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Distinct Effects of Protease and Reverse Transcriptase Inhibition in an Immunological Model of HIV-1 Infection with Impulsive Drug Effects

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We present an immunological model that considers the dynamics of $CD4^+$ T cells interacting with free virions, reverse transcriptase inhibiting drugs and protease inhibiting drugs. We divide the T cells into multiple classes and use impulsive differential equations to describe the drug activity. As expected, we find that insufficient dosing of either drug corresponds to high viral load and a large population of infectious T cells. The model further predicts that, in the absence of physiological limits on tolerable drug concentrations, sufficiently frequent dosing with the reverse transcriptase inhibitor alone could theoretically maintain the $CD4^+$ T cell count arbitrarily close to the T cell count in the uninfected immune system. However, for frequent dosing of the protease inhibitor alone, the limiting T cell populations may not be enough to maintain the immune system. Furthermore, frequent dosing of both drugs has the same net effect on the T cell population as frequent dosing of the reverse transcriptase inhibitor only. Thus, the two drug classes can have fundamentally different effects on the long-term dynamics and the reverse transcriptase inhibitor, in particular, plays a crucial role in maintaining the immune system. We also provide estimates for the dosing intervals of each drug that could theoretically sustain the T cell population at a desired level.

1. INTRODUCTION

The best current therapy for HIV involves the simultaneous administration of two or more anti-viral drugs, potent inhibitors of HIV-1 replication *in vivo*. Although several new classes of drugs are now in clinical trials (Moyle, 2003), these drugs are typically chosen from two major classes of anti-virals, reverse transcriptase inhibitors and protease inhibitors. Reverse transcriptase inhibitors block the translation of viral RNA into DNA for incorporation into the host genome, thus preventing the infection of new cells; in contrast protease inhibitors interfere with

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essential steps of protein cleavage in new virions, thus preventing infected cells from producing infectious viral particles (Janeway *et al*., 2001).

Mathematical models have been developed to study the dynamics of viral replication during HIV-1 infection [see Covert and Kirschner (2000), Nowak and May (2000), Perelson (2002) for review articles], including the effects of drug treatment (Nowak *et al*., 1997; Kepler and Perelson, 1998; Nowak and May, 2000; Wahl and Nowak, 2000; Nelson *et al*., 2001; Calloway and Perelson, 2002; Nelson and Perelson, 2002; Perelson, 2002). The effects of different drug classes, however, have typically been aggregated in these modelling approaches (Nowak and May, 2000; Calloway and Perelson, 2002), with some notable exceptions (Nelson and Perelson, 2002; Perelson, 2002). We present here a model in which the two major classes of anti-virals are treated separately, allowing us to examine the (possibly different) effects of each drug on viral dynamics.

The distinguishing feature of our model is that the immune cells infected by the virus, $CD4^+$ T cells, are divided into multiple classes, depending on whether a cell has been infected or has absorbed either of the drugs. We also make use of impulsive differential equations to model the change in drug concentration which occurs when a new dose is administered. These techniques allow us to make a number of interesting predictions: we find, for example, that the reverse transcriptase inhibitor has the potential to maintain immune function, in the sense that it is possible to choose a small enough dosing interval so that the population of T cells is arbitrarily close to the level for the uninfected immune system, whether or not the protease inhibitor is also present. However, the protease inhibitor alone may not be enough to ensure a sufficient immune response, regardless of the dosing frequency.

This paper is organised as follows. In Section 2 we develop the model. In Section 3 we examine the model without drugs. In Section 4 we state some preliminary results for the model with drugs. In Section 5, we consider the extreme cases where the dosing intervals of one or both drugs shrink to zero. In Section 6 we provide some numerical simulations to illustrate the predictions of the model. In Section 7 we provide estimates on the dosing intervals to guarantee a suitably healthy immune system. Finally, in Section 8 we discuss the biological implications of the model predictions.

2. THE MODEL

2.1. ***T cells.*** We would like to examine the various possible fates of a $CD4^+$ T cell in some detail. At any time, a T cell may come into contact with (1) an infectious virion, (2) the reverse transcriptase inhibitor or (3) the protease inhibitor. Naïvely, it seems that we might need a very large number of T cell classes to capture all of these possible effects, since in its lifetime each cell may undergo several (or none) of these interactions, and the order in which the interactions occur

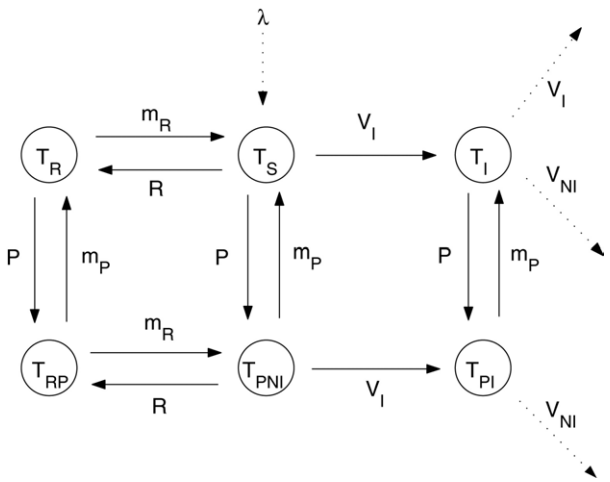

Figure 1. The various drug classes and their interactions. The six classes of T cells are susceptible, infected, reverse transcriptase inhibited, doubly inhibited, noninfected protease inhibited and infected protease inhibited cells. Each cell may come into contact with an infectious virion, the reverse transcriptase inhibitor or the protease inhibitor. Once infected, cells cannot move into the class of reverse transcriptase inhibited cells. Cells with either drug will eventually revert back to their appropriate drug-free state. Healthy cells are produced at a rate λ. Infected cells will produce infectious or defective (noninfectious) virions. Infected protease inhibited cells will produce noninfectious virus.

may also be important (encountering reverse transcriptase followed by virus will be different from encountering the virus first). By careful consideration of the possible combinations, however, we find that T cells can be classified into six populations, described below in paragraphs (a) through (f) and pictured in Fig. 1.

Throughout these classifications, we define a 'reverse transcriptase inhibited' T cell as a cell in which the intracellular concentration of the drug is sufficiently high that the probability of viral RNA being transcribed to DNA is negligible. Likewise a 'protease inhibited' T cell is a cell in which the chance of producing infectious virions is negligible. A 'doubly inhibited' T cell is one which has absorbed sufficient quantities of both drugs.

We assume that each drug affects the relevant T cells for a certain period of time, after which the T cells revert to their appropriate state. Thus, reverse transcriptase inhibited cells and uninfected protease inhibited cells will revert to susceptible cells, doubly inhibited cells will revert to either reverse transcriptase inhibited cells or uninfected protease inhibited cells, while infected protease inhibited cells will revert to infected T cells. This feature of the model may be important because, for example, lamivudine has an intracellular half-life of 12 h (Rodman *et al*., 1996) and zidovudine has a half-life of 4 h (Cammack *et al*., 1992), whereas the lifetime of an uninfected cell is of the order of days (Nelson and Perelson, 2002).

(a) Let T_S be the population of susceptible (noninfected) $CD4^+$ T cells. These cells are produced at a constant rate, λ. There are four possible fates for these

T cells: they may die at natural death rate d_S; they may (b) become infected; or they may absorb either (c) the reverse transcriptase inhibitor or (d) the protease inhibitor.

(b) We use T_I to denote the population of infected CD4$^+$ T cells. These cells produce infectious virus, V_I, or defective (noninfectious) virus V_{NI}, and have a significantly higher death rate, d_I (Ho *et al.*, 1995). Like the healthy cells, these cells may later absorb either the reverse transcriptase inhibitor or the protease inhibitor. If the protease inhibitor is absorbed, the cell will join the population of (f) infected, protease-inhibited cells (T_{PI}, described below). Since the viral genome has already been transcribed into the host DNA, absorbing the reverse transcriptase inhibitor has no effect on these infected cells.

