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Predicting the potential impact of a cytotoxic T-lymphocyte HIV vaccine: How often should you vaccinate and how strong should the vaccine be?

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Abstract

To stimulate the immune system's natural defenses, a post-infection HIV vaccination program to regularly boost cytotoxic T-lymphocytes has been proposed. We develop a mathematical model to describe such a vaccination program, where the strength of the vaccine and the vaccination intervals are constant. We apply the theory of impulsive differential equations to show that the model has an orbitally asymptotically stable periodic orbit, with the property of asymptotic phase. We show that, on this orbit, the vaccination frequency can be chosen so that the average number of infected $CD4^+$ T cells can be made arbitrarily low. We illustrate the results with numerical simulations and show that the model is robust with respect to both the parameter choices and the formulation of the model as a system of impulsive differential equations.
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1. Introduction

A vaccine that stimulates the cytotoxic T-lymphocyte (CTL) response represents the best hope for control of HIV [6,4,12]. CTLs are host cells with the ability to identify and destroy virally infected cells in the body [14]. CTLs are activated via specific recognition of viral fragments (called epitopes), presented by cell-surface molecules [18]. With proper stimulation and activation, they can eliminate infected cells and control viral infection [21]. Here, we model the situation where CTLs can effectively control the viral infection (by way of reducing the number of infected cells) when the post-infection vaccine (presenting the correct viral epitopes) is administered at regular intervals. Results from ongoing clinical trials of such vaccines are expected by 2011.

We propose a simple impulsive differential equation model of infected $CD4^+$ T-helper cells and CTLs, with the mechanism of vaccination described by a fixed pulsing at regular intervals that activates CTLs. The use of impulsive differential equations has recently been proposed to model dynamic drug concentrations during antiretroviral therapy [15,16] and is here applied to the question of regular CTL vaccinations. This framework allows us to capitalise on the theory of impulsive differential equations [1–3,13], facilitating our investigation of pulsed vaccination.

The structure of this paper is as follows: In Section 2, we develop the model and briefly discuss impulsive differential equations. In Section 3, we analyse the corresponding non-impulsive system to determine global stability and phase-plane portraits. In Section 4, we describe the impulsive periodic orbit, prove that it is orbitally asymptotically stable and show that the number of infected T cells is driven

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towards zero, if the vaccine is sufficiently strong, or is given sufficiently often. In Section 5, we illustrate our results with numerical simulations and examine the sensitivity of the results with respect to both the model and parameters. We conclude with a discussion of the implications.

2. The model

Let T be the density of infected $CD4^+$ T cells and C be the density of CTLs in the body. We make the simplifying approximation that these infected $CD4^+$ T cells are produced at a constant rate, π. This approximation is reasonable when the amount of free virus is constant, such as in the clinically asymptomatic period of infection; it becomes less reasonable in the earliest or latest stages of infection. Accordingly, we use equilibrium approximation of free virus to estimate parameters. Infected $CD4^+$ T cells die at death rate d [11] and are cleared by CTLs at rate p, proportional to the density of each [20]. CTLs proliferate at rate α, proportional to the density of CTLs and infected $CD4^+$ T cells, and die at death rate δ [19].

We assume that the CTLs are pulsed by the vaccine at fixed times t_k. For the purposes of our model, we assume that the effect of the vaccine is instantaneous, resulting in a system of impulsive differential equations, whereby solutions are continuous for $t \neq t_k$ (satisfying the associated system of ordinary differential equations) and undergo an instantaneous change in state when $t = t_k$. This technique assumes that the change in CTL concentration immediately after a vaccine is administered is nearly instantaneous; that is, the time-to-peak is negligible on the relevant time scale. By neglecting the dispersion and delay as the vaccine enters the body, we overestimate the temporal effects of vaccinating at intervals. The implications of this assumption will be taken up further in Section 5.

Thus, the model is

$$\begin{aligned} \frac{dT}{dt} &= \pi - dT - pCT \qquad t \neq t_k \\ \frac{dC}{dt} &= \alpha CT - \delta C \qquad t \neq t_k \\ \Delta C &= \widetilde{C} \qquad t = t_k, \end{aligned} \tag{2.1}$$

where t_k $(k = 1, 2, \ldots)$ are the vaccination times and where $\widetilde{C}$ is defined to be the strength of the vaccine, which is proportional to the number of CTLs the vaccine stimulates. Here, the impulsive effect is defined as

$$\Delta C \equiv C(t_k^+) - C(t_k^-) = \widetilde{C},$$

where $C(t_k^-)$ is the CTL concentration immediately before the impulsive effect and $C(t_k^+)$ is the CTL concentration immediately after the impulsive effect.

