Published in final edited form as:

Math Biosci. 2012 January ; 235(1): 98–109. doi:10.1016/j.mbs.2011.11.002.

A model of HIV-1 infection with two time delays: mathematical analysis and comparison with patient data

Kasia A. Pawelek^{†,¶}, Shengqiang Liu^{‡,¶}, Faranak Pahlevani[§], and Libin Rong^{†,*}

[†]Department of Mathematics and Statistics, Oakland University, Rochester, MI 48309, USA

[‡]The Academy of Fundamental and Interdisciplinary Science, Harbin Institute of Technology, Harbin 150080, China

[§]Division of Science and Engineering, Penn State University, Abington College, Abington, PA 19001, USA

Abstract

Mathematical models have made considerable contributions to our understanding of HIV dynamics. Introducing time delays to HIV models usually brings challenges to both mathematical analysis of the models and comparison of model predictions with patient data. In this paper, we incorporate two delays, one the time needed for infected cells to produce virions after viral entry and the other the time needed for the adaptive immune response to emerge to control viral replication, into an HIV-1 model. We begin model analysis with proving the positivity and boundedness of the solutions, local stability of the infection-free and infected steady states, and uniform persistence of the system. By developing a few Lyapunov functionals, we obtain conditions ensuring global stability of the steady states. We also fit the model including two delays to viral load data from 10 patients during primary HIV-1 infection and estimate parameter values. Although the delay model provides better fits to patient data (achieving a smaller error between data and modeling prediction) than the one without delays, we could not determine which one is better from the statistical standpoint. This highlights the need of more data sets for model verification and selection when we incorporate time delays into mathematical models to study virus dynamics.

Keywords

Mathematical model; Virus dynamics; Stability analysis; Delays; Data fitting

1 Introduction

Human immunodeficiency virus (HIV) infection is characterized by three different phases, namely the primary infection, clinically asymptomatic stage (chronic infection), and acquired immunodeficiency syndrome (AIDS) or drug therapy. During primary infection, viral load in the peripheral blood experiences a substantial increase to the peak level,

© 2011 Elsevier Inc. All rights reserved.

*Corresponding author: rong2@oakland.edu; Phone: 248-370-3446; Fax: 248-370-4184.

¶These authors contributed equally to this study.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

followed by decline to the steady state, which is referred to as the viral set point [13, 46]. Extremely high viral load during primary infection leads to the activation of CD8⁺ T cells, which are recognized as cytotoxic T cells (CTL) capable of suppressing viral replication. Viral decline from the peak is due to the control by these immune cells and/or limited target cell availability [14]. The viral set point has been shown to be predictive for the pace of disease development [34]. The higher the viral set point, the more quickly disease progresses to full-blown AIDS. A better understanding of the virus dynamics will provide more insights into the viral control during primary infection. Knowledge about the host immune response to HIV infection could be crucial for the development of HIV vaccines and treatment of the infection by enhancing immune response [20].

Mathematical models have made considerable contributions to our understanding of HIV infection, immune responses, and antiretroviral treatment [5, 6, 14, 28, 38, 40, 42, 44, 45, 48, 49, 56, 57, 60] (see reviews in [39, 41, 43, 52, 64]). Time delays have also been incorporated into mathematical models to study virus dynamics. To characterize the time between the initial viral entry into a target cell and subsequent viral production, an intracellular delay was first introduced by Herz et al. [21] to analyze the clinical data. Assuming that the level of target cells is constant and that the protease inhibitor is 100% effective, they obtained the expression of the viral load and explored the effect of the intracellular delay on viral load change [21]. Nelson and Perelson [37] analyzed the delay model with imperfect drug treatment. They provided an analytical expression of the dominant eigenvalue that determines the rate of viral decay. Their result explains why there was no change in the estimate of the infected cell death using the delay model in [21]. The delay effect canceled out due to the assumption of 100% effectiveness of the protease inhibitor. Combining the intracellular delay with less than perfect antiretroviral treatment resulted in a significant increase in the estimated value for the infected cell death compared with the case of perfect drug therapy [37]. Because the conversion of a newly infected cells into a productively infected one is a multi-step process, all infected cells would not finish all these processes in the same time. In [36] and [40], a gamma distribution was introduced to describe a continuous time delay between viral infection and production. By fitting to patient data, they found the estimate of the viral clearance rate using the model without delay was underestimate. However, no change was found in the estimate of the infected cell death due to the assumption of a perfect drug [40]. More within-host HIV models including time delays can be found in [2, 3, 9, 11, 12, 16, 26, 62, 67].

Although time delay representing either the time needed for infected cells to produce virions after viral entry or the time needed for the CD8⁺ T cell immune response to emerge to control viral replication has been included in HIV models, very few, if any, models have considered both delays which are biologically reasonable during HIV infection. In general, including more than one time delay will bring challenges to both mathematical analysis of the models and comparison of model predictions with experimental data. In this paper, we incorporated the two delays into an HIV-1 model. We began model analysis with proving the positivity and boundedness of the solutions, local stability of the steady states, and uniform persistence of the system. By developing a few different Lyapunov functionals, we obtained conditions ensuring global stability of the infection-free and infected steady states. We also fit the model to viral load data from 10 patients during primary HIV infection and estimated parameter values. We used an F-test to compare the data fits using both the two-delay model and the model without delays from a statistical viewpoint. This work presents mathematical properties of the solutions of the two-delay model, and provides more information for model verification and selection when delays are included to study virus dynamics.

2 Model description

Ciupé et al. [9] incorporated one time delay into an HIV-1 model to account for the time needed to activate the CD8⁺ T cell response, i.e., the immune cells at time t were activated by infected cells at time $t - \tau_2$, where τ_2 is a constant. Here we include this immune delay as well as an intracellular delay, τ_1 , between viral entry and viral production (this phase is referred to as the eclipse phase). The model is described by a system of differential equations (1). It includes four variables: uninfected target cells $T(t)$, productively infected cells $T^*(t)$, free virus $V(t)$, and effector cells $E(t)$. The parameter s represents the rate at which target cells are created, d is the death rate of target cells, k is the infection rate, and δ is the death rate of productively infected cells. As described in [37], we assume $k_1 = ke^{-a\tau_1}$, where a ($d < a < \delta$) is the death rate of infected cells before viral production commences. Thus, $e^{-a\tau_1}$ is the probability that an infected cell survives the eclipse phase to produce virions. The constant d_x represents the killing rate of infected cells by effector cells. N is the number of virions produced by an infected cell during its lifespan, and c is the viral clearance rate constant. Effector cells are assumed to be generated at a rate proportional to the level of productively infected cells, and die at a rate d_E . Note that the generation of effector cells was described using a mass action term pET^* in other studies [31, 68]. However, it generates a steady state of infected cells, $T^* = d_E/p$, which is independent of any viral parameters. Here, we assumed the same generation rate for effector cells as in [9]. The model including the two delays is given by

$$\begin{aligned} \frac{d}{dt}T(t) &= s - dT - kVT \\ \frac{d}{dt}T^*(t) &= k_1V(t - \tau_1)T(t - \tau_1) - \delta T^* - d_xET^* \\ \frac{d}{dt}V(t) &= N\delta T^* - cV \\ \frac{d}{dt}E(t) &= pT^*(t - \tau_2) - d_EE \end{aligned} \quad (1)$$

Model (1) has two steady states: the infection-free steady state $E_0 = (\tilde{T}, 0, 0, 0)$ with $\tilde{T} = s/d$ and the infected steady state $E_1 = (\bar{T}, \bar{T}^*, \bar{V}, \bar{E})$ where

$$\begin{aligned} \bar{T} &= \frac{c^2 d_x p}{2kk_1 N^2 \delta^2 d_E} \left[\frac{kN\delta^2 d_E}{cd_x p} - d + \sqrt{\left(\frac{kN\delta^2 d_E}{cd_x p} - d \right)^2 + 4s \frac{kk_1 N^2 \delta^2 d_E}{c^2 d_x p}} \right] \\ \bar{T}^* &= \frac{d_E}{d_x p} \left(\frac{k_1 N \delta \bar{T}}{c} - \delta \right) \\ \bar{V} &= \frac{N\delta}{c} \bar{T}^* \\ \bar{E} &= \frac{p}{d_E} \bar{T}^* \end{aligned} \quad (2)$$

From $T^* > 0$, we have that the infected steady state exists if and only if $k_1 N \bar{T} / c > 1$, which is equivalent to $k_1 N s / (dc) > 1$, i.e., $k_1 N \tilde{T} / c > 1$. Note that $\mathcal{R}_0 = k_1 N s / (dc)$ is the basic reproductive ratio of the basic model (without the immune response) [49]. Because $k_1 = ke^{-a\tau_1}$, $\mathcal{R}_0$ is intracellular delay-dependent.

We will study the mathematical properties of the solutions of model (1). We will also fit both the two-delay model and the model without delays to the data from 10 patients during primary HIV infection [60], and compare the data fits using a statistical test.

3 Analysis of the delay model (1)

3.1 Initial conditions

We denote by $X=C([- \tau, 0], \mathbb{R}_+^4)$ the Banach space of continuous functions mapping the interval $[- \tau, 0]$ into $\mathbb{R}_+^4$ equipped with the sup-norm, where $\tau = \max\{\tau_1, \tau_2\}$. By the standard theory of functional differential equations (see [19]) we know that for any $\varphi \in C([- \tau, 0], \mathbb{R}_+^4)$ there exists a unique solution

$$Y(t, \varphi) = (T(t, \varphi), T^*(t, \varphi), V(t, \varphi), E(t, \varphi))$$

of the system (1), which satisfies $Y_0 = \varphi$. The initial conditions are given by

$$T(\theta) = \varphi_1(\theta), \quad T^*(\theta) = \varphi_2(\theta), \quad V(\theta) = \varphi_3(\theta), \quad E(\theta) = \varphi_4(\theta), \quad \theta \in [- \tau, 0] \quad (3)$$

where $\varphi = (\varphi_1, \dots, \varphi_4) \in \mathbb{R}_+^4$ with $\varphi_i(\theta) \geq 0$ ($\theta \in [- \tau, 0]$, $i = 1, \dots, 4$) and $\varphi_2(0), \varphi_3(0), \varphi_4(0) > 0$.

3.2 Positiveness and boundedness of solutions

Proposition 1—Let $Y(t, \varphi)$ be the solution of the delayed system (1) with the initial condition (3). $T(t), T^*(t), V(t), E(t) > 0$, ($\forall t \geq 0$) are ultimately bounded. Moreover, there exists an $\varepsilon > 0$ such that $\liminf_{t \rightarrow \infty} T(t) \geq \varepsilon$.

