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A Mathematical Model of HIV dynamics Treated with a Population of Gene Edited Hematopoietic Progenitor Cells Exhibiting Threshold Phenomenon

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The use of CRISPR/Cas gene editing technology has the potential to excise the CCR5 gene from hematopoeitic progenitor cells, rendering their differentiated CD4+ T cell descendants HIV resistant. In this manuscript, we describe the development of a mathematical model to mimic the therapeutic potential of CRISPR/Cas gene editing of hematopoietic progenitor cells to produce a class of HIV resistant CD4+ T cells. We define the requirements for the permanent suppression of viral infection using gene editing as a novel therapeutic approach. We develop nonlinear ordinary differential equation (ODE) models to replicate HIV production in an infected host, incorporating the most appropriate aspects found in the many existing clinical models of HIV infection, and extend this model to include compartments representing immune cells made resistant to HIV infection by the CRISPR/Cas intervention. Through an analysis of model equilibria and stability and computation of R_0 for both treated and untreated infections, we show that the proposed therapy has the potential to suppress HIV infection indefinitely and return CD4-positive (CD4+) T cell counts to normal levels. A computational study for this treatment shows the potential for a successful "functional cure" of HIV. A sensitivity analysis illustrates the consistency of numerical results with theoretical results and highlights the parameters requiring better biological justification. Simulations of varying levels production of HIV resistant CD4+ T cells and varying immune enhancements as the result of these indicate a clear threshold response of the model, and a range of treatment parameters resulting in a return to normal CD4+ T cell counts.

Keywords: CRISPR/Cas, HIV, Mathematical Model, gene editing, hematopoietic progenitor cell, sensitivity analysis, threshold phenomena

1. Introduction

HIV infection of CD4-positive (CD4+) T cells leads to their rapid decline and compromises the host's immune system leading to death of the infected patient from infection or cancer (Okoye &

Picker (2013)). In addition, HIV infection of resting memory CD4+ T cells and some subsets of macrophages results in the development of latent reservoirs of HIV-infected cells that remain dormant in tissues such as the gut and brain, but can become reactivated to produce new viral particles (Sebastian & Collins (2014), Persaud et al. (2000)).

Anti-viral drugs cause the suppression of viral replication and have allowed most HIV-infected individuals to live a normal life-span (Collaboration et al. (2008)). However, many of the drugs in the anti-viral cocktail are toxic to major organs (Carr (2003)). Moreover, none of the current drugs can remove the integrated viral genome, so patients must remain on anti-retroviral therapy for life because even brief interruptions of this therapy result in the resumption of viral replication leading to high levels of circulating virus (for Management of Antiretroviral Therapy (SMART)). There is an urgent need to develop therapies that can allow patients to safely cease anti-viral therapy.

Gene editing using CRISPR/Cas has emerged as an exciting new mechanism to cleave the integrated HIV genome in infected cells, and also to destroy cellular genes required for viral binding and internalization including CCR5 (Lebbink et al. (2017), Tebas et al. (2014), Kaminski et al. (2016), Huang & Nair (2017)). Several methodologies to achieve gene editing have been developed including the use of zinc-finger nuclease (ZFN)(Durand & Siliciano (2014)), transcription activator-like effector nuclease (TALEN) (Strong et al. (2015)), and clustered repetitive interspersed palindromic repeats /CRISPR-associated (CRISPR/Cas) (Huang & Nair (2017)). Both ZFN and TALEN require a unique enzyme to cleave a specific gene sequence, whereas CRISPR/Cas makes use of a complementary sequence of RNA, called a guide RNA, together with the DNA cleaving enzyme (Cas) (Hsu et al. (2014)). CRISPR/Cas has emerged as the most efficient approach to achieve gene editing as it only requires the development of unique guide RNA sequences to bind to regions within the targeted gene sequence, and this RNA molecule guides the Cas enzyme to mediate a double-stranded cleavage cut essentially mutating the DNA and preventing gene transcription.

In the case of HIV-1, CRISPR/Cas has been developed to target and destroy viral genes such as the integrated HIV genome, to prevent the production of new viral particles. The permanent integration of the CRISPR/Cas guide RNA and Cas sequences targeting the integrated HIV pro-virus in a healthy cell also protects these cells from becoming infected with HIV-1. Although the guide RNA and the Cas components of CRISPR/Cas can be delivered to a target cell in any number of methods, the use of a viral vector such as a lentiviral vector leads to the permanent integration of the vector's transgene into the target cell genome to constitutively transcribe both the guide RNA and the Cas (Ortinski et al. (2017)). The guide RNA is a single stranded RNA molecule of approximately 20 nucleotides in length designed to bind to a specific target gene region. In the case of HIV-1, we have designed guide RNA sequences to bind to regions within the HIV-1 pro-virus such that a cleavage cut within this region would permanently disable the production of new viral particles.