3. The system without vaccination

First, we shall analyse the model when there is no vaccination. In this case, the system has no impulses, so the dynamics are continuous in both state variables. The non-impulsive system has two equilibria,

$$(\hat{T}, \hat{C}) = \left(\frac{\pi}{d}, 0\right)$$
$$(\overline{T}, \overline{C}) = \left(\frac{\delta}{\alpha}, \frac{\alpha\pi - \delta d}{p\delta}\right),$$

which we shall refer to as the trivial and non-trivial equilibria, respectively. The nullclines of the non-impulsive model are given by

$$C = 0$$
$$T = \frac{\delta}{\alpha}$$
$$T = \frac{\pi}{d + pC}.$$

Lemma 3.1. *The trivial equilibrium is globally asymptotically stable if and only if the non-trivial equilibrium does not exist in the positive plane. The non-trivial equilibrium of the non-impulsive model is globally asymptotically stable whenever it exists in the positive plane.*

Proof. First, note that the non-trivial equilibrium is only positive if

$$\alpha\pi - \delta d > 0. \tag{3.1}$$

The Jacobian matrix for the non-impulsive model is

$$J = \begin{bmatrix} -d - pC & -pT \\ \alpha C & \alpha T - \delta \end{bmatrix}$$
$$J|_{(\hat{T},\hat{C})} = \begin{bmatrix} -d & -\frac{p\pi}{d} \\ 0 & \frac{\alpha\pi}{d} - \delta \end{bmatrix}.$$

Hence, the trivial equilibrium is unstable if and only if

$$\frac{\alpha\pi}{d} - \delta > 0.$$

This is equivalent to existence of the non-trivial equilibrium, as shown by Eq. (3.1).

Next,

$$J|_{(\overline{T},\overline{C})} = \begin{bmatrix} -d - p\overline{C} & -p\overline{T} \\ \alpha\overline{C} & 0 \end{bmatrix}.$$

This matrix has negative trace and positive determinant. It follows that both eigenvalues are negative and hence that $(\overline{T}, \overline{C})$ is asymptotically stable whenever it exists in the positive plane.

Finally, we have

$$\frac{\partial}{\partial T}\left[\frac{T'}{CT}\right] + \frac{\partial}{\partial C}\left[\frac{C'}{CT}\right] = \frac{\partial}{\partial T}\left[\frac{\pi - dT - pCT}{CT}\right] + \frac{\partial}{\partial C}\left[\frac{\alpha CT - \delta C}{CT}\right] = -\frac{\pi}{CT^2}.$$

Since this value is not identically zero and does not change sign in the positive plane, it follows from the Dulac criterion [10] that there are no periodic orbits in the positive plane. Since the non-impulsive model is a two-dimensional

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model, there is no higher-order behaviour. It follows that the stable fixed point is globally asymptotically stable. □

Sample phase-plane portraits for the positive plane are shown in Fig. 1, illustrating the case where only the trivial equilibrium is stable (Fig. 1A) and the case where the non-trivial equilibrium is stable (Fig. 1B).

4. The system with vaccination

The impulsive effect continually 'interrupts' the trajectories in the non-impulsive system, by moving solutions a fixed distance to the right (see Fig. 2). Define τ to be the duration of time between successive vaccine administrations. Then we can define

$$T_{\text{int}} = e^{\int_0^\tau (\alpha T(u) - \delta) du}. \tag{4.1}$$

T_{int} is a measure of the difference in CTLs between the beginning and the end of an impulsive cycle. For a periodic vaccine administered at times t_k $(k = 1, 2, \ldots)$, we have $\tau \equiv t_{k+1} - t_k$, a constant.

Theorem 4.1. *Model (2.1) has a positive impulsive periodic orbit, with one impulse per period. At times of impulse* $\{t_n\}_{n=0}^{\infty}$, *the endpoints of this impulsive periodic orbit satisfy*

$$C(t_n^-) = \frac{\widetilde{C} T_{\text{int}}}{1 - T_{\text{int}}}$$
$$C(t_n^+) = \frac{\widetilde{C}}{1 - T_{\text{int}}}.$$

Furthermore, this periodic orbit is orbitally asymptotically stable and has the property of asymptotic phase.

Proof. From the second equation in model (2.1), we can write

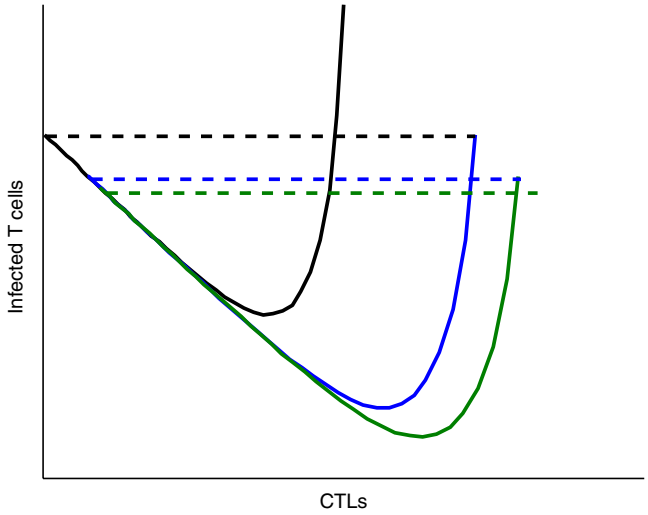

Fig. 2. Schematic representation of the impulsive effect. The impulsive effect 'interrupts' the continuous trajectories, moving them a fixed distance $\widetilde{C}$ horizontally to the right (dashed lines), before resuming their progress. Consequently, the cycling converges to an impulsive periodic orbit with one impulse per cycle.