Proof: From (1) we obtain

$$\begin{aligned} T(t) &= T(0)e^{-\int_0^t (d+kV(\zeta)) d\zeta} + \int_0^t s e^{-\int_\gamma^t (d+kV(\zeta)) d\zeta} d\gamma \\ T^*(t) &= T^*(0)e^{-\int_0^t (\delta+d_x E(\zeta)) d\zeta} + \int_0^t k_1 T(\gamma - \tau_1) V(\gamma - \tau_1) e^{-\int_\gamma^t (\delta+d_x E(\zeta)) d\zeta} d\gamma \\ V(t) &= V(0)e^{-ct} + \int_0^t N \delta T^*(\gamma) e^{-c(t-\gamma)} d\gamma \\ E(t) &= E(0)e^{-d_E t} + \int_0^t p T^*(\gamma - \tau_2) e^{-d_E(t-\gamma)} d\gamma \end{aligned} \quad (4)$$

Using (3) we know that the solution of model (1) is positive for all $t \geq 0$.

Next, we show that the solution is ultimately bounded. From the $T(t)$ equation in (1), we have

$$\frac{dT}{dt} \leq s - dT$$

Thus, $\limsup_{t \rightarrow \infty} T(t) \leq \frac{s}{d}$ and $T(t)$ is ultimately bounded. We define a Lyapunov functional

$$U(t) = T(t) + \frac{k}{k_1} T^*(t + \tau_1)$$

Thus, $U(t) \geq 0$ for $t \geq 0$. Differentiating $U(t)$ along the solution of system (1), we obtain

$$\frac{dU(t)}{dt} \leq s - dT(t) - \frac{\delta k}{k_1} T^*(t + \tau_1) = s - dT(t) + \delta T(t) - \delta U(t) \leq s + \delta T(t) - \delta U(t) \leq C_1 - \delta U(t),$$

where $C_1 = s + \frac{\delta s}{d} > 0$. Thus, $\limsup_{t \rightarrow \infty} U(t) \leq \frac{C_1}{\delta}$ and $T^*(t)$ is ultimately bounded. It follows from the third and fourth equation of (4) that $V(t)$ and $E(t)$ are also ultimately bounded. From the first equation of (1), one can show that

$$\dot{T}(t) \geq s - T \cdot (d + kV_u), \quad \text{for a large } t,$$

where V_u is the upper bound of $V(t)$. This shows that $T(t)$ is uniformly bounded away from zero.

3.3 Local stability of the steady states

To study the local stability of the steady states of model (1), we linearized the system and obtained the characteristic equation, given by the following determinant:

$$\begin{vmatrix} -d - k\bar{V} - \lambda & 0 & -k\bar{T} & 0 \\ k_1 \bar{V} e^{-\lambda \tau_1} & -\delta - d_x \bar{E} - \lambda & k_1 \bar{T} e^{-\lambda \tau_1} & -d_x \bar{T}^* \\ 0 & N\delta & -c - \lambda & 0 \\ 0 & p e^{-\lambda \tau_2} & 0 & -d_E - \lambda \end{vmatrix} = 0 \quad (5)$$

where λ is an eigenvalue. We have the following result for the infection-free steady state.

Theorem 1—The infection-free steady state of model (1) is locally asymptotically stable when $\mathcal{R}_0 < 1$ and unstable when $\mathcal{R}_0 > 1$.

Proof: We first prove the local stability when $\mathcal{R}_0 < 1$. At the infection-free steady state, the characteristic equation becomes

$$(\lambda + c)(\lambda + \delta) = c\delta e^{-\lambda \tau_1} \mathcal{R}_0 \quad (6)$$

If λ has a nonnegative real part, then the modulus of the left-hand side of (6) satisfies

$$|(\lambda + c)(\lambda + \delta)| \geq c\delta$$

while the modulus of the right-hand side of (6) satisfies $|c\delta e^{-\lambda\tau_1}\mathfrak{R}_0| \leq c\delta\mathfrak{R}_0 < c\delta$. This leads to a contradiction. Thus, all the eigenvalues have negative real parts, and hence the infection-free steady state is locally asymptotically stable when $\mathfrak{R}_0 < 1$.

When $\mathfrak{R}_0 > 1$, we define a function $f(\lambda) = (\lambda + c)(\lambda + \delta) - c\delta e^{-\lambda\tau_1}\mathfrak{R}_0$. It is clear that $f(0) < 0$ and $f(\lambda) \rightarrow \infty$ when $\lambda \rightarrow \infty$. By the continuity we know there exists at least one positive root. Thus, the infection-free steady state is unstable if $\mathfrak{R}_0 > 1$.

At the infected steady state, the characteristic equation (5) can be simplified to

$$(\lambda + d + k\bar{V})(\lambda + c)[(\lambda + \mathfrak{R}_0\delta)(\lambda + d_E) + (\mathfrak{R}_0 - 1)d_E\delta e^{-\lambda\tau_2}] = (\lambda + d)(\lambda + d_E)\mathfrak{R}_0c\delta e^{-\lambda\tau_1} \quad (7)$$

For a special case of $\tau_2 = 0$, we have the following theorem for the stability of the infected steady state.

Theorem 2—The infected steady state of model (1) is locally asymptotically stable when $\mathfrak{R}_0 > 1$ in the case of $\tau_2 = 0$.

Proof: In the case of $\tau_2 = 0$, the characteristic equation is

$$(\lambda + d + k\bar{V})(\lambda + c)[(\lambda + \mathfrak{R}_0\delta)(\lambda + d_E) + (\mathfrak{R}_0 - 1)d_E\delta] = (\lambda + d)(\lambda + d_E)\mathfrak{R}_0c\delta e^{-\lambda\tau_1} \quad (8)$$

Obviously, equation (8) does not have a nonnegative real solution. Now we prove that (8) does not have any complex root λ with a nonnegative real part. Suppose, by contradiction, that $\lambda = x + iy$ with $x \geq 0$ is a root of (8). Because its complex conjugate $\lambda = x - iy$ is also a root of (8), we can assume that $y > 0$.

When $\mathfrak{R}_0 \rightarrow 1$, we have $\bar{V} \rightarrow 0$. Thus, equation (8) reduces to $(\lambda + \delta)(\lambda + c) = c\delta e^{-\lambda\tau_1}$. Using the same arguments as above, we can show that it does not have any root with a nonnegative real part.

By the continuous dependence of roots of the characteristic equation on $\mathfrak{R}_0$, we know that the curve of the roots must cross the imaginary axis as $\mathfrak{R}_0$ decreases sufficiently close to 1. That is, the characteristic equation (8) has a pure imaginary root, say, iy_0 , where $y_0 > 0$. From (8), we have

$$(d + k\bar{V} + iy_0)(c + iy_0)[(\mathfrak{R}_0\delta + iy_0)(d_E + iy_0) + (\mathfrak{R}_0 - 1)d_E\delta] = (d + iy_0)(d_E + iy_0)\mathfrak{R}_0c\delta e^{-i\tau_1 y_0} \quad (9)$$

We claim that the following inequality holds:

$$|(\mathfrak{R}_0\delta + iy_0)(d_E + iy_0) + (\mathfrak{R}_0 - 1)d_E\delta| > \mathfrak{R}_0\delta|d_E + iy_0|. \quad (10)$$

In fact, after straightforward computations, we have

$$\begin{aligned}
& |(\Re_0 \delta + i y_0)(d_E + i y_0) + (\Re_0 - 1)d_E \delta|^2 - (\Re_0 \delta)^2 |d_E + i y_0|^2 \\
&= [y_0^2 - (\Re_0 - 1)d_E \delta]^2 + 2\Re_0(\Re_0 - 1)(d_E \delta)^2 + (d_E y_0)^2 \\
&> 0.
\end{aligned}$$

Thus, (10) holds. It follows from $|d + kV + i y_0| \geq |d + i y_0|$, $|c + i y_0| > c$, and the inequality (10) that the modulus of the left-hand side of (9) is greater than the modulus of the right-hand side. This leads to the contradiction. Therefore, we conclude that the characteristic equation (8) does not have any root with a nonnegative real part. Thus, the infected steady state is locally asymptotically stable when $\Re_0 > 1$ in the case of $\tau_2 = 0$.

In the case of $\tau_2 > 0$, the analysis of the characteristic equation (7) is challenging. When $\tau_1 = 0$ and $\tau_2 > 0$, Ciupe et al. [9] used the Routh-Hurwitz criteria to obtain a sufficient condition for the local stability of the infected steady state. We will show below that a positive immune delay τ_2 is able to destabilize the infected steady state even for a simplified model of (1). To reduce the dimension of the system (i.e., the order of the characteristic equation), we simplified model (1) by assuming the level of target cells is a constant, T_0 . We will show that for this simplified model, the infected steady state is locally stable for $\tau_2 < \tau_2^*$ and bifurcation occurs when $\tau_2 = \tau_2^*$ in the case of $\tau_1 = 0$, where $\tau_2^* > 0$ is a threshold of the immune delay.

The simplified model is

$$\begin{aligned}
\frac{d}{dt} T^*(t) &= k_1 V(t - \tau_1) T_0 - \delta T^* - d_x E T^* \\
\frac{d}{dt} V(t) &= N \delta T^* - c V \\
\frac{d}{dt} E(t) &= p T^*(t - \tau_2) - d_E E
\end{aligned} \tag{11}$$

The simplified model has two steady states: the infection-free steady state $(0, 0, 0)$ and the infected steady state $(T^*, V, \bar{E})$, where

$$\bar{T}^* = \frac{d_E \delta (\Re_0 - 1)}{d_x p}, \quad \bar{V} = \frac{N \delta \bar{T}^*}{c}, \quad \bar{E} = \frac{p \bar{T}^*}{d_E}$$

Here, $\Re_0 = \frac{k_1 N T_0}{c}$ is the basic reproductive ratio of model (11). The infected steady state exists if and only if $\Re_0 > 1$.

The characteristic equation for the linearized system is

$$\begin{vmatrix}
-\delta - d_x \bar{E} - \lambda & k_1 T_0 e^{-\lambda \tau_1} & -d_x \bar{T}^* \\
N \delta & -c - \delta & 0 \\
p e^{-\lambda \tau_2} & 0 & -d_E - \lambda
\end{vmatrix} = 0 \tag{12}$$

where λ is an eigenvalue.

At the infection-free steady state, the characteristic equation becomes

$$(\lambda + d_E)[(\lambda + c)(\lambda + \delta) - \mathfrak{R}_0 c \delta e^{-\lambda \tau_1}] = 0$$

Using the similar arguments in Theorem 1, we know the infection-free steady state is locally asymptotically stable when $\mathfrak{R}_0 < 1$ and unstable when $\mathfrak{R}_0 > 1$.