(c) T_R denotes noninfected cells which have absorbed the reverse transcriptase inhibitor, but not the protease inhibitor. Like T_S cells, these cells may also come into contact with either infectious virus or the protease inhibitor. Since the cell cannot be infected while in this state, the former has no consequence for the cell. If the protease inhibitor is absorbed, the cell will join the population of (d) noninfected doubly inhibited cells (T_{RP}, described below).

(d) T_{RP} denotes noninfected cells which have absorbed both the reverse transcriptase inhibitor and the protease inhibitor. Like T_R cells, these cells cannot be infected while in this state.

As we will demonstrate shortly, the T_R and T_{RP} classes of cells prove to be crucial for the maintenance of the immune system, even if the population of healthy susceptible cells, T_S, approaches zero.

(e) T_{PNI} denotes noninfected cells which have absorbed the protease inhibitor but not the reverse transcriptase inhibitor. These cells may subsequently absorb infectious virus or the reverse transcriptase inhibitor. In the former case, the cell will join the population of (f) infected protease-inhibited cells (T_{PI}, described below). In the latter case, the cell will join the population of (d) noninfected doubly inhibited cells, T_{RP}.

(f) Finally, T_{PI} denotes infected cells which have absorbed the protease inhibitor. Unlike the population T_I, these cells release only noninfectious virus, V_{NI}. Like T_I, however, these cells die at the higher death rate d_I, and are unaffected by subsequent absorption of the reverse transcriptase inhibitor.

Infectious virions, V_I, are produced by infected T cells and are removed by clearance, infection of susceptible cells and infection of protease inhibited cells. Nonfectious virions, V_{NI}, are produced by protease inhibited cells or represent deficient virions produced by infected cells and are removed by clearance. We ignore the loss of virions due to reinfection of infected cells or infection of reverse transcriptase inhibited cells. This has the effect of overestimating the virion count and plays no important role in the estimates below.

2.2. ***Drugs.*** We use R to denote the intracellular concentration of the reverse transcriptase inhibitor and its active metabolites (Hoggard and Back, 2002), while P denotes the intracellular concentration of the protease inhibitor. We assume that

drugs are given at independent times t_k for the reverse transcriptase inhibitor and s_k for the protease inhibitor (not necessarily fixed, although later we shall assume that they are). The effect of the drugs is assumed to be instantaneous, resulting 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 $t = s_k$. The model thus consists of a system of ordinary differential equations together with two difference equations. According to impulsive theory, we can describe the nature of the impulse at time r_k via the difference equation

$$\Delta y \equiv y(r_k^+) - y(r_k^-) = f(r_k, y(r_k^-)). \tag{2.1}$$

We refer the interested reader to Bainov and Simeonov (1989, 1993, 1995) and Lakshmikantham *et al.* (1989) for more details on the theory of impulsive differential equations.

Approximation by impulsive differential equations is typical when a period of rapid change occurs on a timescale that is short, compared to the timescale of the remainder of the cycle. The application of this technique here assumes that the change in intracellular drug concentration immediately after a dose is taken is nearly instantaneous, that is, the time-to-peak is negligible compared to the timescale of the intracellular activity. We explored the effects of relaxing this assumption numerically, and found that differences in therapy outcome were negligible over the timescales of interest (weeks or years of infection), for realistic time-to-peak values from the pharmacokinetics literature (see Section 6 for simulation details).

By neglecting the known dispersion and delay as the drug enters the intracellular space, we overestimate the temporal effects of dosing at intervals. The implications of this assumption will be taken up further in the discussion. For a fuller treatment of the effects of spatially distinct compartments, see Kepler and Perelson (1998); for a detailed model of the kinetics of drug action, see Austin *et al.* (1998).

2.3. ***Combining T cell populations with virus and drugs.*** The dynamics of virus and T cell concentrations are thus given by:

$$\begin{aligned}
\frac{dV_I}{dt} &= n_I \omega T_I - d_V V_I - r_I T_S V_I - r_I T_{PNI} V_I \\
\frac{dV_{NI}}{dt} &= n_I T_{PI} + n_I (1-\omega) T_I - d_V V_{NI} \\
\frac{dT_S}{dt} &= \lambda - r_I T_S V_I - d_S T_S - r_R T_S R - r_P T_S P + m_R T_R + m_P T_{PNI} \\
\frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I - r_P T_I P + m_P T_{PI}
\end{aligned}$$

$$\begin{aligned}
\frac{dT_R}{dt} &= r_R T_S R - d_S T_R + m_P T_{RP} - m_R T_R - r_P T_{RP} P \\
\frac{dT_{RP}}{dt} &= r_R T_{PNI} R - d_S T_{RP} - m_P T_{RP} - m_R T_{RP} + r_P T_R P \\
\frac{dT_{PNI}}{dt} &= r_P T_S P - d_S T_{PNI} - r_I T_{PNI} V_I - r_R T_{PNI} R - m_P T_{PNI} + m_R T_{RP} \\
\frac{dT_{PI}}{dt} &= r_I T_{PNI} V_I - d_I T_{PI} + r_P T_I P - m_P T_{PI}
\end{aligned} \tag{2.2}$$

for $t \neq t_k$ and $t \neq s_k$ (see impulsive conditions below).

Here t is time in days, n_I is the number of virions produced per infected cell per day, ω is the fraction of virions produced by an infected T cell which are infectious, d_V is the rate at which free virus is cleared, d_S is the noninfected CD4$^+$ T cell death rate, d_I is the infected CD4$^+$ T cell death rate, r_I is the infection rate of noninfected T cells, r_R is the rate at which the reverse transcriptase inhibitor inhibits the T cells, r_P is the rate at which the protease inhibitor inhibits the T cells, m_R is the rate at which the reverse transcriptase inhibitor is cleared from the intracellular compartment, m_P is the rate at which the protease inhibitor is similarly cleared, and λ represents a source of susceptible cells. All death rates, rates of infection and λ are assumed to be positive and we assume $0 \ll \omega \leq 1$. Furthermore, $d_S < d_I < d_V$ (Ho *et al.*, 1995).

In addition, the dynamics of the two drugs, R and P, are given by

$$\begin{aligned}
\frac{dR}{dt} &= -d_R R \qquad t \neq t_k \\
\frac{dP}{dt} &= -d_P P \qquad t \neq s_k.
\end{aligned} \tag{2.3}$$

The impulsive conditions are

$$\begin{aligned}
\Delta R &= R^i \qquad t = t_k \\
\Delta P &= P^i \qquad t = s_k.
\end{aligned} \tag{2.4}$$

Here, d_R is the rate at which the reverse transcriptase inhibitor is cleared, d_P is the rate at which the protease inhibitor is cleared, P^i is the dose of protease inhibitor and R^i is the dose of reverse transcriptase inhibitor. In general $s_k \neq t_k$, so that the two drugs are taken at different times. Thus (2.2)–(2.4) describe a system of impulsive differential equations.

Note that using (2.1), we have

$$R(t_k^+) = R(t_k^-) + R^i \tag{2.5}$$

$$P(s_k^+) = P(s_k^-) + P^i. \tag{2.6}$$

The impulse times t_k, s_k can be assumed fixed, reflecting regular dosing periods, although we can set t_1 to be significantly large to reflect the fact that drugs are not taken until after the infection has been diagnosed. We will likewise assume that $R(0) = P(0) = 0$.

