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Modelling Mutation to a Cytotoxic T-lymphocyte HIV Vaccine

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Resistance to a postinfection HIV vaccine that stimulates cytotoxic T-lymphocytes (CTLs) depends on the relationship between the vaccine strength, the fitness cost of the mutant strain, and the rate of mutant escape. If the vaccine is strong enough, both strains of the virus should be controlled by administering the vaccine sufficiently often. However, if escape mutation to the vaccine occurs, then either the wild type or the mutant can outcompete the other strain. Imperfect adherence may result in the persistence of the mutant, while fluctuations in the vaccination time—even if no vaccines are missed—may result in the mutant outcompeting the wild type.

1. INTRODUCTION

Virus-specific cytotoxic T-lymphocytes (CTLs) control HIV-1 replication in humans and SIV replication in rhesus monkeys, thereby delaying the onset of disease and progression to AIDS (Klein et al., 1998; Jin et al., 1999; Schmitz et al., 1999; Amara et al., 2002; Barouch et al., 2003). Controls against SIV during trials of vaccines that elicit CTL responses (Barouch et al., 2000; Amara et al., 2001,

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2002) provide hope that a T-cell-based vaccine will be efficacious against HIV/AIDS, and have shown preliminary efficacy. However, despite the CTL responses, HIV-infected individuals progress to AIDS in most cases, and there is evidence of the failure of such vaccines (Barouch et al., 2002, 2003). The role of CTLs during the course of HIV-1 infection is still unclear.

Experimental results show that one of the main reasons for CTL response failure is viral escape from CTLs (Barouch et al., 2002, 2003; Asquith, 2008). CTLs lyse infected cells by recognizing viral peptide epitopes displayed on the cell surface and bind to class I major histocompatibility complex (MHC) molecules (Collins et al., 1998; Klein et al., 1998; Smith, 2004). Due to a high mutation rate in both HIV-infected humans and SIV-infected monkeys, viral mutation can develop in CTL epitopes, resulting in escape of these viruses from CTL recognition (Barouch et al., 2003; Jamieson et al., 2003; Goulder and Watkins, 2004; Smith, 2004). For example, Asquith (2008) observed 21 different epitope locations where escape mutation occurs. These mutants show a lower level of fitness than wild-type virus (Smith, 2004). However, selection pressure exerted by CTLs on viral replication may cause escape mutants to outcompete the wild-type virus (Barouch et al., 2003). Here, we investigate the effect of viral mutation on the ability of CTLs to control the viral infection when a postinfection vaccine is administered at regular intervals.

Currently, the major control measure to combat HIV consists of combinations of antiretroviral drugs, which are expensive and have a high pill burden and debilitating side effects, producing nonadherence and drug resistance (Altice and Friedland, 1998; Bartlett, 2002; Smith, 2006; Miron and Smith?, 2010). Unlike antiretroviral drugs, a single drug holiday may be critical because the intervals between subsequent vaccinations are significantly longer than the intervals between subsequent drug doses.

Korthals Altes et al. (2002) and Ball et al. (2007) have theorized the potential of HIV to evade a CTL vaccine. Other diseases show antigenic variation to annual vaccination, suggesting that a CTL vaccine is unlikely to be all-encompassing (Gerdil, 2003). Here, we assume that cells infected with the mutant strain can be partially controlled by CTLs but less efficiently than control of the wild-type strain. As with drug resistance, we assume a trade-off for mutation in terms of the rate of infection or the total number of virions each infected cell can produce. We assume that the wild type will outcompete the mutant in the absence of the vaccine but that the CTL vaccine may reduce the wild type's fitness, giving an advantage to the mutant.

Smith? and Schwartz (2008) modelled a CTL vaccine in the absence of resistance and showed that the virus could be eliminated if the vaccine were sufficiently strong, assuming perfect adherence and no viral mutation in response to the vaccine. We extend that model to incorporate mutation and address the following questions. Can we determine the critical vaccine threshold for eradication of the virus? Under what conditions can a mutant strain of the virus persist in the presence of a CTL vaccine? How does vaccine frequency affect the emergence of resistance?

2. THE MODEL

Let T denote the density of susceptible CD4$^+$ T cells. We consider two virus strains, the wild-type virus V_1 and the mutant V_2. CD4$^+$ T cells infected with wild-type and mutant strains are denoted by T_1 and T_2. The density of specialized CTL cells produced by the body's immune system to kill infected CD4$^+$ T cells is denoted by C.

Susceptible CD4$^+$ T cells are produced with constant rate π and die with rate δ_T. They are infected by wild-type or mutant strains at rates r_1 and r_2. New virus particles are produced at rates n_1 and n_2. The chance of *de novo* mutation is ε. Free-virus particles and infected T cells die at rates δ_V and δ_I. Infected T cells are also cleared by the body's defensive CTLs; this happens at rates p_1 and p_2. CTLs reproduce in the presence of infected T cells at rate α and die at rate δ_C. At fixed vaccination times t_k, $k = 1, 2, \ldots$, vaccination increases CTL cells by a fixed amount $\widetilde{C}$, which is proportional to the total number of CTLs the vaccine stimulates.

For $t \neq t_k$, the model is:

$$T'(t) = \pi - r_1 V_1 T - r_2 V_2 T - \delta_T T \tag{1}$$

$$V_1'(t) = n_1 T_1 - \delta_V V_1 \tag{2}$$

$$V_2'(t) = n_2 T_2 - \delta_V V_2 \tag{3}$$

$$T_1'(t) = (1 - \varepsilon) r_1 V_1 T - p_1 T_1 C - \delta_I T_1 \tag{4}$$

$$T_2'(t) = \varepsilon r_1 V_1 T + r_2 V_2 T - p_2 T_2 C - \delta_I T_2 \tag{5}$$

$$C'(t) = \alpha(T_1 + T_2)C - \delta_C C, \tag{6}$$

and for $t = t_k$:

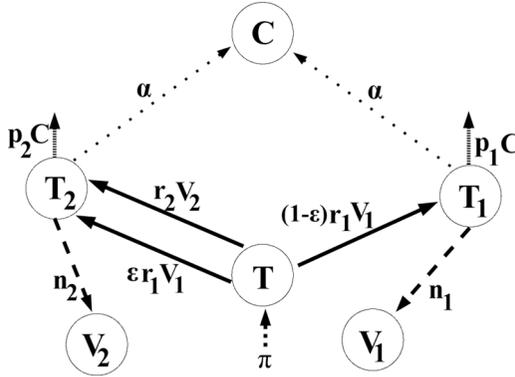$$\Delta C \equiv C(t_k^+) - C(t_k^-) = \widetilde{C}, \tag{7}$$

FIGURE 1 The model. Susceptible T cells are produced at a constant rate π and die at rate δ_T. Once infected by the wild-type virus V_1 or the mutant strain V_2, they start to produce new virus particles at rates n_1 and n_2. Due to mutation, some proportion ε of T cells infected by the wild type become resistant. Infected T cells die at rate δ_I. The production of CTL cells is stimulated by infected T cells at rate α. CTLs then kill productively infected T cells at rates p_1 and p_2, respectively.

where $C(t_k^-)$ is the CTL concentration immediately before the impulse and $C(t_k^+)$ is the CTL concentration immediately after the impulse. The model is represented in Figure 1.

To understand the effect of CTL killing of infected cells on the viral dynamics, we could add more epitope mutations and corresponding specific CTL cells. However, as our primary objective is to evaluate the activation of CTLs due to vaccine administration, we assume only one type of CTL cell. In our simulations, the CTL concentration growth is dominated by vaccination. For the sake of simplicity, we take the same rate of CTL clone activation for both virus strains.

The mutant strain possesses a fitness cost resulting in a reduced infectivity and a reduced replication capacity (Smith, 2004), while the *de novo* mutation rate is 3×10^{-5} per nucleotide (Mansky and Temin, 1995), so $(1 - \varepsilon)r_1 n_1 > r_2 n_2$.

During *in vitro* experiments, in which the ability of the mutants Gag p11C, Env TL9, Env p41A, and Pol p68A to bind to MHC class I molecule Mamu-A*01 and be recognized by epitope-specific CTLs were assessed, Barouch et al. (2002, 2003) observed partial CTL responses against mutant viruses. This implies that $p_2 > 0$. Many escape mutations often result in a reduced recognition of mutant epitope by CTLs (Barouch et al., 2003; Jamieson et al., 2003; Goulder and Watkins, 2004; Smith, 2004). For example, compared with the

wild type, the mutant p68A peptide had more than 2 log-fold lower affinity for Mamu-A*01 and more than 3 log-fold lower recognition by CD8+ T-lymphocytes (Barouch et al., 2002). This implies $p_1 > p_2$.