$$\int_0^t \frac{dC}{C} = \int_0^t (\alpha T(u) - \delta) du$$
$$C(t) = C(0) e^{\int_0^t (\alpha T(u) - \delta) du}. \tag{4.2}$$

It follows that

$$C(\tau^-) = C(0) T_{\text{int}}$$
$$C(\tau^+) = C(0) T_{\text{int}} + \widetilde{C}.$$

Suppose $C(\tau^+) = C(0)$. Then

$$C(0) = \frac{\widetilde{C}}{1 - T_{\text{int}}}.$$

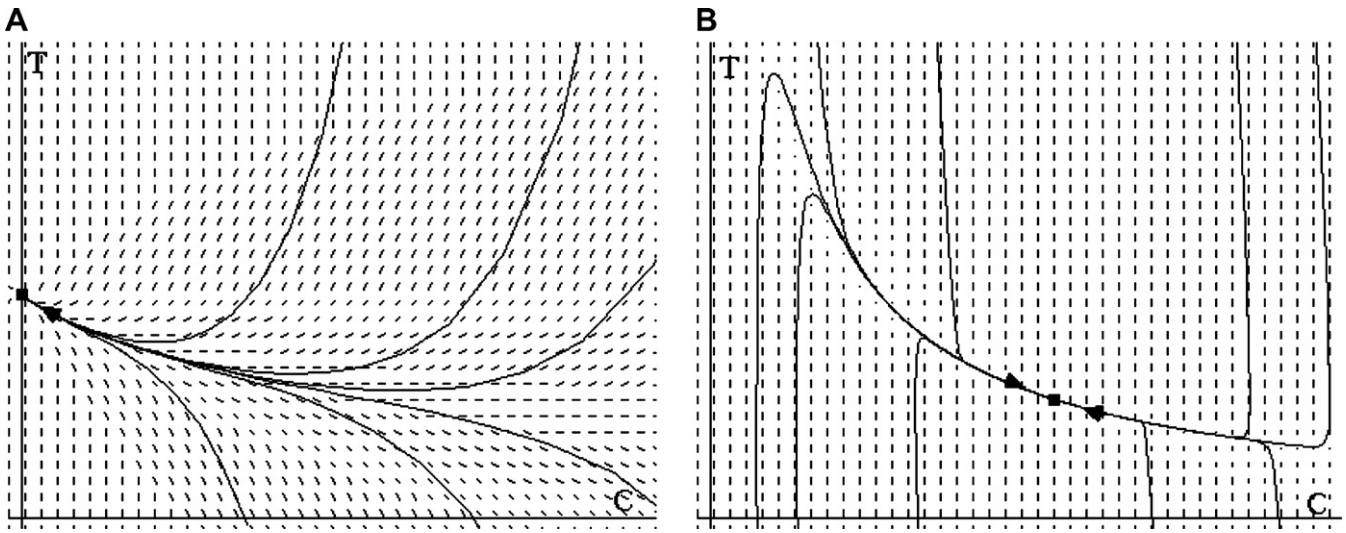

Fig. 1. Sample phase-plane portraits illustrating stable equilibria in the positive plane, for the system without vaccination. (A) When the non-trivial equilibrium is not in the positive plane, the trivial equilibrium is stable. (B) When the non-trivial equilibrium is in the positive plane, the trivial equilibrium is unstable and the non-trivial equilibrium is stable. Note that, for visual accessibility, the CTLs are on the x-axis and the infected T cells are on the y-axis.

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Thus,

$$C(t) = \frac{\widetilde{C}e^{\int_0^t(\alpha T(u)-\delta)du}}{1-T_{\text{int}}} \tag{4.3}$$

and hence

$$\begin{aligned} C(\tau^-) &= \frac{\widetilde{C}T_{\text{int}}}{1-T_{\text{int}}} \\ C(\tau^+) &= \frac{\widetilde{C}T_{\text{int}}}{1-T_{\text{int}}} + \widetilde{C} \\ &= C(0). \end{aligned}$$

It follows that (4.3) defines a impulsive periodic orbit on the range $0 < t < \tau$, with one impulse per cycle, whose endpoints are $\frac{\widetilde{C}}{1-T_{\text{int}}}$ and $\frac{\widetilde{C}T_{\text{int}}}{1-T_{\text{int}}}$.

Next, we show that $T_{\text{int}} < 1$. Suppose $T_{\text{int}} \geqslant 1$. Then $\int_0^\tau(\alpha T(u)-\delta)du \geqslant 0$. It follows from (4.2) that $C(\tau^-) \geqslant C(0)$. But

$$\begin{aligned} & C(\tau^+) = C(\tau^-) + \widetilde{C} \\ \Rightarrow \quad & C(0) = C(\tau^-) + \widetilde{C} \\ & \geqslant C(0) + \widetilde{C} \\ \Rightarrow \quad & \widetilde{C} \leqslant 0. \end{aligned}$$

This is a contradiction, so $T_{\text{int}} < 1$. It follows that the orbit is positive.

