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HIV-1 dynamics revisited: biphasic decay by cytotoxic T lymphocyte killing?

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The biphasic decay of blood viraemia in patients being treated for human immunodeficiency virus type 1 (HIV-1) infection has been explained as the decay of two distinct populations of cells: the rapid death of productively infected cells followed by the much slower elimination of a second population the identity of which remains unknown. Here we advance an alternative explanation based on the immune response against a single population of infected cells. We show that the biphasic decay can be explained simply, without invoking multiple compartments: viral load falls quickly while cytotoxic T lymphocytes (CTL) are still abundant, and more slowly as CTL disappear. We propose a method to test this idea, and develop a framework that is readily applicable to treatment of other infections.

Keywords: human immunodeficiency virus (HIV); hepatitis C virus (HCV); mathematical model; antiviral treatment; cytotoxic T lymphocytes (CTL); cytotoxicity

1. INTRODUCTION

Successful antiviral treatment of human immunodeficiency virus type 1 (HIV-1) infection results in a biphasic exponential decline of blood viraemia (Perelson *et al.* 1997). The first phase is steep, with > 99% of virus disappearing in the first 1–2 weeks ($t_{1/2}$ of 1–2 days); the second is more gradual ($t_{1/2}$ of 10–40 days), continuing for a month or more until virus falls below detection limits. Because virus is known to infect several cell types *in vivo* (Schrager & D'Souza 1998), early studies assumed the two phases reflected the decay of two different populations of infected cells (Notermans *et al.* 1998; Perelson *et al.* 1997). According to this conventional explanation, the first phase results from the decay of short-lived infected cells, while the second results from the slower decay of a minor population of longer-lived cells, perhaps macrophages or immune-privileged cells, the exact identity of which remains unknown (Finzi & Silliciano 1998).

This explanation assumes that the immune system exerts a constant antiviral effect during treatment. However, it is now known that treatment results in a profound reduction of cytotoxic T lymphocytes (CTL), which kill infected cells (Gray *et al.* 1999; Nixon *et al.* 1999; Ogg *et al.* 1999). In light of increasing evidence that CTL play an important role in controlling infection (Jin *et al.* 1999; Ogg *et al.* 1998; Schmitz *et al.* 1999), we here advance an alternative explanation for the biphasic decay based on the effects of the immune response. Using simple mathematical models, we show that the first phase of decay may reflect CTL killing, while the second may reflect the natural death rate of infected cells. This would imply that infected cells may be longer lived *in vivo* than generally thought; that HIV-1 may be relatively non-cytotoxic; and that CTL killing may be responsible for more infected-cell death than virus-mediated cytolysis. We conclude by proposing a simple experiment to test this idea.

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2. THE MODEL

We consider the immune response against a single pool of infected cells. This interaction may be described by the following simple system of differential equations, adapted from the standard model of virus dynamics (De Boer & Perelson 1998; Nowak & Bangham 1996):

$$\mathrm{d}x/\mathrm{d}t = \lambda - dx - \beta xy, \tag{1a}$$

$$\mathrm{d}y/\mathrm{d}t = \beta xy - ay - pyz, \tag{1b}$$

$$\mathrm{d}z/\mathrm{d}t = cy - bz. \tag{1c}$$

The variables x, y, and z denote uninfected cells, infected cells, and the lytic immune response (e.g. CTL), respectively. Uninfected cells are produced at a rate λ, die at a per capita rate d, and are infected with a rate constant β, the viral infectivity. Infected cells die at a per capita rate a and are killed by CTL with a rate constant p. (Note that a reflects the combined effects of the natural death rate of uninfected cells, d, and any additional cytotoxic effects the virus may have.) CTL proliferate proportional to the number of infected cells with a rate constant c and die at a per capita rate b. (Formally, b reflects all pathways by which CTL are lost, which may include reversion to quiescent or memory phenotypes as well as death. We refer to it as a death rate only for simplicity.) Pre-treatment equilibrium infected cell and CTL frequencies are given by $y^* = (b/c)[\beta x^* - a)/p]$ and $z^* = (\beta x^* - a)/p$, respectively, where x^*, the equilibrium concentration of uninfected cells, is the positive root of the expression $[a\beta b - dcp \pm [(dcp - a\beta b)^2 + 4\beta^2 b\lambda cp]^{1/2}]/(2\beta^2 b)$. This general framework is applicable to treatment of many different infections.

This system makes a common simplifying assumption relative to the standard model. Because free virus is thought to be short-lived relative to infected cells (Perelson *et al.* 1996; Wei *et al.* 1995), viral load can be treated as proportional to the number of infected cells (Bonhoeffer *et al.* 1997). This means that the

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equilibrium expression for infected-cell frequency also describes baseline viral load. Relaxing this assumption changes neither the arguments nor the conclusions we present.

Antiviral treatment decreases the rate of new infection; this is reflected in the model by a decrease in infectivity, β (De Boer & Perelson 1998). The extent of this decrease depends on the effectiveness of the regimen, which we denote s. Values of s range from 0 to 1 depending on both host factors and treatment regimen. A value of $s = 0$ corresponds to a completely effective treatment, i.e. a regimen that reduces new infection to zero; so, for example, a value of $s = 0.05$ would describe a regimen that reduces infectivity to 5% of its pre-treatment value. We incorporate this into the model by substituting $s\beta$ for β in equation (1*a*,*b*). This yields

$$\mathrm{d}x/\mathrm{d}t = \lambda - dx - s\beta xy, \tag{2a}$$

$$\mathrm{d}y/\mathrm{d}t = s\beta xy - ay - pyz, \tag{2b}$$

$$\mathrm{d}z/\mathrm{d}t = cy - bz. \tag{2c}$$

Now equilibrium infected-cell and CTL frequencies are given by $y^* = (b/c)[(s\beta x^* - a)/p]$ and $z^* = (s\beta x^* - a)/p$, respectively, with x^* being the positive root of the expression $[as\beta b - dcp \pm [(dcp - as\beta b)^2 + 4s^2\beta^2 b\lambda cp]^{1/2}]/(2s^2\beta^2 b)$. If $s < a/(\beta x^*) \equiv s_\tau$, there is too little new infection to maintain the infected-cell population, and treatment will reduce viral load to zero (Bonhoeffer *et al.* 1997). This threshold corresponds to the basic reproductive ratio (Anderson & May 1991), and defines the minimum effectiveness that the treatment must have in order to lead to viral clearance. Note that even if effectiveness falls short of this threshold ($s > s_\tau$), as long as effectiveness is near the threshold ($s \approx s_\tau$), treatment may reduce viral load to arbitrarily low levels (see § 3(c)).

