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A mathematical model of cell-to-cell spread of HIV-1 that includes a time delay

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Abstract. We consider a two-dimensional model of cell-to-cell spread of HIV-1 in tissue cultures, assuming that infection is spread directly from infected cells to healthy cells and neglecting the effects of free virus. The intracellular incubation period is modeled by a gamma distribution and the model is a system of two differential equations with distributed delay, which includes the differential equations model with a discrete delay and the ordinary differential equations model as special cases. We study the stability in all three types of models. It is shown that the ODE model is globally stable while both delay models exhibit Hopf bifurcations by using the (average) delay as a bifurcation parameter. The results indicate that, differing from the cell-to-free virus spread models, the cell-to-cell spread models can produce infective oscillations in typical tissue culture parameter regimes and the latently infected cells are instrumental in sustaining the infection. Our delayed cell-to-cell models may be applicable to study other types of viral infections such as human T-cell leukaemia virus type 1 (HTLV-1).

1. Introduction

In the last decade, several theories have been presented in attempt to explain the mechanisms that lead to the depletion of $CD4^+$ lymphocytes in an infected individual. Since majority of infection occurs in the lymphatic tissues where 98% of $CD4^+$ lymphocytes reside (Rosemberg and Janossy [38]), understanding the dynamics within lymphatic tissues is vital to uncovering information regarding cellular infection and viral production. Many mathematical models have been developed to describe the immunological response to HIV-1 infection. Most of these models focus on cell-free viral spread in a compartment such as the bloodstream,

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see, for example, Callaway and Perelson [3]. Kirschner, Lenhart and Serbin [15], Kirschner and Webb [16–18], McLean and Kirkwood [24], McLean and Nowak [25], Müller et al. [27], Nowak and Bangham [31], Nowak and May [32,33], Perelson, Kirschner and De Boer [35], Perelson [34], Perelson and Nelson [36], Wodarz et al. [46], etc.

There is precedent for studying *in vitro* cell-to-cell spread of HIV-1 (as well as that of other viruses) since many features are easier to determine experimentally in tissue cultures than in, for example, a more complex medium such as the bloodstream. Also, HIV-1 is thought to be active in areas such as the lymph nodes and the brain where cell-to-cell spread would be a much more important mode of infection than cell-free viral spread. In fact, it has been reported (Dimitrov et al. [6] and Sato et al. [40]) that cell-to-cell spread of virus is favored over infections with cell-free virus inocula. The data of Gummuluru et al. [11] support the hypothesis that cell-to-cell spread og HIV-1 is the *predominat* route of viral spread since viral replication in a system with rapid cell turnover kinetics depends on cell-to-cell transfer of virus. See also Bailey et al. [1], Bajaria et al. [2], Chun [4], Finzi and Siliciano [7], Haase et al. [12,13], Philips et al. [37], Schacker et al. [41], etc.

In [43], Spouge et al. have studied HIV-1 cell-to-cell infection kinetics in tissue cultures in terms of mathematical models and observed that the asymptotic behavior is similar to that of a model representing cell-free viral spread. That is, in ordinary differential equations models, under all realistic parameter ranges, the system tends toward an "infected equilibrium", in which healthy cells and infected cells co-exist.

Upon infection with HIV-1, there is a short intracellular "eclipse phase" (often referred to as "latency" in the literature), during which the cell is infected but has not yet begun producing virus. Spouge et al. admitted that their system "does not include a latent period after cells have been infected, ... latency might be modeled either by a delay or by an explicit class of latently infected cells. ... We have omitted latency because it is fast (about a day) on the time scales of interest here (at least a week)". They also pointed out that there are two methods to model this eclipse phase, by a time delay or by an explicit class of latently infected cells. Perelson et al. [35] studied a system with an explicit class of latently infected cells. Herz et al. [14] assumed that cells become productively infected τ time units after initial infection. They reported that including an intracellular delay did change the estimates of the viral clearance rate but did not change the productively infected T cell loss rate. Culshaw and Ruan [5] showed that such an intracellular delay did not change the stability of the infected steady state for clinically reported parameter values. Tam [45] investigated the delay effect in a model which describes the interaction between a replicating virus and host cells. Mittler et al. [26] assumed that the intracellular delay was continuous and varied according to a gamma distribution and observed dramatical changes in the estimates of viral clearance. Using the method of stages, Grossman [8,9] found that including a delay model for the death of infected cells resulted in different conclusions about residual transmission of infection in the presence of drugs that effectively reduce viral load. Nelson et al. [28–30] extended the development of delay models of HIV-1 infection and treatment to more general cases of combination antiviral therapy that is less than completely efficacious. Lloyd [22] observed

that the models neglecting the intracellular delay before virion production can lead to severe underestimates of the reproductive number and to overly optimistic predictions of how efficacious treatment must be in order to prevent the disease.

In this paper, we consider the cell-to-cell spread of HIV-1 in tissue cultures (in vitro) and model the intracellular eclipse phase by a gamma distribution, that is, a distributed delay representing the lag between the time a cell becomes infected and when it begins to infect other cells. The model is then described by a system of differential equations with distributed delay. When the distribution takes the form of a delta function at a positive number τ, the model becomes a system of differential equations with a discrete delay. When $\tau = 0$, the model reduces to a system of ordinary differential equations (ODE) considered by Spouge et al. [43].

Does the cellular eclipse phase affect the qualitative properties of the model? If so, how? We try to answer these questions and find that in fact the cellular eclipse phase does change the dynamics of the model: it can cause the model to lose its stability and induce fluctuations in the cell concentrations. This result indicates that we must exercise caution when extrapolating such a model's qualities to the cell-free (or the *in vivo*) case.

In general, the model can have at most three steady states – trivial, healthy, and infected. A transcritical bifurcation occurs when the fraction of infected cells surviving the incubation period surpasses a critical value. If this fraction is too small the healthy steady state is stable and the infected steady state is unfeasible. When this fraction increases and passes through a critical value, the healthy steady state becomes unstable and the infected steady state exists (and is stable under certain conditions). A new result we obtain for the ODE model is the global stability of the steady state. This is established via Liapunov's method.

For the model with a discrete delay we examine under what conditions the infected equilibrium retains stability. By using the results in [39], we find that the fraction of infected cells surviving the incubation period also determines regions of absolute and conditional stability in the delay model. For a small range of its possible values, the infected equilibrium is asymptotically stable for all delay values, but once the fraction of cells surviving the incubation period increases beyond a *new* critical value, the equilibrium is only conditionally stable, in the sense that stability depends on the size of the delay. Moreover, the larger the fraction of cells surviving the eclipse phase, the smaller the delay may be for the equilibrium to retain stability.

We also study the model with distributed delay. By choosing a specific distribution (the weak kernel) and using the so-called linear chain trick, we carry on linear stability analysis of the model. It is shown that the infected steady state is asymptotically stable if the average delay is small and unstable if it is large enough. As in the discrete delay case, a Hopf bifurcation occurs as the average delay passes through a critical value and a periodic solution bifurcates from the infected equilibrium. This causes fluctuations in the concentrations of both cell populations. Numerical simulations are presented in all three cases to illustrate the stability and bifurcation results.