At the infected steady state, the characteristic equation is

$$(\lambda + c)[(\lambda + \mathfrak{R}_0 \delta)(\lambda + d_E) + (\mathfrak{R}_0 - 1)d_E \delta e^{-\lambda \tau_2}] = (\lambda + d_E)\mathfrak{R}_0 c \delta e^{-\lambda \tau_1} \quad (13)$$

Similar to Theorem 2, we can show that the infected steady state of model (11) is locally asymptotically stable when $\mathfrak{R}_0 > 1$ in the case of $\tau_2 = 0$. We show in the following theorem that bifurcation occurs when $\tau_2 > 0$.

Theorem 3—In the case of $\tau_1 = 0$ and $\tau_2 > 0$, the infected steady state is locally asymptotically stable when $\tau_2 < \tau_2^*$, where $\tau_2^* = \min_{j \in \mathbb{N}} \{\tau_2^{1,j}, \tau_2^{2,j}\}$ with $\tau_2^{1,j}$ and $\tau_2^{2,j}$ defined below by (22) and (23), respectively. Moreover, a Hopf bifurcation occurs at the infected steady state when $\tau_2 = \tau_2^*$.

Proof: In the case of $\tau_1 = 0$ and $\tau_2 > 0$, the characteristic equation (13) is

$$\lambda(\lambda + d_E)(\lambda + c + \mathfrak{R}_0 \delta) + (\lambda + c)(\mathfrak{R}_0 - 1)d_E \delta e^{-\lambda \tau_2} = 0 \quad (14)$$

For the ease of notation, we rewrite (14) as

$$\lambda^3 + \alpha_1 \lambda^2 + \alpha_2 \lambda + \alpha_3 (\lambda + c) e^{-\lambda \tau_2} = 0 \quad (15)$$

where $\alpha_1 = d_E + c + \mathfrak{R}_0 \delta$, $\alpha_2 = d_E(c + \mathfrak{R}_0 \delta)$, $\alpha_3 = (\mathfrak{R}_0 - 1)d_E \delta$. Note that these coefficients are independent of the immune delay.

We will determine if the solution curve of the characteristic equation (15) crosses the imaginary axis. Suppose that $i\omega$ ($\omega > 0$) is a root of (15). Substituting $\lambda = i\omega$ into (15) and separating the real and imaginary parts, we have

$$\alpha_1 \omega^2 = \alpha_3 [c \cos(\omega \tau_2) + \omega \sin(\omega \tau_2)] \quad (16)$$

$$\omega(\omega^2 - \alpha_2) = \alpha_3 [\omega \cos(\omega \tau_2) - c \sin(\omega \tau_2)] \quad (17)$$

Taking squares and adding the two equations, we have

$$\alpha_1^2 \omega^4 + \omega^2 (\omega^2 - \alpha_2)^2 = \alpha_3^2 (c^2 + \omega^2) \quad (18)$$

Letting $y = \omega^2$, the above equation can be simplified to

$$y^3 + y^2 (\alpha_1^2 - 2\alpha_2) + y (\alpha_2^2 - \alpha_3^2) - \alpha_3^2 c^2 = 0 \quad (19)$$

We define a function $F(y)$ as the left-hand side of the above equation.

It is easy to verify that $\alpha_1^2 - 2\alpha_2 = d_E^2 + (c + \Re_0 \delta)^2 > 0$, and that $\alpha_2^2 - \alpha_3^2 = d_E^2 (c + \delta)(c + 2\Re_0 \delta - \delta) > 0$. By Descartes' rule of signs, there is one positive root of equation (19). We denote the positive root by y^* . Thus, the corresponding value of ω is $\omega^* = \sqrt{y^*}$.

Next, we solve for $\sin(\omega \tau_2)$ and $\cos(\omega \tau_2)$ from (16) and (17), and obtain

$$\cos(\omega \tau_2) = \frac{\alpha_1 c \omega^2 + \omega^2 (\omega^2 - \alpha_2)}{\alpha_3 (c^2 + \omega^2)} \quad (20)$$

$$\sin(\omega \tau_2) = \frac{\alpha_1 \omega^3 + c \omega (\alpha_2 - \omega^2)}{\alpha_3 (c^2 + \omega^2)} \quad (21)$$

The unique solution $\theta = \omega \tau_2 \in [0, 2\pi]$ of (20) and (21) is $\theta = \arccos(\alpha_1 c \omega^2 + \omega^2 (\omega^2 - \alpha_2) / (\alpha_3 (c^2 + \omega^2)))$ if $\sin(\theta) > 0$, i.e., if $\alpha_1 \omega^2 + \alpha_2 c - c \omega^2 > 0$, and $\theta = 2\pi - \arccos(\alpha_1 c \omega^2 + \omega^2 (\omega^2 - \alpha_2) / (\alpha_3 (c^2 + \omega^2)))$ if $\sin(\theta) \leq 0$, i.e., if $\alpha_1 \omega^2 + \alpha_2 c - c \omega^2 \leq 0$. Therefore, for the imaginary root $\lambda = i\omega$ of (15) we have two sequences $\{\tau_2^{1,j}\}$ and $\{\tau_2^{2,j}\}$ for $j \in \mathbb{N}$

$$\tau_2^{1,j} = \frac{1}{\omega} \left[\arccos \left(\frac{\alpha_1 c \omega^2 + \omega^2 (\omega^2 - \alpha_2)}{\alpha_3 (c^2 + \omega^2)} \right) + 2j\pi \right] \quad (22)$$

and

$$\tau_2^{2,j} = \frac{1}{\omega} \left[2\pi - \arccos \left(\frac{\alpha_1 c \omega^2 + \omega^2 (\omega^2 - \alpha_2)}{\alpha_3 (c^2 + \omega^2)} \right) + 2j\pi \right]. \quad (23)$$

Assuming $\tau_2^* = \min_{j \in \mathbb{N}} \{\tau_2^{1,j}; \tau_2^{2,j}\}$, i.e., τ_2^* is the minimum value associated with the imaginary solution $i\omega^*$ of the characteristic equation (15) we found above, we determine

$\text{sign} \left\{ \frac{d\text{Re}(\lambda)}{d\tau_2} \Big|_{\tau_2 = \tau_2^*} \right\}$, where sign is the sign function and $\text{Re}(\lambda)$ is the real part of λ . We assume that $\lambda(\tau_2) = \nu(\tau_2) + i\omega(\tau_2)$ is a solution of (15). Thus, $\nu(\tau_2^*) = 0$ and $\omega(\tau_2^*) = \omega^*$. Taking derivative of (15) with respect to τ_2 , we have

$$\left[3\lambda^2 + 2\alpha_1\lambda + \alpha_2 + \alpha_3(1 - \tau_2(\lambda + c))e^{-\lambda\tau_2} \right] \frac{d\lambda}{d\tau_2} = \alpha_3\lambda(\lambda + c)e^{-\lambda\tau_2}$$

From (15), we have

$$e^{\lambda\tau_2} = - \frac{\alpha_3(\lambda + c)}{\lambda^3 + \alpha_1\lambda^2 + \alpha_2\lambda}$$

Thus,

$$\left(\frac{d\lambda}{d\tau_2} \right)^{-1} = - \frac{3\lambda^2 + 2\alpha_1\lambda + \alpha_2}{\lambda^2(\lambda^2 + \alpha_1\lambda + \alpha_2)} + \frac{1}{\lambda(\lambda + c)} - \frac{\tau_2}{\lambda}$$

Evaluating $\left(\frac{d\lambda}{d\tau_2} \right)^{-1}$ at $\tau_2 = \tau_2^*$ (i.e., $\lambda = i\omega^*$) and taking the real part, we have

$$\operatorname{Re} \left[\left(\frac{d\lambda}{d\tau_2} \right)^{-1} \Big|_{\tau_2 = \tau_2^*} \right] = \frac{(\alpha_2 - 3\omega^{*2})(\alpha_2 - \omega^{*2}) + 2\alpha_1^2\omega^{*2}}{\omega^{*2}[(\alpha_2 - \omega^{*2})^2 + \alpha_1^2\omega^{*2}]} - \frac{1}{c^2 + \omega^{*2}}$$

From (18), we have

$$\omega^{*2}[(\alpha_2 - \omega^{*2})^2 + \alpha_1^2\omega^{*2}] = \alpha_3^2(c^2 + \omega^{*2})$$

Thus,

$$\operatorname{Re} \left[\left(\frac{d\lambda}{d\tau_2} \right)^{-1} \Big|_{\tau_2 = \tau_2^*} \right] = \frac{(\alpha_2 - 3\omega^{*2})(\alpha_2 - \omega^{*2}) + 2\alpha_1^2\omega^{*2} - \alpha_3^2}{\alpha_3^2(c^2 + \omega^{*2})}$$

From the definition of $F(y) = y^3 + y^2(\alpha_1^2 - 2\alpha_2) + y(\alpha_2^2 - \alpha_3^2) - \alpha_3^2c^2$, we find the numerator of the right-hand side of the above equation is exactly $F'(y)$ evaluated at $y = \omega^{*2}$. Thus,

$$\operatorname{Re} \left[\left(\frac{d\lambda}{d\tau_2} \right)^{-1} \Big|_{\tau_2 = \tau_2^*} \right] = \frac{F'(\omega^{*2})}{\alpha_3^2(c^2 + \omega^{*2})}$$

Therefore,

$$\operatorname{sign}\left\{\frac{d\operatorname{Re}(\lambda)}{d\tau_2}\Big|_{\tau_2=\tau_2^*}\right\}=\operatorname{sign}\left\{\operatorname{Re}\left[\left(\frac{d\lambda}{d\tau_2}\right)^{-1}\Big|_{\tau_2=\tau_2^*}\right]\right\}=\operatorname{sign}\{F'(\omega^{*2})\}$$

Because

$$F'(\omega^{*2})=3\omega^{*4}+2\omega^{*2}[d_E^2+(c+\Re_0\delta)^2]+d_E^2(c+\delta)(c+2\Re_0\delta-\delta)>0$$

we know $\frac{d\operatorname{Re}(\lambda)}{d\tau_2}$ is positive at $\tau_2=\tau_2^*$. Thus, the solution curve of the characteristic equation (15) crosses the imaginary axis. This shows that a Hopf bifurcation occurs at $\tau_2=\tau_2^*>0$. When $\tau_2<\tau_2^*$ the infected steady state is locally asymptotically stable by continuity.

3.4 Global stability of the steady states of model (1)

3.4.1 Infection-free steady state

Theorem 4: If the basic reproductive number $\Re_0=\frac{k_1 N_S}{dc}<1$, then the infection-free equilibrium E_0 of model (1) is globally asymptotically stable.

