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Can the viral reservoir of latently infected CD4$^+$ T cells be eradicated with antiretroviral HIV drugs?

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Abstract The majority of cells infected with the human immunodeficiency virus are activated CD4$^+$ T cells, which can be treated with antiretoviral drugs. However, an obstacle to eradication is the presence of viral reservoirs, such as latently infected CD4$^+$ T cells. Such cells may be less susceptible to antiretroviral drugs and may persist at low levels during treatment. We introduce a model of impulsive differential equations that describe T cell and drug interactions. We make the extreme assumption that latently infected cells are unaffected by drugs, in order to answer the research question: Can the viral reservoir of latently infected cells be eradicated using current antiretroviral therapy? We analyse the model in both the presence and absence of drugs, showing that, if the frequency of drug taking is sufficiently high, then the number of uninfected CD4$^+$ T cells approaches the number of T cells in the uninfected immune system. In particular, this implies that the latent reservoir will be eliminated. It follows that, with sufficient application of drugs, latently infected cells cannot sustain a viral reservoir on their own. We illustrate the results with numerical simulations.

Keywords Latently infected cells · HIV therapy · Mathematical model · Viral reservoirs · Impulsive differential equations

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1 Introduction

HIV primarily attacks memory $CD4^+$ T helper cells. Once infected, cells produce a great many virus particles. In turn, the immune system mounts a response, creating antibodies that control, to some extent, the number of virus particles. Further control is available in the form of antiretroviral drugs, primarily drawn from two major classes, reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs) (Janeway et al. 2001). If drugs are taken with sufficient frequency, the virus is largely controlled and remains below the level of detection (Chun and Fauci 1999). However, antiretroviral drugs cannot eradicate the virus, as viral rebound occurs when drugs are stopped (Arlen et al. 2006). It has been suggested that HIV has a number of reservoirs where it will lay dormant. Primary among these reservoirs are latently infected $CD4^+$ T cells (Blankson et al. 2002). Virus that resides in latently infected $CD4^+$ T cells has been shown to be virologically quiescent and lacking the ability to produce multiply spliced HIV RNA or viral particles (Chun et al. 2005). Latently infected cells have low frequency, less than one latently infected cell per million resting $CD4^+$ T cell (Chun et al. 1997). The size of the total viral reservoir is not large; approximately 10^3–10^6 cells per patient (Ramratnam et al. 2000). However, this reservoir appears to decay slowly, with a halflife of 6–44 months (Ramratnam et al. 2000; Finzi et al. 1999). Hence, although infected, these cells do not produce virus until activated, thus potentially providing a longer-lived hiding place where virus may evade control by either the immune system or treatment (Blankson et al. 2002; Wodarz and Nowak 2002).

RTIs prevent infection of cells, whereas PIs can transform an infected cell into cells that, while nevertheless infected, will only produce noninfectious virus (Janeway et al. 2001). However, latently infected cells may result in the regular, albeit low-level, creation of newly infected cells (Blankson et al. 2002). Consequently, in this paper, we analyse a mathematical model that includes latent infection, as well as control of infection by drugs and an immune-system response. We assume that, since the viral genome has already been transcribed into the host DNA, latently infected cells are unaffected by RTIs. While we expect that latently infected cells may absorb PIs and that such cells, when activated, will result in activated cells that produce noninfectious virus, we will instead assume that antiretroviral drugs have no effect on the proportion of cells that are latently infected. This is in line with some experimental findings, that suggest that antiretroviral drugs do not effectively block replication of virus from the latent viral reservoir (Chun et al. 2003). We also assume that such cells live significantly longer than productively infected $CD4^+$ T cells. This is the most extreme case.

A number of mathematical models have included latently infected cells. Culshaw et al. (2003) modelled cell-to-cell spread of HIV with a time delay, although they neglected free virus; they demonstrated that latently infected cells may be instrumental in sustaining infective oscillations. Curlin et al. (2007) modelled induction-maintenance (IM) therapy, with latent cells included; they suggested that both IM and antiretroviral therapy may fail if the latent pool was too large. Hadjiandreou et al. (2007) constructed a model of the complete timecourse of HIV/AIDS, including immunological compartments such as macrophages, latently infected cells and cyto-

toxic T-lymphocytes. Jones and Perelson (2007) modelled viral blips and showed that a latent reservoir could produce viral transients when activated by opportunistic infections. Sedaghat et al. (2007) employed a simple model for the dynamics of the latent reservoir to show that the stability of the latent reservoir was unlikely to arise from ongoing replication during antiretroviral therapy. Shi et al. (2008) formulated a cellular automata model for HIV dynamics and drug treatment that showed that the chronic phase of infection is sustained by the activation of latently infected cells.

Recently, it has been shown that the mechanics of HIV drug interaction should be included in mathematical models (Smith? 2008). Here, the effect of the drugs is assumed to be instantaneous at dosing times t_k and s_k, for RTI and PI dosing, respectively. This results in a system of impulsive differential equations, whereby solutions are continuous for $t \neq t_k$ and $t \neq s_k$ (satisfying the associated system of ordinary differential equations) and undergo an instantaneous change in state when $t = t_k$ or $t = s_k$ (see Bainov and Simeonov 1989, 1993, 1995; Lakshmikantham et al. 1989 for more details). Impulsive differential equations have been used to model HIV dynamics in a variety of settings (Krakovska and Wahl 2007; Smith? and Wahl 2004; Smith? and Schwartz 2008), and have been used for general disease models (d'Onofrio 2002). Despite the discontinuities in solutions in some variables (as well as the discontinuities in the derivatives of most others), impulsive differential equations have been shown to have good approximations to nonimpulsive models (Smith? and Schwartz 2008).