The central thesis of this paper is how dramatically our results differ from those presented in [43]. Their paper analyzed an ODE model and determined that its

features were qualitatively similar to those of an ODE model representing cell-free viral spread. However, the delayed cell-to-cell model exhibits markedly different behavior from its ODE representation. While the infected equilibrium is globally asymptotically stable in the ODE model, it is only conditionally stable in the delay models. Moreover, given realistic parameter values this equilibrium is in fact unstable and surrounded by a family of periodic orbits born via the Hopf (delay-induced) bifurcation. This shows that the delayed models of cell-to-cell spread produce sustained infective oscillations in typical tissue culture parameter regimes. It is important to note that in similar delayed models of cell-free viral spread (see [5] and [29]), the infected equilibrium often remains stable under realistic parameter regimes.

The paper is organized as follows: the general model is described in section 2. The ODE model is considered in section 3. Section 4 deals with the model with a discrete delay. The model with the distributed delay model is analyzed in section 5. The paper ends with a discussion in section 6.

2. The general model

Let $C(t)$ represent the concentration of healthy cells and $I(t)$ be the concentration of infected cells. We consider the following system modeling the interaction of the healthy and infected cells:

$$\begin{aligned} \frac{dC}{dt} &= r_C C(t)\left(1 - \frac{C(t)+I(t)}{C_M}\right) - k_I C(t) I(t), \\ \frac{dI}{dt} &= k_I' \int_{-\infty}^{t} C(u) I(u) F(t-u)\,du - \mu_I I(t), \end{aligned} \tag{2.1}$$

where r_C is the effective reproductive rate of healthy cells (the term is the total reproductive rate for healthy cells r minus the death rate for healthy cells μ_C), C_M is the effective carrying capacity of the system, k_I represents the infection of healthy cells by the infected cells in a *well-mixed* system, $\frac{k_I'}{k_I}$ is the fraction of cells surviving the incubation period, μ_I is the death rate of the infected cells. The interpretation of the variables and parameters and the values of the parameters are given in Table 1.

The initial values of system (2.1) are

$$C(s) = \phi(s) \geq 0, \quad I(s) = \psi(s) \geq 0, \quad s \in (-\infty, 0],$$

where ϕ and ψ are continuous functions on $(-\infty, 0]$.

We assume that the cells, which are productively infectious at time t, were infected u time units ago, where u is distributed according to a probability distribution $F(u)$, called the *delay kernel*. Throughout this paper, we use the family of generic delay kernels of the form

$$F(u) = \frac{\alpha^{n+1} u^n}{n!} e^{-\alpha u},$$

Table 1. Variables and parameters for cell-to-cell spread

Parameters and Variables		Values	Ref.
Dependent Variables			
C	concentration of healthy cells	5×10^5/ mL	[43]
I	concentration of infected cells	500/ mL	[43]
Parameters and Constants			
C_M	effective carrying capacity of healthy cells	2×10^6/ mL	[19]
k_I	rate constant for cell-to-cell spread	2×10^{-6}/ mL/day	[43]
r	healthy cell reproductive rate	0.7/day	[6]
μ_c	death rate of healthy cells	0.02/day	[35]
μ_I	death rate of infected cells	0.3/day	[21]
Derived Quantities			
r_C	$(= r - \mu_C)$ effective healthy cell reproductive rate	0.68/day	[43]
k_I'	k_I'/k_I fraction of cells surviving the incubation period	varies	

where $\alpha > 0$ is a constant and $n \geq 0$ is an integer. According to MacDonald [23], n is called the *order* of the delay kernel and the *average delay* is defined by

$$\tau = \int_0^\infty uF(u)du = \frac{n+1}{\alpha}.$$

In the literature, the kernels with $n = 0$ and $n = 1$, i.e.,

$$F(u) = \alpha e^{-\alpha u} \quad \text{and} \quad F(u) = \alpha^2 u e^{-\alpha u},$$

are called the *weak* and *strong* kernels, respectively, and are frequently used in biological modeling. Such kernels were also used in mathematical models of HIV-1 infections by Mittler et al. [26].

The system (2.1) has three equilibria: the trivial equilibrium $E_0 = (0, 0)$, the healthy equilibrium $E_1 = (C_M, 0)$, and the infected equilibrium $\overline{E} = (\overline{C}, \overline{I})$, where

$$\overline{C} = \frac{\mu_I}{k_I'}, \quad \overline{I} = \frac{r_C(k_I' C_m - \mu_I)}{k_I'(k_I C_m + r_C)}$$

if $k_I' > \mu_I / C_M$.

Notice that system (2.1) has some special cases. When

$$F(u) = \delta(u),$$

the delta function, we have the following ordinary differential equations (ODE):

$$\begin{aligned} \frac{dC}{dt} &= r_C C(t)\left(1 - \frac{C(t) + I(t)}{C_M}\right) - k_I I(t) C(t), \\ \frac{dI}{dt} &= k_I' I(t) C(t) - \mu_I I(t). \end{aligned} \tag{2.2}$$

The initial conditions are

$$C(0) = C_0 \geq 0, \quad I(0) = I_0 \geq 0,$$

where C_0 and I_0 are constants.

When the kernel takes the following form

$$F(u) = \delta(u - \tau),$$

where $\tau \geq 0$ is a constant, then system (2.1) becomes the following delay differential equations (DDE) with a discrete delay:

$$\begin{aligned} \frac{dC}{dt} &= r_C C(t)\left(1 - \frac{C(t) + I(t)}{C_M}\right) - k_I I(t)C(t), \\ \frac{dI}{dt} &= k_I' I(t-\tau)C(t-\tau) - \mu_I I(t). \end{aligned} \tag{2.3}$$

The initial conditions are

$$C(s) = \phi(s) \geq 0, \quad I(s) = \psi(s) \geq 0, \quad s \in [-\tau, 0],$$

where ϕ and ψ are continuous functions on $[-\tau, 0]$. Note that the ODE model (2.2) is also a special case of the DDE model (2.3) with $\tau = 0$.

In the following sections, we will consider the ODE model (2.2), the model (2.3) with a discrete delay, and the following distributed model with a weak kernel

$$\begin{aligned} \frac{dC}{dt} &= r_C C(t)\left(1 - \frac{C(t) + I(t)}{C_M}\right) - k_I C(t)I(t), \\ \frac{dI}{dt} &= k_I' \int_{-\infty}^{t} \alpha e^{-\alpha(t-u)} C(u)I(u)du - \mu_I I(t), \end{aligned} \tag{2.4}$$

for which the initial values are

$$C(s) = \phi(s) \geq 0, \quad I(s) = \psi(s) \geq 0, \quad s \in [-\infty, 0],$$

where ϕ and ψ are continuous functions on $[-\infty, 0]$.

3. The ODE model

In this section, we discuss the ODE model (2.2). Notice that the system has the same three equilibria as the general system (2.1) has: the trivial equilibrium $E_0 = (0, 0)$, the healthy equilibrium $E_1 = (C_M, 0)$, and the infected equilibrium $\overline{E} = (\overline{C}, \overline{I})$. Stability analysis of these three equilibria reveals two possible scenarios:

(i) When $C_M < \frac{\mu_I}{k_I}$ (which, under parameter ranges given, usually is not the case), the healthy cells predominate and infected cells die exponentially. In this case E_0 is unstable, E_1 is asymptotically stable, and $\overline{E}$ is unstable. We note that the condition for E_1 to be stable is that $k_I < 1.5 \times 10^{-7}$, or that *less than* 7.5% of infected cells survive the incubation period to become infectious. In this case E_1 is asymptotically stable. We note, however, that in reality it is unlikely that so few cells would survive latency, and that the following case is more likely.