3. LOCAL STABILITY

Because of the impulsive effect in C, there are no classical equilibria for System (1)–(7). As this system is autonomous, we investigate impulsive orbits (or orbits, for short) defined by $T' = V_1' = V_2' = T_1' = T_2' = 0$ and $C \neq 0$. We use the abbreviation:

$$a_j = a_j(C) = p_j C + \delta_I \quad \text{for } j = 1, 2. \tag{8}$$

The disease-free orbit is:

$$(\overline{T}, \overline{V}_1, \overline{V}_2, \overline{T}_1, \overline{T}_2) = \left(\frac{\pi}{\delta_T}, 0, 0, 0, 0 \right). \tag{9}$$

The mutant-only orbit is in the form $(\widehat{T}, 0, \widehat{V}_2, 0, \widehat{T}_2)$, where:

$$\widehat{T} = \frac{\delta_V a_2}{r_2 n_2}$$

$$\widehat{V}_2 = \frac{r_2 n_2 \pi - \delta_T \delta_V a_2}{r_2 a_2 \delta_V}$$

$$\widehat{T}_2 = \frac{\delta_C}{\alpha} - \frac{r_2 n_2 \pi - \delta_T \delta_V a_2}{r_2 n_2 a_2}.$$

Due to mutation, there is no wild-type-only orbit.

For both strains of the virus to be eradicated, the disease-free orbit must be locally stable. Otherwise, both strains of the virus can re-emerge even if the total number of virus particles and infected cells is already low, with the potential effect that the mutant strain gains an advantage. The latter scenario is of particular importance if the mutant-only orbit is locally stable. If both the disease-free and mutant-only orbits are locally unstable, then the two virus strains will coexist in the presence of the vaccine.

Definition 3.1. Let C_1^* be the value of C so that:

$$\delta_T \delta_V a_1 = (1 - \varepsilon) r_1 n_1 \pi; \quad \text{that is, } C_1^* \equiv \frac{1}{p_1} \left(\frac{(1 - \varepsilon) r_1 n_1 \pi}{\delta_T \delta_V} - \delta_I \right). \tag{10}$$

Let C_2^* be the value of C so that:

$$\delta_T \delta_V a_2 = r_2 n_2 \pi; \quad \text{that is, } C_2^* \equiv \frac{1}{p_2} \left(\frac{r_2 n_2 \pi}{\delta_T \delta_V} - \delta_I \right). \tag{11}$$

Denote $C^* \equiv \max\{C_1^*, C_2^*\}$. Finally, let C_3^* be the value of C so that:

$$r_2 n_2 a_1 = (1-\varepsilon)r_1 n_1 a_2; \quad \text{that is, } C_3^* = \frac{(1-\varepsilon)r_1 n_1 - r_2 n_2}{r_2 n_2 p_1 - (1-\varepsilon)r_1 n_1 p_2}. \quad (12)$$

For reasonable parameter values, C_1^* and C_2^* are positive. C_1^* determines the long-term behavior of the wild-type strain, while C_2^* determines the long-term behavior of the mutant strain. The parameter C_3^* can take any sign and is critical in the analysis of the competition between the two virus strains.

Lemma 3.2. *Either*

$$C_1^* = C_2^* = C_3^* \quad or \quad C_3^* > C_1^* > C_2^* \quad or \quad C_2^* > C_1^* > C_3^*. \quad (13)$$

Proof. *Case* 1: $C_1^* = C_2^*$. Set $C = C_1^* = C_2^*$ and conclude that

$$\frac{\pi}{\delta_T \delta_V} = \frac{a_1}{(1-\varepsilon)r_1 n_1} \quad \text{and} \quad \frac{\pi}{\delta_T \delta_V} = \frac{a_2}{r_2 n_2}. \quad (14)$$

Thus,

$$\frac{a_1}{(1-\varepsilon)r_1 n_1} = \frac{a_2}{r_2 n_2} \Rightarrow r_2 n_2 a_1 = (1-\varepsilon)r_1 n_1 a_2 \Rightarrow C = C_3^*. \quad (15)$$

Case 2: $C_1^* > C_2^*$. Set $C = C_2^*$. Then

$$C < C_1^* \Leftrightarrow a_1 < \frac{(1-\varepsilon)r_1 n_1 \pi}{\delta_T \delta_V} \Leftrightarrow r_2 n_2 a_1 < (1-\varepsilon)r_1 n_1 \frac{r_2 n_2 \pi}{\delta_T \delta_V} \quad (16)$$

$$\Leftrightarrow r_2 n_2 a_1 < (1-\varepsilon)r_1 n_1 a_2$$

$$\Leftrightarrow C < C_3^*, \quad (17)$$

and hence $C_3^* > C_2^*$.
Set $C = C_3^* > C_2^*$. Then

$$r_2 n_2 a_1 = (1-\varepsilon)r_1 n_1 a_2 \Rightarrow r_2 n_2 a_1 > (1-\varepsilon)r_1 n_1 \frac{r_2 n_2 \pi}{\delta_T \delta_V} \quad (18)$$

$$\Rightarrow \delta_T \delta_V a_1 > (1-\varepsilon)r_1 n_1 \pi \Rightarrow C > C_1^*, \quad (19)$$

and so $C_3^* > C_1^*$.
Case 3: $C_1^* < C_2^*$. Set $C = C_1^*$. Then

$$C < C_2^* \Leftrightarrow a_2 < \frac{r_2 n_2 \pi}{\delta_T \delta_V} \Leftrightarrow (1-\varepsilon)r_1 n_1 a_2 < r_2 n_2 \frac{(1-\varepsilon)r_1 n_1 \pi}{\delta_T \delta_V} \quad (20)$$

$$\Leftrightarrow (1-\varepsilon)r_1 n_1 a_2 < r_2 n_2 a_1 \Leftrightarrow C > C_3^*. \quad (21)$$

Thus, $C_1^* > C_3^*$ and $C_2^* > C_3^*$.

As all the parameters are subject to slight fluctuations, the case $C_1^* = C_2^* = C_3^*$ is of no practical importance.

Theorem 3.3. *The disease-free orbit is locally stable if and only if $C > C^*$, that is, $C > C_1^*$ and $C > C_2^*$. The mutant-only orbit is locally stable if and only if $C_2^* > C > C_3^*$.*

Proof. We use the abbreviations:

$$G_1 = G_1(\lambda) = \det\begin{pmatrix} -\delta_V - \lambda & n_1 \\ (1 - \varepsilon)r_1 T & -a_1 - \lambda \end{pmatrix}$$

$$G_2 = G_2(\lambda) = \det\begin{pmatrix} -\delta_V - \lambda & n_2 \\ r_2 T & -a_2 - \lambda \end{pmatrix}.$$

The Jacobian matrix of our system is:

$$J(T, V_1, V_2, T_1, T_2)$$
$$= \begin{pmatrix} -r_1 V_1 - r_2 V_2 - \delta_T & -r_1 T & -r_2 T & 0 & 0 \\ 0 & -\delta_V & 0 & n_1 & 0 \\ 0 & 0 & -\delta_V & 0 & n_2 \\ (1 - \varepsilon)r_1 V_1 & (1 - \varepsilon)r_1 T & 0 & -a_1 & 0 \\ \varepsilon r_1 V_1 + r_2 V_2 & \varepsilon r_1 T & r_2 T & 0 & -a_2 \end{pmatrix}.$$

For the disease-free orbit, we have $\overline{V}_1 = \overline{V}_2 = \overline{T}_1 = \overline{T}_2 = 0$, so that the associated characteristic polynomial

$$\det(J - \lambda I) = -(\delta_T + \lambda)G_1 G_2 - (\delta_V + \lambda)\big[(1 - \varepsilon)r_1 V_1((a_1 + \lambda)G_2 \quad (22)$$
$$+ \varepsilon r_1 n_1 T(a_2 + \lambda)) + (\varepsilon r_1 V_1 + r_2 V_2)(a_2 + \lambda)G_1\big], \quad (23)$$

simplifies to

$$\det\left(J\left(\frac{\pi}{\delta_T}, 0, 0, 0, 0\right) - \lambda I\right) = -(\delta_T + \lambda)G_1 G_2. \quad (24)$$

With $T = \frac{\pi}{\delta_T}$, we have

$$G_1 = \lambda^2 + (a_1 + \delta_V)\lambda + \delta_V a_1 - (1 - \varepsilon)r_1 n_1 \frac{\pi}{\delta_T}, \quad (25)$$

and

$$G_2 = \lambda^2 + (a_2 + \delta_V)\lambda + \delta_V a_2 - r_2 n_2 \frac{\pi}{\delta_T}. \quad (26)$$

An orbit is locally asymptotically stable if and only if all the zeros of the characteristic polynomial have negative real part. We use the

Routh-Hurwitz criterion to check this condition for both G_1 and G_2. The disease-free orbit is locally asymptotically stable if and only if:

$$\delta_T \delta_V a_1 > (1 - \varepsilon) r_1 n_1 \pi \quad \text{and} \quad \delta_T \delta_V a_2 > r_2 n_2 \pi. \tag{27}$$

This is equivalent to $C > C^*$.