We now apply impulsive Floquet theory to system (2.1) to establish orbital asymptotical stability of the periodic orbit and asymptotic phase. We calculate the non-trivial impulsive Floquet multiplier (see Bainov and Simeonov [2]). Define

$$\begin{aligned} P(T,C) &= \pi - dT - pCT \\ Q(T,C) &= \alpha CT - \delta C \\ a(T,C) &= 0 \\ b(T,C) &= \widetilde{C}, \end{aligned}$$

with the (differentiable) function ϕ implicitly defined by $\{\phi(T(t),C(t)) = 0 : t = t_k\}$. Let $(\xi(t),\eta(t))$ define the impulsive periodic orbit. Then

$$\begin{aligned} \xi(t_k^-) &= \xi(t_k^+) = T(\tau) \\ \eta(t_k^-) &= \frac{\widetilde{C}T_{\text{int}}}{1-T_{\text{int}}} \\ \eta(t_k^+) &= \frac{\widetilde{C}}{1-T_{\text{int}}}. \end{aligned}$$

Therefore, the non-trivial Floquet multiplier is

$$\mu_2 = \Delta_1 \exp\left[\int_0^\tau \left(\frac{\partial P}{\partial T}(\xi(t),\eta(t)) + \frac{\partial Q}{\partial C}(\xi(t),\eta(t))\right)dt\right], \tag{4.4}$$

where

$$\Delta_1 = \frac{P_+\left(\frac{\partial b}{\partial C}\frac{\partial \phi}{\partial T} - \frac{\partial b}{\partial T}\frac{\partial \phi}{\partial C} + \frac{\partial \phi}{\partial T}\right) + Q_+\left(\frac{\partial a}{\partial T}\frac{\partial \phi}{\partial C} - \frac{\partial a}{\partial C}\frac{\partial \phi}{\partial T} + \frac{\partial \phi}{\partial C}\right)}{P\frac{\partial \phi}{\partial T} + Q\frac{\partial \phi}{\partial C}};$$

P, Q, $\frac{\partial a}{\partial T}$, $\frac{\partial b}{\partial T}$, $\frac{\partial a}{\partial C}$, $\frac{\partial b}{\partial C}$, $\frac{\partial \phi}{\partial T}$ and $\frac{\partial \phi}{\partial C}$ are computed at the point $(\xi(t_k^-),\eta(t_k^-))$ and $P_+ = P(\xi(t_k^+),\eta(t_k^+))$, $Q_+ = Q(\xi(t_k^+),\eta(t_k^+))$. We thus have

$$\begin{aligned} P_+ &= \pi - d\xi(t_k^-) - p\eta(t_k^+)\xi(t_k^-) \quad Q_+ = \alpha\xi(t_k^-)\eta(t_k^+) - \delta\eta(t_k^+) \\ P &= \pi - d\xi(t_k^-) - p\eta(t_k^-)\xi(t_k^-) \quad Q = \alpha\xi(t_k^-)\eta(t_k^-) - \delta\eta(t_k^-), \end{aligned}$$

since $\xi(t_k^+) = \xi(t_k^-)$. In particular,

$$\begin{aligned} Q &= T_{\text{int}}Q_+ \\ P_+T_{\text{int}} &= (\pi - d\xi(t_k^-))T_{\text{int}} - p\eta(t_k^+)T_{\text{int}}\xi(t_k^-) \\ &= (\pi - d\xi(t_k^-))T_{\text{int}} - p\eta(t_k^-)\xi(t_k^-), \end{aligned} \tag{4.5}$$

since $\eta(t_k^-) = \eta(t_k^+)T_{\text{int}}$. From the nullclines, $T'(t_k^-) > 0$. Thus, $\pi - d\xi(t_k^-) > p\eta(t_k^-)\xi(t_k^-) > 0$. Hence,

$$P_+T_{\text{int}} < P, \tag{4.6}$$

since $T_{\text{int}} < 1$. It follows, from (4.5) and (4.6), that

$$\begin{aligned} \Delta_1 &= \frac{P_+\frac{\partial \phi}{\partial T} + Q_+\frac{\partial \phi}{\partial C}}{P\frac{\partial \phi}{\partial T} + Q\frac{\partial \phi}{\partial C}} \\ &< \frac{1}{T_{\text{int}}}. \end{aligned}$$

We thus have

$$\begin{aligned} \mu_2 &< \frac{1}{T_{\text{int}}}\exp\int_0^\tau \left(\frac{\partial P}{\partial T}(\xi(t),\eta(t)) + \frac{\partial Q}{\partial C}(\xi(t),\eta(t))\right)dt \\ &= \frac{1}{T_{\text{int}}}\exp\int_0^\tau (-d - p\eta(t) + \alpha\xi(t) - \delta)dt \\ &= \frac{1}{T_{\text{int}}}\exp\left(-\int_0^\tau (d + p\eta(t))dt\right)\exp\left(\int_0^\tau (\alpha\xi(t) - \delta)dt\right) \\ &= \frac{1}{T_{\text{int}}}\exp\left(-\int_0^\tau (d + p\eta(t))dt\right)T_{\text{int}} \\ &< 1. \end{aligned}$$