3. RESULTS

(a) *Virus decay*

We first consider the case where treatment is completely effective, i.e. $s = 0$. This reduces equation (2*a*) to $\mathrm{d}x/\mathrm{d}t = \lambda - dx$ and equation (2*b*) to $\mathrm{d}y/\mathrm{d}t = ay - pyz$. Solving for $y(t)$ from equation (2*b*,*c*) yields $y(t) = y^* \mathrm{e}^{-(at + p\int z(t)\,\mathrm{d}t)}$, which describes a biphasic exponential decay in viral load (figure 1*a*). This can be understood as follows.

At equilibrium, two mechanisms control infection: CTL killing ($-pyz$) and natural infected-cell death ($-ay$), which includes viral cytotoxic effects. Before treatment, these two drains are balanced by infection of new cells (βxy). Treatment prevents new infection, upsetting this balance. As a result, the number of infected cells falls from the combination of CTL killing and natural death; this accounts for the first phase. The loss of infected cells results in a loss of CTL, since CTL depend on the antigenic stimulus provided by infected cells for their maintenance (the cy term in equation (2*c*)). If CTL fall rapidly—specifically, if their per capita death rate (b) is large relative to that of infected cells (a)—killing soon becomes insignificant, and the remaining infected cells will decay at a rate dominated by natural death (a), yielding the second phase. Because viral load is proportional to the number of infected cells, it too decays in two phases: a steep first phase due to the combined effects of CTL killing and the natural death of infected cells, and a slower second phase driven by natural death alone. The analogy is of turning off the tap on a split-basin sink (figure 2).

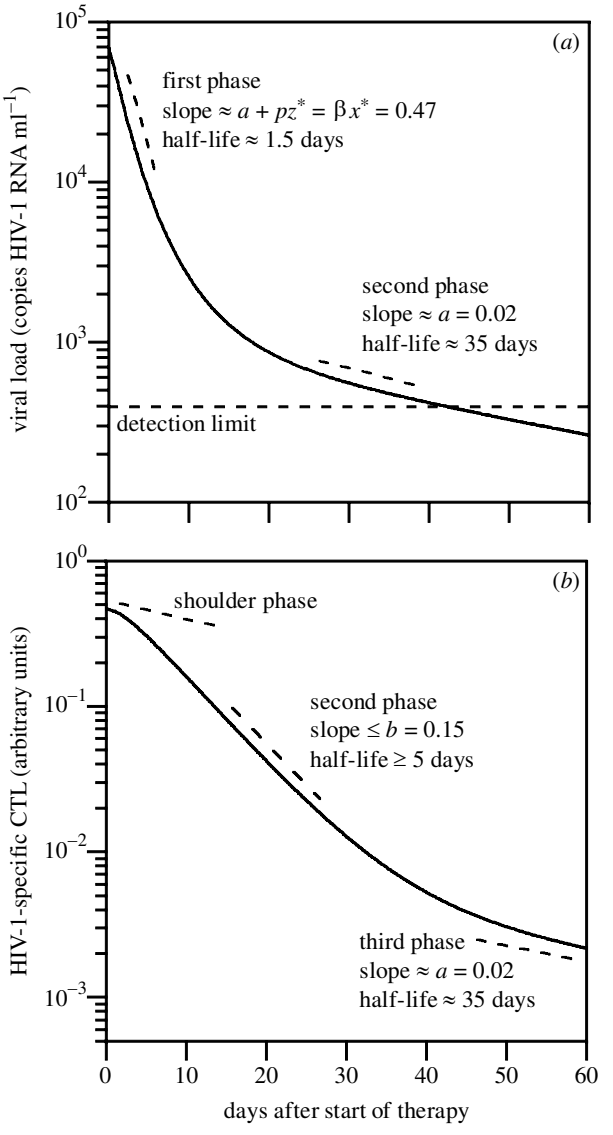

Figure 1. Viral load and virus-specific CTL under antiviral therapy. (*a*) Initially, high CTL levels result in a steep decline in viral load; this is the first phase of viral load decay. As infected cells are cleared, CTL levels fall, and infected cells are killed more slowly, resulting in the second phase. The rate of decline decreases from $a + pz^* = \beta x^*$ to a over time (all parameters as defined in text). (*b*) Meanwhile, CTL decay rapidly, following a short shoulder phase, as antigenic stimulation disappears. Interestingly, it is possible that this rapid decay will bring CTL into quasi-equilibrium with what remains of the infected-cell population. This means that the CTL population will eventually decay at the natural infected-cell death rate. Simulation was for the system described by equation (2) with viral load = $10^6 y(t)$ and parameter values $\lambda = 0.05$, $a = d = 0.02$, $p = c = 1.0$, $b = 0.15$, and $\beta = 0.5$ before therapy and 0.0 thereafter ($s = 0.0$).