This paper is organised as follows. In Sect. 2, we introduce the mathematical model. In Sect. 3, we analyse the stability of the disease-free equilibrium in the absence of drugs, the disease-free impulsive orbit in the presence of drugs and determine the outcomes when there is sufficiently frequent dosing of either RTIs or PIs. In Sect. 4, we perform numerical simulations to compare our theoretical predictions with realistic dosing regimens. We conclude with a discussion.

2 The model

We include CD4$^+$ T cells that may be susceptible, infected or latently infected. A fraction of infected cells will be latently infected; such cells do not produce infectious virus and may have longer life than productively infected cells. Upon activation, latently infected cells become productively infected cells. Infected T cells produce infectious virus and, in turn, the immune system responds to the presence of infectious virus by creating antibodies.

In our model, we describe latently infected cells by a separate compartment, rather than via a delay (Mittler et al. 1998; Nelson et al. 2001, 2000; Nelson and Perelson 2002). This has the advantage of explicitly accounting for the viral dynamics (Smith? 2008), although it has the disadvantage of introducing more parameters. We assume that some cells become latently infected upon contact with the virus at rate α_L, but that they are not productively infected (Kirschner et al. 1997) until they leave the latent state (Wu and Ding 1999) with rate p_L.

Susceptible cells may be inhibited with either RTIs, PIs or may be infected. Infected cells may be inhibited with PIs, and cells inhibited with one drug may be inhibited with the other. Drug effects may wear off, and the rate of such waning may differ for each drug. Drugs are modelled via impulsive differential equations, so that they

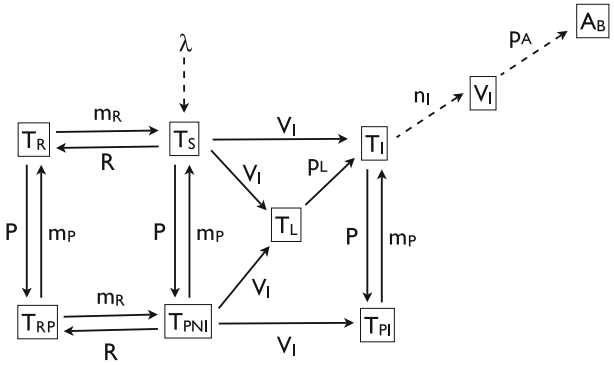

Fig. 1 The model. Susceptible T cells (T_S) are produced from the lymphic source at rate λ. Uninfected cells may be inhibited with RTIs (T_R), PIs (T_{PNI}) or both drugs (T_{RP}). Drug effects wear off at rates m_R and m_P. Infected cells (T_I) produce infectious virus (V_I); the presence of such virus also prompts an antibody response (A_B) from the immune system. Infected cells may be subsequently inhibited by PIs (T_{PI}), whereupon they no longer produce infectious virus. Susceptible cells and cells inhibited with PIs may become infected; a fraction of these will be latently infected (T_L). Uninfected cells inhibited with RTIs cannot become infected while they remain in this state; conversely, RTIs have no effect on already-infected cells. We assume that neither drug has an effect on latently infected cells, thus modelling the most extreme case. See Table 1 for the complete list of coefficients

decay exponentially during each cycle as they are metabolised and then undergo an instantaneous change in state when the next dose is received. In order to assess the effects of the viral reservoir of latently infected cells, we will assume that such cells are (a) unaffected by either drug and (b) live as long as susceptible cells (Kirschner et al. 1997). The model is illustrated in Fig. 1.

Thus, the model is

$$
\begin{aligned}
\frac{dV_I}{dt} &= n_I T_I - d_V V_I \\
\frac{dA_B}{dt} &= p_A V_I - d_A A_B \\
\frac{dT_S}{dt} &= \lambda - r_I T_S V_I - d_S T_S - r_R T_S R - r_P T_S P + m_R T_R + m_P T_{PN} \\
\frac{dT_I}{dt} &= q_I T_S V_I - d_I T_I + p_L T_L - \delta_A A_B T_I - r_P T_I P + m_P T_{PI} \\
\frac{dT_L}{dt} &= \alpha_L T_S V_I + \alpha_L T_{PN} V_I - d_L T_L - p_L T_L \\
\frac{dT_R}{dt} &= r_R T_S R - d_S T_R + m_P T_{RP} - m_R T_R - r_P T_R P \\
\frac{dT_{RP}}{dt} &= r_R T_{PN} R - d_S T_{RP} - m_P T_{RP} - m_R T_{RP} + r_P T_R P \\
\frac{dT_{PN}}{dt} &= r_P T_S P - d_S T_{PN} - r_I T_{PN} V_I - r_R T_{PN} R - m_P T_{PN} + m_R T_{RP} \\
\frac{dT_{PI}}{dt} &= q_I T_{PN} V_I - d_I T_{PI} - \delta_A A_B T_{PI} + r_P T_I P - m_P T_{PI}
\end{aligned}
\tag{1}
$$

for $t \neq t_k, s_k$. The behaviour of drugs is given by

$$\begin{aligned} \frac{dR}{dt} &= -d_R R \qquad t \neq t_k \\ \frac{dP}{dt} &= -d_P P \qquad t \neq s_k. \end{aligned} \tag{2}$$

The impulsive conditions are

$$\begin{aligned} \Delta R &= R^i \qquad t = t_k \\ \Delta P &= P^i \qquad t = s_k. \end{aligned} \tag{3}$$

The parameters are summarised in Table 1. All parameters and initial conditions are assumed to be nonnegative.