Thus, the non-trivial impulsive Floquet multiplier lies inside the unit circle, so the τ-periodic orbit is orbitally asymptotically stable and has the property of asymptotic phase. □

Remark. While condition (4.2) holds for all solutions, we have not demonstrated that the periodic orbit is unique. In particular, there may be periodic orbits with more than one impulse per cycle. If we generalise (4.1) by defining

$$T_{\text{int}}^j \equiv e^{\int_{j\tau}^{(j+1)\tau}(\alpha T(u)-\delta)du}$$

and define

$$a_k(n) = \prod_{k=0}^{n-1} T_{\text{int}}^k,$$

then we have

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$$
\begin{aligned}
C(\tau^+) &= C(0)T^0_{\text{int}} + \widetilde{C}\\
C(2\tau^+) &= C(\tau^+)T^1_{\text{int}} + \widetilde{C}\\
&= C(0)T^0_{\text{int}}T^1_{\text{int}} + \widetilde{C}(T^1_{\text{int}} + 1)\\
C(3\tau^+) &= C(0)T^0_{\text{int}}T^1_{\text{int}}T^2{}_{\text{int}} + \widetilde{C}(T^1_{\text{int}}T^2_{\text{int}} + T^2_{\text{int}} + 1)\\
&\vdots\\
C(n\tau^+) &= C(0)a_0(n) + \widetilde{C}(a_1(n) + a_2(n) + \cdots + a_{n-1}(n) + 1).
\end{aligned}
$$

Thus, it is possible that the sequence $\{C(k\tau^+)\}$ may diverge, or that there is an nth-order impulsive periodic orbit. Such an orbit would satisfy (4.2) and the conditions

$$
\begin{aligned}
C(0) &= \frac{\widetilde{C}(a_1 + a_2 + \cdots + a_{n-1} + 1)}{1 - a_0}\\
C(j\tau^+) &= C(0)a_0(j) + \widetilde{C}(a_1(j) + a_2(j) + \cdots + a_{j-1}(j) + 1)\\
&\quad (j = 1, 2, \ldots, n).
\end{aligned}
$$

However, numerical simulations did not show any higher-order periodic orbits for the parameter range under consideration, so we restrict the remainder of our attention to the first-order periodic orbit.

Theorem 4.2. *Define the average infected T cell strength to be the average number of infected CD4$^+$ T cells during a single cycle of the impulsive periodic orbit, given by*

$$T_{\text{av}} \equiv \frac{1}{\tau}\int_0^\tau T(u)du.$$

Then, on the impulsive periodic orbit given in Theorem 4.1, we have

$$\lim_{\tau\to 0} T_{\text{av}} = \lim_{\widetilde{C}\to\infty} T_{\text{av}} = 0. \tag{4.7}$$

Proof. Since $T(t_k^-) = T(t_k^+) = T(t_k)$, it follows that, for any k,

$$
\begin{aligned}
\frac{dT}{dt}(t_k^-) &= \pi - dT(t_k) - pC(t_k^-)T(t_k)\frac{dT}{dt}(t_k^+)\\
&= \pi - dT(t_k) - pC(t_k^+)T(t_k)\\
&= \pi - dT(t_k) - p(C(t_k^-) + \widetilde{C})T(t_k)\\
&= \frac{dT}{dt}(t_k^-) - p\widetilde{C}T(t_k).
\end{aligned}
$$

Thus,

$$\frac{dT}{dt}(t_k^+) < \frac{dT}{dt}(t_k^-). \tag{4.8}$$

Furthermore, from the nullclines, in the positive plane $\frac{dT}{dt} = 0$ only when $T = \frac{\pi}{d+pC}$. Thus, since $\frac{dC}{dt} < 0$ within each cycle, it follows that T has at most one turning point per cycle. From (4.8), this turning point must be a minimum and $\frac{dT}{dt}(t_k^-) > 0$.

Next, note that

$$\lim_{\tau\to 0} C(0^+) = \lim_{\tau\to 0}\frac{\widetilde{C}}{1 - e^{\int_0^\tau(\alpha T(u) - \delta)du}} = \infty$$

and

$$\lim_{\tau\to 0} C(\tau^-) = \lim_{\tau\to 0}\frac{\widetilde{C}e^{\int_0^\tau(\alpha T(u) - \delta)du}}{1 - e^{\int_0^\tau(\alpha T(u) - \delta)du}} = \infty.$$

We have

$$
\begin{aligned}
\lim_{\tau\to 0} T_{\text{av}} &= \lim_{\tau\to 0}\frac{1}{\tau}\int_0^\tau T(u)\,du\\
&= \lim_{\tau\to 0} T(\tau).
\end{aligned}
$$

Suppose that $\lim_{\tau\to 0}T(\tau) \neq 0$. Then

$$
\begin{aligned}
\lim_{\tau\to 0}\frac{dT}{dt}(\tau) &= \lim_{\tau\to 0}(\pi - dT(\tau) - pC(\tau)T(\tau))\\
&= -\infty.
\end{aligned}
$$