For the mutant-only orbit, $V_1 = T_1 = 0$, so that the characteristic polynomial simplifies to:

$$\det(J(T, 0, V_2, 0, T_2) - \lambda I)$$
$$= -G_1 \left((\delta_T + \lambda) G_2 + r_2 V_2 (\delta_V + \lambda)(a_2 + \lambda) \right) \tag{28}$$
$$= -G_1 G_3, \tag{29}$$

where:

$$G_3 = \lambda^3 + (\delta_T + \delta_V + a_2 + r_2 V_2) \lambda^2$$
$$+ ((\delta_V + a_2)(\delta_T + r_2 V_2)) \lambda + r_2 V_2 \delta_V a_2 \tag{30}$$
$$= \lambda^3 + \left(\delta_V + a_2 + \frac{r_2 n_2 \pi}{a_2 \delta_V} \right) \lambda^2 + \left((\delta_V + a_2) \frac{r_2 n_2 \pi}{a_2 \delta_V} \right) \lambda$$
$$+ r_2 n_2 \pi - \delta_T \delta_V a_2. \tag{31}$$

From the Routh-Hurwitz criterion, the mutant-only orbit is locally stable if:

$$\delta_T \delta_V a_2 < r_2 n_2 \pi. \tag{32}$$

This is equivalent to $C < C_2^*$. For the mutant-only orbit, we have $T = \frac{\delta_V a_2}{r_2 n_2}$ and

$$G_1 = \lambda^2 + (a_1 + \delta_V) \lambda + \delta_V \left(a_1 - \frac{(1 - \varepsilon) r_1 n_1}{r_2 n_2} a_2 \right). \tag{33}$$

This gives the second condition for the mutant-only orbit to be stable, namely:

$$r_2 n_2 a_1 > (1 - \varepsilon) r_1 n_1 a_2. \tag{34}$$

That is, $C > C_3^*$.

The mutant strain can only emerge if $C_2^* > C$. For $C > C_2^*$, the trajectory of V_2 does not completely lie in the positive plane, which does not make biological sense. The wild type can only emerge locally around the disease-free orbit if $C > C_1^*$. In the case $C_2^* > C_1^* > C > C_3^*$, it is not only the presence of the CTLs but also the competition with
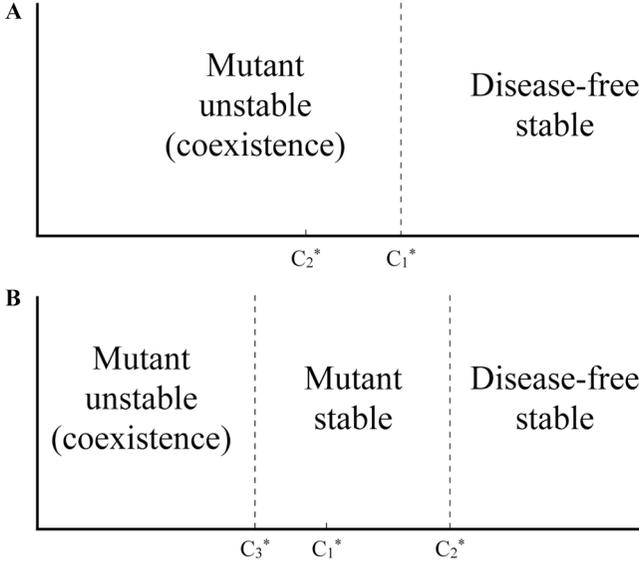
FIGURE 2 Regions of stability. **A.** $C_2^* < C_1^*$. If $C > C_1^*$, the mutant cannot survive on its own, so the disease-free orbit is stable. Conversely, if $C < C_1^*$, both strains coexist. **B.** $C_1^* < C_2^*$. The disease-free orbit is stable if $C > C_2^*$ and unstable otherwise. If $C_3^* < C < C_2^*$, then the mutant survives on its own, while if $0 \leq C < C_3^*$, both strains coexist.

the mutant strain that prevents the emergence of wild-type virus. If neither the disease-free orbit nor the mutant-only orbit are locally stable, then neither the wild-type nor the mutant strain is eliminated. In this case, both strains coexist.

If the vaccine is not strong enough to guarantee $C > C^*$, then it is not possible to eradicate both strains of the virus. If $C_2^* > C_1^*$ and if the vaccine is such that $C_2^* > C > C_3^*$, then the mutant strain may become dominant (Figure 2).

4. GLOBAL STABILITY

The biologically relevant domain $\{(x_1, \ldots, x_n) : x_1 > 0, \ldots, x_n > 0\} \subset \mathbb{R}^n$ $(n \in \mathbb{N})$ is called the positive plane.

4.1. Sufficient Vaccination

From the previous section, we know that a necessary condition for both virus strains to be eradicated is $C > C^*$. Theorem 4.1 tells us that this condition is sufficient.

This result, which was not possible to derive from Smith? and Schwartz (2008), allows us to give a mathematically supported recommendation for the desired amount of CTL cells in the blood to resist HIV.

Theorem 4.1. *The disease-free orbit is globally stable in the positive plane if and only if $C > C^*$, that is, $C > C_1^*$ and $C > C_2^*$.*

Proof. The maximum value of T is the steady-state level before infection, while the minimum value of C must be greater than C^*. Without loss of generality, let T and C equal these values. Thus:

$$T \equiv \frac{\pi}{\delta_T} \tag{35}$$

$$C \equiv C_{\min} > C^*. \tag{36}$$

Fixing T and C at these values ensures optimal growth for the virus. If virus particles are present and if their total number grows, then the amount of healthy T cells falls below its maximal value. This limits the recruitment of new infected cells. As the vaccine is administered at fixed intervals, it stays above its minimal value all the time, only reaching it at that time when the next vaccination occurs.

By fixing T, we lose all orbits made possible by different values of T. However, for $C > C^*$, the mutant-only orbit is not in the positive plane, because $\widehat{V}_2 < 0$. No coexistence orbit can lie in the positive plane as $V_2 < \widehat{V}_2$, due to the competition between the virus strains. Consequently, we restrict the result of this theorem to the positive plane.

As T and C are now fixed, System (1)–(7) simplifies to the linear ordinary differential equation:

$$\begin{pmatrix} V_1' \\ V_2' \\ T_1' \\ T_2' \end{pmatrix} = \begin{pmatrix} -\delta_V & 0 & n_1 & 0 \\ 0 & -\delta_V & 0 & n_2 \\ \frac{(1-\varepsilon)r_1\pi}{\delta_T} & 0 & -a_1 & 0 \\ \frac{\varepsilon r_1\pi}{\delta_T} & \frac{r_2\pi}{\delta_T} & 0 & -a_2 \end{pmatrix} \begin{pmatrix} V_1 \\ V_2 \\ T_1 \\ T_2 \end{pmatrix}. \tag{37}$$

The disease-free orbit is now represented by the only equilibrium $(0, 0, 0, 0)$. A linear system behaves globally just as it behaves locally: a locally stable equilibrium is globally stable. The characteristic function of the matrix of Eq. (37) inherits the eigenvalues from G_1 and G_2; the equilibrium is then globally stable if $C > C_1^*$ and $C > C_2^*$. From Theorem 3.3, the disease-free orbit is not locally stable if $C < C^*$. Then it cannot be globally stable for $C < C^*$.

Regardless of how many HIV virus particles circulate in the patient's blood or what strain these particles are, the minimal required vaccine strength C^* will always be sufficient to control the virus: the vaccine threshold to eradicate both strains of the virus is C^*.

Corollary 4.2. *Whenever $C > C_1^*$, the wild type cannot persist in the positive plane.*

Proof. Following the proof of Theorem 4.1, the differential equations for V_2 and T_2 do not influence the wild type. If we reduce Eq. (37) to:

$$\begin{pmatrix} V_1' \\ T_1' \end{pmatrix} = \begin{pmatrix} -\delta_V & n_1 \\ \frac{(1-\varepsilon)r_1\pi}{\delta_T} & -a_1 \end{pmatrix} \begin{pmatrix} V_1 \\ T_1 \end{pmatrix}, \tag{38}$$

the equilibrium $(0,0)$ is locally stable if $C > C_1^*$. With the over-estimations underlying Eq. (37), the result follows.

4.2. Insufficient Vaccination

We investigate the effect of insufficient vaccination. In the previous section, the mutant strain could become dominant if the vaccine only results in intermediate CTL levels.

Wolkowicz and Lu (1992) describe the competition between the species X_1 and X_2 for the resource S as:

$$S'(t) = (S^0 - S)D - \frac{q_1}{y_1}X_1 S - \frac{q_2}{y_2}X_2 S \tag{39}$$

$$X_1'(t) = (q_1 S - D_1)X_1 \tag{40}$$

$$X_2'(t) = (q_2 S - D_2)X_2. \tag{41}$$

All the parameters, S^0, D, q_i, y_i, D_i, $i = 1, 2$, are positive constants. Given that the competitors have different fitness levels, one expects that the weaker competitor cannot survive.