3 Analysis

3.1 The absence of drugs

In the absence of drugs, there are two equilibria. The disease-free equilibrium is given by

$$(V_I, A_B, T_S, T_I, T_L, T_R, T_{RP}, T_{PN}, T_{PI}) = \left(0, 0, \frac{\lambda}{d_S}, 0, 0, 0, 0, 0, 0\right)$$

The endemic equilibrium is given by

$$(V_I, A_B, T_S, T_I, T_L, T_R, T_{RP}, T_{PN}, T_{PI}) = \left(\bar{V}_I, \bar{A}_B, \bar{T}_S, \bar{T}_I, \bar{T}_L, 0, 0, 0, 0\right),$$

where

$$\begin{aligned} \bar{A}_B &= \frac{p_A}{d_A}\bar{V}_I \\ \bar{T}_L &= \frac{\alpha}{d_L + p_L}\bar{V}_I \bar{T}_S \\ \bar{V}_I &= \frac{d_A n_I}{\delta_A p_A d_V (d_L + p_L)}(q_I(d_L + p_L) + p_L\alpha_L)\bar{T}_S - \frac{d_S d_A}{\delta_A p_A} \end{aligned}$$

and where $\bar{T}_S$ is the positive root of

$$\begin{aligned} &\frac{r_I d_A n_I}{\delta_A p_A d_V (d_L + p_L)}(q_I(d_L + p_L) + p_L\alpha_L)T_S^2 \\ &+ \left[\frac{r_I d_A n_I}{\delta_A p_A d_V (d_L + p_L)}(q_I(d_L + p_L) + p_L\alpha_L) - \frac{d_S d_A}{\delta_A p_A} + d_S\right]\bar{T}_S - \lambda = 0. \end{aligned}$$

Table 1 List of symbols

Parameter	Units	Explanation	Value
V_I	virions μM^{-1}	Infectious virus	–
A_B	antibodies μM^{-1}	Antibodies	–
T_S	cells μM^{-1}	Susceptible $CD4^+$ T cells	–
T_I	cells μM^{-1}	Infected $CD4^+$ T cells	–
T_L	cells μM^{-1}	Latently infected $CD4^+$ T cells	–
T_R	cells μM^{-1}	$CD4^+$ T cells inhibited with RTIs	–
T_{RP}	cells μM^{-1}	$CD4^+$ T cells inhibited with both RTIs and PIs	–
T_{PN}	cells μM^{-1}	Uninfected $CD4^+$ T cells inhibited with PIs	–
T_{PI}	cells μM^{-1}	Infected $CD4^+$ T cells inhibited with PIs	–
λ	cells $days^{-1}$	Production rate of $CD4^+$ T cells	280
d_V	$days^{-1}$	Clearance rate of infectious virus	3
d_A	$days^{-1}$	Clearance rate of antibodies	0.5
d_S	$days^{-1}$	Death rate of uninfected $CD4^+$ cells	0.1
d_I	$days^{-1}$	Death rate of infected $CD4^+$ cells	0.5
d_L	$days^{-1}$	Death rate of latently infected $CD4^+$ cells	0.1
n_I	virions $cells^{-1} days^{-1}$	Rate of production of virions per productively infected cell	265.5
r_I	$cells^{-1}$ day^{-1}	Rate of infection of susceptible cells	0.0032
q_I	$virions^{-1} days^{-1}$	Rate of increase of infected cells	$0.8r_I$
p_A	antibodies $virions^{-1} days^{-1}$	Production rate of antibodies in response to virus	0.01
δ_A	$days^{-1}$	Loss of infected cells due to antibodies	0.01
p_L	$days^{-1}$	Rate at which latently infected cells become productive	0.05
α_L	$virions^{-1} days^{-1}$	Production rate of latently infected cells in response to virions	$0.2r_I$
R	μM	Reverse transcriptase inhibitor (RTI)	–
P	μM	Protease inhibitor (PI)	–
r_R	$\mu M^{-1} days^{-1}$	Rate at which RTIs inhibit $CD4^+$ T cells	20
r_P	$\mu M^{-1} days^{-1}$	Rate at which PIs inhibit $CD4^+$ T cells	20
m_R	$days^{-1}$	Rate at which RTIs are cleared from intracellular compartments	0.6931

Table 1 continued

Parameter	Units	Explanation	Value
m_P	days^{-1}	Rate at which PIs are cleared from intracellular compartments	3.4567
d_R	days^{-1}	Rate at which RTIs are cleared from the body	0.6654
d_P	days^{-1}	Rate at which PIs are cleared from the body	1.3863
R^i	μM	RTI dosage	4.65
P^i	μM	PI dosage	1.85
t_k	days	RTI dosing times	–
s_k	days	PI dosing times	–
τ	days	RTI dosing period	0.5
σ	days	PI dosing period	1

All parameters have sample values listed, whereas state variables (marked by a dash) vary according to model (1)

The Jacobian matrix is $J = [J_1|J_2]$, where

$$J_1 = \begin{bmatrix} -d_V & 0 & 0 & n_I & 0 \\ p_A & -d_A & 0 & 0 & 0 \\ -r_I T_S & 0 & -d_S - r_I V_I - r_R R - r_P P & 0 & 0 \\ q_I T_S & -\delta_A T_I & q_I V_I & -d_I - \delta_A A_B - r_P P & p_L \\ \alpha_L (T_S + T_{PN}) & 0 & \alpha_L V_I & 0 & -d_L - p_L \\ 0 & 0 & r_R R & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ -r_I T_{PN} & 0 & r_P P & 0 & 0 \\ q_I T_{PN} & -\delta_A T_{PI} & 0 & r_P P & 0 \end{bmatrix}$$

and

$$J_2 = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ m_R & 0 & m_P & 0 \\ 0 & 0 & 0 & m_P \\ 0 & 0 & \alpha_L V_I & 0 \\ -d_S - m_R - r_P P & m_P & 0 & 0 \\ r_P P & -m_R - m_P - d_S & r_R R & 0 \\ 0 & m_R & -r_R R - m_P - r_I V_I - d_S & 0 \\ 0 & 0 & q_I V_I & -d_I - \delta_A A_B - m_P \end{bmatrix}.$$