3. THE SYSTEM WITHOUT DRUGS

First we shall analyse the model when there are no drugs present. In this case, model (2.2) becomes a system of (nonimpulsive) ordinary differential equations.

$$\begin{aligned}
\frac{dV_I}{dt} &= n_I\omega T_I - d_V V_I - r_I T_S V_I - r_I T_{PNI} V_I \\
\frac{dV_{NI}}{dt} &= n_I T_{PI} + n_I(1-\omega)T_I - d_V V_{NI} \\
\frac{dT_S}{dt} &= \lambda - r_I T_S V_I - d_S T_S + m_R T_R + m_P T_{PNI} \\
\frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I + m_P T_{PI} \\
\frac{dT_R}{dt} &= -d_S T_R + m_P T_{RP} - m_R T_R \\
\frac{dT_{RP}}{dt} &= -d_S T_{RP} - m_P T_{RP} - m_R T_{RP} \\
\frac{dT_{PNI}}{dt} &= -d_S T_{PNI} - r_I T_{PNI} V_I - m_P T_{PNI} + m_R T_{RP} \\
\frac{dT_{PI}}{dt} &= r_I T_{PNI} V_I - d_I T_{PI} - m_P T_{PI}.
\end{aligned} \tag{3.7}$$

This system has two non-negative steady states, given by

$$\begin{aligned}
(\bar{V}_I, \bar{V}_{NI}, \bar{T}_S, \bar{T}_I, \bar{T}_R, \bar{T}_{RP}, \bar{T}_{PNI}, \bar{T}_{PI}) &= \left(0, 0, \frac{\lambda}{d_S}, 0, 0, 0, 0, 0\right), \\
&\left(\frac{\lambda}{d_V d_I}(n_I\omega - d_I) - \frac{d_S}{r_I}, n_I(1-\omega)\left(\frac{\lambda}{d_I} - \frac{d_V d_S}{r_I(n_I\omega - d_I)}\right), \frac{d_V d_I}{r_I(n_I\omega - d_I)},\right. \\
&\left.\frac{\lambda}{d_I} - \frac{d_S d_V}{r_I(n_I\omega - d_I)}, 0, 0, 0, 0\right)
\end{aligned}$$

which we shall refer to as the trivial and nontrivial equilibria, respectively. We shall refer to the number of T cells in the trivial equilibrium as the number of T cells in the uninfected immune system, since there is no virus present.

The Jacobian matrix for system (3.7), evaluated at the trivial equilibrium is $J(0, 0, \frac{\lambda}{d_S}, 0, 0, 0, 0, 0) = [J_1 \mid J_2]$ where

$$J_1 = \begin{bmatrix} -d_V - r_I\bar{T}_S & 0 & -r_I\bar{V}_I & n_I\omega \\ 0 & -d_V & 0 & n_I(1-\omega) \\ -r_I\bar{T}_S & 0 & -r_I\bar{V}_I - d_S & 0 \\ r_I\bar{T}_S & 0 & r_I\bar{V}_I & -d_I \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

$$J_2 = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & n_I \\ m_R & 0 & m_P & 0 \\ 0 & 0 & 0 & m_P \\ -d_S - m_R & m_P & 0 & 0 \\ 0 & -d_S - m_R - m_P & 0 & 0 \\ 0 & m_R & -d_S - r_I\bar{V}_I - m_P & 0 \\ 0 & 0 & r_I\bar{V}_I & -d_I - m_P \end{bmatrix}$$

where $\bar{V}_I$ and $\bar{T}_S$ are equilibrium values. This matrix has the characteristic equation

$$\begin{aligned} 0 = \det(J - \mu I) \;=\; & (d_V + \mu)(d_S + m_P + m_R + \mu)(d_S + m_R + \mu) \\ & \times (d_s + r_I\bar{V}_I + m_P + \mu)(d_I + m_P + \mu)\, f(\mu) \end{aligned}$$

where

$$f(\mu) = \mu^3 + a\mu^2 + b\mu + c$$

with

$$\begin{aligned} a \;&=\; d_V + r_I\bar{T}_S + r_I\bar{V}_I + d_S + d_I > 0 \\ b \;&=\; (d_V + d_I + r_I\bar{T}_S)(d_S + d_I + r_I\bar{V}_I) - d_I^2 - r_I\bar{T}_S(n_I\omega + r_I\bar{V}_I) \\ c \;&=\; d_V d_I(d_S + r_I\bar{V}_I) + r_I d_S\bar{T}_S(d_I - n_I\omega). \end{aligned}$$

When $\bar{V}_I = 0$ and $\bar{T}_S = \frac{\lambda}{d_S}$, we have

$$b = \left(d_V + d_I + \frac{r_I\lambda}{d_S}\right)(d_S + d_I) - d_I^2 - \frac{r_I n_I\omega\lambda}{d_S} < 0,$$

provided

$$\omega n_I > \frac{1}{r_I\lambda}(d_S(d_V + d_I) + r_I\lambda)(d_S + d_I) - \frac{d_S d_I^2}{r_I\lambda}, \tag{3.8}$$

which will be the case when ω is not too close to zero, since n_I and λ are normally large, compared to the other constants (by several orders of magnitude). Furthermore,

$$c = d_V d_S d_I + r_I\lambda(d_I - n_I\omega) < 0$$

when ω is not too close to zero, since n_I and λ are normally large, compared to the other constants.

The critical points of $f(\mu)$ occur when

$$\mu = -\frac{a}{3} \pm \frac{\sqrt{a^2 - 3b}}{3}$$

and hence it follows that there is a critical point with real part greater than zero. Thus the Jacobian matrix has at least one eigenvalue with positive real part and hence the trivial equilibrium is unstable.

Note that $d_S < d_I < d_V$. When $\bar{V}_I = \frac{\lambda}{d_V d_I}(n_I\omega - d_I) - \frac{d_S}{r_I}$ and $\bar{T}_S = \frac{d_V d_I}{r_I(n_I\omega - d_I)}$, we have

$$b = \frac{d_V d_I d_S}{n_I\omega - d_I} + \frac{\lambda r_I}{d_V}(n_I\omega - d_I) + \frac{\lambda r_I}{d_I}(n_I\omega - d_I) > 0$$

if $n_I\omega > d_I$, which is a necessary condition for the existence of the nontrivial equilibrium. Furthermore, we have

$$c = \lambda r_I(n_I\omega - d_I) - d_V d_S d_I > 0$$

provided

$$n_I\omega > d_I + \frac{d_V d_S d_I}{\lambda r_I}$$

which will be the case if ω is not too close to zero, since n_I and λ are normally large compared to the other constants. Thus the eigenvalues have a negative real part and hence the nontrivial equilibrium is usually stable. If ω is close to zero, then the infectious T cells produce mainly noninfectious virus, so the viral load is relatively low, even in the absence of drugs. Such cases are rare in HIV positive patients.

The existence of a trivial and a nontrivial steady state, as well as their stability properties, correspond to the usual properties of such immunological models without drug effects [see Calloway and Perelson (2002) or Nelson and Perelson (2002)].

4. THE SYSTEM WITH DRUGS

The application of drugs via impulsive differential equations will obviously perturb these steady states. In general, impulsive models do not exhibit steady states, but rather impulsive periodic orbits (periodic orbits with discontinuities). However, it should be noted that in model (2.2) only the drugs will exhibit discontinuities directly. The remaining parameters may have discontinuities in their derivatives, but will have continuous solutions.

We shall use the initial conditions $V_I(0) = V_0 > 0$, $V_{NI}(0) = 0$ and $T_I(0) = 0$. Before therapy, $R(0) = P(0) = 0$. It follows immediately that $T_R(0) = T_{RP}(0) = T_{PNI}(0) = T_{PI}(0) = 0$. We assume V_0 is small compared to the product $n_I\lambda$. We shall also assume $T_S(0) \leq \frac{\lambda}{d_S}$, which includes the possibility that the immune system may not be operating at peak capacity when infection begins. These initial conditions correspond to the very earliest stages of infection, when the system is at the uninfected equilibrium except for a small population of infectious virus. We are therefore assuming that (1) the initial viral load is low compared to the total viral load as the infection progresses, (2) the initial (susceptible) T cell count is usually at the uninfected equilibrium value before infection (Schacker *et al*., 1998), although we allow for the possibility that it may be less, and (3) no drugs are taken before diagnosis. These initial conditions will be assumed hereafter.