Proof: By Theorem 1, it suffices for us to prove the global attractiveness of E_0 . We define a Lyapunov functional

$$M(t)=M_1(t)+M_2(t)+M_3(t)$$

with

$$\begin{aligned}M_1 &= \tilde{T} \left(\frac{T}{\tilde{T}} - \ln \frac{T}{\tilde{T}} - 1 \right) + \frac{k}{k_1} T^* + b_1 V + b_2 E \\M_2 &= k \int_{t-\tau_1}^t V(s) T(s) ds \\M_3 &= b_2 p \int_{t-\tau_2}^t T^*(s) ds\end{aligned}$$

where $\tilde{T}=\frac{s}{d}$, $b_1=\frac{ks}{cd}=\frac{k}{c}\tilde{T}$, and $b_2=\frac{k\delta(c-k_1 N\tilde{T})}{cpk_1}=\frac{k\delta(1-\Re_0)}{pk_1}>0$ since $\Re_0<1$. We know that $\frac{T}{\tilde{T}} - \ln \frac{T}{\tilde{T}} - 1 \geq 0$ for all $T(t)>0$ (the equality holds if and only if $T(t)=\tilde{T}$). From the definition of $M(t)$ and Proposition 1, we know $M(t)$ is well-defined and $M(t)\geq 0$. The equality holds if and only if $T(t)=\tilde{T}$, $T^*(t)=V(t)=E(t)=0$.

Differentiating $M(t)$ along the solution of system (1), we obtain

$$\begin{aligned}
\frac{dM}{dt}\Big|_{(1)} &= \frac{dM_1}{dt}\Big|_{(1)} + \frac{dM_2}{dt}\Big|_{(1)} + \frac{dM_3}{dt}\Big|_{(1)} \\
&= \frac{dT}{dt} - \frac{\tilde{T}}{T} \frac{dT}{dt} + \frac{k}{k_1} \frac{dT^*}{dt} + b_1 \frac{dV}{dt} + b_2 \frac{dE}{dt} + k(VT - V(t - \tau_1)T(t - \tau_1)) + b_2 p(T^* - T^*(t - \tau_2)) \\
&= (s - dT - kVT - s + d\tilde{T}) - \frac{\tilde{T}}{T} (s - dT - kVT) + \frac{k}{k_1} [k_1 V(t - \tau_1)T(t - \tau_1) - \delta T^* - d_x E T^*] + \\
&\quad b_1 (N\delta T^* - cV) + b_2 [pT^*(t - \tau_2) - d_e E] + kVT - kV(t - \tau_1)T(t - \tau_1) + b_2 pT^* - b_2 pT^*(t - \tau_2) \\
&= -d\frac{1}{T}(T - \tilde{T})^2 - \frac{k d_x}{k_1} E T^* - b_2 d_e E + b_2 pT^* + kV\tilde{T} - \frac{k\delta}{k_1} T^* + b_1 N\delta T^* - b_1 cV \\
&= -d\frac{1}{T}(T - \tilde{T})^2 - \frac{k d_x}{k_1} E T^* - b_2 d_e E + T^* \left(\frac{k\delta(c - k_1 N\tilde{T})}{ck_1} + \frac{kN\delta}{c} \tilde{T} - \frac{k\delta}{k_1} \right) + V \left(k\tilde{T} - \frac{ks}{d} \right) \\
&= -d\frac{1}{T}(T - \tilde{T})^2 - \frac{k d_x}{k_1} E T^* - b_2 d_e E \leq 0.
\end{aligned}$$

It follows that $M(t)$ is bounded and non-increasing. Thus $\lim_{t \rightarrow \infty} M(t)$ exists. Note that $\frac{dM}{dt} = 0$ if and only if $T(t) = \tilde{T}$ and $E(t) = 0$. Substituting $T(t) = \tilde{T}$ into the first equation of (1), one can directly obtain $V(t) = 0$. Using $E(t) = V(t) = 0$ in the second equation of (1), we have $T^*(t) =$

0. Therefore, the maximal compact invariant set in $\{\frac{dM}{dt}\Big|_{(1)} = 0\}$ is the singleton E_0 . By the LaSalle invariance principle for delay systems in [19, Theorem 5.3.1], the infection-free steady state E_0 is globally attracting. Further, it was showed in Theorem 1 that E_0 is locally asymptotically stable. Therefore, E_0 is globally asymptotically stable.

3.4.2 Permanence as $\mathfrak{R}_0 > 1$ —We show the uniform persistence of system (1), for which we apply the persistence theory by Smith and Zhao [58] for infinite dimensional systems (also see [66]). The methods and techniques we are using have been recently employed in [29, Theorem 2], [55, Theorem 6.1], [63, Theorem 3.1] for distributed and infinite delay systems and in [30] for a discrete delay system.

To proceed, we introduce the following notation and terminology. Denote by $P(t)$, $t \geq 0$ the family of solution operators corresponding to (1). The ω -limit set $\omega(x)$ of x consists of $y \in X$ such that there exists a sequence $t_n \rightarrow \infty$ as $n \rightarrow \infty$ with $P(t_n)x \rightarrow y$ as $n \rightarrow \infty$.

Theorem 5: Assuming $\mathfrak{R}_0 > 1$ and the initial conditions (3), system (1) is uniformly persistent; that is, there exists $\eta_0 > 0$ such that

$$\liminf_{t \rightarrow \infty} T(t) \geq \eta_0, \quad \liminf_{t \rightarrow \infty} T^*(t) \geq \eta_0, \quad \liminf_{t \rightarrow \infty} E(t) \geq \eta_0, \quad \text{and} \quad \liminf_{t \rightarrow \infty} V(t) \geq \eta_0.$$

Proof: Let

$$\begin{aligned}
X^0 &= \{\varphi \in X : \varphi_2(0) > 0, \varphi_3(0) > 0\}, \\
\partial X &= X \setminus X^0 = \{\varphi \in X : \varphi_2(0) = 0 \text{ or } \varphi_3(0) = 0\},
\end{aligned}$$

which is relatively closed in X .

Now we prove X^0 is positively invariant for $P(t)$. By the second and third equations of (1) we have

$$\frac{dT^*(t)}{dt} \geq -\delta T^*(t), \quad \frac{dV(t)}{dt} \geq -cV(t), \quad \forall t \geq 0. \quad (24)$$

Since $T^*(0, \varphi) = \varphi_2(0) > 0$, we have $V(0, \varphi) = \varphi_3(0) > 0$. It follows from (24) that

$$T^*(t, \varphi) \geq \varphi_2(0) \cdot e^{-\delta t} > 0; \quad V(t, \varphi) \geq \varphi_3(0) \cdot e^{-ct} > 0, \quad \forall t \geq 0.$$

Thus X^0 is positively invariant for $P(t)$.

We set

$$M_{\partial} = \{\varphi \in X : Y(t)\varphi \text{ satisfies (1) and } Y(t)\varphi \in \partial X, \forall t \geq 0\}.$$

We claim that

$$M_{\partial} = \{(T, 0, 0, E)\}. \quad (25)$$

Assuming $Y(t) \in M_{\partial}, \forall t \geq 0$, it suffices to show that $T^*(t) = V(t) = 0, \forall t \geq 0$. If it is not true, then there exists $t_0 > 0$ such that either (a) $T^*(t_0) > 0, V(t_0) = 0$; or (b) $T^*(t_0) = 0, V(t_0) > 0$.

For case (a), from the third equation of (1) we have

$$\left. \frac{dV}{dt} \right|_{t=t_0} = N\delta T^*(t_0) > 0.$$

Hence there is an $\varepsilon_0 > 0$ such that $V(t) > 0, \forall t \in (t_0, t_0 + \varepsilon_0)$. On the other hand, from $T^*(t_0) > 0$ there exists an ε_1 ($0 < \varepsilon_1 < \varepsilon_0$) such that $T^*(t) > 0, \forall t \in (t_0, t_0 + \varepsilon_1)$. Thus, we have $T^*(t) > 0, V(t) > 0, \forall t \in (t_0, t_0 + \varepsilon_1)$, which contradicts the assumption that $(T(t), T^*(t), V(t), E(t)) \in M_{\partial}, \forall t \geq 0$. Similarly, we can obtain a contradiction for case (b). This proves the claim (25).

Let $\mathcal{A} = \bigcap_{x \in A_b} \omega(x)$, where A_b is the global attractor of $P(t)$ restricted to ∂X . We show that $\mathcal{A} = \{E_0\}$. In fact, from $\mathcal{A} \subseteq M_{\partial}$ and the second and first equations of (1), we have $\lim_{t \rightarrow \infty} E(t) = 0$ and $\lim_{t \rightarrow \infty} T(t) = \tilde{T}$. Thus, $\{E_0\}$ is the isolated invariant set in X .

Next we show that $W^s(E_0) \cap X^0 = \emptyset$. If this is not true, then there exists a solution

$(T_t, T_t^*, V, E_t) \in X^0$ such that

$$\lim_{t \rightarrow \infty} T(t) = \tilde{T} = \frac{s}{d}, \quad \lim_{t \rightarrow \infty} T^*(t) = 0, \quad \lim_{t \rightarrow \infty} V(t) = 0, \quad \lim_{t \rightarrow \infty} E(t) = 0.$$

For any sufficiently small constant $\varepsilon > 0$, there exists a positive constant $T_0 = T_0(\varepsilon)$ such that

$$T(t) > \tilde{T} - \varepsilon > 0, \quad E(t) < \varepsilon \quad \text{for all } t \geq T_0.$$

For the constant ε given above, it follows from the second and third equations of (1) that

$$\begin{cases} \frac{dT^*(t)}{dt} \geq k_1(\tilde{T} - \varepsilon)V(t - \tau_1) - \delta T^* - d_x \varepsilon T^*, \\ \frac{dV(t)}{dt} = N\delta T^* - cV, \quad t \geq T_0 + \tau. \end{cases}$$

If $T^*(t), V(t) \rightarrow 0$, as $t \rightarrow \infty$, then by a standard comparison argument and the nonnegativity, the solution $(n_{T^*}(t), n_V(t))$ of the following monotone system

$$\begin{cases} \frac{dn_{T^*}(t)}{dt} = k_1(\tilde{T} - \varepsilon) \cdot n_V(t - \tau_1) - \delta n_{T^*}(t) - d_x \varepsilon n_{T^*}(t), \\ \frac{dn_V(t)}{dt} = N\delta n_{T^*}(t) - cn_V(t), \quad t \geq T_0 + \tau. \end{cases} \quad (26)$$

with the initial condition $n_{T^*}(t) = T^*(t)$, $n_V(t) = V(t)$, $\forall t \in [T_0, T_0 + \tau]$ converges to $(0, 0)$ as well. Thus $\lim_{t \rightarrow \infty} \tilde{W}(t) = 0$, where $\tilde{W}(t) > 0$ is defined by

$$\tilde{W}(t) = n_{T^*}(t) + \frac{k_1(\tilde{T} - \varepsilon)}{c} n_V(t) + k_1(\tilde{T} - \varepsilon) \int_{t-\tau_1}^t n_V(\xi) d\xi.$$

Differentiating $\tilde{W}(t)$ with respect to time gives

$$\left. \frac{d\tilde{W}(t)}{dt} \right|_{(26)} = \left[\frac{1}{c} \cdot N\delta k_1(\tilde{T} - \varepsilon) - \delta - d_x \varepsilon \right] \cdot n_{T^*}(t).$$

Because $\mathfrak{R}_0 > 1$, we have $\frac{1}{c} \cdot N\delta k_1(\tilde{T} - \varepsilon) - \delta - d_x \varepsilon > 0$ for a sufficiently small ε . Therefore $\tilde{W}(t)$ goes to either infinity or a positive number as $t \rightarrow \infty$, which leads to a contradiction with $\lim_{t \rightarrow \infty} \tilde{W}(t) = 0$. Thus we have $W^s(\tilde{E}_0) \cap X^0 = \emptyset$.