If there is no virus and no drugs, then the Jacobian matrix is

$$\begin{bmatrix} -d_V & 0 & 0 & n_I & 0 & 0 & 0 & 0 & 0 \\ p_A & -d_A & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -r_I T_S & 0 & -d_S & 0 & 0 & m_R & 0 & m_P & 0 \\ q_I T_S & 0 & 0 & -d_I & p_L & 0 & 0 & 0 & m_P \\ \alpha_L T_S & 0 & 0 & 0 & -d_L - p_L & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -d_S - m_R & m_P & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -m_R - m_P - d_S & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & m_R & -m_P - d_S & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -d_I - m_P \end{bmatrix}.$$

This matrix has characteristic equation

$$\begin{aligned} 0 = {} & (d_A + \Lambda)(d_S + \Lambda)(d_S + m_R + \Lambda)(m_R + m_P + d_S + \Lambda) \\ & \times (m_P + d_S + \Lambda)(d_I + m_P + \Lambda) \det M, \end{aligned}$$

where

$$M = \begin{bmatrix} -d_V - \Lambda & n_I & 0 \\ q_I T_S & -d_I - \Lambda & p_L \\ \alpha_L T_S & 0 & -d_L - p_L - \Lambda \end{bmatrix}$$

Clearly, the only positive eigenvalues for J will be positive eigenvalues of M. We thus have

$$\begin{aligned} \det M &= -(d_V + \Lambda)(d_I + \Lambda)(d_L + p_L + \Lambda) + n_I p_L \alpha_L T_S + (d_L + p_L + \Lambda) n_I q_I T_S \\ &= -\Lambda^3 - \Lambda^2 [d_V + d_I + d_L + p_L] - \Lambda [d_I (d_L + p_L) + d_V (d_L + p_L) \\ &\quad + d_V d_I - n_I q_I T_S] - d_V d_I (d_L + p_L) + n_I p_L \alpha_L T_S + (d_L + p_L) n_I q_I T_S. \end{aligned}$$

Since n_I is large compared to the death rates, M (and hence J) will have a positive eigenvalue. It follows that the disease-free equilibrium is unstable in the absence of drugs.

3.2 The presence of drugs

When drugs are present, there are no equilibria, due to impulses in the drug dynamics. However, we can still calculate equilibrium-like orbits that exhibit no variation in any state variables except for the drugs. These orbits are the impulsive analogue of equilibria.

If drugs are present and there is no virus, then the disease-free impulsive orbit is

$$(V_I, A_B, T_S, T_I, T_L, T_R, T_{RP}, T_{PN}, T_{PI}) = \left(0, 0, \hat{T}_S, 0, 0, \hat{T}_R, \hat{T}_{RP}, \hat{T}_{PN}, 0\right),$$

where

$$\hat{T}_{PN} = \frac{r_P P \hat{T}_S + m_R \hat{T}_{RP}}{d_S + r_R R + m_P} \tag{4}$$

$$\hat{T}_R = \frac{r_R R \hat{T}_S + m_P \hat{T}_{RP}}{d_S + m_R + r_P P} \tag{5}$$

$$\hat{T}_S = \frac{f_1}{f_2} T_{RP} \tag{6}$$

$$\hat{T}_{RP} = \frac{\lambda}{f_3} \tag{7}$$

with

$$\begin{aligned} f_1 &= d_S(d_S + r_P P + m_R)(d_S + r_R R + m_P) + m_P(d_S + m_R)(d_S + r_R R + m_P) \\ &\quad + m_R(d_S + m_P)(d_S + r_P P + m_R) \end{aligned} \tag{8}$$

$$f_2 = r_R R r_P P(2d_S + m_R + m_P + r_R R + r_P P) \tag{9}$$

$$\begin{aligned} f_3 &= \left[d_S + r_R R + r_P P - \frac{m_R r_R R}{d_S + r_P P + m_R} - \frac{m_P r_P P}{d_S + r_R R + m_P} \right] \frac{f_1}{f_2} \\ &\quad - \frac{m_R m_P}{d_S + r_P P + m_R} - \frac{m_R m_P}{d_S + r_R R + m_P} \end{aligned} \tag{10}$$

Define $\tau \equiv t_{k+1} - t_k$ and $\sigma \equiv s_{k+1} - s_k$. Then, the drugs satisfy the impulsive period orbits

$$R(t) = \frac{R^i e^{-d_R t}}{1 - e^{-d_R \tau}}$$

$$\text{and} \quad P(t) = \frac{P^i e^{-d_P t}}{1 - e^{-d_P \sigma}}$$

for $t_k < t < t_{k+1}$, with endpoints

$$\begin{aligned} R(t_k^+) &= \frac{R^i}{1 - e^{-d_R \tau}} \qquad R(t_{k+1}^-) = \frac{R^i e^{-d_R \tau}}{1 - e^{-d_R \tau}} \\ \text{and} \quad P(s_k^+) &= \frac{P^i}{1 - e^{-d_P \sigma}} \qquad P(s_{k+1}^-) = \frac{P^i e^{-d_P \tau}}{1 - e^{-d_P \sigma}}, \end{aligned} \tag{11}$$

respectively.

The following lemmas are straightforward, but useful.

Lemma 1 *Suppose x is a variable satisfying*

$$x'(t) < c - q(\phi)x(t),$$

where c is a constant and $q(\phi)$ is independent of x and t. Then

(a) *If $x(0) < \frac{c}{q(\phi)}$, it follows that*

$$x(t) < \frac{c}{q(\phi)}$$

for all t.