Suppose the drugs are given at fixed intervals. Let $\tau = t_{k+1} - t_k$ be the period of the reverse transcriptase inhibitor and $\sigma = s_{k+1} - s_k$ be the period of the protease inhibitor (for $k \geq 1$). For t satisfying $t_k < t \leq t_{k+1}$ and $s_j < t \leq s_{j+1}$, we have

$$R(t) = R(t_k^+)e^{-d_R(t-t_k)}$$
$$P(t) = P(s_j^+)e^{-d_P(t-s_j)}.$$

The impulsive effect means we have a recursion relation at the moments of impulse, given by

$$R(t_k^+) = R(t_k^-) + R^i$$
$$P(s_j^+) = P(s_j^-) + P^i.$$

Thus

$$R(t_k^+) = R^i\frac{1-e^{-kd_R\tau}}{1-e^{-d_R\tau}} \to \frac{R^i}{1-e^{-d_R\tau}}$$

as $k \to \infty$. Similarly

$$P(s_j^+) \to \frac{P^i}{1-e^{-d_P\sigma}}$$

as $j \to \infty$.

However, if $R(t_k^+) = \frac{R^i}{1-e^{-d_R\tau}}$, then $R(t_{k+1}^-) = \frac{R^i}{1-e^{-d_R\tau}}e^{-d_R\tau}$ and so

$$R(t_{k+1}^+) = \frac{R^i}{1-e^{-d_R\tau}}e^{-d_R\tau} + R_i$$
$$= \frac{R^i}{1-e^{-d_R\tau}}.$$

Furthermore, note that

$$R(t_k^+) - \frac{R^i}{1-e^{-d_R\tau}} = R^i\frac{1-e^{-kd_R\tau}}{1-e^{-d_R\tau}} - \frac{R^i}{1-e^{-d_R\tau}}$$
$$= -\frac{R^i e^{-kd_R\tau}}{1-e^{-d_R\tau}}.$$

It follows that the impulse points $\frac{R^i}{1-e^{-d_R\tau}}$ and $\frac{R^i e^{-d_R\tau}}{1-e^{-d_R\tau}}$ define the ends of a positive impulsive periodic orbit in the reverse transcriptase inhibitor, to which the endpoints of each cycle monotonically increase.

Similarly, the impulse points $\frac{P^i}{1-e^{-d_P\sigma}}$ and $\frac{P^i e^{-d_P\sigma}}{1-e^{-d_P\sigma}}$ define the ends of a positive impulsive periodic orbit in the protease inhibitor, to which the endpoints of each cycle monotonically increase.

The following lemma is straightforward, but will be used quite frequently.

LEMMA 4.1. *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. By linearity we have

$$\begin{aligned}
\frac{d}{dt}(e^{q(\phi)t}x) &< ce^{q(\phi)t} \\
e^{q(\phi)t}x(t) &< x(0) + \frac{c}{q(\phi)}e^{q(\phi)t} - \frac{c}{q(\phi)} \\
x(t) &< \left(x(0) - \frac{c}{q(\phi)}\right)e^{-q(\phi)t} + \frac{c}{q(\phi)} \qquad (4.9)\\
&< \frac{c}{q(\phi)} \\
&\to 0
\end{aligned}$$

as $\phi \to 0$ if $\lim_{\phi\to 0} q(\phi) = \infty$, thus proving parts (a) and (b). □

Remark. Lemma 4.1 also holds if the inequalities are reversed.

Next, recall that the death rate for noninfected cells is much less than the death rate for infected cells. If we let

$$T_{\text{tot}} \equiv T_S + T_R + T_{RP} + T_{PNI} + T_I + T_{PI}$$

then

$$\begin{aligned} T'_{\text{tot}} &= \lambda - d_S(T_S + T_R + T_{RP} + T_{PNI}) - d_I(T_I + T_{PI}) \\ &\leq \lambda - d_S(T_{\text{tot}}) \\ T_{\text{tot}}(t) &\leq \left(T_{\text{tot}}(0) - \frac{\lambda}{d_S}\right) e^{-d_S t} + \frac{\lambda}{d_S} \\ &\leq \frac{\lambda}{d_S} \end{aligned} \tag{4.10}$$

for all t, since $T_{\text{tot}}(0) \leq \lambda/d_s$. Thus the limiting value of the total number of T cells with infection is less than or equal to the number of T cells in the uninfected immune system. If there is no infection then $T_I = T_{PI} = 0$ and we have equality. In practice, $T_{\text{tot}}(t)$ will be less than λ/d_S when infection is present. By similar reasoning,

$$T_{\text{tot}}(t) \geq \frac{\lambda}{d_I}. \tag{4.11}$$

Note that these results are independent of drug activity.

5. Extreme Cases

We consider four extreme cases to demonstrate the different long-term outcomes that can occur, depending on the dosing intervals. A small dosing interval corresponds to frequent drug administration. Intuitively, we expect that small dosing intervals should provide the most effective therapy, whereas large dosing intervals should have little effect on the virus. To illustrate, we shall examine the four extreme cases, when there are no drugs (corresponding to an infinitely large dosing interval) and as each dosing interval shrinks to zero.

As the infection progresses, the susceptible T cell numbers are reduced as they become infected or receive drugs, while the infected T cells die off at a faster rate than the noninfected T cells. Intuitively, this suggests that T_R, T_{RP} and T_{PNI} will be the dominant populations as time goes on, and correspondingly, these cells will ultimately be responsible for maintaining the health of the immune system.

Furthermore, although there may be high levels of noninfectious virus, this is irrelevant to therapy outcome, given that these virus particles can play no further part in infection.

The initial conditions on the drug concentrations and the monotonicity of the impulsive trajectories imply that

$$R(t) < \frac{R^i}{1 - e^{-d_R \tau}} \qquad \text{and} \qquad P(t) < \frac{P^i}{1 - e^{-d_P \sigma}} \tag{5.12}$$

for all t. Since the impulsive drug orbits are asymptotically stable, it follows that for any $\epsilon > 0$, there exists t_1 such that

$$R(t) > \frac{R^i e^{-d_R \tau}}{1 - e^{-d_R \tau}} - \epsilon \qquad \text{and} \qquad P(t) > \frac{P^i e^{-d_P \sigma}}{1 - e^{-d_P \sigma}} - \epsilon \tag{5.13}$$

for all $t > t_1$.

We shall also assume that R^i and P^i are not both zero, so the results below apply to systems with one or both drugs. Note that if $R^i = 0$, then $R(t) \equiv 0$ and so $T_R' = -d_S T_R$. Thus, since $T_R(0) = 0$, $T_R \equiv 0$.

LEMMA 5.1. *V_I is ultimately bounded and satisfies*

$$V_I < \frac{n_I \omega \lambda}{d_S d_V}.$$

Proof. Using (4.10), we have

$$\begin{aligned} V_I' &= n_I \omega T_I - d_V V_I - r_I T_S V_I - r_I T_{PNI} V_I \\ &\leq \frac{n_I \omega \lambda}{d_S} - d_V V_I. \end{aligned}$$

Using Lemma 4.1(a), we have

$$V_I(t) < \frac{n_I \omega \lambda}{d_S d_V}$$

since $V_I(0)$ is small compared to n_I and λ. □

LEMMA 5.2. *The susceptible T cells satisfy*

$$T_S(t) > \frac{\lambda}{\alpha(\tau, \sigma)}$$

where $\alpha(\tau, \sigma) \to \infty$ as $\tau \to 0$ or $\sigma \to 0$.

Proof. Using Lemma 5.1 and (5.12), we have

$$\begin{aligned} T_S' &> \lambda - r_I T_S \frac{n_I \omega \lambda}{d_S d_V} - d_S T_S - r_R T_S \frac{R^i}{1 - e^{-d_R \tau}} - r_P T_S \frac{P^i}{1 - e^{-d_P \sigma}} \\ &= \lambda - \alpha(\tau, \sigma) T_S, \end{aligned}$$

where

$$\alpha(\tau, \sigma) = \frac{r_I n_I \omega \lambda}{d_S d_V} + d_S + \frac{r_R R^i}{1 - e^{-d_R \tau}} + \frac{r_P P^i}{1 - e^{-d_P \sigma}}$$

as $\tau \to 0$ or $\sigma \to 0$.