However, this is a contradiction, since $\frac{dT}{dt}(\tau) > 0$ for $\tau > 0$. It follows that $\lim_{\tau\to 0}T(\tau) = 0$ and hence

$$\lim_{\tau\to 0} T_{\text{av}} = 0.$$

Finally, note that, on $0 < t < \tau$, $C(t) > C(\tau^-)$. Thus,

$$
\begin{aligned}
\lim_{\widetilde{C}\to\infty} C(t) &\geqslant \lim_{\widetilde{C}\to\infty} C(\tau^-)\\
&= \lim_{\widetilde{C}\to\infty}\frac{\widetilde{C}T_{\text{int}}}{1 - T_{\text{int}}}\\
&= \infty.
\end{aligned}
$$

Thus,

$$\lim_{\widetilde{C}\to\infty}\frac{dT}{dt} = \lim_{\widetilde{C}\to\infty}(\pi - dT - pCT) = -\infty$$

unless $\lim_{\widetilde{C}\to\infty} T = 0$. Hence,

$$
\begin{aligned}
\lim_{\widetilde{C}\to\infty} T_{\text{av}} &= \frac{1}{\tau}\int_0^\tau \lim_{\widetilde{C}\to\infty} T(u)\,du\\
&= 0. \quad \square
\end{aligned}
$$

Remark. It follows that the number of infected T cells can theoretically be kept as low as desired, by appropriate choice of vaccine strength $\widetilde{C}$ and/or period τ. Thus, infection can theoretically be kept arbitrarily low (ignoring latently infected cells and other reservoirs), assuming a sufficiently strong vaccine or sufficiently frequent vaccinations (although the impulsive assumptions break down as the limit approaches zero).

5. Simulations

To illustrate the theorem, parameter values from the literature were used (see Table 1). All parameters can be found in published studies, except π. In a model of de Boer and Perelson [8], the production of infected CD4$^+$ T cells is

Table 1
Parameters used

Parameter	Value	Units	Reference
π	1.5	cells day^{-1} μL^{-1}	[8]
d	0.5	day^{-1}	[9]
p	0.05	μL cells^{-1} day^{-1}	[5]
α	0.067	μL cells^{-1} day^{-1}	[8]
δ	0.2	day^{-1}	[8]

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given by the term βV, where $\beta = 0.015$ is the infection rate and V represents the (non-constant) density of infectious virions (Eq. (9) in that reference). At equilibrium values, $V = 100$, suggesting that $\pi = \beta\overline{V} = (0.015)(100) = 1.5$ cells day^{-1} μL^{-1}. Thus, our estimates assume approximate equilibrium concentrations of free virus.

A CTL boost of 35 cells/μL administered every 120 days produces an average of 2.02 infected CD4$^+$ T cells/μL (Fig. 3). Conversely, the same CTL boost administered every 14 days produces an average of 1.07 CD4$^+$ T cells/μL (Fig. 4). All other parameters are as in the Table 1.

It should be noted that these results depend primarily upon two factors: (a) the choice of an impulsive model to approximate the CTL boost and (b) sensitivity to parameter variation. Consequently, we explore the sensitivity of our results to each factor.

Activation of CTLs may be delayed by up to ten days after HIV infection [7]. Thus, the impulsive approximation by instantaneous activation may be too coarse. To explore this, we ran numerical simulations that overestimated this delay to 14 days (see Fig. 5). Specifically, the non-impulsive model (dashed curve) activated linear growth of CTLs 14 days before the instantaneous effect of the impulsive model (solid curve). While the trajectories were out of phase during this delay period, they came back into phase quite rapidly afterwards. Furthermore, the average infected T cell concentration was lower in the non-impulsive model (dashed horizonal line) than for the impulsive model (solid horizontal line).

To explore sensitivity of our results to parameter variation, we varied each parameter individually, while holding all others at mean values. Our output was the average number of infected T cells (see Fig. 6). In each case, the mean

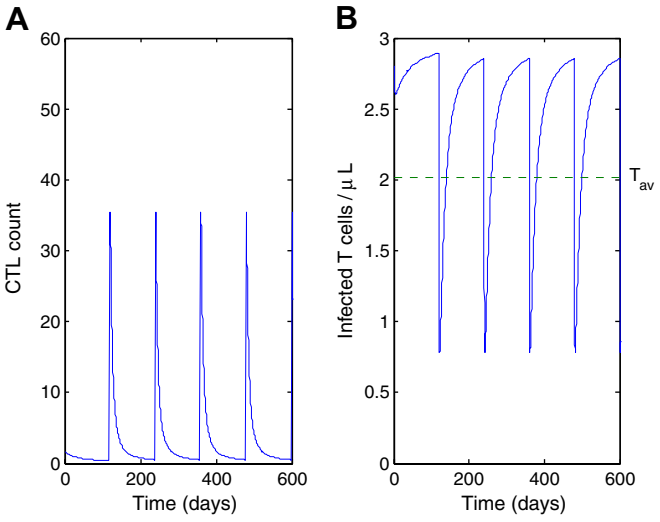

Fig. 3. A CTL boost of 35 cells/μL administered every 120 days produces an average infected T cell concentration of 2.02 cells/μL. (A) CTL concentrations, with initial values of 1.7 cells/μL, showing impulsive (discontinuous) vaccination and approximately exponential decay. (B) Infected T cell concentrations, with initial values of 2.8 cells/μL. Note that tractories in this case are continuous (although their derivatives are not).