(b) *If $x(0) < \frac{c}{q(\phi)}$ and $\lim_{\phi \to 0} q(\phi) = \infty$, it follows that*

$$x(t) \to 0$$

as $\phi \to 0$ for all t.

Proof See Lemma 4.1 in Smith? and Wahl (2004).

Lemma 2 *Suppose x is a variable satisfying*

$$x'(t) > c(t) - qx(t),$$

where q is a constant and $c(t)$ is bounded as $t \to \infty$. Then

$$x(t) > x(0)e^{-qt} + \frac{c(t)}{q} - \frac{1}{q}\int_0^t c'(u)e^{-q(t-u)}du \to \frac{c_\infty}{q}$$

as $t \to \infty$, with

$$c_\infty = \lim_{t \to \infty} c(t).$$

Proof We have

$$x'(t) + qx(t) > c(t)$$

$$\frac{d}{dt}\left[e^{qt}x(t)\right] > c(t)e^{qt}$$

$$e^{qt}x(t) - x(0) > \int_0^t c(u)e^{qu}du$$

$$x(t) > x(0)e^{-qt} + \frac{c(t)}{q} - \frac{1}{q}\int_0^t c'(u)e^{-q(t-u)}du$$

□

Theorem 1 *If RTIs are taken with sufficient frequency, then cells inhibited with RTIs approach the levels of CD4$^+$ T cells in the uninfected immune system, while all other cells approach zero. That is,*

$$T_R + T_{RP} \to \frac{\lambda}{d_S}$$
$$\text{and } T_S, T_I, T_L, T_{PN}, T_{PI} \to 0,$$

as $t \to \infty$ *and* $\tau \to 0$*, for any fixed* σ.

Proof First, note that, since $d_I, d_L \geq d_S$, we have

$$T_S' + T_I' + T_L' + T_R' + T_{RP}' + T_{PN}' + T_{PI}' \leq \lambda - d_S(T_S + T_I + T_L + T_R + T_{RP} + T_{PN} + T_{PI}).$$

Thus,

$$T_S + T_I + T_L + T_R + T_{RP} + T_{PN} + T_{PI} \leq \frac{\lambda}{d_S}. \tag{12}$$

Then

$$V_I' \leq \frac{n_I \lambda}{d_S} - d_V V_I.$$

By Lemma 1,

$$V_I \leq \frac{n_I \lambda}{d_S d_V}. \tag{13}$$

Consequently, using Lemma 1,

$$A_B \leq \frac{p_A n_I \lambda}{d_A d_S d_V}. \tag{14}$$

By Lemma 5.3 of Smith? and Wahl (2004),

$$T_S \leq f(t, \tau, \sigma), \tag{15}$$

where $f(t, \tau, \sigma) \to 0$ as $\tau \to 0$ or $\sigma \to 0$ and $t \to \infty$. Using Theorem 5.1 of Smith? and Wahl (2004),

$$T_{PN} \leq \gamma(t, \tau, \sigma) \tag{16}$$

where $\gamma(t, \tau, \sigma) \to 0$ as $\tau \to 0$ and $t \to \infty$, for each fixed σ.

Hence, using Lemma 1 and (13),

$$T_L \leq \frac{\alpha_L n_I \lambda (f(t, \tau, \sigma) + \gamma(t, \tau, \sigma)}{d_S d_V (d_L + p_L)} \to 0 \tag{17}$$

as $\tau \to 0$ or $\sigma \to 0$ and $t \to \infty$.

We can write

$$T_I' + T_{PI}' = q_I V_I (T_S + T_{PN}) - d_I (T_I + T_{PI}) + p_L T_L - \delta_A A_B T_I.$$

Using Lemma 1, (13), (14) and (17), we have

$$\begin{aligned} T_I + T_{PI} &\leq \frac{q_I \frac{n_I \lambda}{d_S d_V} (f(t,\tau,\sigma) + \gamma(t,\tau\sigma)) + p_L \frac{\alpha_L n_I \lambda}{d_S d_V (d_L + p_L)} (f(t,\tau,\sigma) + \gamma(t,\tau,\sigma))}{d_I + \delta_A \frac{p_A n_I \lambda}{d_A d_S d_V}} \\ &\to 0 \end{aligned}$$

as $\tau \to 0$ and $t \to \infty$, for each fixed σ.

Finally, using (12), (13), (15) and (16) we have

$$\begin{aligned} T_S' + T_R' + T_{RP}' + T_{PN}' &= \lambda - r_I V_I (T_S + T_{PN}) - d_S (T_S + T_R + T_{RP} + T_{PN}) \\ &> \lambda - r_I \frac{n_I \lambda}{d_S d_V} (f(t,\tau,\sigma) + \gamma(t,\tau,\sigma)) - d_S (T_S + T_R + T_{RP} + T_{PN}). \end{aligned}$$

Thus, using Lemma 2, we have

$$\begin{aligned} &T_R + T_{RP} \\ &> \frac{\lambda}{d_S} - \frac{r_I}{d_S} \frac{n_I \lambda}{d_S d_V} (f(t,\tau,\sigma) + \gamma(t,\tau,\sigma)) + (T_R(0) + T_{RP}(0) + T_S(0) + T_{PN}(0)) e^{-d_S t} \\ &\quad + \frac{1}{d_S} \int_0^t \frac{r_I}{d_S} \frac{n_I \lambda}{d_S d_V} \left\{ \left[\frac{\partial}{\partial u} f(u,\tau,\sigma) + \gamma(u,\tau,\sigma) \right] \right\} e^{-q(t-u)} du - T_S - T_{PN} \\ &> \frac{\lambda}{d_S} - \frac{r_I}{d_S} \frac{n_I \lambda}{d_S d_V} (f(t,\tau,\sigma) + \gamma(t,\tau,\sigma)) + (T_R(0) + T_{RP}(0) + T_S(0) + T_{PN}(0)) e^{-d_S t} \\ &\quad + \frac{1}{d_S} \int_0^t \frac{r_I}{d_S} \frac{n_I \lambda}{d_S d_V} \left\{ \left[\frac{\partial}{\partial u} f(u,\tau,\sigma) + \gamma(u,\tau,\sigma) \right] \right\} e^{-q(t-u)} du - f(t,\tau,\sigma) - \gamma(t,\tau,\sigma) \\ &\to \frac{\lambda}{d_S} \end{aligned}$$