Define $\mathfrak{p} : X \rightarrow R_+$ by

$$\mathfrak{p}(\varphi) = \min\{\varphi_2(0), \varphi_3(0)\}, \quad \forall \varphi \in X.$$

It is clear that $X^0 = \mathfrak{p}^{-1}(0, \infty)$ and $\partial X = \mathfrak{p}^{-1}(0)$. Thus by [58, Theorem 3] we have

$$\liminf_{t \rightarrow \infty} (T^*(t), V(t)) \geq (\eta_1, \eta_1)$$

for some constant $\eta_1 > 0$. Moreover, by the fourth equation of (1), we have $\liminf_{t \rightarrow \infty} E(t) \geq \eta_2$ for some constant $\eta_2 > 0$. Let $\eta_0 = \min\{\eta_1, \eta_2, \varepsilon\}$, where ε is from Proposition 1 such that $\liminf_{t \rightarrow \infty} T(t) \geq \varepsilon > 0$. We showed that $\liminf_{t \rightarrow \infty} T(t) \geq \eta_0$, $\liminf_{t \rightarrow \infty} T^*(t) \geq \eta_0$, $\liminf_{t \rightarrow \infty} E(t) \geq \eta_0$, and $\liminf_{t \rightarrow \infty} V(t) \geq \eta_0$. This finishes the proof of Theorem 5.

3.4.3 Infected steady state

Theorem 6: If the basic reproductive number satisfies $1 < \mathfrak{R}_0 \leq 1 + d_x \eta_0 / (2\delta)$ where η_0 was defined in Theorem 5, then the infected equilibrium E_1 of model (1) is globally attracting.

Proof: We define a Lyapunov functional

$$W(t) = W_1(t) + W_2(t) + W_3(t)$$

with

$$\begin{aligned} W_1(t) &= \bar{T} \cdot H\left(\frac{T}{\bar{T}}\right) + \frac{k}{k_1} \bar{T}^* \cdot H\left(\frac{T^*}{\bar{T}^*}\right) + a_1 \bar{V} \cdot H\left(\frac{V}{\bar{V}}\right) + a_2 \bar{E} \cdot H\left(\frac{E}{\bar{E}}\right), \\ W_2(t) &= k \bar{V} \bar{T} \int_{t-\tau_1}^t H\left(\frac{V(s)T(s)}{\bar{V}\bar{T}}\right) ds, \\ W_3(t) &= a_2 p \int_{t-\tau_2}^t T^*(s) ds, \end{aligned}$$

where $H(x) = x - 1 - \ln x$, $a_1 = \frac{k d_x}{k_1 p} \bar{E}$, and $a_2 = \frac{k d_x}{k_1 p} \bar{E}$. Proposition 1 implies that $W(t)$ is well-defined and that $W(t) \geq 0$, $\forall t \geq 0$. The equality holds if and only if $T(t) = \bar{T}$, $T^*(t) = \bar{T}^*$, $V(t) = \bar{V}$ and $E(t) = \bar{E}$. We calculate the derivatives of W_1 , W_2 , and W_3 separately as follows

$$\begin{aligned} \frac{dW_1}{dt} \Big|_{(1)} &= \left(1 - \frac{\bar{T}}{T}\right) \frac{dT}{dt} + \frac{k}{k_1} \left(1 - \frac{\bar{T}^*}{T^*}\right) \frac{dT^*}{dt} + a_1 \left(1 - \frac{\bar{V}}{V}\right) \frac{dV}{dt} + a_2 \left(1 - \frac{\bar{E}}{E}\right) \frac{dE}{dt} \\ &= \left(1 - \frac{\bar{T}}{T}\right) (s - dT - kVT - s + d\bar{T} + k\bar{V}\bar{T}) + \\ &\quad \frac{k}{k_1} \left(1 - \frac{\bar{T}^*}{T^*}\right) (k_1 V(t - \tau_1) T(t - \tau_1) - \delta T^* - d_x E T^*) + \\ &\quad a_1 \left(1 - \frac{\bar{V}}{V}\right) (N\delta T^* - cV) + a_2 \left(1 - \frac{\bar{E}}{E}\right) (pT^*(t - \tau_2) - d_E E) \\ &= -d \frac{1}{T} (T - \bar{T})^2 + (k\bar{V}\bar{T} + a_1 c\bar{V}) + (kV\bar{T} - a_1 cV) - kVT - \frac{k\bar{V}\bar{T}^2}{T} - \frac{k\delta}{k_1} T^* + \\ &\quad kV(t - \tau_1) T(t - \tau_1) - \frac{k d_x}{k_1} E T^* - k \frac{\bar{T}^* V(t - \tau_1) T(t - \tau_1)}{\bar{T}^*} + \frac{k\delta}{k_1} \bar{T}^* + a_1 N\delta T^* - \\ &\quad a_1 N\delta \frac{\bar{V} T^*}{V} + a_2 p T^*(t - \tau_2) + \left(\frac{k d_x}{k_1} \bar{T}^* E - a_2 d_E E\right) - a_2 p \frac{\bar{E} T^*(t - \tau_2)}{E} + a_2 d_E \bar{E} \\ &= -d \frac{1}{T} (T - \bar{T})^2 + 2k\bar{V}\bar{T} - kVT - \frac{k\bar{V}\bar{T}^2}{T} + kV(t - \tau_1) T(t - \tau_1) - \\ &\quad \frac{k\delta}{k_1} T^* - \frac{a_2 p}{E} E T^* - k \frac{\bar{T}^* V(t - \tau_1) T(t - \tau_1)}{\bar{T}^*} + \frac{k\delta}{k_1} \bar{T}^* + \\ &\quad a_1 N\delta T^* - a_1 N\delta \frac{\bar{V} T^*}{V} + a_2 p T^*(t - \tau_2) - a_2 p \frac{\bar{E} T^*(t - \tau_2)}{E} + a_2 p \bar{T}^* \\ &= -\Delta_0 + k\bar{V}\bar{T} \Delta_1 + a_2 p \bar{T}^* \Delta_2 + T^* \left(a_1 N\delta - \frac{k\delta}{k_1}\right) + \frac{k\delta}{k_1} \bar{T}^* - a_2 p \bar{T}^* \end{aligned}$$

where

$$\begin{aligned} \Delta_0 &= d \frac{1}{T} (T - \bar{T})^2 \\ \Delta_1 &= 2 - \frac{VT}{\bar{V}\bar{T}} - \frac{\bar{T}}{T} + \frac{V(t - \tau_1) T(t - \tau_1)}{\bar{V}\bar{T}} - \frac{T^* \bar{V}}{\bar{T}^* V} - \frac{\bar{T}^* V(t - \tau_1) T(t - \tau_1)}{T^* \bar{V}\bar{T}} \\ \Delta_2 &= 2 - \frac{ET^*}{E \bar{T}^*} + \frac{T^*(t - \tau_2)}{\bar{T}^*} - \frac{\bar{E} T^*(t - \tau_2)}{E \bar{T}^*} \end{aligned}$$

Next, we calculate the derivative of W_2 .

$$\begin{aligned}
& \frac{dW_2}{dt} \Big|_{(1)} + k\overline{VT}\Delta_1 = k\overline{VT}\Delta_1 + k\overline{VT} \left[\frac{VT - V(t-\tau_1)T(t-\tau_1)}{\overline{VT}} + \ln \frac{V(t-\tau_1)T(t-\tau_1)}{\overline{VT}} \right] \\
& = k\overline{VT} \left[2 - \frac{VT}{\overline{VT}} - \frac{\overline{T}}{\overline{T}} + \frac{V(t-\tau_1)T(t-\tau_1)}{\overline{VT}} - \frac{T^*\overline{V}}{\overline{T}^*V} - \frac{\overline{T}^*V(t-\tau_1)T(t-\tau_1)}{T^*\overline{VT}} \right] + k\overline{VT} \left[\frac{VT - V(t-\tau_1)T(t-\tau_1)}{\overline{VT}} + \ln \frac{V(t-\tau_1)T(t-\tau_1)}{\overline{VT}} \right] \\
& = k\overline{VT} \left[2 - \frac{\overline{T}}{\overline{T}} - \frac{T^*\overline{V}}{\overline{T}^*V} - \frac{\overline{T}^*V(t-\tau_1)T(t-\tau_1)}{T^*\overline{VT}} + \ln \frac{V(t-\tau_1)T(t-\tau_1)}{\overline{VT}} \right] \\
& = k\overline{VT}(\Omega_0 + \Omega_1 + \Omega_2) - k\overline{VT}
\end{aligned}$$

where

$$\begin{aligned}
\Omega_0 &= 1 - \frac{\overline{T}^*V(t-\tau_1)T(t-\tau_1)}{T^*\overline{VT}} + \ln \frac{\overline{T}^*V(t-\tau_1)T(t-\tau_1)}{T^*\overline{VT}} \\
\Omega_1 &= 1 - \frac{\overline{T}}{\overline{T}} + \ln \frac{\overline{T}}{\overline{T}} \\
\Omega_2 &= 1 - \frac{T^*\overline{V}}{\overline{T}^*V} + \ln \frac{T^*\overline{V}}{\overline{T}^*V}
\end{aligned}$$

Because $1 + \ln x - x \leq 0$ for all $x > 0$ we have $\Omega_0, \Omega_1, \Omega_2 \leq 0$.