(ii) When $\frac{\mu_I}{k_I'} < C_M < \frac{r_C}{C_M}$, healthy cells and infected cells co-exist. This would correspond to the case where, in models representing cell-free viral spread, we have an endemically infected steady state. This means that infection is present but it does not grow out of bound, and levels of healthy cells do not crash to zero. In

this case E_0 remains unstable, E_1 is now also unstable and $\overline{E}$ has become asymptotically stable. A transcritical bifurcation occurs at $C_M > \mu_I/k'_I$, corresponding to $k'_I = 1.5 \times 10^{-7}$. With parameter values given in Table 1, numerical simulations show that the positive equilibrium $\overline{E}$ is asymptotically stable (see Figure 3.1). In the $(C, I)-$plane, trajectories spiral towards the equilibrium (see Figure 3.2).

The equilibrium $\overline{E}$ is, in fact, globally stable for $\frac{\mu_I}{k'_I} < C_M < \frac{r_C}{C_M}$. We can see this by applying Liapunov's theorem. We choose the following Liapunov function:

$$V(C, I) = c_1\left(-\overline{C}\log\frac{C}{\overline{C}} + C - \overline{C}\right) + c_2\left(-\overline{I}\log\frac{I}{\overline{I}} + I - \overline{I}\right) \qquad (3.1)$$

This function is clearly positive if we choose c_1, c_2 to be positive constants, and it equals zero for $E = \overline{E}$. We have

$$\begin{aligned}\frac{dV}{dt} &= c_1\frac{dC/dt}{C}(C-\overline{C}) + c_2\frac{dI/dt}{I}(I-\overline{I})\\ &= -c_1\frac{r_C}{C_M}(C-\overline{C})^2 + \left[c_2k'_I - c_1\frac{(r_C - k_IC_M)}{C_M}\right](C-\overline{C})(I-\overline{I}).\end{aligned}$$

Assume that $C_M < \frac{r_C}{k_I}$ and choose $c_1 = k'_I,\ c_2 = \frac{(r_C - k_IC_M)}{C_M} > 0$. We have

$$\frac{dV}{dt} = -\frac{k'_Ir_C}{C_M}(C-\overline{C})^2 < 0,$$

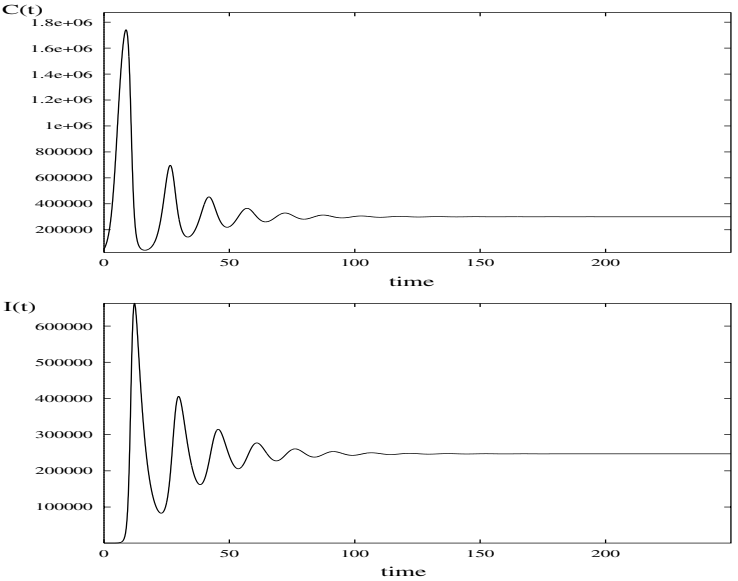

Fig. 3.1. $C(t)$ and $I(t)$ converge to the steady state values.

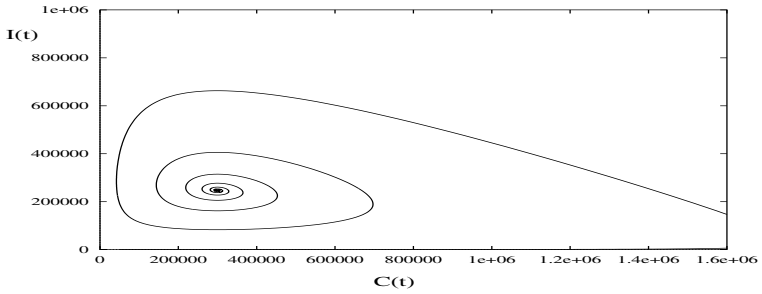

Fig. 3.2. The infected equilibrium is asymptotically stable.

which implies that the equilibrium $\overline{E}$ is globally asymptotically stable for $\frac{\mu_I}{k_I'} < C_M < \frac{r_C}{k_I}$. We thus have proved

Proposition 3.1 *If*

$$\frac{\mu_I}{k_I'} < C_M < \frac{r_C}{k_I}, \tag{3.2}$$

then the infected equilibrium $\overline{E}$ of the ODE model (2.2) is globally asymptotically stable.

4. The discrete delay model

In this section we consider the delay differential equation model with a discrete delay, namely, system (2.3). Notice that the model has the same equilibria given in section 2, $E_0 = (0, 0)$, $E_1 = (C_M, 0)$, and $\overline{E} = (\overline{C}, \overline{I})$.

We are interested in the stability of the infected equilibrium $\overline{E}$. The characteristic equation of the linearized system is given by:

$$\Delta(\lambda) = \lambda^2 + p\lambda + r + (s\lambda + q)e^{-\lambda\tau} = 0, \tag{4.1}$$

where

$$\begin{aligned} p &= \frac{\mu_I(k_I'C_M + r_C)}{k_I'C_M} \\ q &= r_C\mu_I\frac{(k_I'C_M - 2\mu_I)}{k_I'C_M} \\ r &= \frac{r_C\mu_I^2}{k_I'C_M} \\ s &= -\mu_I. \end{aligned}$$

Characteristic equations of this form have been extensively examined in [39]. Certain conditions on the coefficients p, q, r and s will ensure either all roots of the characteristic equation have negative real part or at least one root has positive real part. The results of interest to us are as follows:

Lemma 4.1 *Consider a characteristic equation of the form (4.1).*

(i) If $p+s>0$ and $q+r>0$, then all roots of the characteristic equation have negative real part in the absence of delay.

(ii) If $p+s>0$, $q+r>0$, and either ($s^2-p^2+2r<0$ and $r^2-q^2>0$) or $(s^2-p^2+2r)^2<4(r^2-q^2)$, then all roots of the characteristic equation have negative real part for all delay values, that is, the equilibrium is absolutely stable.