Since λ and n_I are large compared to the other constants, it follows that $\frac{\lambda}{\alpha(\tau,\sigma)}$ is small in general. It is thus reasonable to expect that $T_S(0) > \frac{\lambda}{\alpha(\tau,\sigma)}$, since the body already has a sizable number of T cells when initially infected. Thus, by the analogue of Lemma 4.1(a), we have

$$T_S(t) > \frac{\lambda}{\alpha(\tau, \sigma)}. \quad \square$$

For simplicity of notation, define $m \equiv m_R + m_P$.

LEMMA 5.3. (1) *If $R^i \neq 0$, then there exists t_1 such that*

$$T_S(t) < \frac{\lambda(1 + \frac{m}{d_S})(1 - e^{-d_R \tau})}{r_R R^i e^{-d_R \tau}} + \delta(t, \tau, \sigma)$$

for $t > t_1$, where $\delta(t, \tau, \sigma) \to 0$ as $t \to \infty$ or $\tau \to 0$ or $\sigma \to 0$.

(2) *If $P^i \neq 0$, then there exists t_1 such that*

$$T_S(t) < \frac{\lambda(1 + \frac{m}{d_S})(1 - e^{-d_P \sigma})}{r_P P^i e^{-d_P \sigma}} + \delta(t, \tau, \sigma)$$

for $t > t_1$.

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

Proof. (1) Using (5.13), if ϵ is any positive number satisfying

$$(r_R + r_P)\epsilon < \min\left\{d_S, \frac{r_P P^i e^{-d_P \sigma}}{1 - e^{-d_P \sigma}}\right\}$$

then there exists t_1 such that

$$\begin{aligned} T_S' &< \lambda - d_S T_S - r_R T_S R - r_P T_S P + \frac{\lambda m_R}{d_S} + \frac{\lambda m_P}{d_S} \\ &< \lambda\left(1 + \frac{m}{d_S}\right) - d_S T_S - \frac{r_R R^i e^{-d_R \tau}}{1 - e^{-d_R \tau}} T_S - \frac{r_P P^i e^{-d_P \sigma}}{1 - e^{-d_P \sigma}} T_S + (r_R + r_P)\epsilon T_S \end{aligned}$$

for $t > t_1$. We thus have, using (4.9)

$$T_S(t) < \left(T_S(0) - \frac{\lambda(1+\frac{m}{d_S})}{\beta(\tau,\sigma)}\right) e^{-\beta(\tau,\sigma)t} + \frac{\lambda(1+\frac{m}{d_S})}{\beta(\tau,\sigma)}$$

where

$$\begin{aligned}\beta(\tau,\sigma) &= d_S + \frac{r_R R^i e^{-d_R\tau}}{1-e^{-d_R\tau}} + \frac{r_P P^i e^{-d_P\sigma}}{1-e^{-d_P\sigma}} - (r_R + r_P)\epsilon \\ &> \frac{r_R R^i e^{-d_R\tau}}{1-e^{-d_R\tau}}.\end{aligned}$$

Thus

$$T_S(t) < \delta(t,\tau,\sigma) + \frac{\lambda(1+\frac{m}{d_S})(1-e^{-d_R\tau})}{r_R R^i e^{-d_R\tau}}$$

where

$$\delta(t,\tau,\sigma) \equiv \left(T_S(0) - \frac{\lambda(1+\frac{m}{d_S})}{\beta(\tau,\sigma)}\right) e^{-\beta(\tau,\sigma)t}.$$

Note that $\delta(t,\tau,\sigma) \to 0$ as $t \to \infty$ or $\tau \to 0$ or $\sigma \to 0$. The proof of part 2 is similar. □

THEOREM 5.1. *If $R^i \neq 0$, then $T_I \to 0$, $T_{PNI} \to 0$, $T_{PI} \to 0$ and $T_R + T_{RP} \to \frac{\lambda}{d_S}$ as $t \to \infty$ and $\tau \to 0$, for any fixed σ.*

Proof. Using part 2 of Lemma 5.3 and (5.12), there exists t_1 such that

$$\begin{aligned}T'_{PNI} \leq\ & r_P\lambda\left(1+\frac{m}{d_S}\right)e^{d_P\sigma} + \frac{\delta(t,\tau,\sigma)r_P P^i}{1-e^{-d_P\sigma}} - \frac{r_R R^i e^{-d_R\tau}}{1-e^{-d_R\tau}}T_{PNI} \\ & + r_R\epsilon T_{PNI} + \frac{m_P\lambda}{d_S}\end{aligned}$$

for $t > t_1$, where ϵ is any positive number such that

$$\epsilon < \frac{R^i e^{-d_R\tau}}{1-e^{-d_R\tau}}.$$

Thus, using Lemma 4.1,

$$\begin{aligned}T_{PNI} \ &\leq\ \frac{r_P\lambda(1+\frac{m}{d_S})e^{d_P\sigma} + \frac{\delta(t,\tau,\sigma)r_P P^i}{1-e^{-d_P\sigma}} + \frac{\lambda m_P}{d_S}}{r_R R^i e^{-d_R\tau} - r_R\epsilon(1-e^{-d_R\tau})}(1-e^{-d_R\tau}) \equiv \gamma(t,\tau,\sigma) \\ &\to 0\end{aligned}$$

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

Using Lemma 5.1, part 1 of Lemma 5.3 and Theorem 5.1, we have

$$\begin{aligned} T_I' + T_{PI}' &= r_I(T_S + T_{PNI})V_I - d_I(T_I + T_{PI}) \\ &\leq \frac{r_I n_I \omega \lambda}{d_S d_V}\left[\frac{\lambda(1+\frac{m}{d_S})(1-e^{-d_R\tau})}{r_R R^i e^{-d_R\tau}} + \delta(t,\tau,\sigma) + \gamma(t,\tau,\sigma)\right] \\ &\quad -d_I(T_I + T_{PI}) \\ T_I + T_{PI} &\leq \frac{r_I n_I \omega \lambda}{d_S d_V d_I}\left[\frac{\lambda(1+\frac{m}{d_S})(1-e^{-d_R\tau})}{r_R R^i e^{-d_R\tau}} + \delta(t,\tau,\sigma) + \gamma(t,\tau,\sigma)\right] \\ &\to 0 \end{aligned}$$

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

Using Lemma 5.1 and part 1 of Lemma 5.3, we have

$$\begin{aligned} T_S' + T_R' + T_{RP}' + T_{PNI}' &= \lambda - r_I(T_S + T_{PNI})V_I - d_S(T_S + T_R + T_{RP} + T_{PNI}) \\ &> \lambda - \frac{r_I n_I \omega \lambda}{d_S d_V}\left(\frac{\lambda(1+\frac{m}{d_S})(1-e^{-d_R\tau})}{r_R R^i e^{-d_R\tau}} + \delta(t,\tau,\sigma)\right. \\ &\quad \left. + \gamma(t,\tau,\sigma)\right) - d_S(T_S + T_R + T_{RP} + T_{PNI}). \end{aligned}$$

By the analogue of (4.9), we have

$$\begin{aligned} T_R + T_{RP} &> \frac{\lambda}{d_S} - \frac{r_I n_I \lambda}{d_S^2 d_I}\left(\frac{\lambda(1+\frac{m}{d_S})(1-e^{-d_R\tau})}{r_R R^i e^{-d_R\tau}} + \delta(t,\tau,\sigma) + \gamma(t,\tau,\sigma)\right) \\ &\quad + \left[T_S(0) - \frac{\lambda}{d_S} + \frac{r_I n_I \lambda}{d_S^2 d_I}\left(\frac{\lambda(1+\frac{m}{d_S})(1-e^{-d_R\tau})}{r_R R^i e^{-d_R\tau}} + \delta(t,\tau,\sigma)\right.\right. \\ &\quad \left.\left. + \gamma(t,\tau,\sigma)\right)\right] e^{-d_s t} - T_S - T_{PNI} \end{aligned}$$

$\to \frac{\lambda}{d_S}$ as $t \to \infty$ and $\tau \to 0$, for any fixed σ, using the first part of this proof and Lemma 5.3. □

These results are summarised in the following four cases.