as $t \to \infty$ and $\tau \to 0$, for any fixed σ. □

Theorem 2 *If PIs are taken with sufficient frequency, then uninfected cells inhibited with PIs approach the levels of CD4$^+$ T cells in the uninfected immune system, while all other cells approach zero. That is,*

$$\begin{aligned} T_{RP} + T_{PN} &\to \frac{\lambda}{d_S} \\ \text{and } T_S, T_I, T_L, T_R, T_{PI} &\to 0, \end{aligned}$$

as $t \to \infty$ and $\sigma \to 0$, for any fixed τ.

Proof From (11), it follows that as $\sigma \to 0$, $r_P P \to \infty$. Thus, from (8)–(9), we have

$$\lim_{r_P P \to \infty} f_1 = \infty$$

$$\lim_{r_P P \to \infty} f_2 = \infty$$

$$\lim_{r_P P \to \infty} \frac{f_1}{f_2} = 0.$$

We also have

$$r_P P \frac{f_1}{f_2} = \frac{d_S(d_S + r_R R + m_P)(d_S + r_P P + m_R)}{r_R R(2d_S + m_P + m_R + r_P P + r_R R)} + \frac{m_R(d_S + m_P)(d_S + r_P P + m_R) + m_P(d_S + m_R)(d_S + r_R R + m_P)}{r_R R(2d_S + m_P + m_R + r_P P + r_R R)}.$$

Thus, using L'Hôpital's rule,

$$\lim_{r_P P \to \infty} r_P P \frac{f_1}{f_2} = \frac{d_S(d_S + r_R R + m_P) + m_R(d_S + m_P)}{r_R R}.$$

Using (10), we have

$$\begin{aligned}
\lim_{r_P P \to \infty} f_3 &= \lim_{r_P P \to \infty} \left[r_P P \frac{f_1}{f_2} - \frac{m_P r_P P}{d_S + r_R R + m_P} \frac{f_1}{f_2} - \frac{m_P m_R}{d_S + r_R R + m_P} \right] \\
&= \lim_{r_P P \to \infty} \left[\frac{(d_S + r_R R) r_P P f_1 / f_2}{d_S + r_R R + m_P} - \frac{m_P m_R r_R R}{r_R R(d_S + r_R R + m_P)} \right] \\
&= \frac{d_S(d_S + r_R R)(d_S + r_R R + m_P) + m_R(d_S + r_R R)(d_S + m_P)}{r_R R(d_S + r_R R + m_P)} \\
&\quad - \frac{m_P m_R r_R R}{r_R R(d_S + r_R R + m_P)} \\
&= \frac{d_S(d_S + r_R R)(d_S + r_R R + m_P) + m_R d_S(d_S + m_P + r_R R)}{r_R R(d_S + r_R R + m_P)} \\
&= \frac{d_S(d_S + r_R R + m_R)}{r_R R}.
\end{aligned}$$

Consequently,

$$\begin{aligned}
\lim_{r_P P\to\infty} T_S &= \lim_{r_P P\to\infty} \frac{f_1}{f_2} \lim_{r_P P\to\infty} T_{RP} \\
&= 0 \\
\lim_{r_P P\to\infty} T_R &= \lim_{r_P P\to\infty} \frac{r_R R T_S}{d_S + r_P P + m_R} + \lim_{r_P P\to\infty} \frac{m_P T_{RP}}{d_S + r_P P + m_R} \\
&= 0 \\
\lim_{r_P P\to\infty} T_{RP} &= \frac{\lambda r_R R}{d_S(d_S + r_R R + m_R)} \\
\lim_{r_P P\to\infty} T_{PN} &= \lim_{r_P P\to\infty} \frac{r_P P f_1/f_2 + m_R}{d_S + r_R R + m_P} T_{RP} \\
&= \frac{(d_S + m_R)}{r_R R} T_{RP}.
\end{aligned}$$

Thus

$$\begin{aligned}
\lim_{r_P P\to\infty} (T_{RP} + T_{PN}) &= \frac{d_S + m_R}{r_R R} T_{RP} + T_{RP} \\
&= \frac{d_S + r_R R + m_R}{r_R R} T_{RP} \\
&= \frac{\lambda}{d_S}.
\end{aligned}$$

□

Remark Note that, since $\tau \to 0$ implies that $r_R R \to \infty$, we have

$$\begin{aligned}
\lim_{r_R R\to\infty} \left[\lim_{r_P P\to\infty} T_{PN} \right] &= 0 \\
\lim_{r_R R\to\infty} \left[\lim_{r_P P\to\infty} T_{RP} \right] &= \frac{\lambda}{d_S}.
\end{aligned}$$

Thus, if either, or both, drugs are taken with sufficient frequency, then nonzero cells inhibited with drugs can maintain $CD4^+$ T cell counts at uninfected levels.

4 Numerical simulations

While the disease-free equilibrium is globally asymptotically stable if either RTIs or PIs are taken with sufficient frequency, in practice there are limitations on the frequency of dosing and the dosage. Too much drug will be toxic for the patient. Thus, we ran numerical simulations for realistic dosing regimens and dosages. In this case, we simulated a realistic drug, the nucleoside RTI Didanosine, supplemented by a low-level PI.