(iii) If $p+s>0$, $q+r>0$, and either $r^2-q^2<0$ or ($s^2-p^2+2r>0$ and $(s^2-p^2+2r)^2=4(r^2-q^2)$), then there is a critical value τ_0 defined by:

$$\tau_0=\frac{1}{\omega_+}\arccos\frac{q(\omega_+^2-r)-ps\omega_+^2}{s^2\omega_+^2+q^2}, \tag{4.2}$$

where ω_+ satisfies

$$2\omega_+^2=(s^2-p^2+2r)+\sqrt{(s^2-p^2+2r)^2-4(r^2-q^2)}, \tag{4.3}$$

when $\tau\in[0,\tau_0)$, all roots of the characteristic equation have negative real part; when $\tau=\tau_0$, there is a pair of purely imaginary roots $\pm i\omega_+$; and when $\tau>\tau_0$, the characteristic equation has at least one root with positive real part.

We will use the above results to analyze the stability of the infected equilibrium. Checking the first two conditions, we note that $p+s>0$ holds if

$$\mu_I\left(\frac{k_I'C_M+r_C}{k_I'C_M}-1\right)>0$$

which is obviously the case, since r_C is positive. The second condition, $q+r>0$, holds whenever $k_I'>\mu_I/C_M$, which is exactly the condition for the feasibility of the interior equilibrium in the ODE model. This is not surprising, because the preceding two conditions are simply conditions for stability of the system in the absence of delay.

Consider the third condition for the characteristic equation to have only roots with negative real part. For this to be true, we require that *both* of the following conditions hold:

$$r^2-q^2>0, \tag{4.4}$$

$$s^2-p^2+2r<0. \tag{4.5}$$

The second condition holds for all values of parameters. However, the first condition is somewhat more interesting. Notice that for $r^2-q^2>0$, we require the following inequality to be satisfied:

$$C_M^2k_I'^2-4\mu_IC_Mk_I'+3\mu_I^2<0.$$

This is true when

$$\frac{\mu_I}{C_M}<k_I'<\frac{3\mu_I}{C_M}.$$

We summarize the conditions on stability as follows:

Proposition 4.2 *The positive equilibrium $\overline{E}$ of system (4.1) is asymptotically stable for all delay τ when*

$$\frac{\mu_I}{C_M} < k_I' < \frac{3\mu_I}{C_M}. \tag{4.6}$$

Thus, there is a region of absolute stability for the infected equilibrium. Notice that this region corresponds to only between 7.5% and 22.5% of infected cells surviving the latent period. The obvious question to ask is, what happens when more cells survive (which, in realistic situations, is likely)?

We note that for $k_I' > 3\mu_I/C_M$, $r^2 - q^2 < 0$, and delay-induced instability may occur because the characteristic equation has a root with positive real part. Define

$$A = \sqrt{((k_I' C_M)^2 - \mu_I)((k_I' C_M)^2 - 3\mu_I)}.$$

We summarize the conditions for bifurcation as follows:

Proposition 4.3 *Assume that*

$$k_I' > \frac{3\mu_I}{C_M}. \tag{4.7}$$

Then there is a critical value τ_0 given by

$$\tau_0 = \frac{1}{\omega_+} \arccos \frac{1}{k_I' C_M} \left[\frac{(k_I' C_M (r_C + \mu_I) - r_C \mu_I) A - 2 r_C \mu_I k_I' C_M (k_I' C_M - 2\mu_I)}{\mu_I A + 2 r_C (k_I' C_M - 2\mu_I)^2} \right],$$

where

$$\omega_+ = \frac{1}{2 k_I' C_M} \sqrt{2 r_C \mu_I (2A - r_C \mu_I)},$$

such that the infected equilibrium $\overline{E}$ of system (4.1) is asymptotically stable when $\tau \in [0, \tau_0)$ and unstable when $\tau > \tau_0$. A Hopf bifurcation occurs at $\overline{E}$ when $\tau = \tau_0$; that is, a family of periodic solutions bifurcates from $\overline{E}$ when τ passes through the critical value τ_0.

Notice that τ_0 depends on k_I'. In the following, we will see that for larger values of k_I', the critical value τ_0 gets smaller, whereas the periods and amplitudes of the oscillatory solutions get larger.

Using values of k_I' corresponding to 25%, 50%, 75% of cells surviving incubation, we obtain the following results for the critical value of the delay.

Suppose that 25% of infected cells survive incubation. This corresponds to a value of $k_I' = 5 \times 10^{-7}$. In this case, using the formulas given above, we obtain a critical value of the delay to be $\tau_0 = 6.23$ days. Since the actual incubation period is one day, we do not expect this to be of biological significance. Numerical simulations show that both C and I are stable for realistic values of all other parameters, when $k_I' = 5 \times 10^{-7}$.

Now suppose that half the infected cells survive incubation. In this case, the critical value for τ_0 obtained analytically is 0.82 days, which *is* of biological significance. Numerical simulations show that for $k_I' = 10^{-6}$ and $\tau = 0.4 < \tau_0$,

the components $C(t)$ and $I(t)$ are converging to the steady state values as time increases (see Figure 4.1). In the $(C, I)-$plane, trajectories spiral towards the equilibrium (see Figure 4.2).

When the delay is increased to $\tau = 1 > \tau_0$, the components $C(t)$ and $I(t)$ oscillate with increasing time (see Figure 4.3). In the $(C, I)-$plane, trajectories are approaching the periodic solution as the time increases (see Figure 4.4).

If 75% of the infected cells survive, numerical analysis shows that when k_I' is smaller the oscillations are more frequent (i.e., the periods are shorter) and the amplitudes are smaller. Thus, increasing the value of k_I' will increase the periods and the amplitudes of the periodic solutions. There appears to be an interplay between the value of the delay and the fraction of infected cells surviving incubation. Specifically, the more cells survive incubation, the smaller the critical value of the delay must be to induce instability of the interior equilibrium.

5. The distributed delay model

Finally we consider the distributed delay model with a weak kernel, that is, system (2.4). To study the stability of the infected equilibrium, let

$$X(t) = \int_{-\infty}^{t} \alpha e^{-\alpha(t-u)} C(u) I(u) du. \tag{5.1}$$

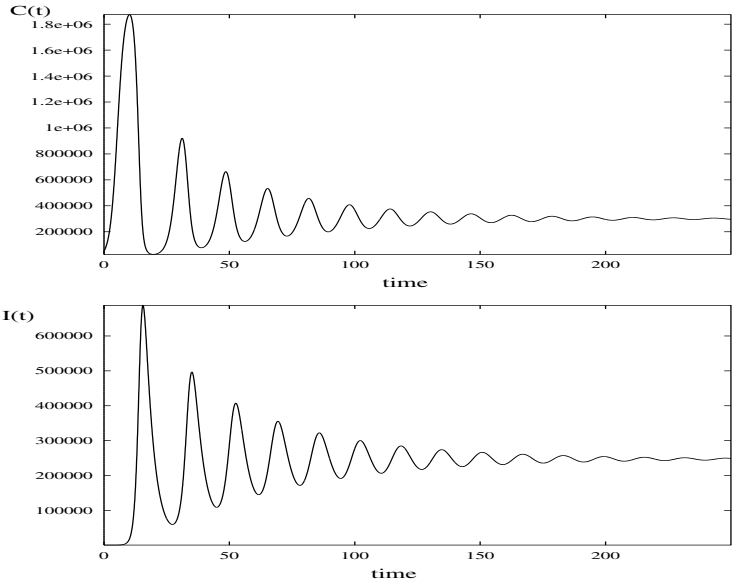

Fig. 4.1. $C(t)$ and $I(t)$ converge to the steady state values when $\tau < \tau_0$, here $\tau = 0.4$.