The derivative of W_3 gives

$$\begin{aligned}
\frac{dW_3}{dt} \Big|_{(1)} + a_2p\overline{T}^*\Delta_2 &= a_2p(T^* - T^*(t-\tau_2)) + a_2p\overline{T}^* \left(2 - \frac{ET^*}{\overline{ET}^*} + \frac{T^*(t-\tau_2)}{\overline{T}^*} - \frac{\overline{ET}^*(t-\tau_2)}{\overline{ET}^*} \right) \\
&= a_2pT^* - a_2p\frac{ET^*}{\overline{E}} + 2a_2p\overline{T}^* - a_2p\frac{\overline{ET}^*(t-\tau_2)}{\overline{E}}
\end{aligned}$$

Assuming $\widehat{\Omega} = -\Delta_0 + k\overline{VT}(\Omega_0 + \Omega_1 + \Omega_2)$ and combining the derivatives of W_1, W_2 , and W_3 , we have

$$\begin{aligned}
& \frac{dW}{dt} \Big|_{(1)} = \frac{dW_1}{dt} \Big|_{(1)} + \frac{dW_2}{dt} \Big|_{(1)} + \frac{dW_3}{dt} \Big|_{(1)} \\
& = -\Delta_0 + k\overline{VT}(\Omega_0 + \Omega_1 + \Omega_2) - k\overline{VT} + T^* \left(a_1N\delta - \frac{k\delta}{k_1} \right) + \frac{k\delta}{k_1}\overline{T}^* - a_2p\overline{T}^* + a_2pT^* - a_2p\frac{ET^*}{\overline{E}} + 2a_2p\overline{T}^* - a_2p\frac{\overline{ET}^*(t-\tau_2)}{\overline{E}} \\
& \leq \widehat{\Omega} + a_2pT^* + a_2p\overline{T}^* - k\overline{VT} + T^* \left(a_1N\delta - \frac{k\delta}{k_1} \right) + \frac{k\delta}{k_1}\overline{T}^* - a_2p\frac{ET^*}{\overline{E}} \\
& = \widehat{\Omega} + T^* \left(a_2p + a_1N\delta - \frac{k\delta}{k_1} \right) + \left(\frac{k\delta}{k_1}\overline{ET}^* - k\overline{VT} + \frac{k\delta}{k_1}\overline{T}^* \right) - a_2p\frac{ET^*}{\overline{E}} \\
& = \widehat{\Omega} + T^* \left(\frac{2k\overline{N}\delta}{c} - \frac{2k\delta}{k_1} \right) + \overline{T}^* \left[\frac{k}{k_1} \left(\frac{k_1N\delta}{c}\overline{T} - \delta \right) - \frac{kN\delta}{c}\overline{T} + \frac{k\delta}{k_1} \right] - a_2p\frac{ET^*}{\overline{E}} \\
& = \widehat{\Omega} + 2k\delta T^* \left(\frac{\overline{T}N}{c} - \frac{1}{k_1} \right) - a_2p\frac{ET^*}{\overline{E}} \\
& = \widehat{\Omega} + \frac{2k\delta}{k_1} T^* (\Re_0 - 1) - a_2p\frac{ET^*}{\overline{E}} \\
& = \widehat{\Omega} + \frac{k}{k_1} T^* (2\delta(\Re_0 - 1) - d_x E)
\end{aligned}$$

From Theorem 5 we know that there exists $t_M > 0$ such that $E(t) \geq \eta_0$ for all $t > t_M$. Thus,

$$\frac{dW}{dt} \Big|_{(1)} \leq \widehat{\Omega} + \frac{k}{k_1} T^* (2\delta(\Re_0 - 1) - d_x \eta_0) \leq \frac{k}{k_1} T^* (2\delta(\Re_0 - 1) - d_x \eta_0) \leq 0, \quad \forall t > t_M.$$

Because $\frac{dW}{dt} \Big|_{(1)} \leq \widehat{\Omega} \leq 0$, $\frac{dW}{dt} \Big|_{(1)} = 0$ implies that

$$T = \bar{T}, \quad T^* \bar{V} = \bar{T}^* V.$$

Substituting $T(t) = \bar{T}$ into the first equation of (1), one can get $V(t) = \bar{V}$. From $T^* \bar{V} = \bar{T}^* V$, we have $T^* = \bar{T}^*$. Substituting the above equalities into the second equation of (1), we have

$E(t) = \bar{E}$. Therefore, the largest compact invariant set in $\left\{ \frac{dW}{dt} \Big|_{(1)} = 0 \right\}$ is the infected steady state E_1 .

Using the similar arguments in Theorem 4, we prove that

$$\lim_{t \rightarrow \infty} (T(t), T^*(t), V(t), E(t)) = E_1.$$

Theorem 7: If $\tau_2 = 0$ in system (1) and $\mathcal{R}_0 > 1$, then the infected steady state E_1 is globally attracting.

Proof: We replace the term $a_2 p \frac{\bar{E} T^* (t - \tau_2)}{E}$ in $\frac{dW}{dt} \Big|_{(1)}$ of Theorem 6 with $a_2 p \frac{\bar{E} T^*}{E}$, and get

$$\begin{aligned} \frac{dW}{dt} \Big|_{(1)} &= -\Delta_0 + T^* \left(a_1 N \delta - \frac{k\delta}{k_1} \right) + \frac{k\delta}{k_1} \bar{T}^* - a_2 p \bar{T}^* + k \bar{V} T (\Omega_0 + \Omega_1 + \Omega_2) - k \bar{V} T + a_2 p T^* - a_2 p \frac{ET}{E} + 2a_2 p \bar{T}^* - a_2 p \frac{\bar{E} T^*}{E} \\ &\leq a_2 p T^* + a_2 p \bar{T}^* - k \bar{V} T + T^* \left(a_1 N \delta - \frac{k\delta}{k_1} \right) + \frac{k\delta}{k_1} \bar{T}^* - a_2 p \frac{ET^*}{E} - a_2 p \frac{\bar{E} T^*}{E} \\ &= T^* \left(a_1 N \delta - \frac{k\delta}{k_1} + a_2 p \right) - a_2 p T^* \left(\frac{E}{E} + \frac{\bar{E}}{E} \right) + a_2 p \bar{T}^* - k \bar{V} T + \frac{k\delta}{k_1} \bar{T}^* \\ &\leq T^* \left(a_1 N \delta - \frac{k\delta}{k_1} + a_2 p \right) - 2a_2 p T^* + \bar{T}^* \left[\frac{k}{k_1} \left(\frac{k_1 N \delta}{c} \bar{T} - \delta \right) - \frac{k N \delta}{c} \bar{T} + \frac{k\delta}{k_1} \right] \\ &= T^* \left(a_1 N \delta - \frac{k\delta}{k_1} - a_2 p \right) \end{aligned}$$

Considering $a_1 = \frac{k}{c} \bar{T}$ and $a_2 = \frac{k\delta}{k_1 p} \bar{E}$, we have $a_1 N \delta - \frac{k\delta}{k_1} - a_2 p = 0$. Thus, for $\tau_2 = 0$, $\frac{dW}{dt} \Big|_{(1)} \leq 0$. Again using the similar arguments in Theorem 4, we show that E_1 is globally attracting.

4 Comparison with patient data

We compared modeling predictions with/without time delays to the plasma viral load data obtained from 10 patients during primary HIV-1 infection [60]. For most of the patients, the time between initial infection and the time of the first data point is unknown and needs to be estimated. Here, we used the same time shift as in [60]. Because CD4⁺ T cell data are not available for these patients, we fixed the parameters, such as s , d , and k , at the same values as in [60] as well as in a later study [9] for each patient. Although the current estimate of the viral clearance rate c is higher [47], we chose an earlier estimate from [44], $c = 3 \text{ day}^{-1}$, for comparisons because the same value was used in the data fitting using the model without a delay in [60].

The precise values of the intracellular time delay τ_1 and the immune delay τ_2 are unknown. Previous estimates of τ_1 suggested values of ≤ 1.5 days [35–37, 44]. The CTL immune response to HIV infection is observed in the first few weeks of infection, coincident with the initial decline in the plasma viral load [22]. The best-fit values for τ_2 range from 19 to 32 days in [9]. Here, we assumed that τ_1 is ≤ 1.5 days and τ_2 is ≤ 5 weeks, and estimated both

of them by data fitting. The ratio k_1/k represents the proportion of new infections that progress to the productive state. When performing data fitting, we found that by setting k_1 equal to k the resulting error was very similar to the error obtained with k_1 fitted. Thus, we assumed k_1 is equal to k during data fitting, reducing the number of fitted parameters by one. In fact, k is a good approximation of k_1 because both α and τ_1 are small.

The initial target cell, infected cell, and viral concentrations were set to be $T_0 = 10,000$ cells/ml, $T_0^* = 0$ cells/ml, and $V_0 = 10^{-6}$ RNA copies/ml, respectively [60]. We also assumed that no virus-specific immune cells exist before virus infection, i.e., $E_0 = 0$ cells/ml. The rest of the parameters were estimated by fitting the predictions of the two-delay model to the viral load data from 10 patients during primary HIV infection [60]. The data fitting was performed using a commercial software package Berkeley Madonna. The program generates parameter values that give the best fit of the model to the data set.

Data fits using the two delay model are shown in Figure 2. For comparison, we also included the fits using the model without delays to the same viral load data in the same figure. The delay model (green solid) seems to fit the data better than the model without delays (red dotted) for some patients. For example, for patient 2 and patient 4, the delay model captured most of the data points, although the prediction using the delay model exhibited frequent oscillations for patient 2. However, for some other patients such as patients 3 and 5, the two fits using models with and without delays generated very similar fits. The parameter values corresponding to the best fits using the model without and with delays are given in Table 1 and Table 2, respectively.

For each patient, we also calculated the RMS (root mean square) error, shown in Table 3, which represents the deviation between patient data and the best fit. We found that the delay model achieved a smaller error than the model without time delays for each patient. However, this does not mean that the delay model is better in fitting to the data because it has two more parameters than the model without delays. To further compare the best fits using the two models, we performed an F-test, which is able to determine which one of the two nested models provides a better fit to the same data set from a statistical standpoint. Notice that the model without delays is a special case of the delay model. Thus, they are nested models with 7 parameters for the delay model and 5 for the model without delays.

We define the F-test statistic

$$F = \frac{[RSS_{non} - RSS_{two}]/[df_{non} - df_{two}]}{RSS_{two}/df_{two}}$$

where RSS is the residual sum of squares, df is the degree of freedom (i.e., the number of data points minus the number of fitted parameters), and the subscripts *non* and *two* represent the model without delays and with two delays, respectively. It is clear that $RSS = n \cdot RMS^2$ where n is the number of data points. To calculate the p -value for the F test, we compute the F value with the numerator degree of freedom $df_{non} - df_{two}$ and the denominator degree of freedom df_{two} . Results were shown in Table 3. We found that the two-delay model provides significantly better fits for patients 7 and 10 (with the p -value < 0.05), although the p -value is close to 0.05 for these patients. For the other patients, there is no significant difference in the fits, although the delay model generates a smaller error.