Analysis of the rate of decay of the first phase raises two points of interest. First, the rate of decay of the first phase is approximately $a + pz^*$. Upon substitution for z^* this is equivalent to βx^*. This yields an interesting observation: although the first phase of decay of viral load is

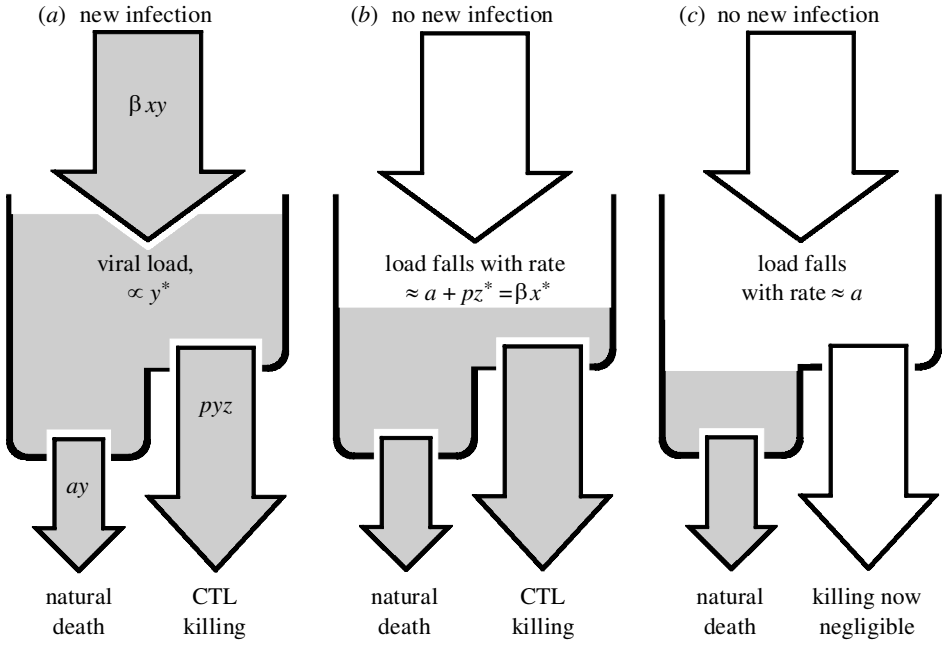

Figure 2. Biphasic decay in viral load during treatment can be likened to draining a split-basin sink. (*a*) During active infection, there are two drains on the infected-cell population: immune-mediated killing, e.g. by CTL, and natural death, which includes virus-mediated cytotoxic effects. (*b*) At the start of treatment, both drains are open, and viral load falls quickly, resulting in a steep first phase. (*c*) The loss of antigen stimulation results in a decrease in CTL killing until only one significant drain remains—the natural death of infected cells. Hence viral load falls more slowly, resulting in the slower second phase.

due to the combination of CTL killing and infected-cell death, the rate of this decay can be described solely in terms of viral infectivity (β) and the equilibrium frequency of uninfected cells (x^*). Hence differences in viral infectivity may account for observed differences between patients in the slope of decay during the first phase. Second, because baseline viral load is given by $(b/c)[(\beta x^* - a)/p]$, a positive correlation between baseline viral load and rate of decay during the first phase, such as has been observed (Notermans *et al.* 1998; Perelson *et al.* 1997), might be explained if patients differ from each other mainly in viral infectivity and uninfected-cell kinetics.

(b) *CTL decay*

Interestingly, CTL may also decay multiphasically in this model (figure 1*b*). At first, decay is slow; the CTL population is maintained near the pre-treatment equilibrium through stimulation by infected cells, which are still abundant. This results in a short shoulder phase. As infected cells decay, the cy term in equation (2*c*) becomes small and the rate of decay approaches b, yielding a second phase. Provided that $b > a$, one may also observe a third phase. In this case, the rapid decay of CTL brings it into quasi-steady-state equilibrium with the infected-cell population, which continues to decline. This is seen mathematically by rearranging equation (2*c*) to yield $z^*_{\mathrm{qss}} = (c/b)y(t)$; the rate of decay of CTL is now set by the natural infected-cell death rate, a. Interestingly, then, the CTL population may decay at a rate much slower than their per capita death rate, b. Figure 3*a–c* and table 1 show how the prominence of these phases depends on the relative death rates of CTL and infected cells and on other parameters.

We note that the behaviour of the CTL population depends on the particular mathematical form chosen for the CTL response. However, the possibility—depending on viral and immune parameters, as shown in figure 3*a–c* and table 1—of a biphasic decay of viral load is a general feature of treatment in the presence of a (lytic) immune response. More complicated models that include latently infected cells, longer-lived infected cells (figure 4 and § 4), and/or CTL precursors retain this feature (not shown).

(c) *Imperfect treatment*

For the more realistic case where $s > 0$, provided that $s < s_\tau \equiv a/(\beta x^*)$, treatment will result in viral clearance with behaviour similar to that for the case where treatment is perfect and $s = 0$ (figure 3*d*). The rate of decay during the first phase is now approximately $a + pz^* - s\beta x^*$, which upon substitution yields $(1-s)\beta x^*$. One might expect from this that more potent treatment regimens (i.e. those that are more effective at reducing new infection and hence lead to lower values of s) cause steeper first-phase decays than less potent ones; indeed, this has been seen (Nowak *et al.* 1997). More likely is that imperfect treatment will have an effect on the rate of decay of the second phase. If $s < s_\tau$ but is still small enough to lower viral load to very low levels, the rate of decay of the second phase will be smaller than a, and the half-life of viral decay during this phase will be correspondingly longer (figure 3*d*).