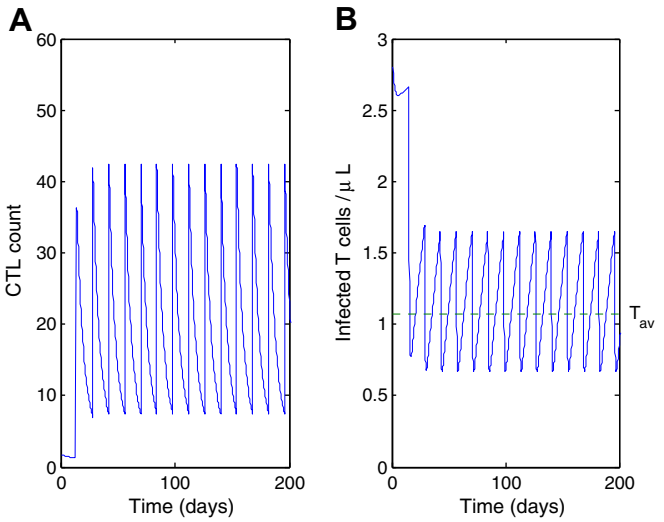

Fig. 4. A CTL boost of 35 cells/μL administered every 14 days produces an average infected T cell concentration of 1.07 cells/μL. All other parameters are as in Fig. 3. Note that this figure illustrates the convergence of the impulsive periodic orbit, as well as the fact that the infected T cell concentration can be kept low with sufficiently frequent vaccination. However, it should be noted that this is an extremely short vaccination interval, of the same order of magnitude as the CTL activation period, and thus the impulsive assumptions would break down in reality.

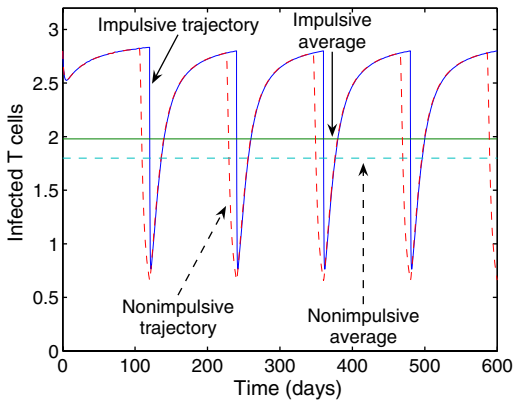

Fig. 5. Comparison of the impulsive model with a non-impulsive activation effect. The impulsive model (solid curve) resembles the infected T cell trajectories from Fig. 3B. The non-impulsive model (dashed curve) followed the same differential equations except that the impulsive effect was replaced by a linear growth function for the CTLs, activated 14 days earlier. Not only do the trajectories come into phase quite quickly, the average infected T cell concentration from the non-impulsive model is lower than that estimated by the impulsive model.

value on the x-axis (dashed line) is the value used in the Table 1. Since the slope of each graph is quite mild, the model is robust with respect to parameter choices. If d, p or α increase, then the average number of infected T cells is reduced slightly, whereas if these parameters are decreased, then the average number of infected T cells is increased slightly.

It is well established that small variations in death rates have large effects on the outcome [17], so the sensitivity curve for δ is not unexpected. Furthermore, of all parame-