When no drugs are taken, the viral load is high (≈5,015 virions/μM) and the reservoir of latently cells is also high (≈132 cells/μM). The dynamics are fast, reaching approximate equilibrium values after only 10 days (Fig. 2).

When RTIs are taken alone, the viral load is reduced to moderate levels (≈740 virions/μM) and the reservoir of latently infected cells drops to low levels (≈8.5 cells/μM) (Fig. 3). In this case, the system does not reach equilibrium, due to impulses, but approaches an impulsive periodic orbit. Parameters in this case simulate the nucleoside RTI Didanosine.

When PIs are taken alone, the viral load is high (≈1,400), but is reduced by a factor of approximately 4 from the case of no drugs (Fig. 4). However, the reservoir of latently infected cells is barely reduced (≈130). Note the large viral load variation in this case.

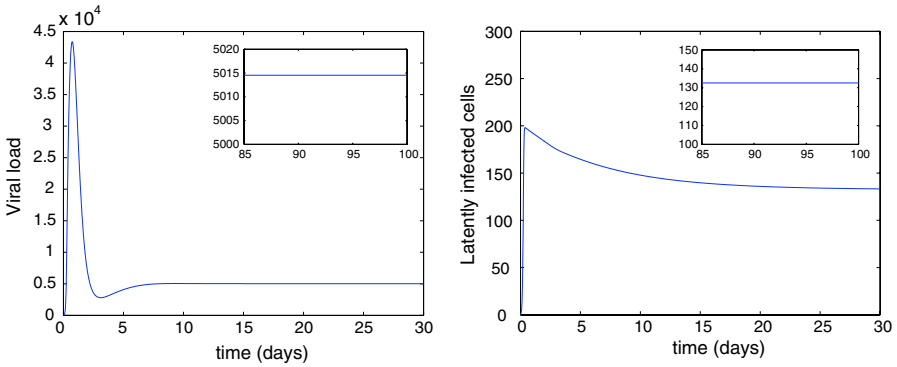

Fig. 2 The case of no drugs. Parameters are as in Table 1, except that $R^i = 0$ and $P_i = 0$. Initial conditions were $V_I(0) = 50$, $T_S(0) = 1,000$ and all other initial conditions zero. Inset: timecourse of solutions from 85 to 100 days, showing the long-term behaviour. The solution settles down into an endemic equilibrium with high viral load and a high reservoir of latently infected cells

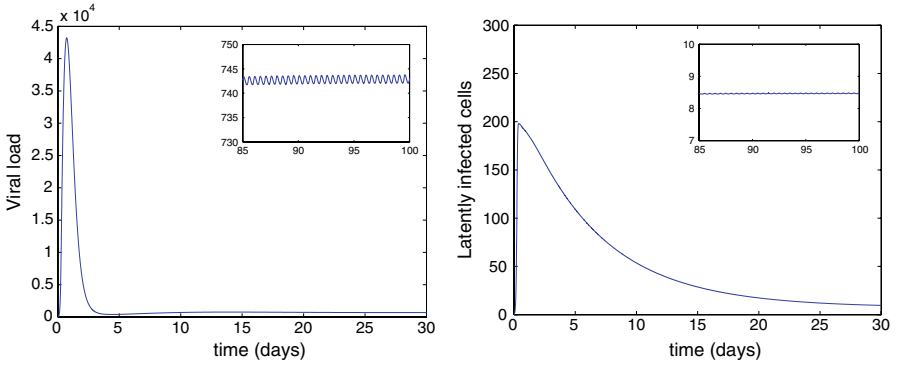

Fig. 3 Presence of RTIs, absence of PIs. All parameters as in Table 1, except that $R_i = 4.65$ and $P^i = 0$. Inset: timecourse of solutions from 85 to 100 days, showing the impulsive trajectories. The solution settles down into an impulsive periodic orbit with high viral load and a low level reservoir of latently infected cells. This level, while not zero, is significantly smaller than the level without drugs

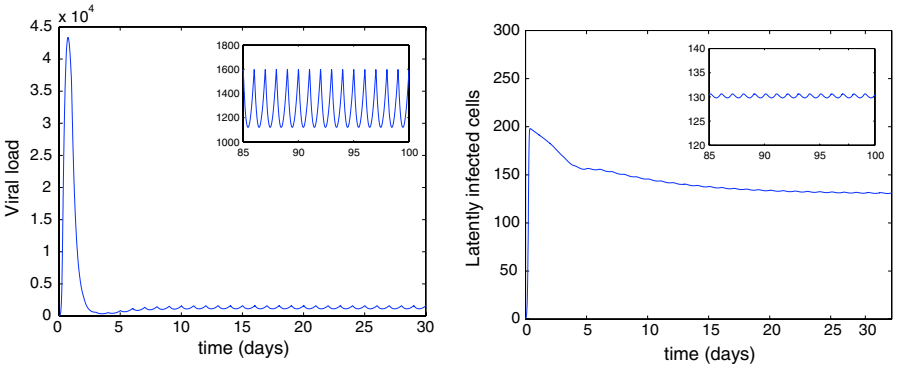

Fig. 4 Presence of PIs, absence of RTIs. All parameters as in Table 1, except that $R^i = 0$ and $P_i = 1.85$. *Inset* timecourse of solutions from 85 to 100 days, showing the impulsive trajectories. The solution settles down into an impulsive periodic orbit with moderately high viral load and a high reservoir of latently infected cells. Note that this reservoir is almost unchanged from the levels without drugs