Definition 4.3. Define λ_i, $i = 1, 2$, as:

$$\lambda_i = \frac{D_i}{q_i}. \tag{42}$$

These positive parameters are called break-even concentrations.

Lemma 4.4. *Assume that $\lambda_2 < \lambda_1 < S^0$. Then the equilibrium value*

$$\left(\lambda_2, 0, \frac{y_2 D(S^0 - \lambda_2)}{D_2} \right), \tag{43}$$

is globally stable in the positive plane.

Proof. In Wolkowicz and Lu (1992).

If $C_2^* > C$, then the break-even concentration of T_2 is smaller than the corresponding S^0, and if $C > C_3^*$ then the break-even concentration of T_2 is smaller than the break-even concentration of T_1.

Theorem 4.5. *The mutant-only orbit is globally stable in the positive plane if and only if the vaccine is taken at intermediate strength, that is, $C_2^* > C > C_3^*$.*

Proof. By Lemma 3.2, either

$$C_2^* > C > C_1^* > C_3^*, \tag{44}$$

or

$$C_2^* > C_1^* > C > C_3^*. \tag{45}$$

If inequality (44) holds, Corollary 4.2 implies that the mutant-only orbit is globally stable for all positive starting values.

If inequality (45) holds, the dynamics of the virus particles are fast compared to the dynamics of T cells (Perelson et al., 1996), so the system is at quasi steady state and $V_i = \frac{n_i}{\delta_V} T_i$, $i = 1, 2$.

Then our model reads:

$$T'(t) = \pi - \frac{r_1 n_1}{\delta_V} T_1 T - \frac{r_2 n_2}{\delta_V} T_2 T - \delta_T T \tag{46}$$

$$T_1'(t) = \left(\frac{(1 - \varepsilon) r_1 n_1}{\delta_V} T - a_1 \right) T_1 \tag{47}$$

$$T_2'(t) = \frac{\varepsilon r_1 n_1}{\delta_V} T_1 T + \left(\frac{r_2 n_2}{\delta_V} T - a_2 \right) T_2. \tag{48}$$

This system describes a competition between T_1 and T_2 for the resource T. An overestimation in favor of the wild type is to drop the mutation term in the differential equation of T_2. This leads to:

$$T'(t) = \pi - \frac{r_1 n_1}{\delta_V} T_1 T - \frac{r_2 n_2}{\delta_V} T_2 T - \delta_T T \tag{49}$$

$$T_1'(t) = \left(\frac{(1 - \varepsilon) r_1 n_1}{\delta_V} T - a_1 \right) T_1 \tag{50}$$

$$T_2'(t) = \left(\frac{r_2 n_2}{\delta_V} T - a_2 \right) T_2, \tag{51}$$

which is of the same form as System (39)–(41).

The break-even concentrations are:

$$\lambda_1 = \frac{a_1 \delta_V}{(1-\varepsilon)r_1 n_1} \quad \text{and} \quad \lambda_2 = \frac{a_2 \delta_V}{r_2 n_2}. \tag{52}$$

Due to $C_1^* > C > C_3^*$, $\lambda_2 < \lambda_1 < S^0$, and, with Lemma 4.4, the wild type is driven extinct.

For the necessity part, Theorem 3.3 implies that if $C_2^* > C > C_3^*$ does not hold, then the mutant-only orbit is not locally stable and cannot be globally stable.

If $C_2^* > C > C_1^* > C_3^*$, the same simplification as in the proof leads to a break-even concentration λ_1 of the wild-type with $\lambda_1 > S^0$. Wolkowicz and Lu (1992) show that again the wild-type is driven extinct.

The values λ_1 and λ_2 are functions of the amount of vaccination, the fitness cost and a measure of escape mutation. If $\lambda_2 < \lambda_1$, then $C > C_3^*$. At intermediate values ($C_2^* > C > C_3^*$), the mutant strain becomes dominant.

Local stability is needed to establish global stability during the proof of Theorem 4.1, because the lack of local stability for the mutant-only orbit when $C > C^*$ implies that global stability is impossible. Similarly, the disease-free orbit is not locally stable if $C < C^*$ and cannot be globally stable. In the proof of Theorem 4.5, the mutant-only orbit is not locally stable when $C_2^* > C > C_3^*$ and hence cannot be globally stable.

5. THE EFFECTS OF PARTIAL ADHERENCE

In the two previous sections, we established the critical level of CTLs necessary to control the virus. We also saw that resistance may emerge if the vaccine results in intermediate CTL levels, where the threshold is determined by a combination of vaccine strength, fitness cost, and escape mutation rate. However, a postinfection CTL vaccine is administered at regular intervals so that the actual CTL amount is not constant. In this section, we determine the maximal time between the vaccinations, depending on the vaccine strength, to ensure that the CTL amount always exceeds the desired level. Establishing this maximal time frame allows us to recommend a CTL vaccine treatment.

As with antiretroviral drugs (Smith, 2006; Miron and Smith?, 2010), taking extended breaks from the vaccine may result in the development of resistance. We examine how many vaccinations may be missed before the virus becomes uncontrolled and how

many vaccinations must then be taken in succession to return to preinterruption CTL levels.

5.1. Perfect Adherence

The moments of vaccination are denoted by t_k and the CTL count immediately after the kth vaccination is denoted by $C(t_k^+)$. From the impulsive differential equation for C, we have:

$$C(t) = C(t_k^+)e^{\int_{t_k}^t (\alpha(T_1(u)+T_2(u))-\delta_C)du} \qquad (t_k < t < t_{k+1}) \qquad (53)$$

$$> C(t_k^+)e^{-\delta_C(t-t_k)}, \qquad (54)$$

as T_1 and T_2 are non-negative.

If the vaccine is successful, then the approximation in Eq. (54) is accurate, as T_1 and T_2 are very small. At the beginning of the vaccine treatment, however, it may be a coarse approximation, as Figure 3 shows.

If the vaccine is taken at regular intervals with length τ, we calculate an upper bound on the length of that interval. The
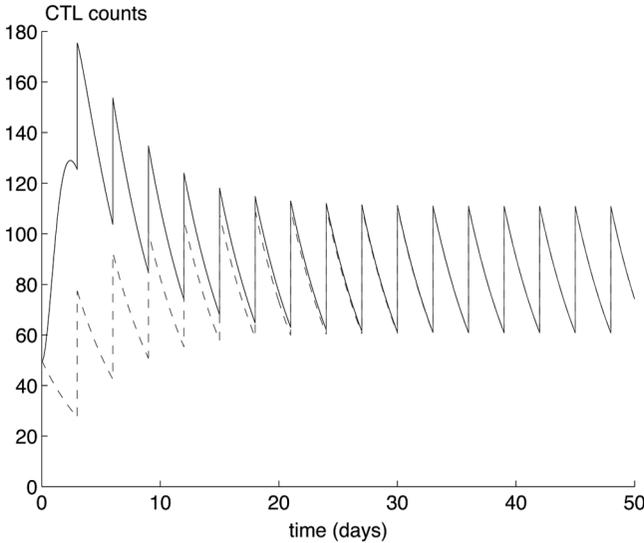

FIGURE 3 CTL counts using the exact value, calculated from Eq. (53) (solid curve) and the approximation, calculated from Eq. (54) (dashed curve). The approximation is a coarse one for initial times, but quickly comes into phase as the virus is controlled.

underestimate

$$C(t) = C(t_k^+)e^{-\delta_C(t-t_k)} \quad t_k < t < t_{k+1}, \tag{55}$$

is a conservative measure of the ability of CTLs to control the virus.