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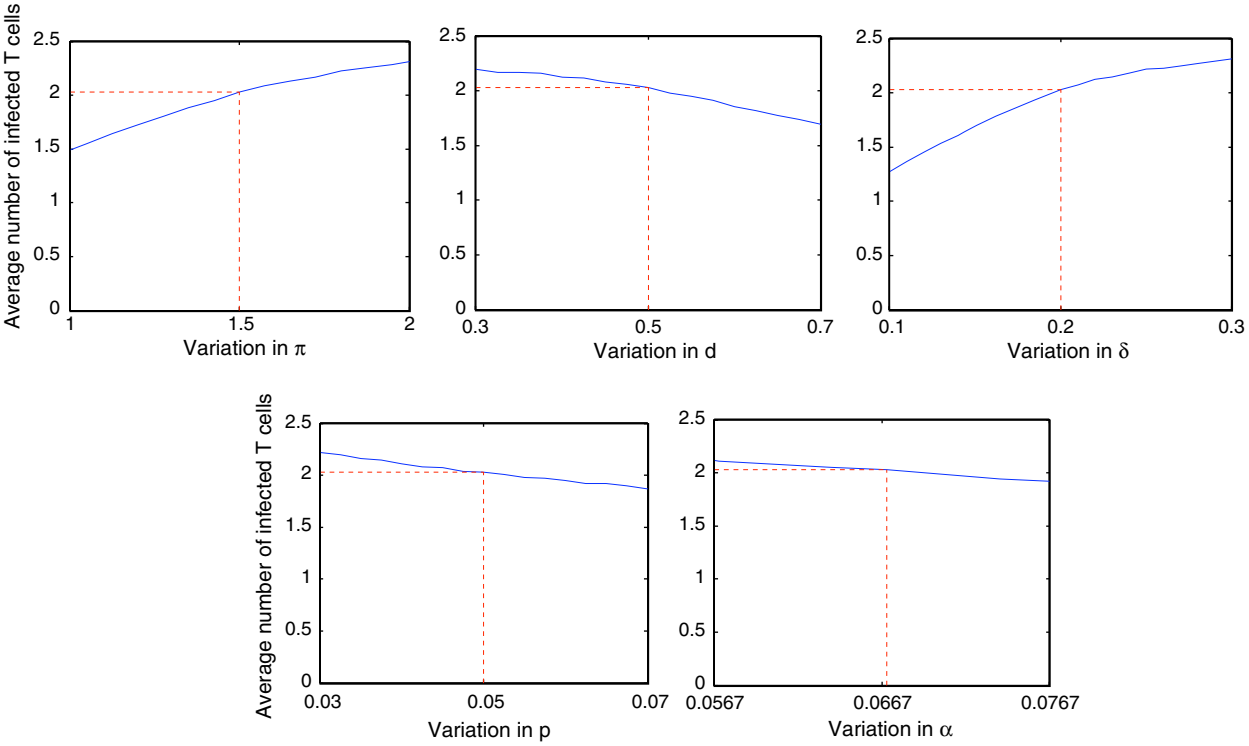

Fig. 6. Sensitivity to parameter variation. Each parameter was varied individually while holding other parameters at median values. The output parameter is the average number of infected T cells. The model is robust to all parameter choices, although the greatest variation occurs in π and δ. The median values on the x-axis are the values given in Table 1 (dashed line).

ters, the death rates have been most carefully analysed in the experimental literature, so we have reasonable faith in the accuracy of these rates.

Increasing π, the production rate of infected T cells, would obviously increase the average number of infected T cells. However, in the context of an infected individual undergoing a CTL vaccination program, it is more likely that we would see a decreased value of π, at least during the long asymptomatic phase the disease. This is because the vaccination program lowers the number of infected cells, which consequently would produce fewer viral particles to infect cells. Subsequently, we would see a reduced average number of infected T cells. Such a reduction of π may offset any variation in the other parameters, further reinforcing the robustness of the parameter choices.

6. Discussion

A CTL vaccine pulsed at regular intervals can keep the average number of infected $CD4^+$ T cells arbitrarily low, by choosing appropriate vaccination intervals and strength of the vaccine.

While impulsive differential equations are a useful tool for mathematical analysis, it should be noted that these results rely upon the assumption that the change in CTL concentration is instantaneous. Such approximations are valid so long as the impulsive cycle time is large compared to the rapid change being approximated. This case, this approximation has the effect of overestimating the average infected $CD4^+$ T cell concentration and hence does not affect our conclusions.

In this case, the approximation is a coarse one: CTLs may take up to 10 days to be stimulated; nevertheless, Fig. 5 demonstrates that this timescale is still short, compared to a vaccination interval of the order of months, or more. Thus, our results are less valid for extremely short vaccination intervals (of the order of weeks). However, vaccination programs with extremely short intervals are unlikely to be implemented in practice.

Although we have shown that the impulsive periodic orbit exists, is positive, is orbitally asymptotically stable and has the property of asymptotic phase, it does not necessarily follow that all trajectories with appropriate initial conditions will necessarily approach this orbit; there may be higher-order periodic orbits or more complex attractors, which are theoretically possible with two-dimensional impulsive orbits. However, numerical simulations did not show any such behaviour. Furthermore, the application of impulsive Floquet multipliers to determine stability is straightforward for two-dimensional systems, as here, or systems that can be reduced to two-dimensional systems (see also [17]), but are less useful for higher-order systems.

The fact that infected $CD4^+$ T cell concentrations vary significantly throughout each impulsive cycle (Figs. 3 and 4) has implications for how patients are monitored and suggests that the progress of patients should be monitored carefully, especially at the time just before vaccination

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when the infected T cell concentration is expected to be maximal.

Furthermore, the assumption that infected $CD4^+$ T cells are produced at a constant rate becomes less valid if the vaccination program is successful. However, the rate of production of infected T cells is likely to decrease due to the effects of the vaccine. This reduction may offset the variation due to random fluctuations in the other parameters.

Further work will investigate the effects of non- or partial-adherence to the vaccine. The effects of full adherence to the vaccine, but with fluctuating vaccination dates will also be investigated. We will also examine the case when the rate of production of infected CTLs varies with the level of CTL killing, as well as the effects that antiretroviral therapy will have in conjunction with such a vaccine.

In conclusion, a post-infection CTL vaccine would be highly desirable, assuming perfect patient adherence. Such a vaccine would, at the very least, offer an alternative to the daily pill burden of antiretroviral drug therapy.

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