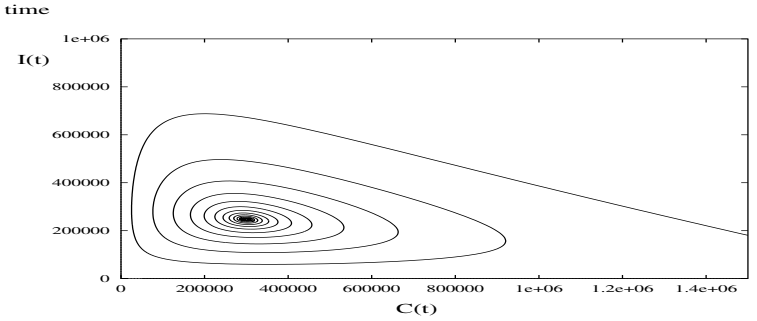

Fig. 4.2. The infected equilibrium is asymptotically stable when $\tau = 0.4 < \tau_0$.

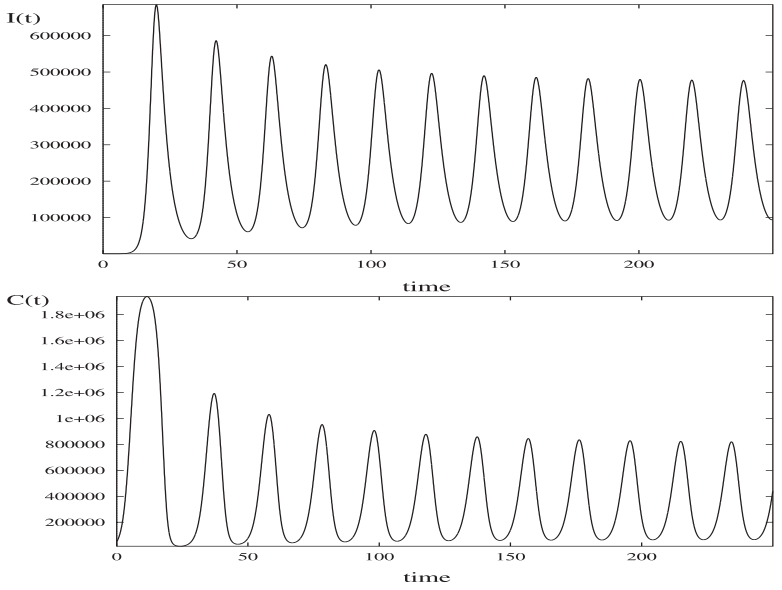

Fig. 4.3. The oscillations of C and I vs. time, $\tau = 1$

Then system (2.4) is equivalent to the following ODE system

$$\begin{aligned}
\frac{dC}{dt} &= r_C C(t)\left(1 - \frac{C(t) + I(t)}{C_M}\right) - k_I C(t) I(t), \\
\frac{dI}{dt} &= k_I' X(t) - \mu_I I(t), \\
\frac{dX}{dt} &= \alpha C(t) I(t) - \alpha X(t).
\end{aligned} \tag{5.2}$$

The positive steady state of system (5.2) is given by $\overline{E} = (\overline{C}, \overline{I}, \overline{X})$, where $\overline{X} = \frac{\mu_I}{k_I'}\overline{I}$. Linearizing the system at the steady state $\overline{E}$, we obtain the characteristic equation

$$\lambda^3 + a_1(\alpha)\lambda^2 + a_2(\alpha)\lambda + a_3(\alpha) = 0, \tag{5.3}$$

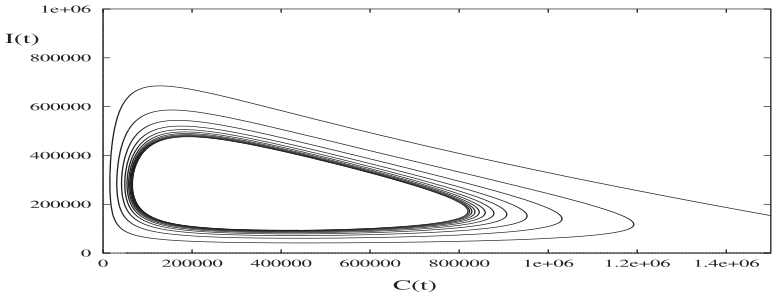

Fig. 4.4. There is an orbitally asymptotically stable periodic solution when $\tau = 1 > \tau_0$.

where

$$a_1(\alpha) = \frac{r_C}{C_M}\overline{C} + \mu_I + \alpha,$$
$$a_2(\alpha) = \alpha(\frac{r_C}{C_M}\overline{C}) + \frac{\mu_I r_C}{C_M}\overline{C},$$
$$a_3(\alpha) = \alpha(k_I' + \frac{r_C}{C_M})\mu_I\overline{I}.$$

By Routh-Hurwitz criteria, the positive steady state $\overline{E}$ is asymptotically stable if and only if

$$a_1(\alpha) > 0, \quad a_3(\alpha) > 0 \quad \text{and} \quad a_1(\alpha)a_2(\alpha) - a_3(\alpha) > 0 \tag{5.4}$$

for all values of α. If there is an $\alpha_0 > 0$ such that

$$a_1(\alpha_0)a_2(\alpha_0) = a_3(\alpha_0), \tag{5.5}$$

then the characteristic equation (5.3) becomes

$$[\lambda + a_1(\alpha_0)][\lambda^2 + a_2(\alpha_0)] = 0,$$

which has roots

$$\lambda_1 = -a_1(\alpha_0) < 0, \quad \lambda_{2,3} = \pm i\sqrt{a_2(\alpha_0)}.$$

If the transversality condition

$$\left.\frac{d\mathrm{Re}\lambda_{2,3}}{d\alpha}\right|_{\alpha=\alpha_0} \neq 0 \tag{5.6}$$

holds, then a Hopf bifurcation occurs at $\overline{E}$ when α passes through the critical value α_0. After some calculations, we have

$$\left.\frac{d\mathrm{Re}\lambda_{2,3}}{d\alpha}\right|_{\alpha=\alpha_0} = -\frac{1}{4[a_1^2(\alpha) + a_2(\alpha)]}\,\frac{d}{d\alpha}[a_1(\alpha)a_2(\alpha) - a_3(\alpha)]|_{\alpha=\alpha_0}.$$

Summarizing the above analysis, we have the following results.

Proposition 5.1 *If conditions in (5.4) are satisfied, then the positive steady state* $\overline{E}$ *of system (2.4) is asymptotically stable. If there is a critical value* $\alpha_0 > 0$ *such that conditions (5.5) and*

$$\frac{d}{d\alpha}[a_1(\alpha)a_2(\alpha) - a_3(\alpha)]|_{\alpha=\alpha_0} \neq 0$$

are satisfied, then a Hopf bifurcation occurs at $\overline{E}$*; that is, a family of periodic solutions bifurcates from* $\overline{E}$ *when* α *passes through the critical value* α_0.