In Figures 3 and 4, we showed how the variations of time delays and other parameter values change the virus dynamics in some patients. Similar changes with varying parameters were

observed in other patients. In Fig. 3A–D, we fixed the intracellular delay τ_1 to be the best fit for each patient and reduced the immune delay τ_2 from the best fit value to 0. We found that the immune delay was able to generate a periodic solution (Patient 2). This further confirms our theoretical prediction in Theorem 3. We also observed that the immune delay did not affect the magnitude of the viral peak and the time to reach the peak. This is due to the small values of the killing rate (d_x) and generation rate (p) of immune cells. Thus, there is only a minor difference in the killing of infected cells by immune cells with and without the immune delay before viral peak. Around viral peak, a high level of infected cells activated a large number of immune cells, which led to an effective killing of infected cells. In Fig. 3E–H, we fixed the immune delay at the best fit and changed the intracellular delay. We found as the intracellular delay increased, the time to reach the peak was delayed. The level of the viral peak was lower for a larger intracellular delay. We also studied the effects of different killing rate (d_x) by immune cells and the viral burst size on the viral load change in Fig. 4. As expected, when the killing rate increases or the viral burst size decreases, the viral set point (or the average viral load in the case of a periodic solution) decreases.

5 Summary and discussion

Mathematical models, in conjunction with experimental data, have provided important insights into virus infection, antiviral therapy, emergence of drug resistance, and immune responses. Time delays are inevitable in many engineering and biological systems [23, 59], and have also been incorporated into viral dynamic models. In this paper, we included two delays in a model to study HIV-1 dynamics. One represents the time needed for infected cells to produce virions after viral entry (intracellular delay), and the other denotes the time needed for the adaptive immune response to emerge to control viral replication (immune delay). We studied how these delays impact the dynamics. We defined a basic reproductive ratio $\mathcal{R}_0$ and showed that this ratio plays an important role in determining the stability of the steady states of the delay model. More specifically, we showed that the infection-free steady state is locally asymptotically stable and globally attracting when $\mathcal{R}_0 > 1$. In the case of $\tau_2 = 0$ (no immune delay), the infected steady state is locally asymptotically stable and globally attracting when $\mathcal{R}_0 > 1$. In the case of $\tau_1 = 0$ (no intracellular delay), we showed that a Hopf bifurcation takes place at a critical threshold of the immune delay ($\tau_2^* > 0$) even for a simplified model assuming the target cell concentration is constant. When $\tau_2 < \tau_2^*$, the infected steady state is still locally asymptotically stable. We also derived conditions under which the infected steady state is globally attracting when both delays are positive. These results suggest that introducing the intracellular delay does not change the stability results (note that the basic reproductive ratio is intracellular delay-dependent in this case). Incorporating the immune response delay into the model generates rich dynamics.

Determining the stability switching regions for a model with two positive delays is challenging. A few papers have studied the mathematical properties of the steady states of a model with two delays [1, 10, 24, 25, 53, 54]. For a general linear scalar system with two delays, Gu et al. [18] showed the stability crossing set can be expressed by a few inequality constraints. Moreover, the crossing curves fall into a few categories of curves, namely, closed curves, open ended curves, and spiral-like curves oriented horizontally, vertically, or diagonally. Identifying the local stability regions when both the intracellular delay and the immune delay vary within their biologically plausible ranges remains a potential topic for future investigation.

We used a simple linear term, $s - dT$, to model the generation and death of target cells. Some studies included a logistic term $\rho T(t)[1 - T(t)/T_{max}]$ to represent the proliferation of target cells [15, 43]. They showed that periodic oscillations can occur through Hopf bifurcation. Another logistic term, $\rho T(t)[1 - (T(t) + T^*(t))/T_{max}]$, was included to describe the

proliferation of target cells in a coupled way [11, 61]. They showed that for an open set of parameter values the infected steady state can be unstable and periodic solutions may exist. Recently, Li and Shu [27] studied the effects of both the intracellular delay and target cell proliferation on virus dynamics.

The model predicts that the infection-free steady state is stable when $\mathcal{R}_0 < 1$, which indicates that the infection can be cleared if antiretroviral therapy is sufficiently efficient in reducing the basic reproductive ratio to below 1. However, current treatment regimens cannot eradicate the virus. The establishment of HIV-1 infection in a few cell populations, such as latently infected CD4⁺ T cells [8,17,65] and hematopoietic progenitor cells [7], represents a major obstacle to viral elimination. Mathematical models have been developed to describe low viral load persistence, slow decay of the latent reservoir, and emergence of intermittent viral blips above the detection limit in the setting of effective combination therapy [50, 51] (see a recent review in [52]). In this paper, we did not include the latent reservoir. The major goal of this study is to examine the effects of both the intracellular and immune delays on virus dynamics.

Model comparison with experimental data showed that including a time delay can affect the estimate of model parameters [9, 21, 37]. We also fit the model with two delays to the viral load data from 10 patients during primary HIV-1 infection. Although we obtained a smaller error between data and fit for each patient, a statistical test suggested that the improvement is not significant for most of the patients. This highlights that more data are needed for model verification and selection when we incorporate time delays into mathematical models to study virus dynamics.

Acknowledgments

Portions of this work were performed during the first three authors' visit to the Fields Institute (Summer 2010 Thematic Program on the Mathematics of Drug Resistance in Infectious Diseases). They would like to acknowledge the hospitality received there. The work is supported in part by the NSF grant DMS-1122290 and NIH P30-EB011339 (LR), the NNSF of China (No. 61075037), the Fundamental Research Funds for the Central Universities (No. HIT.NSRIF.2010052) and Program of Excellent Team in Harbin Institute of Technology (SL). The authors also thank the referees for the comments that improved this manuscript.

References

1. Adimy M, Crauste F, Ruan S. Periodic oscillations in leukopoiesis models with two delays. *J Theor Biol.* 2006; 242:288–99. [PubMed: 16603196]
2. Banks HT, Bortz DM. A parameter sensitivity methodology in the context of HIV delay equation models. *J Math Biol.* 2005; 50:607–25. [PubMed: 15614552]
3. Banks HT, Bortz DM, Holte SE. Incorporation of variability into the modeling of viral delays in HIV infection dynamics. *Math Biosci.* 2003; 183:63–91. [PubMed: 12604136]
4. Beretta E, Kuang Y. Geometric stability switch criteria in delay differential systems with delay dependent parameters. *SIAM J Math Anal.* 2002; 33:1144–65.
5. Bonhoeffer S, Coffin JM, Nowak MA. Human immunodeficiency virus drug therapy and virus load. *J Virol.* 1997; 71:3275–8. [PubMed: 9060694]
6. Bonhoeffer S, May RM, Shaw GM, Nowak MA. Virus dynamics and drug therapy. *Proc Natl Acad Sci USA.* 1997; 94:6971–6. [PubMed: 9192676]
7. Carter CC, Onafuwa-Nuga A, McNamara LA, Riddell J, Bixby D, Savona MR, Collins KL. HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. *Nat Med.* 2010; 16:446–51. [PubMed: 20208541]
8. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, Lloyd AL, Nowak MA, Fauci AS. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA.* 1997; 94:13193–7. [PubMed: 9371822]

9. Ciupe MS, Bivort BL, Bortz DM, Nelson PW. Estimating kinetic parameters from HIV primary infection data through the eyes of three different mathematical models. *Math Biosci.* 2006; 200:1–27. [PubMed: 16469337]
10. Cooke KL, van den Driessche P. Analysis of an SEIRS epidemic model with two delays. *J Math Bio.* 1996; 35:240–60. [PubMed: 9008370]
11. Culshaw RV, Ruan S. A delay-differential equation model of HIV infection of CD4(+) T-cells. *Math Biosci.* 2000; 165:27–39. [PubMed: 10804258]
12. Culshaw RV, Ruan S, Webb G. A mathematical model of cell-to-cell spread of HIV-1 that includes a time delay. *J Math Biol.* 2003; 46:425–44. [PubMed: 12750834]
13. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N Engl J Med.* 1991; 324:961–4. [PubMed: 1823118]
14. De Boer RJ, Perelson AS. Target cell limited and immune control models of HIV infection: a comparison. *J Theor Biol.* 1998; 190:201–14. [PubMed: 9514649]
15. De Leenheer P, Smith HL. Virus dynamics: a global analysis. *SIAM J Appl Math.* 2003; 63:1313–27.
16. Dixit NM, Perelson AS. Complex patterns of viral load decay under antiretroviral therapy: influence of pharmacokinetics and intracellular delay. *J Theor Biol.* 2004; 226:95–109. [PubMed: 14637059]
17. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science.* 1997; 278:1295–300. [PubMed: 9360927]
18. Gu K, Niculescu SI, Chen J. On stability crossing curves for general systems with two delays. *J of Math Anal and Appl.* 2005; 311:231–53.
19. Hale, JK.; Verduyn Lunel, SM. *Introduction to Functional Differential Equations.* Springer-Verlag; 1993.
20. Haynes BF, Pantaleo G, Fauci AS. Toward an understanding of the correlates of protective immunity to HIV infection. *Science.* 1996; 271:324–8. [PubMed: 8553066]
21. Herz AV, Bonhoeffer S, Anderson RM, May RM, Nowak MA. Viral dynamics in vivo: limitations on estimates of intracellular delay and virus decay. *Proc Natl Acad Sci USA.* 1996; 93:7247–51. [PubMed: 8692977]
22. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, Farthing C, Ho DD. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol.* 1994; 68:4650–5. [PubMed: 8207839]
23. Kuang, Y. *Delay Differential Equations with Applications in Population Dynamics.* Academic Press; 1993.
24. Li J, Kuang Y. Analysis of a model of the glucose-insulin regulatory system with two delays. *SIAM J Appl Math.* 2007; 67:757–76.
25. Li J, Kuang Y, Mason C. Modeling the glucose-insulin regulatory system and ultradian insulin secretory oscillations with two time delays. *J Theor Biol.* 2006; 242:722–35. [PubMed: 16712872]
26. Li MY, Shu H. Global dynamics of an in-host viral model with intracellular delay. *Bull Math Biol.* 2010; 72:1492–505. [PubMed: 20087671]
27. Li MY, Shu H. Impact of intracellular delays and target-cell dynamics on in vivo viral infections. *SIAM J Appl Math.* 2010; 70:2434–48.
28. Little SJ, McLean AR, Spina CA, Richman DD, Havlir DV. Viral dynamics of acute HIV-1 infection. *J Exp Med.* 1999; 190:841–50. [PubMed: 10499922]
29. Liu S, Wang S, Wang L. Global dynamics of delay epidemic models with nonlinear incidence rate and relapse. *Nonlinear Anal Real World App.* 2011; 12:119–27.
30. Liu S, Beretta E. Stage-structured Predator-prey Model with the Beddington-DeAngelis functional response. *SIAM J Appl Math.* 2006; 66:1101–29.
31. Liu W. Nonlinear oscillation in models of immune response to persistent viruses. *Theor Popul Biol.* 1997; 52:224–30. [PubMed: 9466963]