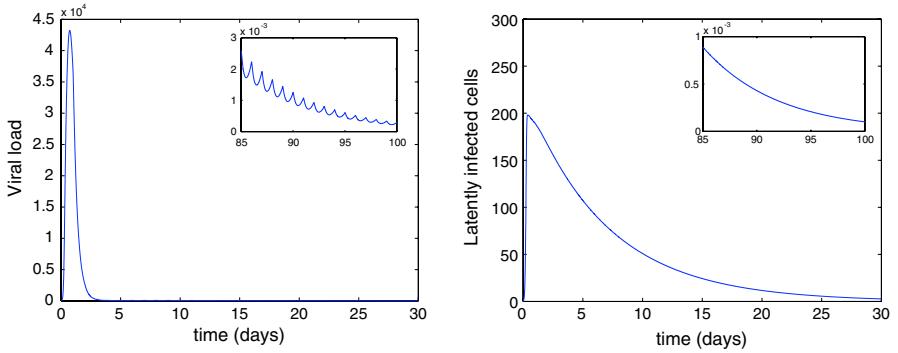

Fig. 5 The case of both drugs. All parameters as in Table 1, including $R_i = 4.65$ and $P_i = 1.85$. *Inset* timecourse of solutions from 85 to 100 days, showing eradication. Both the viral load and the reservoir of latently infected cells approach zero

When both drugs are taken, both the viral load and the reservoir of latently infected cells are driven to zero (Fig. 5). Further simulations demonstrated that, after 200 days, the latently infected cells were of the order of 10^{-13} (not shown) Note that, although the dosing intervals are realistic (twice daily for RTIs and once daily for PIs), the results nevertheless mimic the limiting case of infinitely frequent dosing predicted in Sect. 3.

5 Discussion

Latently infected cells, even if they live for a maximal time and are wholly unaffected by either major class of drug, can be eradicated by sufficiently frequent application of existing antiretroviral drug therapy. While such cells are themselves immune from drug effects in our model, their dependence on the viral load can result in their theoretical elimination, given sufficient drug application. Indeed, such elimination could

theoretically be achieved using only one of the drugs, although in practice realistic dosing regimens may or may not have a significant effect on reducing the latently infected cells (Figs. 3, 4). Using drugs drawn from both major classes, of course, is better at controlling both the virus and the latently infected cell reservoir (Fig. 5). These findings are in line with other models, which suggest that eradication could be achieved, if the drugs are sufficiently potent (Perelson et al. 1997).

When performing numerical simulations, we assumed that the lifespan of latently infected cells was equal to the lifespan of healthy cells (ie $d_L = d_S$). Although viral infection may shorten the lifespan of a latently infected cell, we consider the worst-case scenario of a reservoir of latently infected cells that are exempt from drug inhibition and who live as long as a healthy cell would. Similarly, latently infected cells may be candidates for inhibition by PIs (see Aggarwala 2007; Chun and Fauci 1999), potentially resulting in a new class of cells: latently infected cells inhibited with PIs, T_{PL}, who become T_{PI} cells upon activation. By ignoring such possibilities, we perhaps underestimate the ability of PIs to control the virus. It should also be noted that, for the parameters detailed in Figs. 2, 3, 4 and 5, the PI is significantly less optimal than the RTI. However, even such a weak PI will still contribute to the theoretical eradication of latently infected cells when combined with a standard RTI (Fig. 5).

In attempting to address the question of latently infected cell reservoirs, we have ignored other potential reservoirs of HIV, such as dendritic cells, the brain, eyes, testicles, etc (Chun and Fauci 1999; Curlin et al. 2007). The extent of viral replication in compartments other than resting CD4$^+$ T cells in patients receiving antiretroviral therapy for extended periods of time has yet to be fully delineated (Chun et al. 2005). Consequently, we expect that actual elimination is not possible, but we have demonstrated that latently infected cells, on their own, cannot sustain a reservoir of virus, if treatment is sufficiently aggressive. We have also modelled the immune system merely by production of antibodies, in direct response to viral load. This is an approximation that overlooks other potential reservoirs: cytotoxic T-lymphocytes, for example, which are responsible for cell-mediated killing of infected CD4$^+$ T cells (Smith? and Schwartz 2008), may themselves become infected (albeit at significantly lower rates than CD4$^+$ T cells) and thus provide a home for future emergence of virions.

It should be noted that the equilibrium-like orbits calculated in Sect. 3.2 may not be meaningful in a nonautonomous system. However, since the drug concentrations R and P are bounded as $t \to \infty$, our model is asymptotically autonomous. It follows, by the theory of asymptotically autonomous systems (Thieme 1992) that the stability of these equilibrium-like orbits does reflect the long-term behaviour of the system.

It should also be noted that the methods used here include sufficiently frequent dosing of PIs. In Smith? and Wahl (2004) and Smith? (2008), it was theorised that sufficiently frequent dosing of PIs alone may be insufficient to maintain healthy CD4$^+$ T cell counts. While we have shown here that this will not be true in the limit, we have nevertheless seen that, for realistic dosing regimens, PIs may be unable to reach levels close to those required for viral eradication. Recently, these results were validated, in a clinical study that investigated the possible replacement of triple drug therapy with PI monotherapy, but which showed that such an alternative was not feasible (Fernández-Montero 2008).

Future work will examine the effect of viral blips in the context of explicit drug dynamics, include drug resistance and address the issue of partial adherence to drug regimens on facilitating reservoirs of virus. We will also adapt the model to account for other viral reservoirs and more complex depictions of the immune system.

In conclusion, sufficiently aggressive treatment can control both the viral load and also theoretically eliminate the reservoir of latently infected cells, even under the extreme assumptions that such cells are unaffected by drugs and live as long as uninfected cells. While this follows from the global stability of the disease-free impulsive orbit under infinitely frequent dosing of either drug, similar results also hold for realistic dosing regimens when both drugs are present. It follows that, with sufficient application of existing antiretroviral therapy, latently infected cells cannot sustain a viral reservoir on their own. Consequently, any such reservoir must therefore be supplemented from other viral sources.

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