Notice that for the weak kernel $\alpha e^{-\alpha u}$, the average delay is defined as $\overline{\tau} = \frac{1}{\alpha}$. The above analysis demonstrates that when $\overline{\tau}$ is small (i.e. when α is large), the steady state is stable. When $\overline{\tau}$ is sufficiently large (i.e. as α becomes smaller), the steady state becomes unstable and a Hopf bifurcation occurs. That is, a periodic solution bifurcates from the steady state when α passes a critical value α_0.

With parameter values given in Table 1 and a value of $k_I' = 1.5 \times 10^{-6}$, $\alpha_0 \approx 1.95$. Numerical simulations show that the steady state $\overline{E} = (\overline{C}, \overline{I})$ is asymptotically stable when $\alpha > \alpha_0$ (i.e., $\bar{\tau} < \bar{\tau}_0$)(see Figure 5.1). In the $(C, I)-$plane, trajectories spiral towards the equilibrium (see Figure 5.2).

When $\alpha = \alpha_0$ (i.e., $\bar{\tau} = \bar{\tau}_0$), the steady state $\bar{E}$ loses its stability and Hopf bifurcation occurs. When $\alpha < \alpha_0$ (i.e., $\bar{\tau} > \bar{\tau}_0$), the steady state $\bar{E}$ becomes unstable and there is a periodic solution surrounding $\bar{E}$ (see Figures 5.3 and 5.4).

Similarly, we can analyze system (2.1) with a strong kernel $F(u) = \alpha^2 u e^{-\alpha u}$ and obtain similar results on stability and bifurcation of the model.

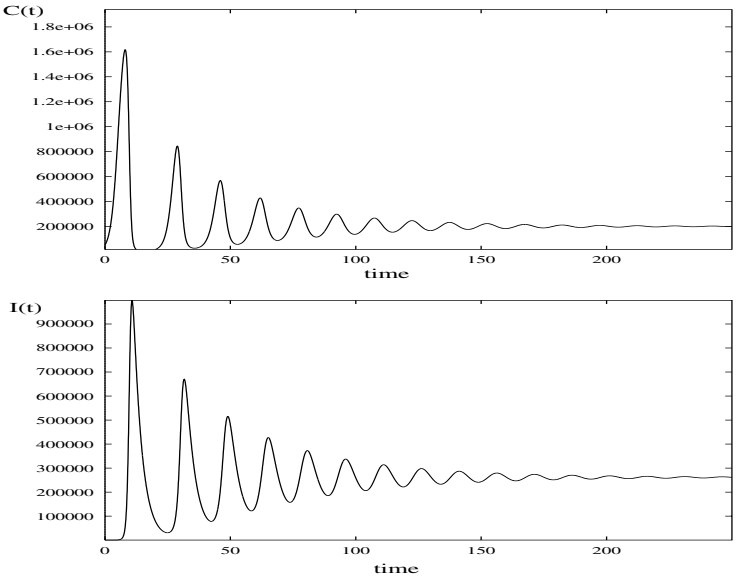

Fig. 5.1. $C(t)$ and $I(t)$ converge to the steady state values when $\alpha > \alpha_0$, here $\alpha = 5$.

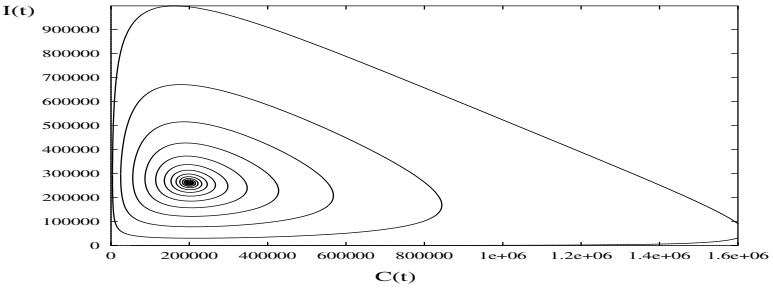

Fig. 5.2. The infected equilibrium is asymptotically stable when $\alpha = 5 > \alpha_0$.

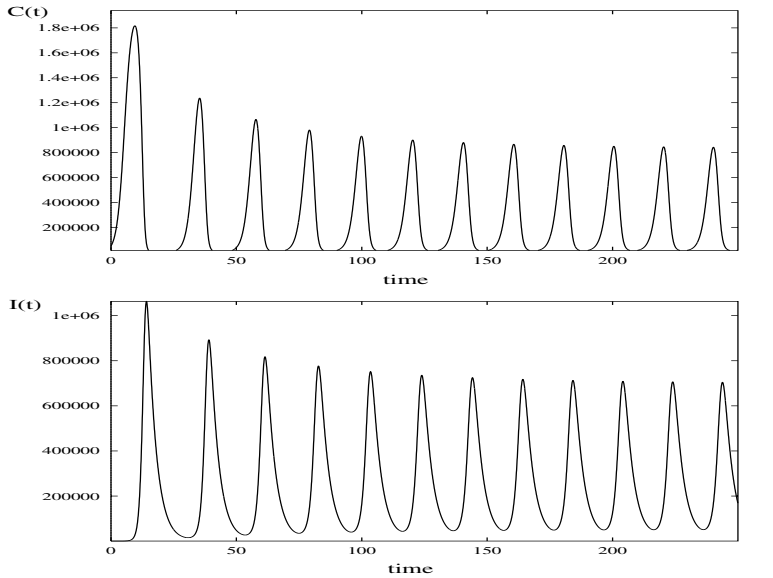

Fig. 5.3. $C(t)$ and $I(t)$ oscillate about the steady state values when $\alpha < \alpha_0$, here $\alpha = 1.5$.

6. Discussion

It is known that cell-to-cell spread may be more effective than cell-to-free virus spread in transmitting HIV-1 ([6,11,37,40]). An infectious HIV-1 virion typically has about one attachment opportunity during a 1-hour incubation in 5×10^5 cells/mL ([42]). For a viral strain taking on the order of 10 hours for spontaneous inactivation, an infectious virion in suspension attaches to a cell before inactivating as long as the cell concentration remains above 5×10^4 cells/mL ([20]). Thus, an increase in cell concentration will increase the frequency of cell-to-cell contacts and the infection rate. Since in lymph nodes the cell concentration is about 10^8 cells/mL, the cell-to-cell spread may be efficient in lymph nodes and such culture models may be helpful in testing antiviral efficacy ([28]).

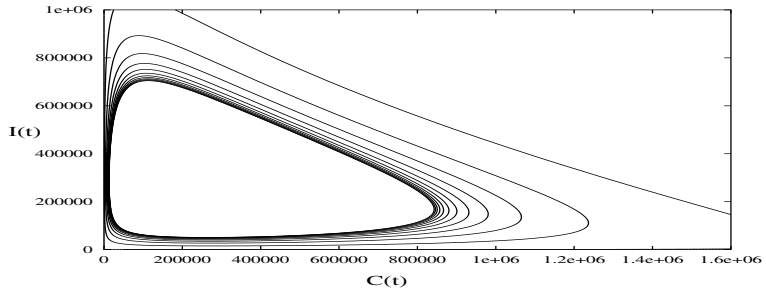

Fig. 5.4. There is a periodic solution when $\alpha = 1.5 < \alpha_0$.