Case (i) Frequent dosing of both drugs. In this case, it follows from Theorem 5.1 that for a suitably small dosing interval of the reverse transcriptase inhibitor, we can make the number of reverse transcriptase inhibited cells and doubly inhibited cells arbitrarily close to the levels of T cells in the uninfected immune system. From Theorem 5.1 and Lemma 5.3, all other cells approach zero. Thus T_R and T_{RP} dominate in this case.

Case (ii) The absence of both drugs. The nontrivial equilibrium is usually stable (for n_I sufficiently large and ω not too close to zero) as shown in Section 3. This corresponds to high levels of V_I and relatively low (or zero) levels of T_S, T_I, T_R, T_{RP}, T_{PNI} and T_{PI}. In this case the virus dominates.

Case (iii) The absence of protease inhibitor, frequent dosing of reverse transcriptase inhibitor. In the absence of the protease inhibitor, it follows from model (2.2) that

$$T'_{RP} + T'_{PNI} < -d_S(T_{RP} + T_{PNI})$$

and hence $T_{RP} \to 0$ as $t \to \infty$. It follows from Theorem 5.1 that for a suitably small dosing interval of the reverse transcriptase inhibitor, we can make the number of reverse transcriptase inhibited cells arbitrarily close to the levels of T cells in the uninfected immune system. From Theorem 5.1, Lemma 5.3 and the above, all other T cells approach zero. Thus T_R dominates in this case.

Case (iv) The absence of reverse transcriptase inhibitor, frequent dosing of protease inhibitor. In this case, it follows from (4.10) and (4.11) that

$$\frac{\lambda}{d_I} \leq T_I + T_{PNI} + T_{PI} \leq \frac{\lambda}{d_S}.$$

However, typically $d_S \ll d_I$. Let μ_0 denote the minimum number of T cells required to maintain the immune system. If μ_0 satisfies

$$\frac{\lambda}{d_I} \leq \lim_{t\to\infty}(T_I + T_{PNI} + T_{PI}) < \mu_0 < \frac{\lambda}{d_S} \tag{5.14}$$

then there is no dosing schedule that will sustain a healthy immune system.

These cases illustrate the patterns of therapy outcome which may be obtained in more realistic situations where there are frequent and infrequent dosing of the drugs, rather than infinite and zero dosing. Case (iii), for example, should approximate the situation where there is reasonably frequent dosing of the reverse transcriptase inhibitor, but infrequent dosing of the protease inhibitor, though not necessarily an absence of the protease inhibitor.

6. NUMERICAL SIMULATIONS

To illustrate these theoretical results, we performed numerical simulations of a typical dosing regimen for a reverse transcriptase inhibitor and a protease inhibitor taken together. We also tested examples of each of the other extreme cases described in the previous section. Equations (2.2)–(2.4) were integrated numerically using a fourth- and fifth-order Runge–Kutta method, ODE45 in MATLAB (The Mathworks, Inc.).

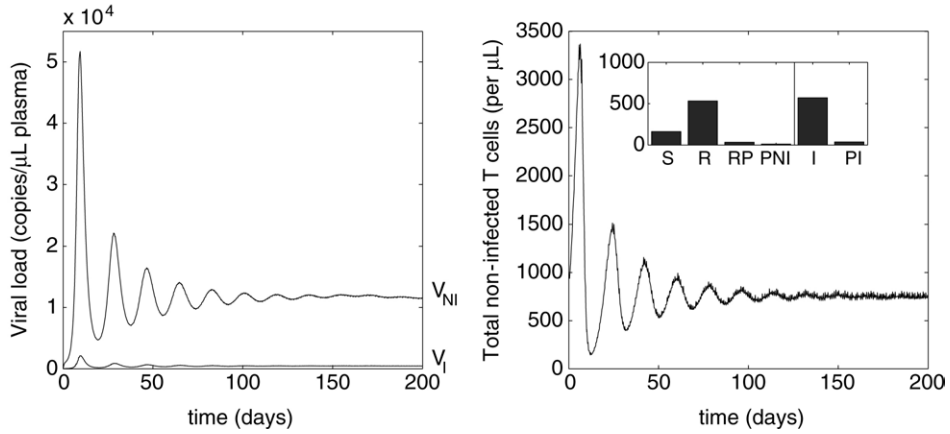

Figure 2. Frequent dosing of both drugs. Typical, frequent dosing of both drugs was simulated by numerical integration as described in the text. Non-infected T cells are maintained close to the healthy equilibrium value (1000 μl^{-1}), largely due to the contribution of T_R. The left panel plots of V_I and V_{NI}; the right panel plots the sum of T_S, T_R, T_{RP} and T_{PNI}. The proportions of each type of T cell at the end of the simulation are shown in the inset. Parameters used were $n_I = 62.5$ day^{-1} (Haase *et al*., 1996), $\omega = 0.05$, $r_I = 0.0032$ day^{-1}, $r_P = 0.127$ μM^{-1} day^{-1}, $r_R = 56.1$ μM^{-1} day^{-1}, $m_R = 4.16$ day^{-1}, $m_P = 8.52$ day^{-1}, $d_V = 3$ day^{-1}, $d_S = 0.1$ day^{-1}, $d_I = 0.5$ day^{-1}, $d_P = 8.32$ day^{-1}, $d_R = 16.6$ day^{-1}, $\lambda = 100$ cells μl^{-1} day^{-1}, $P^i = 11.6$ μM, $R^i = 7.3$ μM, $\tau = 0.5$ days and $\sigma = 0.333$ days. Initial conditions were $V_I(0) = 100$ virions μl^{-1}, $T_S(0) = 1000$ cells μl^{-1} and all other initial conditions zero.

The parameters describing T cell and virus dynamics in our simulations are largely straightforward and were taken from the literature; the reader is referred to Haase *et al*. (1996), Perelson and Nelson (1999) and Wahl and Nowak (2000) for details and to the figure legends for specific values. The parameter r_I, describing the infection rate, was determined such that T_0, the equilibrium value of T_S in the presence of infection but the absence of drug therapy, was about 180 cells μl^{-1}, as determined experimentally (Perelson and Nelson, 1999). The other parameters which are difficult to estimate are r_R and r_P, which give the fraction of susceptible T cells which become inhibited by the drug, per μM drug in plasma, per day. Although the dose–effect curves for these pharmaceuticals are well-established *in vitro*, the relations between plasma concentration, intracellular concentration, and *in vitro* test concentrations are extremely unclear. Therefore, we have made the assumption that $C_{\min}$, the trough concentration of the drug in plasma during a typical dosing regimen, is sufficient to inhibit 100% of the T cells in their lifetime. Note that this assumption will affect the quantitative results for our simulations, but has no effect on the qualitative behaviour as determined in the four analytical cases described in the previous section.