4. DISCUSSION

The conventional explanation of the biphasic decay in treated HIV-1-infected patients—that the two phases

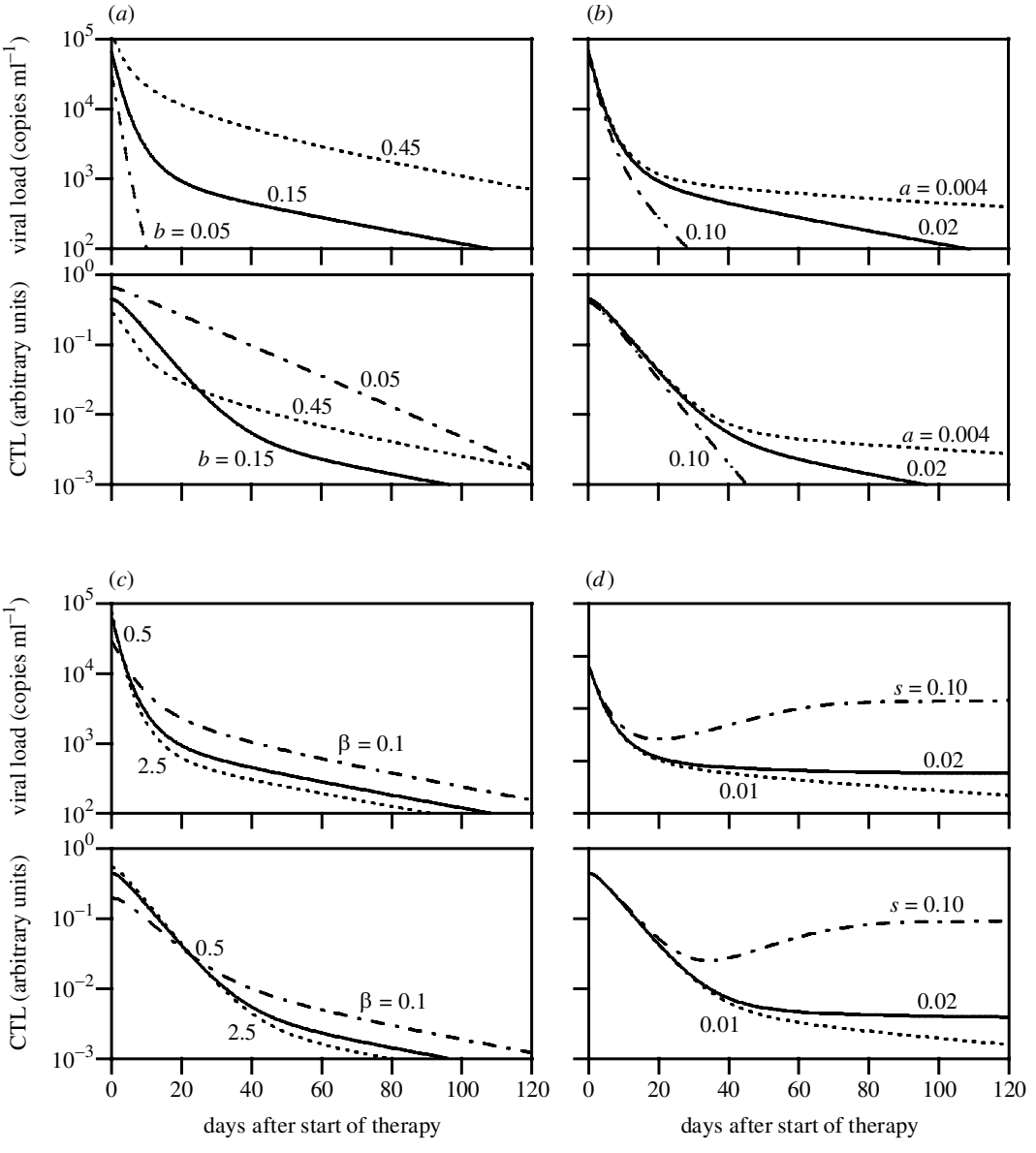

Figure 3. Observation of a biphasic decay depends on model parameters. (*a*) CTL longevity. If the per capita CTL death rate is relatively low (e.g. $b = 0.05\,\text{day}^{-1}$), the CTL population persists at high levels long enough to kill most infected cells. As a result, viral load falls rapidly, and only one phase is observed. However, if the death rate is high ($b = 0.45\,\text{day}^{-1}$), the CTL population falls quickly to quasi-equilibrium, and viral load will decay slowly; in this case a steep first phase may be observed, but its duration will be short. (*b*) Viral cytotoxicity. If the virus is very cytotoxic, or if infected cells are otherwise very short lived, a will be large (e.g. $0.10\,\text{day}^{-1}$) and viral load will fall rapidly irrespective of CTL killing; in this case only a single steep phase is likely to be observed. However, if infected cells are longer lived ($a = 0.02$ or $0.004\ \text{day}^{-1}$), two phases will be observed. (*c*) Viral infectivity. The higher the infectivity, the steeper the first phase. (*d*) Treatment effectiveness. If effectiveness is below a certain threshold (s_τ, see § 3(c); here $s_\tau \approx 0.01$), viral load may bounce back after a transient reduction (e.g. $s = 0.10$). Interestingly, if effectiveness is below but sufficiently near the threshold, viral load may still be reduced to quite low levels (e.g. $s = 0.02$). However, note that in this case the slope of decay of the second phase will be less than a (compare the case for $s = 0.02$ with that for $s = 0.01 < s_\tau$). The effects of differences in λ are similar to those of differences in b (not shown); differences in c and p do not change the shape of viral load or CTL decay (not shown). Viral load and CTL at time zero correspond to equilibrium values. Parameter values are $\lambda = 0.05$, $\beta = 0.5$, $a = d = 0.02$, $p = c = 1.0$, $b = 0.15$, and $s = 0.0$ unless otherwise noted.

correspond to two different infected-cell populations (Perelson *et al.* 1997)—assumes that the immune system exerts a constant antiviral effect during treatment. Recent evidence argues against this assumption: the consensus is that treatment results in a massive decline of CTL, with important implications for disease management (Gray *et al.* 1999; Nixon *et al.* 1999; Ogg *et al.* 1999). Given that CTL are important for control of infection (Borrow *et al.* 1997; Carrington *et al.* 1999; Ogg *et al.* 1998; Schmitz *et al.* 1999), we asked how their loss during treatment might affect viral load.

