil SJD, Sandrin V, Sundquist WI et al. An interferon- α -Induced tethering mechanism inhibits HIV-1 and ebola virus particle release but is counteracted by the HIV-1 Vpu protein. *Cell Host Microbe* 2007;**2**:193–203.
- Nelson PW, Gilchrist MA, Coombs D et al. An age-structured model of HIV infection that allows for variations in the production rate of viral particles and the death rate of productively infected cells. *Math Biosci Eng* 2004;**1**:267–88.
- Nowak MA. HIV mutation rate. *Nature* 1990;**347**:522.
- Nowak MA, May RM. *Virus Dynamics: Mathematical Principles of Immunology and Virology*. Oxford University Press, Oxford, 2000.
- Overbaugh J, Morriss L. The antibody response against HIV-1. *Cold Spring Harb Perspect Med* 2012;**2**:a007039.
- Palmer S, Maldaelli F, Wiegand A et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci USA* 2008;**105**:3879–84.
- Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med* 1993;**328**:327–35.
- Perelson AS. Modeling the Interaction of the Immune System with HIV. In: Castillo-Chavez C (ed). *Mathematical and Statistical Approaches to AIDS Epidemiology*. Heidelberg: Springer-Verlag, 1989, 350–70.
- Perelson AS, Essunger P, Cao Y et al. Decay characteristics of HIV-1 infected compartments during combination therapy. *Nature* 1997;**387**:188–91.
- Petrvac J, Davenport MP. Simian-Human Immunodeficiency Infection – Is the Course Set in the Acute Phase? *PLoS One* 2011;**6**:e17180.
- Petrvac J, Rasmussen TA, Lewin SR et al. Relationship between measures of HIV reactivation and decline of the latent reservoir under latency-reversing agents. *J Virol* 2017;**91**:e02092–02016.
- Petrvac J, Ribeiro RM, Casimiro DR et al. Estimating the Impact of Vaccination on Acute Simian-Human Immunodeficiency Virus/Simian Immunodeficiency Virus Infections. *J Virol* 2008;**82**:11589–98.
- Ramratnam B, Bonhoeffer S, Binley J et al. Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. *Lancet* 1999;**354**:1782–5.
- Reilly C, Wietgreffe S, Sedgewick G et al. Determination of simian immunodeficiency virus production by infected activated and resting cells. *AIDS* 2007;**21**:163–8.
- Ribeiro RM, Hazenberg MD, Perelson AS et al. Naive and memory cell turnover as drivers of CCR5-to-CXCR4 tropism switch in human immunodeficiency virus type 1: implications for therapy. *J Virol* 2006;**80**:802–9.
- Ribeiro RM, Qin L, Chavez LL et al. Estimation of the initial viral growth rate and basic reproductive number during acute HIV-1 infection. *J Virol* 2010;**84**:6096–102.
- Schmitz JE, Kuroda MJ, Santra S et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* 1999;**283**:857–60.
- Schwartz EJ, Biggs KRH, Bailes C et al. HIV Dynamics With Immune Responses: Perspectives From Mathematical Modeling. *Curr Clin Microbiol Rep* 2016;**3**:216–24.
- Sherman KE, Peters MG, Thomas D. Human immunodeficiency virus and liver disease: a comprehensive update. *Hepatology Communications* 2017;**1**:987–1001.
- Sousa AE, Carneiro J, Meier-Schellersheim M et al. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol* 2002;**169**:3400–6.
- Tomaras GD, Lacey SF, McDanal CB et al. CD8+ T cell-mediated suppressive activity inhibits HIV-1 after virus entry with kinetics indicating effects on virus gene expression. *Proc Natl Acad Sci USA* 2000;**97**:3503–8.
- UNAIDS Global HIV & AIDS Statistics - 2018. Fact Sheet, 2018.
- Vaidya NK, Ribeiro RM, Miller CJ et al. Viral dynamics during primary simian immunodeficiency virus infection: effect of time-dependent virus infectivity. *J Virol* 2010;**84**:4302–10.
- Wei X, Ghosh SK, Taylor ME et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995;**373**:117–22.
- Yates A, Stark J, Klein N et al. Understanding the slow depletion of memory CD4+ T cells in HIV infection. *PLoS Med* 2007;**4**:e177.
- Zhang L, Dailey PJ, Gettie A et al. The liver is a major organ for clearing Simian Immunodeficiency Virus in rhesus monkeys. *J Virol* 2002;**76**:5271–3.
- Zhang L, Dailey PJ, He T et al. Rapid clearance of simian immunodeficiency virus particles from plasma of rhesus macaques. *J Virol* 1999;**73**:855.