Results for a typical dosing regimen are shown in Fig. 2. We model the protease inhibitor indinavir, taken three times daily, and the reverse transcriptase inhibitor AZT, taken twice a day. The figure shows the viral load for both infectious and

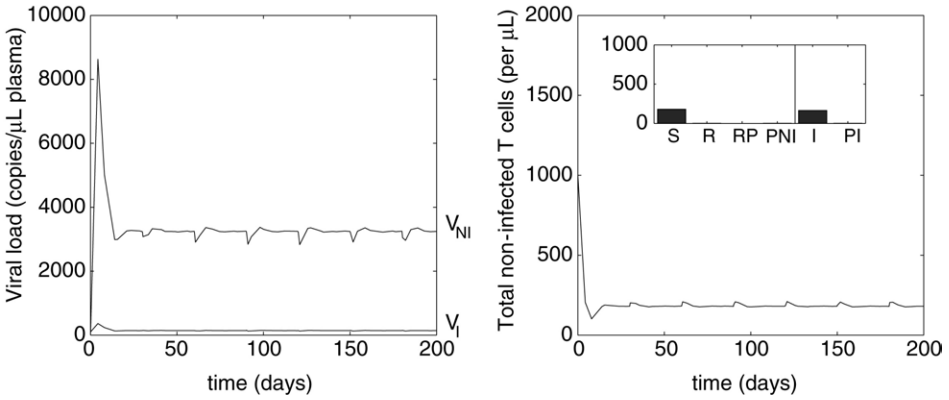

Figure 3. Infrequent dosing of both drugs. The total number of healthy T cells is significantly reduced in the absence of T_R and T_{PNI}. All parameters and initial conditions were the same as in Fig. 2, except for the dosing intervals, which were $\tau = 30$ days and $\sigma = 7$ days.

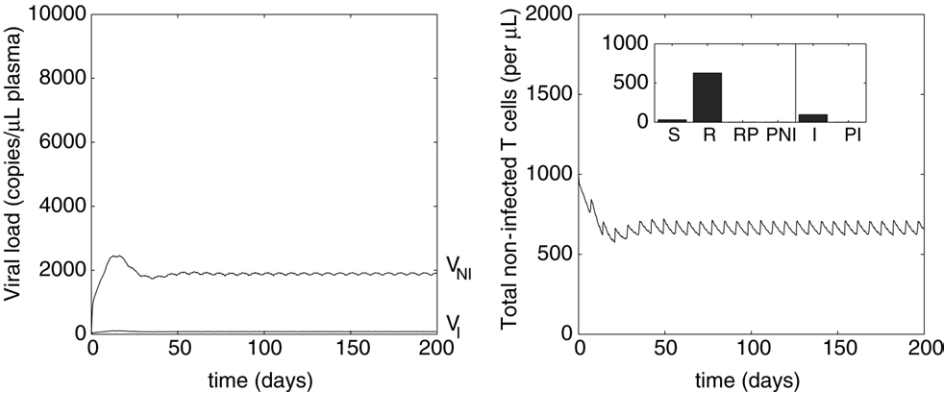

Figure 4. Frequent dosing of the reverse transcriptase inhibitor and infrequent dosing of the protease inhibitor. As in Fig. 2, non-infected T cells are maintained close to the healthy equilibrium value due to the large contribution of T_R. All parameters and initial conditions were the same as in Fig. 2, except for the dosing intervals, which were $\tau = 0.5$ days and $\sigma = 7$ days.

non-infectious virions in the left panel, and the total number of non-infected T cells, per μl plasma, on the right. The inset shows the fraction of T cells in each of the six possible states. We see that for these parameter values, a healthy T cell count is maintained, mainly due to a large population of reverse transcriptase inhibited cells. Note that this picture reflects the best-case scenario for this drug regimen, with perfect adherence to the prescribed regimen and in the absence of resistance mutations. This figure corresponds to our theoretical description of case (i), frequent dosing with both drugs.

Results for the other extreme cases described in the previous section are shown in Figs. 3–5. In all cases, parameters were the same as for Fig. 2, except for

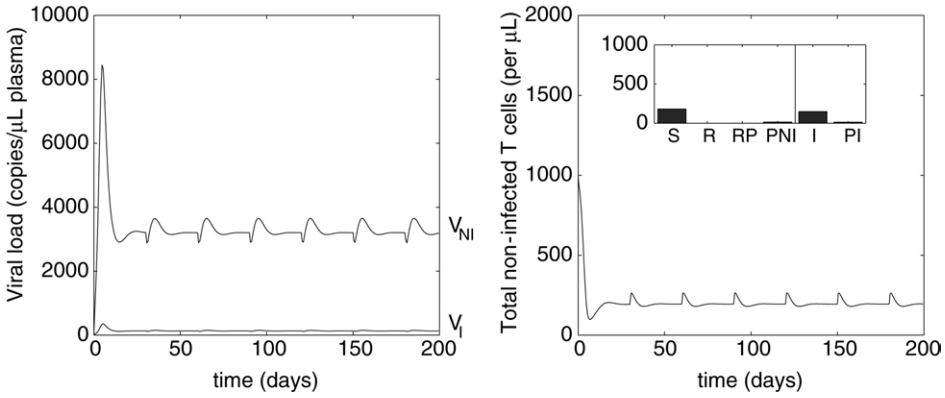

Figure 5. Frequent dosing of the protease inhibitor, with infrequent dosing of the reverse transcriptase inhibitor. Despite the presence of the protease inhibitor at high concentrations, the total healthy T cell population is small. All parameters and initial conditions were the same as in Fig. 2, except for the dosing intervals, which were $\tau = 30$ days and $\sigma = 0.333$ days.

the dosing intervals. In Figs. 2 and 4, T cell counts are not far from the healthy equilibrium value $\frac{\lambda}{d_S} = 1000$, as predicted on theoretical grounds. Nonetheless, the total number of non-infected cells is higher when both drugs are taken frequently. In Fig. 5, despite frequent dosing of the protease inhibitor, the T_{PI} levels remained very low. If $\mu_0 = 200$ cells per μl in this example, then there is no dosing schedule of protease inhibitor which will maintain a healthy immune system.

For case (i) $\tau = 0.5$ days and $\sigma = 0.33$ days. For case (ii), $\tau = 30$ days and $\sigma = 7$ days. For case (iii), $\tau = 0.5$ days and $\sigma = 7$ days. For case (iv), $\tau = 30$ days and $\sigma = 0.33$ days.

7. Estimates of Suitable Dosing Intervals

From (4.10), Lemma 4.1(a), (5.12) and (5.13), if ϵ satisfies

$$r_R \epsilon < \frac{r_R R^i e^{-d_R \tau}}{1 - e^{-d_R \tau}}$$

for $t > t_1$, then we have

$$\begin{aligned} T_S + T_{PNI} &\leq \frac{\lambda}{d_S} - T_R - T_{RP} \\ T_R' + T_{RP}' &= r_R R(T_S + T_{PNI}) - d_S(T_R + T_{RP}) - m_R(T_R + T_{RP}) \\ &\leq r_R R\left(\frac{\lambda}{d_S} - T_R - T_{RP}\right) - (d_S + m_R)(T_R + T_{RP}) \end{aligned}$$

$$< \frac{r_R R^i}{1 - e^{-d_R \tau}} \left(\frac{\lambda}{d_S} - T_R - T_{RP} \right) - (d_S + m_R)(T_R + T_{RP})$$

$$= \frac{r_R R^i \lambda}{(1 - e^{-d_R \tau}) d_S} - \left(d_S + m_R + \frac{r_R R^i}{1 - e^{-d_R \tau}} \right) (T_R + T_{RP})$$

$$T_R + T_{RP} < \frac{r_R R^i \lambda}{d_S((d_S + m_R)(1 - e^{-d_R \tau}) + r_R R^i)}.$$

Note that this is a reasonable upper bound, since it satisfies

$$\frac{r_R R^i \lambda}{d_S((d_S + m_R)(1 - e^{-d_R \tau}) + r_R R^i)} \to \frac{\lambda}{d_S}$$

as $\tau \to 0$.

Using Lemma 5.2, the analogue of Lemma 4.1(a), (5.13) and Theorem 5.1, if $R^i \neq 0$, there exists t_1 such that for any sufficiently small $\epsilon > 0$, we have

$$T_R' + T_{RP}' > \frac{r_R \lambda R^i e^{-d_R \tau}}{(1 - e^{-d_R \tau}) \alpha(\tau, \sigma)} - \frac{\epsilon r_R \lambda}{\alpha(\tau, \sigma)} - (d_S + m_R)(T_R + T_{RP})$$

$$T_R + T_{RP} > \frac{r_R \lambda R^i e^{-d_R \tau}}{(d_S + m_R)(1 - e^{-d_R \tau}) \alpha(\tau, \sigma)} - \frac{\epsilon r_R \lambda}{(d_S + m_R) \alpha(\tau, \sigma)}$$

for $t > t_1$. Since ϵ is small and $\alpha(\tau, \sigma)$ is usually large (since n_I is usually large) we shall assume that last term is negligible.