Our results suggest an alternative explanation for the biphasic decay. Using the standard model of virus dynamics and parameter values consistent with experimental observations, we show that the CTL response against a single population of infected cells is sufficient to account for the observed biphasic decay in viral load (figure 1*a*). According to this new interpretation, multiple

Table 1. *Post-treatment decay patterns for viruses of different cytotoxicities*

(According to the model described by equation (2), patterns of decay for viral load and CTL are expected to differ depending on whether or not the per capita death rate of CTL, b, is of the same magnitude as the per capita natural death rate of infected cells, a. It is important to note that sensitive measurement techniques (i.e. precise quantitation and low detection limits) may be required to observe such patterns experimentally.)

condition	viral load decay	CTL decay
$b \approx a$; true for more cytotoxic viruses	monophasic: fast, at a rate set by $a + pz^*$ (first phase dominant); second phase unlikely to be observed	monophasic: slow, at a rate set by b (shoulder and second phases dominant over first phase)
$b > a$; true for less cytotoxic viruses	biphasic: fast at first, at a rate set by $a + pz^* = \beta x^*$ (first phase), then slower, at a rate set by a (second phase; figure 1*a*)	multiphasic: short shoulder phase, followed by a fast decay at a rate $\leqslant b$ (second phase), and then by a slow decay at a rate $\approx a$ (third phase; figure 1*b*)

infected populations do not have to be invoked to explain the biphasic decline. Instead, the model shows that the two phases may simply reflect the two pathways by which infected cells die: CTL killing and natural death (figure 2*a*). At the start of treatment, both pathways are operative. Killing and death combine to drain the infected-cell pool quickly, and viral load falls accordingly fast; this results in the steep first phase (figure 2*b*). But the loss of infected cells means a loss of antigenic stimulation, which results in a relative loss of CTL and hence of CTL killing. The natural death pathway now plays the dominant role. With only one drain on the infected cell pool, viral load falls more slowly, resulting in the slower second phase (figure 2*c*). The argument holds for the more realistic case of imperfect treatment (§ 3 and figure 3*d*).

(a) *Reinterpreting the rates of decay*

Our results suggest a redefinition of the measured half-lives of the two phases of decay. The conventional explanation proposes that the one- to two-day half-life of the first phase corresponds to a one- to two-day half-life of infected cells at the start of treatment; however, it says nothing about the relative contributions of CTL killing and natural death. By contrast, the new explanation proposes that most of the death of infected cells during this phase is due to CTL killing. The conventional explanation says the second phase corresponds to the decay of a second population of cells whose half-life is 10–40 days. The new explanation suggests that this phase may instead reflect the natural death rate of infected cells, i.e. the death rate in the absence of CTL (which would correspond to $a \approx 0.02$–0.07 in the model). In other words, the new explanation predicts that, were it not for the immune response, infected cells would live on average for $1/a \approx 14$–50 days.

It is important to be clear that our results do not argue against the existence of reservoirs of viral replication (figure 4). However, in showing that the biphasic decay can be explained with only one infected population, we do challenge the view that the second phase must result purely from the decay of such a reservoir. This means that the second phase may be a poor reflection of the size and decay rate of any long-lived reservoir (figure 4*c*,*d*), contrary to previous reports (Perelson *et al*. 1997).

Variability in slopes (Ding & Wu 1999; Notermans *et al*. 1998; Perelson *et al*. 1997) may be due to both virus and host factors. Interpatient differences in many parameters may contribute to variability in the first phase (figure 3*a*–*c*). However, variability in the second phase is due principally to interpatient differences in viral cytotoxicity (a) and treatment effectiveness (s) (figure 3*b*,*d*). In light of recent work that suggests that virus becomes increasingly pathogenic over the course of infection (Kimata *et al*. 1999), one might expect rates of decay of the second phase to be greater in patients who have progressed to AIDS. However, pathogenicity depends on viral replication rate, infectivity, and tropism as well as on cytotoxicity, and so this expectation may not be borne out by conventional experimental observations. As for effectiveness, as mentioned above, if treatment is not perfect ($s > 0$), the rate of decay of the second phase will underestimate the natural death rate of infected cells (a) (figure 3*d*).

(b) *Viral cytotoxicity*

Our findings also have implications for whether HIV-1 is cytotoxic or not *in vivo*. Under the conventional explanation, either conclusion is possible. If one ascribes the dominant role to cytotoxicity, the conclusion is that virus is cytotoxic for the vast majority of cells (the so-called 'actively infected' pool, the decay of which accounts for the first phase in the conventional explanation) but is non-cytotoxic for others (the 'long-lived' pool, the decay of which accounts for the second phase). If instead one ascribes the dominant role to CTL killing, the conclusion is that any long-lived population must be immune privileged. Our model suggests a middle road: in the simplest case, the short- and long-lived populations of the conventional explanation may be one and the same. In the presence of CTL, infected cells are short lived ($t_{1/2} = 1$–2 days); in their absence, they live much longer ($t_{1/2} = 10$–40 days). Assuming that activated uninfected $CD4^+$ cells have a natural half-life of 40–50 days (Wang *et al*. 1998), the new explanation suggests that HIV-1 may be relatively non-cytotoxic *in vivo*. This is consistent with the virus' known anti-apoptotic properties (Aillet *et al*. 1998; Conti *et al*. 1998; Meinl *et al*. 1998; Sandstrom *et al*. 1996; Scheuring *et al*. 1999; Wang *et al*. 1999) and various other observations (Klenerman & Zinkernagel 1997; Zinkernagel & Hengartner 1994).

The new explanation requires that the death (or reversion) rate of CTL exceeds the natural death rate of infected cells ($b > a$) for the decay in viral load to be biphasic. In other words, infected cells must on average persist longer than CTL, otherwise infected cells will die out naturally before CTL levels fall, and there will be no second phase (figure 3*a*,*b*). Which is the case for HIV-1?