Alternatively, cellular gene excision has been proposed using gene editing techniques. The most likely cellular gene target is that which encodes the CCR5 receptor, as this receptor is dispensable for immune protection. A natural mutation in the CCR5 gene, termed delta-32, is commonly found in from 1 to 5 percent of individuals with no apparent negative effect (Novembre et al. (2005)). Targeting the CCR5 cellular gene is attractive for several reasons. In this way one can eliminate the CCR5 gene in hematopoietic progenitor cells such that all resultant progeny will be CCR5-negative. The CCR5 receptor is required for HIV infection, but is not absolutely required for immune cell function. Thus, eliminating the CCR5 receptor will have no negative consequences on the health or immune responses of individuals. In alternative studies, we have also developed guide RNA sequences that target the cellular gene for CCR5, a cell membrane receptor required in addition to CD4, for HIV-1 infection of target cells.

Whichever gene is targeted, the delivery of the guide RNA and Cas sequences via a lentiviral vector can result in the constitutive expression of the guide RNA and Cas molecules due to the permanent integration of these sequences into a target cell genome. In the case of targeting the

integrated viral genome known as the pro-virus, delivery of the guide RNA and Cas sequences results in both the cleavage of the integrated HIV genome present in an infected cell, and also protects uninfected cells from ever becoming infected with HIV.

Targeting the cellular gene CCR5 has been studied in both mature T cells as well as in the hematopoietic progenitor cell, where CCR5 gene disruption will occur in all progeny during hematopoietic differentiation (cf Peterson et al. (2014)). Genetically modifying hematopoietic progenitor cells that produce HIV resistant CD4+ T cells is a particularly promising intervention, as these progenitor cells have a long lifespan and therefore can potentially have a sustained effect on HIV suppression. This specific intervention is the one modeled here.

Our hypothesis is that, by modifying some proportion of the hematopoietic progenitor cell population, all CD4+ progeny cells will be CCR5-negative. Defining the numbers of CCR5 gene-cleaved progenitor cells necessary to provide a sufficient number of CCR5-negative, HIV resistant, immune cells is a critical aspect to the eventual development of a therapy consisting of gene-modified cells. If the CCR5 gene can be cleaved in a critical proportion of hematopoietic progenitor cells in HIV-infected patients, these gene modified cells will produce HIV resistant cells, allowing patients to eventually cease taking anti-retroviral drug therapies. Currently lacking, however, are the tools necessary to predict how this therapy will function in a patient, and the impact that changes in the numbers of gene-modified cells will have on the immune system. Refinement to the model developed here to accurately predict the outcome of such therapy will hasten the development of a clinical trial.

Mathematical modeling of HIV has been documented since the early 1990's. In addition to several review articles discussing the various mathematical models (Buratto et al. (2014), Baggaley et al. (2005), Stafford et al. (2000)), models of HIV and the dynamics of viral replication with the host immune system go back to early efforts to understand the dynamics of the system (Perelson et al. (1996), Perelson & Nelson (1999), Perelson et al. (1993), Nowak & May (1991), Pandit & De Boer (2015)).

To understand how models are currently used to represent the progression of this disease, we considered 40 recent models of within-host dynamics, all of which are expressed as nonlinear systems of differential equations. Of these models, one used stochastic differential equations Wang, Liu, Xu & Zhang (2015) and eight used delayed differential equations (Huang et al. (2016), Elaiw & Almuallem (2015), Li & Wang (2015), Li et al. (2015), Alshorman et al. (2016), Pitchaimani & Monica (2015), Sahani (2016), Balasubramaniam et al. (2015), Elaiw & Almuallem (2016)). In addition, the focus of several of these papers was somewhat different from ours. Twenty seven models resembled the approach taken in this study. All of these models include T-cells. One includes free virus but not infected T cells (Joly et al. (2016)). Two include infected T cells but not free virus (Balasubramaniam et al. (2015), Rana et al. (2015)). The rest include both. Four models include multiple viral strains (Chen et al. (2015), Pankavich & Shutt (2015), Luwanda & Mwambi (2016), Ke et al. (2015)).