If $C(0) = 0$, we have:

$$C(t_1^+) = \widetilde{C} \tag{56}$$

$$C(t_2^-) = \widetilde{C}e^{-\delta_C\tau} \tag{57}$$

$$C(t_2^+) = \widetilde{C}(1 + e^{-\delta_C\tau}) \tag{58}$$

$$C(t_3^-) = \widetilde{C}(1 + e^{-\delta_C\tau})e^{-\delta_C\tau} \tag{59}$$

$$C(t_3^+) = \widetilde{C}(1 + e^{-\delta_C\tau} + e^{-2\delta_C\tau}) \tag{60}$$

$$\vdots \tag{61}$$

$$C(t_p^+) = \widetilde{C}(1 + e^{-\delta_C\tau} + \cdots + e^{-(p-1)\delta_C\tau}) = \widetilde{C}\frac{1 - e^{-p\delta_C\tau}}{1 - e^{-\delta_C\tau}} \tag{62}$$

$$\lim_{p \to \infty} C(t_p^+) = \frac{\widetilde{C}}{1 - e^{-\delta_C\tau}}. \tag{63}$$

Trajectories converge to an impulsive periodic orbit with endpoints:

$$\frac{\widetilde{C}}{1 - e^{-\delta_C\tau}} \quad \text{and} \quad \frac{\widetilde{C}e^{-\delta_C\tau}}{1 - e^{-\delta_C\tau}}. \tag{64}$$

Assuming perfect adherence, CTL levels after the nth vaccination are approximately:

$$C(t_n^+) = \frac{\widetilde{C}}{1 - e^{-\delta_C\tau}}. \tag{65}$$

For perfect adherence, to control the virus and avoid resistance, the minimum value of the periodic orbit must exceed the threshold. Thus,

$$e^{-\delta_C\tau} > \frac{C^*}{\widetilde{C} + C^*}.$$

So resistance is avoided if

$$\tau < \tau_{\max} \equiv \frac{1}{\delta_C} \ln\left(\frac{\widetilde{C} + C^*}{C^*}\right). \tag{66}$$

We have an upper bound for the maximum time that individuals can wait between vaccinations: a CTL vaccine taken at regular time intervals with the length of τ, with $\tau < \tau_{\max}$, can theoretically

eradicate both strains of the virus. This is consistent with the less specific finding in Smith? and Schwartz (2008). Moreover, if the time τ between two vaccinations satisfies $\tau > \tau_{\max}$, then the mutant strain may persist. The upper bound $\tau_{\max}$ depends on the eradication threshold $C^* = \max\{C_1^*, C_2^*\}$ and on the parameters for both the wild-type and mutant strains.

5.2. Imperfect Adherence

We assume that $\tau \le \tau_{\max}$ and that sufficient vaccinations have been taken so that C is approximately on the periodic orbit. If h vaccinations are subsequently missed, we have:

$$C(t_{n+h}^-) = \frac{\widetilde{C}e^{-h\delta_C\tau}}{1 - e^{-\delta_C\tau}}. \tag{67}$$

Resistance is avoided if $C(t_{n+h}^-) > C^*$. Hence,

$$\frac{\widetilde{C}e^{-h\delta_C\tau}}{1 - e^{-\delta_C\tau}} > C^* \Rightarrow h < \frac{1}{\delta_C\tau} \ln \frac{\widetilde{C}}{C^*(1 - e^{-\delta_C\tau})}. \tag{68}$$

This gives an upper bound on the total number of vaccinations that may be missed before resistance emerges.

To return to CTL levels approximating preinterruption values when k vaccinations are taken in succession, we require:

$$C(t_{n+h+k}^-) > \frac{\widetilde{C}e^{-\delta_C\tau}}{1 - e^{-\delta_C\tau}} - \nu, \tag{69}$$

for some level of tolerance ν. Using impulsive theory,

$$C(t_{n+h}^+) = \widetilde{C} + \frac{\widetilde{C}e^{-h\delta_C\tau}}{1 - e^{-\delta_C\tau}} \tag{70}$$

$$C(t_{n+h+1}^-) = \widetilde{C}e^{-\delta_C\tau}\left(1 + \frac{e^{-h\delta_C\tau}}{1 - e^{-\delta_C\tau}}\right) \tag{71}$$

$$C(t_{n+h+1}^+) = \widetilde{C}\left(1 + e^{-\delta_C\tau} + \frac{e^{-(h+1)\delta_C\tau}}{1 - e^{-\delta_C\tau}}\right) \tag{72}$$

$$C(t_{n+h+2}^-) = \widetilde{C}e^{-\delta_C\tau}\left(1 + e^{-\delta_C\tau} + \frac{e^{-(h+1)\delta_C\tau}}{1 - e^{-\delta_C\tau}}\right) \tag{73}$$

$$\vdots \tag{74}$$

$$C(t_{n+h+k}^-) = \widetilde{C}e^{-\delta_C\tau}\left(1 + e^{-\delta_C\tau} + \cdots + e^{-(k-1)\delta_C\tau} + \frac{e^{-(h+k-1)\delta_C\tau}}{1 - e^{-\delta_C\tau}}\right) \tag{75}$$

$$= \widetilde{C}e^{-\delta_C \tau}\left(\frac{1-e^{-k\delta_C \tau}}{1-e^{-\delta_C \tau}} + \frac{e^{-(h+k-1)\delta_C \tau}}{1-e^{-\delta_C \tau}}\right). \tag{76}$$

Hence:

$$C(t_{n+h+k}^{-}) - \frac{\widetilde{C}e^{-\delta_C \tau}}{1-e^{-\delta_C \tau}} = \frac{\widetilde{C}e^{-(k+1)\delta_C \tau}(e^{-(h-1)\delta_C \tau}-1)}{1-e^{-\delta_C \tau}} > -\nu. \tag{77}$$

The required number of vaccinations to return to within ν of perfect adherence satisfies

$$k > \frac{1}{\delta_C \tau}\ln\left(\frac{\widetilde{C}(1-e^{-(h-1)\delta_C \tau})}{\nu(1-e^{-\delta_C \tau})}\right) - 1, \tag{78}$$

which is the minimum number of vaccinations which must be taken after a vaccination break. Thus, a patient who missed h vaccinations must take at least k vaccinations in succession, in order to return to the periodic orbit (Miron and Smith?, 2010).

6. SIMULATIONS

Table 1 presents the parameters used in the simulations. These parameters give a viral load a little higher than HIV levels in humans but closer to SIV level in rhesus monkeys.

In the absence of vaccination, the CTL count approaches a stable equilibrium (Figure 4A). Under regular vaccinations, the CTL count oscillates in an impulsive periodic orbit (Figure 4B). The results are qualitatively unchanged between low-level and no vaccination.

When $C_2^* < C_1^*$, the mutant and wild type can coexist if vaccination is low, but nonzero. Both values approach a stable orbit (Figures 5A and 6A). When the vaccine is taken regularly, both wild type and mutant oscillate in an impulsive periodic orbit. The disease-free equilibrium is unstable, but the mutant exists only at low levels. Uninfected T cell counts oscillate at around 1,721 cells per microliter, less than the 1,800 cells per microliter for the uninfected patient.

When $C_2^* > C_1^*$, the mutant persists at high levels, while the wild type is driven to extinction if vaccination is low or zero. The mutant oscillates in an impulsive periodic orbit (Figures 5B and 6B). The disease-free equilibrium is also unstable, in this case. Uninfected T cell counts oscillate at around 1,726 cells per microliter, less than the 1,800 cells per microliter for the uninfected patient, but slightly higher than for low vaccination. These results show that, for low-level vaccination, a combination of fitness cost and escape rate determines which viral type dominates.