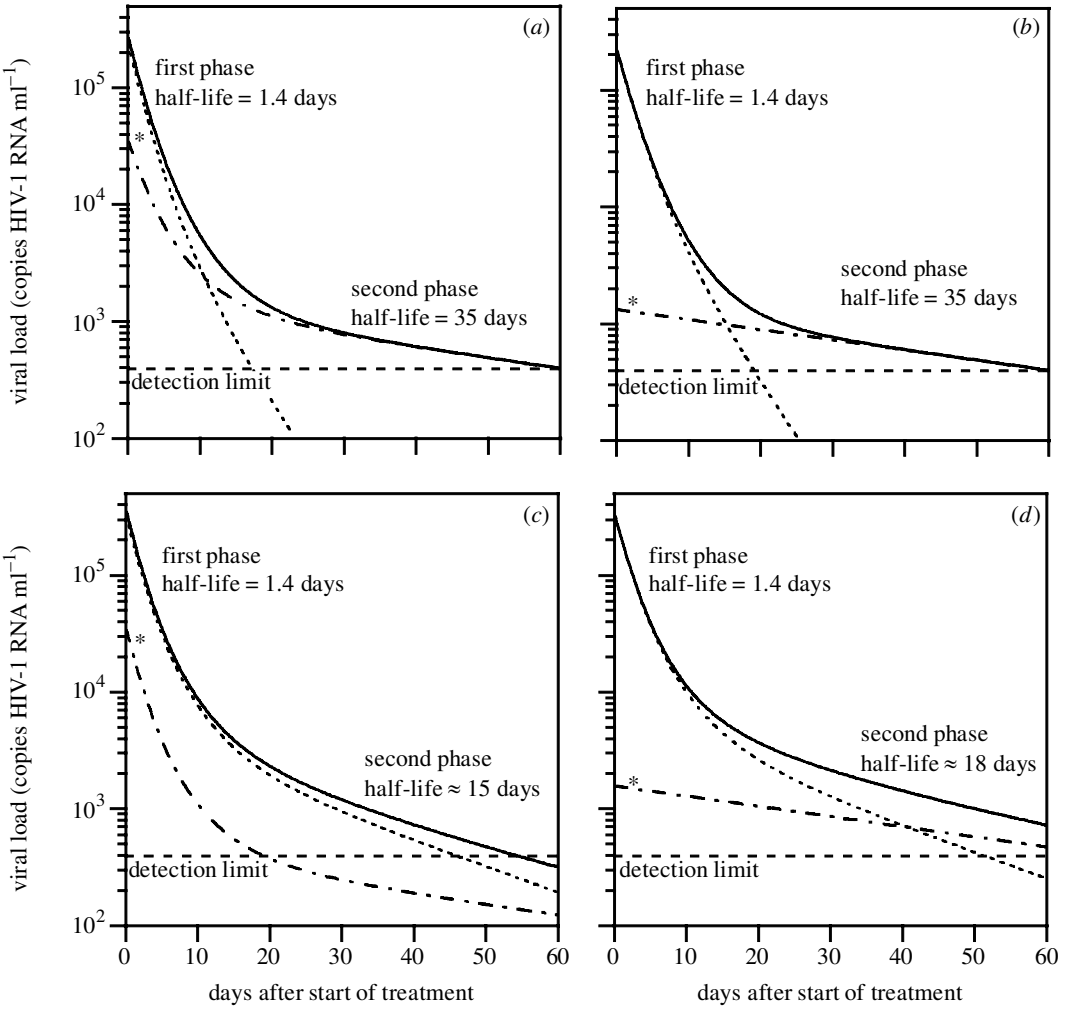

Figure 4. Contribution of minor compartments (dotted–dashed lines) to total viral load (solid lines) during the second phase depends both on whether they are immune privileged and on whether the cells of the major compartment (dotted lines) are naturally short lived (large a) or long lived (small a). (a, b) If the majority of cells are naturally relatively short lived, the second phase will reflect the size of the minor compartment. However, if the minor compartment is immune susceptible ((a) and (c)), the size of this compartment at equilibrium (denoted by an asterisk) may be quite large. (c, d) If the majority of cells are naturally long lived, viral load during the second phase will overestimate the size of the minor compartment. Simulations for (a) and (b) were for the system described by the following equations: $dy_1/dt = \beta x_1^*(y_1 + y_2) - a_1 y_1 - p y_1 z$; $dy_2/dt = \beta x_2^*(y_1 + y_2) - a_2 y_2 - p y_2 z$; $dz/dt = c(y_1 + y_2) - bz$. Here y_1 and y_2 are major and minor infected-cell compartments, respectively, x_1^* and x_2^* denote the equilibrium frequencies of uninfected cells in these compartments, and other parameters are as defined in the text. Parameter values were $x_1^* = 1.0$, $x_2^* = 0.1$, $a_2 = 0.02$, $c = p = 1.0$, $b = 0.15$, $\beta = 0.5$ before treatment and 0.0 thereafter, and $a_1 = 0.20$ in (a) and $a_1 = 0.05$ in (b) ($s = 0.0$). Simulations for (c) and (d) were for the system $dx_2/dt = \lambda d x_2 - \beta x_2(y_1 + y_2)$; $dy_1/dt = \beta x_1^*(y_1 + y_2) - a_1 y_1 - p y_1 z$; $dy_2/dt = \beta x_2(y_1 + y_2) - a_2 y_2$, $dz/dt = c(y_1 + y_2) - bz$. Parameter values for ($c$) and ($d$) were the same as those in (a) and (b), respectively, with $\lambda = 1 \times 10^{-5}$ and $d = 0.02$ ($s = 0.0$). Viral loads were calculated as $5 \times 10^6 y_n(t)$.