We have modified the ODE model for cell-to-cell infection of HIV-1 in tissue cultures proposed by Spouge et al. [43] by incorporating a distributed time delay to model the cellular incubation period (the amount of time between when a cell is infected and when it actually begins to infect other cells). When the mean delay $\tau = 0$, we have the original ODE model whose interior (infected) equilibrium is globally asymptotically stable provided $k_I' > \mu_I / C_M$; that is, so long as at least 7.5% of newly infected cells survive the incubation period.

We then considered the delayed system with both discrete and distributed delays. In the case when the delay kernel is the delta function centered at τ, we have a DDE system with a discrete delay. By using stability analysis, we first determined that the infected equilibrium would only be feasible if, under parameter ranges given, more than 7.5% of newly infected cells survive the eclipse phase. In other words, if fewer cells survive, the only two equilibria would be trivial (no cells at all), or healthy (no infection, healthy cells at their carrying capacity). In this case, the trivial equilibrium is unstable and the healthy equilibrium is asymptotically stable.

However, if more than 7.5% of newly infected cells survive, the dynamics become much more interesting. We discovered that there is a small range for the number of infected cells surviving the eclipse phase within which the infected equilibrium (which now exists) is stable regardless of the value of the delay. Specifically, if between 7.5% and 22.5% of newly infected cells do go on to become infectious, the system tends to the infected steady state no matter the value of the delay. However, we notice that absolute stability ceases to hold for a critical value of k_I'. This value, with parameter ranges given, corresponds to 22.5% of cells surviving the eclipse phase. If a larger proportion survive, the equilibrium becomes conditionally stable and the delay is now a bifurcation parameter. Moreover, we notice that the more cells survive, the smaller the delay is allowed to be for stability to be retained. Once half or more of these cells survive and become infectious, this critical value for τ_0 becomes one day or smaller, which is significant biologically since the intracellular "latent period" in vitro has been estimated at about one day [43]. Numerical solutions confirm that, for various proportions of cells becoming infectious, when the delay passes through its critical values, bifurcations occur whereby the equilibrium loses stability and sustained oscillations occur in

both components. We also noted that the more infected cells survive, the larger the periods and amplitudes of the oscillations.

These results hold also for the case where the delay is distributed. That is, if the average delay passes through its critical value, a family of periodic solutions arises via a Hopf bifurcation. So we conclude that, depending on the amount of infected cells surviving the cellular eclipse phase, the infected equilibrium is only stable if the average delay is quite small.

Numerical simulations confirmed that in realistic parameter regimes if a typical tissue culture infection starts with a small fraction on cells infected, then the cell-to-cell transmission (instantaneous (Fig. 3.1) or slightly delayed (Figs. 4.1 and 5.1)) gives an initial phase of exponential viral growth, followed by transient damped oscillations, and eventually steady states. If the cellular eclipse phase is sufficiently long (i.e. the average delay is large), then the cell-to-cell model exhibits oscillatory modes; i.e. it produces infective oscillations. These results are significant in terms of how much they differ from the results in the original paper by Spouge et al. If cell-to-cell spread in tissue cultures is being used to, in some sense, "approximate" the effects of cell-free viral spread, we must be sure that the qualities of the models are similar in both cases. The existence of bifurcation and instability in this delay model where none exists in the non-delay model indicates that we must carefully examine both models so as to determine the most realistic mathematical representation of HIV-1 spread.

HIV-1 may sustain itself in the body at different stages either by continuously replicating in the $CD4^+$ T cell population or by periodically maintaining the capacity of tissues to activate and infect $CD4^+$ T cells. Our results on the delayed models indicate that latently infected cells may be instrumental in sustaining the infection, as reported by Grossman et al. [10], in the form of infective oscillations (Spouge et al. [43]).

Our cell-to-cell models may be applicable to study the within-host dynamics of other types of viral infections such as human T-cell leukaemia virus type 1 (HTLV-1), hepatitis B, hepatitis C, etc. For example, human T-cell leukaemia virus type 1 (HTLV-1) infection is linked to the development of adult T-cell leukaemia and HTLV-1 associated myelopathy/tropical spastic paraparesis. Previous work in modeling dynamics of immune responses to persistent viruses has been presented by Nowak and Bangham [31]. Tam [45] incorporated a discrete delay into one of Nowak and Bangham's models. Stilianakis and Seydel [44] took a more specific approach to model the HTLV-1 infection with the infection process taking place through cell-to-cell contact between actively infected cells and uninfected cells. Wodarz, Nowak and Bangham [47] proposed a mathematical model for the *in vivo* dynamics of HTLV-1 infection and showed that a high rate of viral replication is consistent with the relative sequence invariance of HTLV-1 and might be necessary to maintain a persistent infection. We leave the modeling and study of the cell-to-cell HTLV-1 infection for future consideration.

We should note that, although these results are interesting, this model is still a very simple one. Some realistic modifications can be made. For example, we have assumed that the system is well-mixed, which may not be the case in tissue cultures. One modification might be to replace the loss/gain term IC by another function,

such as a Michaelis-Menten response function, to more accurately reflect the fact that tissue cultures are not well-mixed. Another possible modification would be to incorporate diffusion effects into the delayed model.

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References

[1] Bailey, J.J., Fletcher, J.E., Chuck, E.T., Shrager, R.I.: A kinetic model of CD4+ lymphocytes with the human immunodeficiency virus (HIV). BioSystems **26**, 177–183 (1992)
[2] Bajaria, S., Webb, G., Cloyd, M., Kirschner, D.: Dynamics of näive and memory $CD4^+$ lymphocytes in HIV-1 disease progression. JAIDS **30**, 41–58 (2002)
[3] Callaway, D.S., Perelson, A.S.: HIV-1 infection and low steady state viral loads. Bull. Math. Biol. **64**, 29–64 (2002)
[4] Chun, T.W., Carruth, L., Finzi, D. et al.: Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature **387**, 183–188 (1997)
[5] Culshaw, R.V., Ruan, S.: A delay-differential equation model of HIV infection of CD4+ T cells. Math. Biosci. **165**, 27–39 (2000)
[6] Dimitrov, D.S., Willey, R.L., Sato, H., Chang, L.-J., Blumenthal, R., Martin, M.A.: Quantitation of human immunodeficiency virus type 1 infection kinetics. J. Virol. **67**, 2182–2190 (1993)
[7] Finzi, D., Siliciano, R.F.: Viral dynamics in HIV-1 infection. Cell **93**, 665–671 (1998)
[8] Grossman, Z., Feinberg, M., Kuznetsov, V., Dimitrov, D., Paul, W.: HIV infection: how effective is drug combination treatment? Immunol. Today **19**, 528–532 (1998)
[9] Grossman, Z., Polis, M., Feinberg, M.B. et al.: Ongoing HIV dissemination during HAART. Nat. Med. **5**, 1099–1104 (1999)
[10] Grossman, Z., Feinberg, M., Paul, W.E.: Muiple modes of cellular activation and virus transmission in HIV infection: A role for cronically and latently infected cells in sustaining viral replication. Proc. Natl. Acad. Sci. USA **95**, 6314–6319 (1998)
[11] Gummuluru, S., Kinsey, C.M., Emerman, M.: An in vitro rapid-turnover assay for human immunodeficiency virus type 1 replication selects for cell-to-cell spread of virua. J. Virol. **74**, 10882–10891 (2000)
[12] Haase, A.T.: Population biology of HIV-1 infection: viral and $CD4^+$ T cell demographics and dynamics in lymphatic tissues. Annu. Rev. Immunol. **17**, 625–656 (1999)
[13] Haase, A.T., Henry, K., Zupancic, M. et al.: Quantitative image analysis of HIV-1 infection in lymphoid tissue. Science **274**, 985–989 (1996)
[14] Herz, A.V.M., Bonhoeffer, S., Anderson, R.M., May, R.M., Nowak, M.A.: Viral dynamics in vivo: Limitations on estimates of intracellular delay and virus decay. Proc. Natl. Acad. Sci. USA **93**, 7247–7251 (1996)
[15] Kirschner, D.E., Lenhart, S., Serbin, S.: Optimal control of the chemotherapy of HIV. J. Math. Biol. **35**, 775–792 (1997)
[16] Kirschner, D.E., Webb, G.: A model for treatment strategy in the chemotherapy of AIDS. Bull. Math. Biol. **58**, 167–190 (1996)
[17] Kirschner, D.E., Webb, G.F.: Understanding drug resistance for monotherapy treatment of HIV infection. Bull. Math. Biol. **59**, 763–785 (1997)