TABLE 1 Parameter Values

| Parameter	Value	Description	Reference
π	$180\,\text{cells}\,\mu l^{-1}\,\text{day}^{-1}$	Production rate of T cells	Smith and Wahl (2005)
p_1	$0.05\,\text{cells}^{-1}\,\mu l\,\text{day}^{-1}$	Wild-type clearance rate	Boer (de) and Perelson (1998)
p_2	0.04–$0.045\,\text{cells}^{-1}\,\mu l\,\text{day}^{-1}$	Mutant clearance rate	Varied
r_1	$2.4 \times 10^{-5}\,\text{cells}^{-1}\,\mu l\,\text{day}^{-1}$	Wild-type infection rate	Rong et al. (2007)
r_2	$2.28 \times 10^{-5}\,\text{cells}^{-1}\,\mu l\,\text{day}^{-1}$	Mutant infection rate	Set at $0.95r_1$
δ_T	$0.1\,\text{day}^{-1}$	Death rate of susceptible T cells	Smith and Wahl (2005)
δ_I	$0.5\,\text{day}^{-1}$	Death rate of infected T cells	Essunger and Perelson (1994)
δ_V	$3\,\text{day}^{-1}$	Clearance rate of the virus	Perelson and Nelson (1999)
δ_C	$0.2\,\text{day}^{-1}$	Death rate of CTLs	Boer (de) and Perelson (1998)
ε	3×10^{-5}	Mutation rate	Mansky and Temin (1995)
α	$0.067\,\text{cells}^{-1}\,\mu l\,\text{day}^{-1}$	CTL proliferation rate	Boer (de) and Perelson (1998)
n_1	$262.5\,\text{day}^{-1}$	Production rate of wild-type virus	Smith and Wahl (2005)
n_2	$249.375\,\text{day}^{-1}$	Production rate of mutant virus	Set at $0.95n_1$
$\tilde{C}$	0–$50\,\text{cells}\,\mu l^{-1}$	Vaccine strength	Varied
τ	3–5 days	Vaccine period	Varied
C_1^*	$65.60\,\text{cells}\,\mu l^{-1}$	Threshold for the wild-type strain behavior	Definition 3.1
C_2^*	64.70–$72.79\,\text{cells}\,\mu l^{-1}$	Threshold for the mutant strain behavior	Definition 3.1, varied
C^*	65.60–$72.79\,\text{cells}\,\mu l^{-1}$	$\max\{C_1^*, C_2^*\}$	Definition 3.1, varied
C_3^*	19.01–$771.43\,\text{cells}\,\mu l^{-1}$	Threshold for the competition between the virus strains	Definition 3.1, varied
$\tau_{\max}$	0–2.83 day(s)	Maximal vaccination period	Eq. (66)
h	3.18–3.67	Maximal number of doses that can be missed before resistance emerges (for $\tau = 2$)	Eq. (68), varied
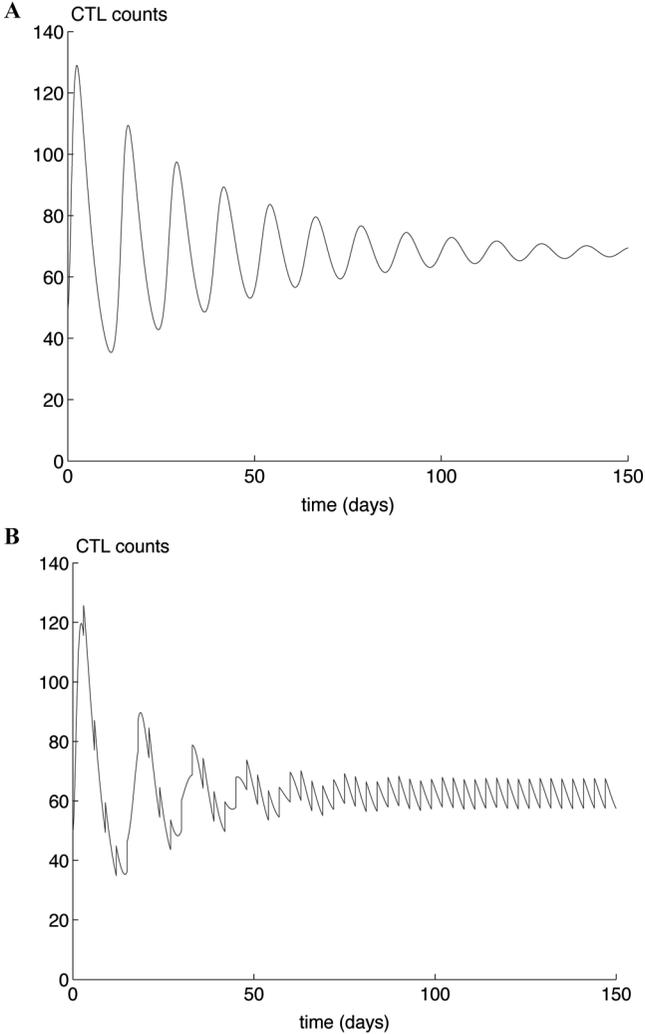
FIGURE 4 CTL counts when $C_2^* < C_1^*$ with and without low-level vaccination. **A.** In the absence of the vaccine, the CTL counts oscillate and approach an equilibrium value of approximately 61 cells/μL. All parameters are as in Table 1, with $p_2 = 0.045$ and $C^i = 0$. **B.** When the vaccine is taken every three days, stimulating 10 cells/μL, the CTL count stabilizes in an impulsive periodic orbit, oscillating around an average of approximately 63 cells/μL.
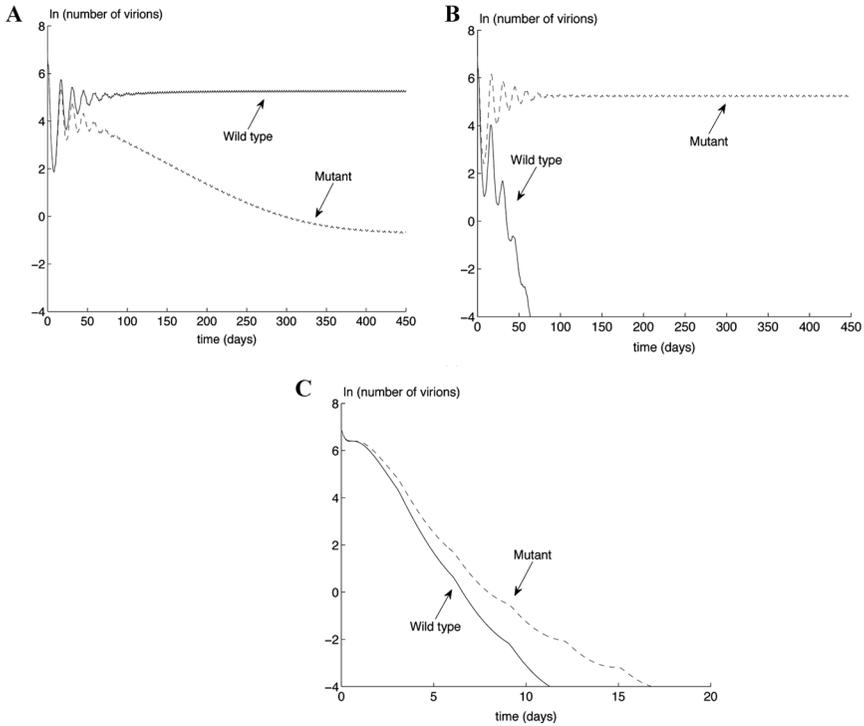
FIGURE 5 Vaccination may result in either the wild-type or mutant strains dominating, or both strains may be eradicated. **A.** The case $C_2^* < C_1^*$, with low-level vaccination. The two populations coexist, with high levels (approximately 190 virions/μL) of wild-type virus and low, but nonzero, levels of mutant (approximately 0.5 virions/μL). All parameters are as in Table 1, with $p_2 = 0.045\,\mu l^{-1}\,\text{day}^{-1}$ and $C^i = 10\,\text{cells}/\mu L$. Both populations oscillate around their averages, in an impulsive periodic orbit. **B.** The case $C_1^* < C_2^*$, with low-level vaccination. The mutant exists at high levels (approximately 191 virions/μL), but the wild type is eradicated. The mutant population oscillates around its average, in an impulsive periodic orbit. All parameters are as in the previous case, except that $p_2 = 0.04\,\mu l^{-1}\,\text{day}^{-1}$. **C.** If the vaccination frequency is increased, then we have eradication of both strains. Parameters were the same as the previous case, except that the vaccine strength was increased to 50 cells/μL.

If the vaccine is sufficiently strong, both strains are driven to extinction, assuming perfect adherence (Figures 5C and 6C). In this case, the total number of uninfected T cells dips briefly, but then quickly returns to preinfection levels.
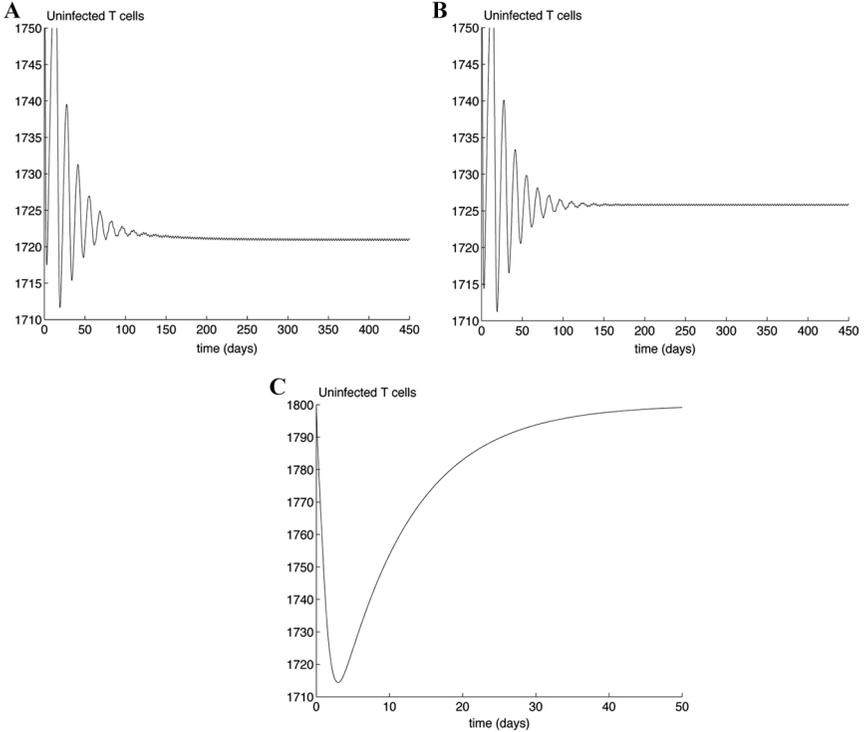
FIGURE 6 Uninfected T cell counts corresponding to the three cases in Figure 5. **A.** The case $C_2^* < C_1^*$, with low-level vaccination. **B.** The case $C_1^* < C_2^*$, with low-level vaccination. **C.** If the vaccination frequency is increased, then the number of uninfected cells returns to maximal values.