Recent studies that followed the decay of HIV-1-specific CTL in treated individuals differ in their conclusions, but on balance suggest that effectors die rapidly ($t_{1/2} < 1$ week; $b > 0.10$ day^{-1}). One study (Nixon *et al*. 1999) found that activated effectors, as measured by a functional assay, fell in frequency from 60 to 4 in 10^6 peripheral blood mononuclear cells in seven days, and stayed at low levels thereafter. This is consistent with a half-life of less than two days, and a value for b of > 0.35 day^{-1} (figure 3a). Using tetramer staining (Altman *et al*. 1996), another study (Ogg *et al*. 1999) found that the CTL population expressing the activation marker CD38 (Lund *et al*. 1998) decayed with a half-life that ranged from 15 to 60 days, depending on the patient, sometimes preceded by a marked early fluctuation (which the authors theorize may be due to tissue redistribution (Ogg *et al*. 1999)) during the first week of treatment. This suggests a value for b of only 0.01–0.05 day^{-1} (figure 3a). A third study (Gray *et al*. 1999) also found that the tetramer-positive population declined slowly, but concluded that this population comprised predominantly memory CTL; effectors, it suggested, are lost very rapidly. These differences may be reconciled as more is learned about the functional differences between CTL subsets. Still, it is important to keep in mind that effector activity, usually as measured by direct *ex vivo* specific lysis (Lau *et al*. 1994), is known to fall rapidly following control of viral load in many viral infections; the same may be true following treatment of HIV-1.

By contrast, infected cells may live many weeks in the absence of host immune factors. One recent study (Wang *et al*. 1998) showed that in peripheral blood T lymphocytes that had been depleted of $CD8^+$ T cells, stimulated with phytohaemagglutinin, and infected with different HIV-1 strains, the percentage of viable cells decayed with a half-life of 25–50 days, depending on the strain of virus, corresponding to $a = 0.014$–$0.028\ \text{day}^{-1}$ in our model (figure 3*b*). This value is consistent with previously reported slopes of the second phase of biphasic decline (Notermans *et al*. 1998; Perelson *et al*. 1997), supporting our new explanation (figure 1*a*). Hence it is plausible that effector CTL die (or revert to resting or memory status) quicker than infected cells (i.e. that $b > a$) and that the biphasic decay results from different rates of CTL killing.

(c) *Testing the model*

The model proposes that CTL killing is primarily responsible for the first phase of viral load decay in treated HIV-1-infected patients; the same should be true for treated simian immunodeficiency virus (SIV)-infected macaques (Kuroda *et al*. 1999). Hence the model should be readily testable by treating animals that have been depleted of $CD8^+$ T cells (Schmitz *et al*. 1999). If CTL killing is indeed primarily responsible for the first phase, the viral decay rate in these animals should be far less than in controls; in this case the decay rate should approximate the natural death rate of infected cells (*a*). If, on the other hand, little change in decay kinetics is observed, it is possible that the CTL response in HIV-1 infection is predominantly non-lytic, and is mediated by secreted soluble factors that prevent new infection (Barker *et al*. 1998; Wodarz & Nowak 1998). Hence a 'deplete, then treat' experiment could help determine the relative contributions of CTL killing and viral cytotoxicity to infected cell turnover *in vivo*.

In summary, our results represent a new interpretation of the effects of combination therapy that suggests that CTL killing is more important, and HIV-1 is less cytotoxic, *in vivo* than previously thought. Because therapy may be reducing viraemia at the price of reducing the CTL response, strategies that also prime the CTL response—i.e. that keep the CTL population high during the administration of antiviral drugs—may prove worthwhile if drugs are to be discontinued without long-term harm to the patient (Wodarz & Nowak 1999). The framework we present may possibly be useful in analysing treatment of other viral infections, such as, perhaps, with hepatitis C virus (Neumann *et al*. 1998), in the presence of a changing immune response.

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REFERENCES

Aillet, F., Masutani, H., Elbim, C., Raoul, H., Chene, L., Nugeyre, M. T., Paya, C., Barre-Sinoussi, F., Gougerot-Pocidalo, M. A. & Israel, N. 1998 Human immunodeficiency virus induces a dual regulation of Bcl-2, resulting in persistent infection of CD4(+) T- or monocytic cell lines. *J. Virol.* **72**, 9698–9705.

Altman, J. D., Moss, P. A. H., Goulder, P. J. R., Barouch, D. H., McHeyzer-Williams, M. G., Bell, J. I., McMichael, A. J. & Davis, M. M. 1996 Phenotypic analysis of antigen-specific T lymphocytes. *Science* **274**, 94–96. [Erratum in *Science* 1998 **280**, 1821.]

Anderson, R. M. & May, R. M. 1991 *Infectious diseases of humans: dynamics and control. Oxford science publications.* Oxford University Press.

Barker, E., Bossart, K. N. & Levy, J. A. 1998 Primary CD8+ cells from HIV-infected individuals can suppress productive infection of macrophages independent of beta-chemokines. *Proc. Natl Acad. Sci. USA* **95**, 1725–1729.

Bonhoeffer, S., May, R. M., Shaw, G. M. & Nowak, M. A. 1997 Virus dynamics and drug therapy. *Proc. Natl Acad. Sci. USA* **94**, 6971–6976.

Borrow, P. (and 10 others) 1997 Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus [see comments]. *Nat. Med.* **3**, 205–211.

Carrington, M., Nelson, G. W., Martin, M. P., Kissner, T., Vlahov, D., Goedert, J. J., Kaslow, R., Buchbinder, S., Hoots, K. & O'Brien, S. J. 1999 HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science* **283**, 1748–1752.

Conti, L., Rainaldi, G., Matarrese, P., Varano, B., Rivabene, R., Columba, S., Sato, A., Belardelli, F., Malorni, W. & Gessani, S. 1998 The HIV-1 vpr protein acts as a negative regulator of apoptosis in a human lymphoblastoid T cell line: possible implications for the pathogenesis of AIDS. *J. Exp. Med.* **187**, 403–413.

De Boer, R. J. & Perelson, A. S. 1998 Target cell limited and immune control models of HIV infection: a comparison. *J. Theor. Biol.* **190**, 201–214.

Ding, A. A. & Wu, H. 1999 Relationships between antiviral treatment effects and biphasic viral decay rates in modeling HIV dynamics. *Math. Biosci.* **160**, 63–82.

Finzi, D. & Silliciano, R. F. 1998 Viral dynamics in HIV-1 infection. *Cell* **93**, 665–671.