Thus if μ_0 is the minimum number of T cells required to maintain a healthy immune system, then we need to choose dosing intervals τ and σ to ensure that

$$\mu_0 < \frac{r_R \lambda R^i e^{-d_R \tau}}{(d_S + m_R)(1 - e^{-d_R \tau})} \left[\frac{r_I n_I \omega \lambda}{d_S d_V} + d_S + \frac{r_R R^i}{1 - e^{-d_R \tau}} + \frac{r_P P^i}{1 - e^{-d_p \sigma}} \right]^{-1}. \tag{7.15}$$

For example, using parameters $n_I = 200$ virions cell^{-1} day^{-1}, $\omega = 0.5$, $r_I = 1/25$ cell^{-1} day^{-1}, $r_P = 1/10$ μM^{-1} day^{-1}, $r_R = 15$ μM^{-1} day^{-1}, $d_V = 2.77$ day^{-1}, $d_S = 0.05$ day^{-1}, $d_P = 1$ day^{-1}, $d_R = 1$ day^{-1}, $m_R = 0.1$ day^{-1}, $\lambda = 100$ cells day^{-1}, $P^i = 15$ μM, $R^i = 18$ μM, we find that for a frequency of 4 doses per day for both drugs, $T_R(t) + T_{RP}(t) > 154$ cells μl^{-1}. In contrast when the dosing frequency is six doses per day, we have $T_R(t) + T_{RP}(t) > 213$ cells μl^{-1}. Thus for these parameter values, doses should be taken about six times per day to guarantee a T cell count above $\mu_0 = 200$ cells μl^{-1}.

Finally, we can assume that $\sigma = k\tau$, where $k \in \mathbb{Q}^+$. This reflects the fact that the dosing intervals usually come into phase over a 24 hour period. In this case, the right-hand side of (7.15) approaches

$$T_{R,\min} \equiv \frac{\lambda r_R R^i d_P k}{(d_S + m_R)(r_R R^i d_P k + r_P P^i d_R)} \tag{7.16}$$

as $\tau \to 0$. Note that $T_{R,\min}$ is strictly less than λ/d_S if the protease inhibitor is present. This implies that the number of T cells will fall between $T_{R,\min}$ and λ/d_S when there are suitably small dosing intervals of both drugs.

8. Summary and Discussion

We present an immunological model of HIV infection in which the different effects of reverse transcriptase inhibitors and protease inhibitors on the $CD4^+$ T cell population are considered. The T cells are classified into six classes, as illustrated in Fig. 1 and described in paragraphs (a) through (f) in Section 2.1.

We use impulsive differential equations to model the kinetics of drug action. Framing our model in these terms allows us to make use of a fairly sophisticated mathematical literature (Bainov and Simeonov, 1989, 1993, 1995; Lakshmikantham *et al*., 1989): we find that drug concentrations monotonically approach an (impulsive) periodic orbit and that for both drugs, this orbit is globally asymptotically stable. This model of drug action, however, compels us to make a number of simplifying assumptions about the uptake and egress of the drugs. First, we approximate the change in drug concentration when a new dose is taken as an instantaneous increase. Although the time-to-peak of plasma drug concentration is arguably negligible on the timescales we consider, the time course of drug entry to the intracellular space (for example to cells in lymph tissue) is almost certainly slower. This assumption has the effect of overestimating the temporal effects of dosing at intervals. We argue that our conclusions regarding the existence of various upper and lower bounds will not be affected by this assumption, although more accurate quantitative estimates will be possible once the intracellular pharmacokinetics are better understood. Finally, we assume that the anti-viral *effect* of the dose decays exponentially after a dose is taken; while exponential decay is typical for drug concentrations in plasma, this assumption is clearly less accurate when the dose–effect curve is non-linear, i.e., for very large or small doses.

We consider four extreme cases to examine the effects that large or small dosing intervals of each drug can have. These four cases demonstrate the importance of sufficiently frequent dosing intervals, and also illustrate the important differences between reverse transcriptase and protease inhibitors.

For example, the model predicts that insufficient dosing with both drugs corresponds to a high viral load and a large population of infectious T cells, as we would expect. More surprisingly, we predict that sufficiently frequent dosing with the reverse transcriptase inhibitor alone could theoretically maintain the $CD4^+$ T cell count arbitrarily close to the T cell count in the uninfected immune system. This limit would only be achievable, however, in the absence of physiological limits on tolerable drug concentrations. This interesting result suggests that new drugs with a mechanism of action similar to the reverse transcriptase inhibitor (i.e., in preventing new T cells from becoming infected), but with negligible side effects,

would hold the promise of maintaining 'normal' T cell counts over long periods of time.

In contrast, for frequent dosing of the protease inhibitor alone, even if the drug is perfectly efficacious and there are no physiological limits on tolerable drug concentrations, it may be impossible to maintain adequate T cell counts for immune system function. This implies that for drugs with a mechanism of action similar to the protease inhibitor (i.e., in preventing infected cells from releasing infectious virions) even extremely high drug concentrations may not be able to adequately protect and maintain the immune response.

Furthermore, we predict that very frequent dosing of both drugs has the same net effect on the T cell population as frequent dosing of the reverse transcriptase inhibitor only. Although the protease inhibitor is critically important in the prevention of antiviral resistance (or when the dosing of the reverse transcriptase inhibitor is infrequent) there is a sense in which its role is secondary. Intuitively, this is not surprising, since reverse transcriptase inhibited cells are immune to viral infection, whereas protease inhibited T cells are not. These results demonstrate the maxim that prevention is better than cure, in the sense that prevention of infection via reverse transcriptase inhibition is more important than production of noninfectious virus via protease inhibition.

These theoretical predictions may appear to be at odds with clinical evidence that protease inhibitors control HIV more effectively (Ghani *et al*., 2001). This is because our result pertains to all possible drugs which either prevent infection or prevent virion production, and compares these two strategies of defence, in the absence of resistance. We do not mean to discount the effects of resistance, but rather we examine dosing regimes for the different drug classes in the hope that future drugs may be used to combat resistance as effectively as the protease inhibitor now does.

Finally, we were able to find a condition [equation (7.15)] to relate the periods of the dosing intervals to the minimum number of T cells required to sustain an adequate immune response. This is an overestimate, so that if the drugs satisfy this condition, then the predicted T cell count will be higher than the minimum. Given quantitative estimates of immune system and drug parameters, this condition may be used to estimate the dosing frequency of a given combination of drugs which could, in principle, maintain T cell counts above a desired threshold. Once again, this dosing frequency may not be attainable due to physiological intolerance at high drug concentrations.

The classification of the T cells that we propose offers a novel means of elucidating the complex effects of antiviral drug classes and their interactions. There is much work that can be done using this classification and the impulsive description of the drug behaviour. Specifically, we would like to examine the effect of adherence to the drug regime and the emergence of resistance to one or both types of drug (Wahl and Nowak, 2000), the effects of drug resistance due to other factors and the possibly different effects of new classes of antivirals (Moyle, 2003). In the

latter case, the initial descriptions of the new classes of drugs suggests that they may behave in a similar manner to the class of T_R cells (preventing viral infection of T cells). If such drugs can be used in combination to overcome resistance then the major use of the protease inhibitor (namely preventing the emergence of drug resistance) may be redundant and 'preventative' drugs such as reverse transcriptase inhibitors, integrase inhibitors and fusion inhibitors may be used exclusively, leading to long-term benefits for the treatment of HIV positive patients.

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