[18] Kirschner, D.E., Webb, G.F.: A mathematical model of combined drug therapy of HIV infection. J. Theor. Med. **1**, 25–34 (1997)
[19] Layne, S.P., Merges, M.J., Dembo, M., Spouge, J.L., Nara, P.L.: HIV requires multiple gp120 molecules for CD4-mediated infection. Nature **346**, 277–279 (1990)
[20] Layne, S.P., Merges, M.J., Spouge, J.L., Dembo, M., Nara, P.L.: Blocking of human immunodeficiency virus infection depends on cell density and viral stock age. J. Virol. **65**, 3293–3300 (1991)
[21] Leonard, R., Zagury, D., Desports, I., Bernard, J., Zagury, J.-F., Gallo, R.C.: Cytopathic effect of human immunodeficiency virus in T4 cells is linked to the last stage of virus infection. Proc. Natl. Acad. Sci. USA **85**, 3570–3574 (1988)
[22] Lloyd, A.L.: The dependence of viral parameter estimates on the assumed viral life cycle: limitations of studies of viral load data. Proc. R. Soc. Lond. B **268**, 847–854 (2001)
[23] MacDonald, N.: Time Delays in Biological Models. Spring-Verlag, Heidelberg, 1978
[24] McLean, A.R., Kirkwood, T.B.L.: A model of human immunodeficiency virus infection in T helper cell clones. J. Theor. Biol. **147**, 177–203 (1990)
[25] McLean, A.R., Nowak, M.A.: Models of interaction between HIV and other pathogens. J. Theor. Biol. **155**, 69–102 (1992)
[26] Mittler, J.E., Sulzer, B., Neumann, A.U., Perelson, A.S.: Influence of delayed viral production on viral dynamics in HIV-1 infected patients. Math. Biosci. **152**, 143–163 (1998)
[27] Müller, V., Marée, A.F.M., De Boer, R.J.: Small variations in multiple parameters account for wide variations in HIV-1 set-points: a novel modelling approach. Proc. R. Soc. Lond. B **268**, 235–242 (2001)
[28] Nelson, P.W., Mittler, J.E., Perelson, A.S.: Effect of drug efficacy and eclipse phase of the viral life cycle on estimates of HIV viral dynamic parameters. JAIDS **26**, 405–412 (2001)
[29] Nelson, P.W., Murray, J.D., Perelson, A.S.: A model of HIV-1 pathogenesis that includes an intracellular delay. Math. Biosci. **163**, 201–215 (2000)
[30] Nelson, P.W., Perelson, A.S.: Mathematical analysis of delay differential equation models of HIV-1 infection. Math. Biosci. **179**, 73–94 (2002)
[31] Nowak, M.A., Bangham, R.M.: Population dynamics of immune responses to persistent viruses. Science **272**, 74–79 (1996)
[32] Nowak, M.A., May, R.M.: Mathematical biology of HIV infections: Antigenic variation and diversity threshold. Math. Biosci. **106**, 1–21 (1991)
[33] Nowak, M.A., May, R.M.: AIDS Pathogenesis: mathematical models of HIV and SIV infections. AIDS **7**, S3–S18 (1993)
[34] Perelson, A.S.: Modeling the interaction of the immune system with HIV. In: Mathematical and Statistical Approaches to AIDS Epidemiology. C. Castillo-Chavez (ed). Springer-Verlag, New York, 1989, pp. 350–370
[35] Perelson, A.S., Kirschner, D.E., De Boer, R.: Dynamics of HIV infection of CD4+ T cells: Math. Biosci. **114**, 81–125 (1993)
[36] Perelson, A.S., Nelson, P.W.: Mathematical analysis of HIV-1 dynamics in vivo. SIAM Rev. **41**, 3–44 (1999)
[37] Philips, D.M.: The role of cell-to-cell transmission in HIV infection. AIDS **8**, 719–731 (1994)
[38] Rosenberg, Y., Janossy, G.: The importance of lymphocyte trafficking in regulating blood lymphocyte levels during HIV and SIV infections. Seminars in Immunol. **11**, 139–154 (1999)
[39] Ruan, S.: Absolute stability conditional stability and bifurcation in Kolmogorov-type predator-prey systems with discrete delays. Quart. Appl. Math. **59**, 159–173 (2001)

[40] Sato, H., Orenstein, J., Dimitrov, D.S., Martin, M.A.: Cell-to-cell spread of HIV-1 occurs with minutes and may not involve the participation of virus particles. Virology **186**, 712–724 (1992)
[41] Schacker, T., Little, S., Connick, E. et al.: Rapid accumulation of HIV in lymphatic tissue reservoirs during acute and early HIV infection: implications for timing of antiretroviral therapy. J. Infect. Dis. **181**, 354–357 (2000)
[42] Spouge, J.L.: Viral multiplicity of attachment and its implications for HIV therapies. J. Virol. **68**, 1782–1789 (1994)
[43] Spouge, J.L., Shrager, R.I., Dimitrov, D.S.: HIV-1 infection kinetics in tissue cultures. Math. Biosci. **138**, 1–22 (1996)
[44] Stilianakis, N.I., Seydel, J.: Modeling the T-cell dynamics and pathogenesis of HTLV-I infection. Bull. Math. Biol. **61**, 935–947 (1999)
[45] Tam, J.: Delay effect in a model for virus replication. IMA J. Math. Appl. Med. Biol. **16**, 29–37 (1999)
[46] Wodarz, D., Lloyd, A.L., Jansen, V.A.A., Nowak, M.A.: Dynamics of macrophage and T cell infection by HIV. J. Theor. Biol. **196**, 101–113 (1999)
[47] Wodarz, D., Nowak, M.A., Bangham, C.R.M.: The dynamics of HTLV-1 and the CTL response. Immunol. Today **20**, 220–227 (1999)