Imperfect adherence may allow the mutant to persist at low, but nonnegligible levels (Figure 7). In this case, the wild type is controlled, while the mutant can rebound. Here, vaccination occurred every three days, but every fourth vaccination was extended by two further days. All other parameters are identical to those of Figure 5C.

Figure 8 shows that, in some situations when the vaccine is less effective against mutant strains, the frequency of vaccine administration becomes important. Figure 8 shows the densities of wild-type and mutant viruses for 2-week, 10-day, and 1-week intervals of vaccine adminstration. Wild-type and mutant viruses compete at the beginning and eventually one of them becomes dominant. For longer vaccine intervals (Figure 8, top), the wild type dominates. If the frequency of vaccine administration is increased, due to higher
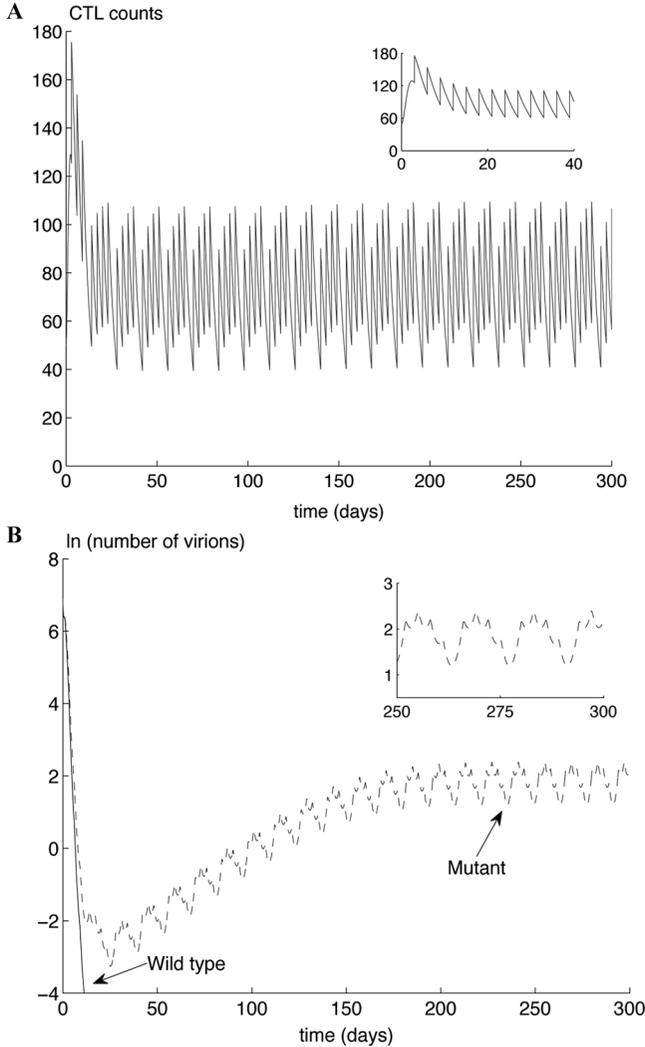
FIGURE 7 Imperfect adherence may cause the mutant to persist. **A.** CTL counts, using the same data as Figure 4B, except that every fourth vaccination happened five days apart, rather than three. Inset: CTL counts for perfect adherence. The CTL counts are barely affected by the treatment interruption. **B.** Virion counts for the case of imperfect adherence. The same data was used as Figure 5C, except for the treatment interruption. Rather than eradication, the mutant now persists at low, but nonzero, levels (approximately $7.4\,\text{virions}/\mu L$). Inset: Long-term behavior of the mutant.
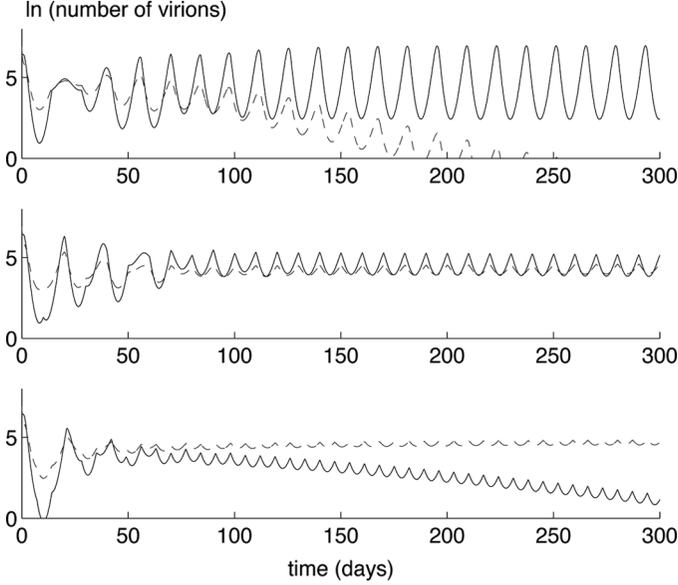
FIGURE 8 Frequency of vaccine administration plays an important role. Dynamics of wild-type (solid curve) and mutant (dashed curve) viruses with intervals of vaccine administration lasting fourteen days (top), ten days (middle) and seven days (bottom). All parameters are as in Table 1, except that $p_2 = 0.015\,\mu l^{-1}\,\text{day}^{-1}$, $r_2 = 1.5 \times 10^{-5}\,\mu l\,\text{day}^{-1}$ and $n_2 = 165.6\,\text{day}^{-1}$.

selection pressure of CTLs, the mutant virus dominates (Figure 8, bottom).

For Figure 9, the data are identical to those of Figure 5A, except that the moment of vaccination fluctuates. The variation in vaccination times are drawn from a normally distributed random variable, scaled so that the mean is the prescribed vaccination time and the standard deviation is half a day. The fluctuations can give the mutant a competitive advantage.

7. CONCLUSION

A CTL vaccine can theoretically eradicate both the wild-type and resistant strains of the virus, if taken with sufficient frequency, at regular intervals. However, if the vaccine is not taken at regular intervals, then the total number of vaccinations that can be missed is given by Eq. (68), and the total number of subsequent vaccinations which must be taken is given by Eq. (78).
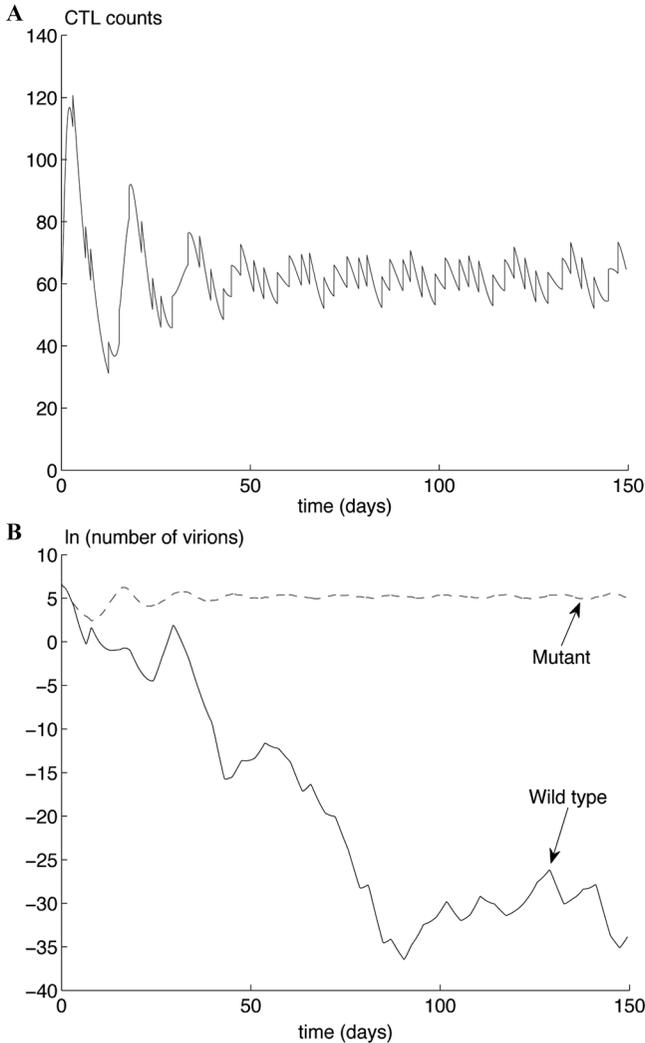
FIGURE 9 Fluctuations in the vaccination time may reverse the competition. **A.** CTL counts. The average is the same as Figure 4B, although the standard deviation is not. **B.** Viral load, using the same data as Figure 5A and perfect adherence, except that the vaccination time fluctuates with standard deviation of 12 hours (one sixth of the vaccination interval). In this case, the fluctuations result in the mutant outcompeting the wild type, reversing the outcome.

Approximating the time to peak by an instantaneous change is a reasonable approximation (Smith? and Schwartz, 2008). While CTLs themselves may be infected, CTLs are infected on a significantly smaller scale than $CD4^+$ T cells, so we ignore such effects here. We assume homogeneity within the body so that every milliliter of blood behaves the same. We have also ignored back mutation and assumed that the mutant strain can be partially controlled by the vaccine.