Gray, C. M., Lawrence, J., Schapiro, J. M., Altman, J. D., Winters, M. A., Crompton, M., Loi, M., Kundu, S. K., Davis, M. M. & Merigan, T. C. 1999 Frequency of class I HLA-restricted anti-HIV CD8+ T cells in individuals receiving highly active antiretroviral therapy (HAART). *J. Immunol.* **162**, 1780–1788.

Jin, X. (and 13 others) 1999 Dramatic rise in plasma viraemia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J. Exp. Med.* **189**, 991–998.

Kimata, J. T., Kuller, L., Anderson, D. B., Dailey, P. & Overbaugh, J. 1999 Emerging cytopathic and antigenic simian immunodeficiency virus variants influence AIDS progression [see comments]. *Nature Med.* **5**, 535–541.

Klenerman, P. & Zinkernagel, R. M. 1997 What can we learn about human immunodeficiency virus infection from a study of lymphocytic choriomeningitis virus? *Immunol. Rev.* **159**, 5–16.

Kuroda, M. J., Schmitz, J. E., Charini, W. A., Nickerson, C. E., Lifton, M. A., Lord, C. I., Forman, M. A. & Letvin, N. L. 1999 Emergence of CTL coincides with clearance of virus during primary simian immunodeficiency virus infection in rhesus monkeys. *J. Immunol.* **162**, 5127–5133.

Lau, L. L., Jamieson, B. D., Somasundaram, T. & Ahmed, R. 1994 Cytotoxic T-cell memory without antigen [see comments]. *Nature* **369**, 648–652.

Lund, F. E., Cockayne, D. A., Randall, T. D., Solvason, N., Schuber, F. & Howard, M. C. 1998 CD38: a new paradigm

in lymphocyte activation and signal transduction. *Immunol. Rev.* **161**, 79–93.

Meinl, E., Fickenscher, H., Thome, M., Tschopp, J. & Fleckenstein, B. 1998 Anti-apoptotic strategies of lymphotropic viruses. *Immunol. Today* **19**, 474–479.

Neumann, A. U., Lam, N. P., Dahari, H., Gretch, D. R., Wiley, T. E., Layden, T. J. & Perelson, A. S. 1998 Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* **282**, 103–107.

Nixon, D. F., Douek, D., Kuebler, P. J., Jin, X., Vesanen, M., Bonhoeffer, S., Cao, Y., Koup, R. A., Ho, D. D. & Markowitz, M. 1999 Molecular tracking of a human immunodeficiency virus nef specific cytotoxic T-cell clone shows persistence of clone-specific T-cell receptor DNA but not mRNA following early combination antiretroviral therapy. *Immunol. Lett.* **66**, 219–228.

Notermans, D. W., Goudsmit, J., Danner, S. A., de Wolf, F., Perelson, A. S. & Mittler, J. 1998 Rate of HIV-1 decline following antiretroviral therapy is related to viral load at baseline and drug regimen. *AIDS* **12**, 1483–1490.

Nowak, M. A. & Bangham, C. R. 1996 Population dynamics of immune responses to persistent viruses. *Science* **272**, 74–79.

Nowak, M. A., Bonhoeffer, S., Shaw, G. M. & May, R. M. 1997 Anti-viral drug treatment: dynamics of resistance in free virus and infected cell populations. *J. Theor. Biol.* **184**, 203–217.

Ogg, G. S. (and 14 others) 1998 Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* **279**, 2103–2106.

Ogg, G. S. (and 13 others) 1999 Decay kinetics of human immunodeficiency virus-specific effector cytotoxic T lymphocytes after combination antiretroviral therapy. *J. Virol.* **73**, 797–800.

Perelson, A. S., Neumann, A. U., Markowitz, M., Leonard, J. M. & Ho, D. D. 1996 HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science* **271**, 1582–1586.

Perelson, A. S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., Markowitz, M. & Ho, D. D. 1997 Decay characteristics of HIV-1-infected compartments during combination therapy [see comments]. *Nature* **387**, 188–191.

Sandstrom, P. A., Pardi, D., Goldsmith, C. S., Chengying, D., Diamond, A. M. & Folks, T. M. 1996 bcl-2 expression facilitates human immunodeficiency virus type-1 mediated cytopathic effects during acute spreading infections. *J. Virol.* **70**, 4617–4622.

Scheuring, U. J., Sabzevari, H., Corbeil, J. & Theofilopoulos, A. N. 1999 Differential expression profiles of apoptosis-affecting genes in HIV-infected cell lines and patient T cells. *AIDS* **13**, 167–175.

Schmitz, J. E. (and 15 others) 1999 Control of viraemia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* **283**, 857–860.

Schrager, L. K. & D'Souza, M. P. 1998 Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. *J. Am. Med. Assoc.* **280**, 67–71.

Wang, L., Klimpel, G. R., Planas, J. M., Li, H. & Cloyd, M. W. 1998 Apoptotic killing of CD4+ T lymphocytes in HIV-1-infected PHA-stimulated PBL cultures is mediated by CD8+ LAK cells. *Virology* **241**, 169–180.

Wang, Z., Morris, G. F., Reed, J. C., Kelly, G. D. & Morris, C. B. 1999 Activation of bcl-2 promoter-directed gene expression by the human immunodeficiency virus type-1 tat protein. *Virology* **257**, 502–510.

Wei, X. (and 11 others) 1995 Viral dynamics in human immunodeficiency virus type 1 infection [see comments]. *Nature* **373**, 117–122.

Wodarz, D. & Nowak, M. A. 1998 The effect of different immune responses on the evolution of virulent CXCR4-tropic HIV. *Proc. R. Soc. Lond.* B **265**, 2149–2158.

Wodarz, D. & Nowak, M. A. 1999 Specific therapy regimes could lead to long-term immunological control of HIV. *Proc. Natl Acad. Sci. USA* **96**, 14 464–14 469.

Zinkernagel, R. M. & Hengartner, H. 1994 T-cell-mediated immunopathology versus direct cytolysis by virus: implications for HIV and AIDS. *Immunol. Today* **15**, 262–268.

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