32. Markowitz M, Louie M, Hurley A, Sun E, Di Mascio M, Perelson AS, Ho DD. A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo. *J Virol.* 2003; 77:5037–8. [PubMed: 12663814]
33. McCluskey CC. Global stability for an SEIR epidemiological model with varying infectivity and infinite delay. *Math Biosci and Eng.* 2009; 6:603–10.
34. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science.* 1996; 272:1167–70. [PubMed: 8638160]
35. Mittler JE, Markowitz M, Ho DD, Perelson AS. Improved estimates for HIV-1 clearance rate and intracellular delay. *AIDS.* 1999; 13:1415–7. [PubMed: 10449298]
36. Mittler JE, Sulzer B, Neumann AU, Perelson AS. Influence of delayed viral production on viral dynamics in HIV-1 infected patients. *Math Biosci.* 1998; 152:143–63. [PubMed: 9780612]
37. Nelson PW, Murray JD, Perelson AS. A model of HIV-1 pathogenesis that includes an intracellular delay. *Math Biosci.* 2000; 163:201–15. [PubMed: 10701304]
38. Nowak MA, Bangham CR. Population dynamics of immune responses to persistent viruses. *Science.* 1996; 272:74–9. [PubMed: 8600540]
39. Nowak, MA.; May, RM. *Virus Dynamics: Mathematical Principles of Immunology and Virology.* Oxford University Press; 2000.
40. Nelson PW, Perelson AS. Mathematical analysis of delay differential equation models of HIV-1 infection. *Math Biosci.* 2002; 179:73–94. [PubMed: 12047922]
41. Perelson AS. Modeling viral and immune system dynamics. *Nat Rev Immunol.* 2002; 2:28–36. [PubMed: 11905835]
42. Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, Markowitz M, Ho DD. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature.* 1997; 387:188–91. [PubMed: 9144290]
43. Perelson AS, Nelson PW. Mathematical analysis of HIV-1 dynamics in vivo. *SIAM Rev.* 1999; 41:3–44.
44. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science.* 1996; 271:1582–6. [PubMed: 8599114]
45. Phillips AN. Reduction of HIV concentration during acute infection: independence from a specific immune response. *Science.* 1996; 271:497–9. [PubMed: 8560262]
46. Piatak M Jr, Saag MS, Yang LC, Clark SJ, Kappes JC, Luk KC, Hahn BH, Shaw GM, Lifson JD. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science.* 1993; 259:1749–54. [PubMed: 8096089]
47. Ramratnam B, Bonhoeffer S, Binley J, Hurley A, Zhang L, Mittler JE, Markowitz M, Moore JP, Perelson AS, Ho DD. Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. *Lancet.* 1999; 354:1782–5. [PubMed: 10577640]
48. Regoes RR, Wodarz D, Nowak MA. Virus dynamics: the effect of target cell limitation and immune responses on virus evolution. *J Theor Biol.* 1998; 191:451–62. [PubMed: 9631576]
49. Rong L, Feng Z, Perelson AS. Emergence of HIV-1 drug resistance during antiretroviral treatment. *Bull Math Biol.* 2007; 69:2027–60. [PubMed: 17450401]
50. Rong L, Perelson AS. Asymmetric division of activated latently infected cells may explain the decay kinetics of the HIV-1 latent reservoir and intermittent viral blips. *Math Biosci.* 2009; 217:77–87. [PubMed: 18977369]
51. Rong L, Perelson AS. Modeling latently infected cell activation: viral, latent reservoir persistence, and viral blips in HIV-infected patients on potent therapy. *PLoS Comput Biol.* 2009; 5:e1000533. [PubMed: 19834532]
52. Rong L, Perelson AS. Modeling HIV persistence, the latent reservoir, and viral blips. *J Theor Biol.* 2009; 260:308–31. [PubMed: 19539630]
53. Ruan S, Wei J. On the zeros of transcendental functions with applications to stability of delay differential equations with two delays, *Dynamics of Continuous. Discrete and Impulsive Systems.* 2003; 10:863–74.

54. Ruan S, Wei J. Periodic solutions of planar systems with two delays. *Proc R Soc Edinburgh Ser A*. 1999; 129:1017–32.
55. Röst G, Wu J. SEIR epidemiological model with varying infectivity and infinite delay. *Math Biosci and Eng*. 2008; 5:389–402.
56. Sedaghat AR, Dinoso JB, Shen L, Wilke CO, Siliciano RF. Decay dynamics of HIV-1 depend on the inhibited stages of the viral life cycle. *Proc Natl Acad Sci USA*. 2008; 105:4832–7. [PubMed: 18362342]
57. Sedaghat AR, Siliciano JD, Brennan TP, Wilke CO, Siliciano RF. Limits on replenishment of the resting CD4+ T cell reservoir for HIV in patients on HAART. *PLoS Pathog*. 2007; 3:e122. [PubMed: 17784786]
58. Smith H, Zhao X. Robust persistence for semidynamical systems. *Nonlinear Anal*. 2001; 46:6169–79.
59. Smith, HL. *Texts in Applied Mathematics*. Springer; 2011. *An Introduction to Delay Differential Equations With Applications to the Life Sciences*.
60. Stafford MA, Corey L, Cao Y, Daar ES, Ho DD, Perelson AS. Modeling plasma virus concentration during primary HIV infection. *J Theor Biol*. 2000; 203:285–301. [PubMed: 10716909]
61. Wang L, Li MY. Mathematical analysis of the global dynamics of a model for HIV infection of CD4+ T cells. *Math Biosci*. 2006; 200:44–57. [PubMed: 16466751]
62. Wang Y, Zhou Y, Wu J, Heffernan J. Oscillatory viral dynamics in a delayed HIV pathogenesis model. *Math Biosci*. 2009; 219:104–12. [PubMed: 19327371]
63. White M, Zhao X. Threshold dynamics in a time-delayed epidemic model with dispersal. *Math Biosci*. 2009; 218:121–9. [PubMed: 19563742]
64. Wodarz, D. *Killer Cell Dynamics: Mathematical and Computational Approaches to Immunology*. Springer; 2007.
65. Wong JK, Hezareh M, Günthard HF, Havlir DV, Ignacio CC, Spina CA, Richman DD. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science*. 1997; 278:1291–1295. [PubMed: 9360926]
66. Zhao, X. *Dynamical Systems in Population Biology*. Springer; 2003.
67. Zhu H, Zou X. Dynamics of a HIV-1 infection model with cell-mediated immune response and intracellular delay. *Disc Cont Dyan Syst B*. 2009; 12:511–24.
68. Zurakowski R, Teel AR. A model predictive control based scheduling method for HIV therapy. *J Theor Biol*. 2006; 238:368–82. [PubMed: 15993900]

Highlights

- We modeled HIV infection including two time delays.
- We showed the local stability of the steady states.
- We showed the global stability of the steady states.
- We compared model predictions with patient data.
- Delay model achieves a smaller error between data and fit, but is not better from a statistical standpoint.

Figure 1.
Schematic representation of model (1).

Figure 2.
Data fits using the two-delay model (solid curves) and the non-delay model (dashed curves).

Figure 3.
The effect of varying immune delay (A–D) or intracellular delay (E–H) on virus dynamics.

Figure 4.

The effect of varying killing rate due to immune cells (top panels) or the viral burst size (bottom panels) on virus dynamics.

Best-fit parameter values using the non-delay model. The values of d , k , and s were chosen from Stafford et. al. [60].

Table 1

Patient	$d_x \times 10^{-4}$ ml cells-day ⁻¹	p day ⁻¹	d_E day ⁻¹	N viron cells ⁻¹	d day ⁻¹	$k \times 10^{-7}$ ml viron-day ⁻¹	s cells ml-day ⁻¹	δ day ⁻¹
1	2.2	0.07	0.01	5101	0.013	0.46	130	0.75
2	10	2	0.55	2966	0.02	3.6	200	0.80
3	5.4	0.01	0.02	5617	0.0065	6.4	65	0.10
4	6.8	0.01	4.07	668	0.0046	48	46	0.13
5	1.0	0.6	1.13	3843	0.017	6.3	170	0.22
6	7.2	2	2.13	1341	0.012	7.5	120	0.59
7	1.0	1	5.00	4493	0.017	8	170	0.32
8	1.0	0.01	0.97	6689	0.0085	6.6	85	0.10
9	1.0	0.01	2.87	1415	0.006	25	60	0.10
10	9.7	0.01	0.30	18621	0.0043	1.9	43	0.50
Mean	3.8	0.04	1.05	4168	0.010	6.5	103	0.27
SD	3.7	0.8	1.76	5158	0.006	14	57.3	0.28

Best-fit parameter values using the two delay model. Parameters values of d , k , and s were chosen from Stafford et. al. [60].

Table 2

Patient	$dx \times 10^{-4}$ ml cells-day ⁻¹	p day ⁻¹	d_E day ⁻¹	τ_1 days	τ_2 days	N viron cells ⁻¹	d day ⁻¹	$k \times 10^{-7}$ ml viron-day ⁻¹	s cells ml-day ⁻¹	δ day ⁻¹
1	3.9	0.02	0.01	0.1	11.2	5505	0.013	0.46	130	0.26
2	8.8	0.4	0.41	0.4	8.5	6167	0.02	3.6	200	0.53
3	3.7	0.3	1.81	0.1	9.2	2374	0.0065	6.4	65	0.30
4	9.4	0.02	0.81	0.5	16.1	1261	0.0046	48	46	0.10
5	1.0	0.09	0.45	0.1	7.0	3360	0.017	6.3	170	0.30
6	9.9	0.01	0.01	0.1	35.0	2244	0.012	7.5	120	0.35
7	1.0	2	1.65	0.2	20.5	5308	0.017	8	170	0.44
8	2.7	0.02	5.00	0.4	8.7	7381	0.0085	6.6	85	0.14
9	1.3	0.01	0.04	0.6	13.8	2175	0.006	25	60	0.10
10	3.7	0.08	0.01	0.1	29.9	23528	0.0043	1.9	43	0.56
Mean	3.7	0.05	0.43	0.2	12.5	4334	0.010	6.5	103	0.30
SD	3.5	0.6	1.55	0.2	9.7	6508	0.006	14	57.3	0.17

Table 3

Comparison of the fits using models with and without delays.

Patient	Number of data points	Non-delay model RMS	Two delay model RMS	p-value for the F-test
1	11	0.324	0.218	0.205
2	10	0.622	0.291	0.102
3	9	0.254	0.222	0.764
4	8	0.229	0.048	0.210
5	9	0.236	0.193	0.669
6	15	0.647	0.506	0.140
7	11	0.210	0.097	0.046
8	16	0.172	0.155	0.392
9	10	0.259	0.205	0.496
10	15	0.516	0.354	0.049