The results depend critically upon the clearance rate p_2 of the mutant. When $p_2 \to 0$, $C_2^* \to \infty$, $C^* \to \infty$, and the right-hand side of Eq. (66) approaches zero. If the mutant escape results in an inability of the CTLs to sufficiently clear the mutant strain of the virus, then the minimum vaccination interval may become too small for patients to adhere to. In this case, the escape mutation may cause a vaccine failure. If a vaccine cannot control the mutant at all, then it is of little use once mutation occurs.

The total number of uninfected T cells at low levels of vaccination when $C_2^* > C_1^*$ is slightly higher (1,726 per microliter) than the total number of uninfected T cells at low levels of vaccination when $C_1^* > C_2^*$ (1,721 per microliter). Vaccination controls the wild type but not the mutant when $C_2^* > C_1^*$, whereas vaccination fully controls neither the wild type nor the mutant when $C_1^* > C_2^*$. When $C_2^* > C_1^*$, uninfected T cells are only depleted by the mutant. Although the uninfected T cell count is slightly higher in this case, this is not a desirable situation. The development of resistance results in a lowered ability to control the virus, as well as the likelihood of spreading the resistant strain to new partners and subsequent development of further resistant stains that cannot be controlled (Smith? et al., 2010).

It is better to have both higher uninfected T cell counts and lower total viral load $V_1 + V_2$. These levels are affected by the presence of the mutant virus and CTL escape. Rather than looking at total viral levels, it is better to look at viruses one by one, as all viruses are not equally harmful and not equally responsive to the CTL vaccine. We thus evaluate the ability of the impulsive CTL vaccine to control both virus strains. In the absence of clinical data, we offer a possible starting point for new clinical tests to investigate CTL vaccines for HIV therapy.

In the absence of vaccination, the results are analogous. With no impulsive periodic orbits, there is a disease-free equilibrium (with $C = 0$) and a mutant-only equilibrium. Both equilibria are unstable, from Theorem 3.3.

We also examined the influence of the vaccination time. Fluctuations in the vaccination time may reverse the expected dominating strain (Figure 9). This can occur even if no vaccinations

are missed. Because the intervals between vaccinations may be significantly larger than those of antiretroviral drugs, the moment of vaccination may vary substantially; a patient who is supposed to receive a vaccine every Tuesday, but who sometimes receives the vaccine on Monday or Wednesday, may develop resistance even if she never misses a vaccination. This highlights the importance of rigorous adherence not only to the number of vaccinations, but also to the precise timing.

Requiring several thousand (or million) patients to report to a health center at regular intervals for their CTL vaccine could be an enormous drain on the health-care system. Consequently, we recommend that such a vaccine should be available for self-administration by patients, provided patients are fully informed about the consequences of imperfect adherence.

A CTL vaccine can be advantageous in combating both wild-type and mutant strains, even if only partially efficacious. The vaccination pattern is crucial: sufficiently frequent vaccination, taken at precise times, can control the virus. Interruptions in the vaccination schedule for finite intervals need not be disastrous, so long as not too many vaccinations are missed and that sufficient vaccinations are taken subsequently. The timing of vaccinations is critical: too much variation in the vaccination time may lead to the emergence of resistance, even if no vaccinations are missed.

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REFERENCES

Altice, F.L. and Friedland, G.H. (1998). The era of adherence to HIV therapy. *Annals of Internal Medicine*, *129*: 503–505.

Amara, R.R., Villinger, F., Altman, J.D., et al. (2001). Control of a mucosal challenge and prevention of clinical AIDS in rhesus monkeys by a multiprotein DNA/MVA vaccine. *Science*, *292*: 69–74.

Amara, R.R., Villinger, F., Altman, J.D., et al. (2002). Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Vaccine*, *20*: 1949–1955.

Asquith, B. (2008). The evolutionary selective advantage of HIV-1 escape variants and the contribution of escape to the HLA-associated risk of AIDS progression. *PLoS One*, *3*(10): e3486.

Ball, C.L., Gilchrist, M.A., and Coombs, D. (2007). Modeling within-host evolution of HIV: Mutation, competition and strain replacement. *Bulletin of Mathematical Biology*, *69*: 2361–2385.

Barouch, D.H., Kunstman, J., Glowczwskie, J., et al. (2003). Viral escape from dominant simian immunodeficiency virus epitope-specific cytotoxic T lymphocytes in DNA-vaccinated rhesus monkeys. *Journal of Virology*, *77*: 7367–7375.

Barouch, D.H., Kunstman, J., Kuroda, M.J., et al. (2002). Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. *Nature*, *415*: 335–339.

Barouch, D.H., Santra, S., Schmitz, J.E., et al. (2000). Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. *Science*, *290*: 486–492.

Bartlett, J.A. (2002), Addressing the challenges of adherence. *Journal of Acquired Immune Deficiency Syndrome*, *29*(Suppl. 1): 2–10.

Boer (de), R.J. and Perelson, A.S. (1998). Target cell limited and immune control models of HIV infection: A comparison. *Journal of Theoretical Biology*, *190*: 201–214.

Collins, K.L., Chen, B.K., Kalams, S.A., et al. (1998). HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature*, *391*: 397–401.

Essunger, P. and Perelson, A.S. (1994). Modeling HIV infection of CD4$^+$ T-cell subpopulations. *Journal of Theoretical Biology*, *170*(4): 367–391.

Gerdil, C. (2003). The annual production cycle for influenza vaccine. *Vaccine*, *21*(16): 1776–1779.

Goulder, P.J.R. and Watkins, D.I. (2004). HIV and SIV CTL escape: Implications for vaccine design. *Nature Reviews Immunology*, *4*: 630–640.

Jamieson, B.D., Yang, O.O., Hultin, L., et al. (2003). Epitope escape mutation and decay of human immunodeficiency virus type 1-specific CTL responses. *Journal of Immunology*, *171*: 5372–5379.

Jin, X., Bauer, D.E., Tuttleton, S.E., et al. (1999). Dramatic rise in plasma viremia after CD8$^+$ T cell depletion in simian immunodeficiency virus-infected macaques. *Journal of Experimental Medicine*, *189*: 991–998.

Klein, M.R., van der Burg, S.H., Pontesilli, O., et al. (1998). Cytotoxic T lymphocytes in HIV-1 infection: A killing paradox? *Immunology Today*, *19*(7): 317–324.

Korthals Altes, H., Wodarz, D., and Jansen, V.A.A. (2002). The dual role of CD4 T helper cells in the infection dynamics of HIV and their importance for vaccination. *Journal of Theoretical Biology*, *214*(4): 633–646.

Mansky, L.M. and Temin, H.M. (1995). Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *Journal of Virology*, *69*: 5087–5094.

Miron R.E and Smith?, R.J. (2010). Modelling imperfect adherence to HIV induction therapy. *BMC Infectious Diseases*, *10*: 6.

Perelson, A.S. and Nelson, P.W. (1999). Mathematical analysis of HIV-1 dynamics in vivo. *SIAM Review*, *41*: 3–44.

Perelson, A.S., Neumann, A.U., Markowitz, M., et al. (1996). HIV-1 dynamics in vivo: Virion clearance rate, infected cell lifespan, and viral generation time. *Science*, *271*: 1582–1586.

Rong, L., Feng, Z., and Perelson, A.S. (2007). Emergence of HIV-1 drug resistance during antiretroviral treatment. *Bulletin of Mathematical Biology*, *69*: 2027–2060.

Schmitz, J.E., Kuroda, M.J., Santra, S., et al. (1999). Control of viremia in simian immunodeficiency virus infection by CD8$^+$ lymphocytes. *Science*, *283*: 857–860.

Smith, R.J. (2006). Adherence to antiretroviral HIV drugs: How many doses can you miss before resistance emerges? *Proceedings of the Royal Society B*, *273*: 617–624.

Smith?, R.J., Okano, J.T., Kahn, J.S., et al. (2010). Evolutionary dynamics of complex networks of HIV drug-resistant strains: The case of San Francisco. *Science*, *327*(5966): 697–701.

Smith?, R.J. and Schwartz, E.J. (2008). Predicting the potential impact of a cytotoxic T-lymphocyte HIV vaccine: How often should you vaccinate and how strong should the vaccine be? *Mathematical Biosciences*, *212*: 180–187.

Smith, R.J. and Wahl, L.M. (2005). Drug resistance in an immunological model of HIV-1 infection with impulsive drug effects. *Bulletin of Mathematical Biology*, *64*(4): 783–813.

Smith, S.M. (2004). HIV CTL escape: At what cost? *Retrovirology*, *1*: 8.

Wolkowicz, G.S.K. and Lu, Z. (1992). Global dynamics of a mathematical model of competition in the chemostat: General response functions and differential death rates. *SIAM Journal on Applied Mathematics*, *52*(1): 222–233.