To model the production of T cells, five models used a logistic term (Luo et al. (2016), Birger et al. (2015), Ogunlaran & Oukouomi Noutchie (2016), Rahmoun et al. (2015), Ikeda et al. (2015)), while seventeen used constant recruitment balanced by linear death (Mojaver & Kheiri (2015), Joly et al. (2016), Wang et al. (2016*a*), Chen et al. (2015), Jo & Roh (2015), Pankavich & Shutt (2015), Tabit et al. (2016), Luwanda & Mwambi (2016), Arruda et al. (2015), Nakaoka et al. (2016), Wu et al. (2015), Wang et al. (2016*b*), Guo & Ma (2016), Hajizadeh & Shahrokhi (2015), Wang, Xu, Wang & Chen (2015), Ke et al. (2015), Wang et al. (2017)), and one study used a bounded rate dependent on both the T cell and viral populations (Adams et al. (2015)). Of the models reviewed, 8 included a compartment for latent cells (Mojaver & Kheiri (2015), Pankavich & Shutt (2015), Li et al. (2015), Alshorman et al. (2016), Nampala et al. (2015), Wang, Xu, Wang & Chen (2015), Rana et al. (2015), Ke et al. (2015)).

It is known that in the presence of virus, activated T cells responding to the infectious state are both recruited to the site of viral resurgence and also proliferate in response to increasing viral load (Jelley-Gibbs et al. (2000)). Four studies include both a recruitment rate of T cells and a proliferation rate, as we do in this study (Liu (2015), Venkatesh et al. (2016), Croicu (2015), Lekgari (2015)). Due to the specificity of the T cell receptor that engages an HIV-infected host cell, not all T cells are "activated" in response to a given virus, however (Hunt et al. (2003)). The distinction between "resting" T cells and "activated" T cells is important, as it is more likely that activated cells become infected by HIV (Biancotto et al. (2008)). This distinction is made in only one of the studies reviewed (Luwanda & Mwambi (2016)).

None of these recent studies incorporated all of the populations we use in our model for HIV dynamics in an untreated patient: resting, activated, infected and latent T cells, as well as viral load. In addition, the model we have developed describes the potential ongoing effect of gene edited progenitor cells that will produce HIV resistant CD4-positive cells for the duration of their survival. We give a discussion of the default parameters producing the typical disease trajectory of an untreated individual, derived whenever possible from direct biological measurements. We analyze the sensitivity of the model and run a series of numerical experiments showing distinct threshold behavior in the treatment parameters. The model indicates a clear threshold behavior for the percent of gene edited hematopoetic progenitor cells that must be introduced to achieve a functional cure for HIV.

2. Model development

2.1 *Model*

We propose a mathematical model for HIV in an individual that includes five compartments that capture the dynamics of an HIV-infected individual who is not undergoing anti-retroviral therapy and two further compartments representing gene modified populations of CD4-positive/CCR5-negative cells that are resistant to HIV due to lack of the CCR5 receptor. These populations have a therapeutic role in that they can respond to a virus but are themselves resistant to infection. This study includes an analysis of the full model and also the five compartment submodel corresponding to an untreated individual.

Figure 1 illustrates the different populations and their interactions in both stages of modeling. The dotted box in this figure shows the two gene enhanced CD4+ cell populations, P and Q, which are resistant to HIV infection but otherwise immunologically competent. We study the dynamics of the disease in the presence of this therapeutic population in the second stage.

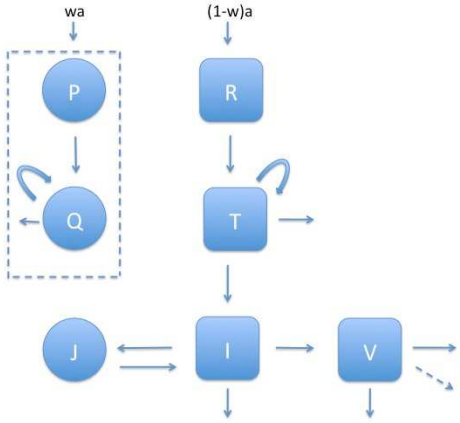


FIG. 1. Hematopoetic progenitor cells produce CD4+ T cells at a constant rate a. A fraction, w, of these will be HIV resistant (P). The rest (R) will be unmodified. These resting T cell populations become activated to produced two classes of T cells (Q and T) of which only the genetically unmodified T cells (T) are susceptible to HIV infection. Infected cells, (I), may become latent, (J) or may produced virions, (V).

2.2 *Model equations*

The model equations are as follows

$$\frac{dR}{dt} = (1-w)a - \left(q + uq\frac{V}{\alpha+V}\right)R \tag{2.1}$$

$$\frac{dT}{dt} = -dT - kTV + \left(q + uq\frac{V}{\alpha+V}\right)R + bT\frac{V}{\alpha+V} \tag{2.2}$$

$$\frac{dI}{dt} = kTV - eI - mI + nJ \tag{2.3}$$

$$\frac{dV}{dt} = epI - cV - \beta QV - kTV \tag{2.4}$$

$$\frac{dJ}{dt} = mI - nJ \tag{2.5}$$

$$\frac{dP}{dt} = wa - \left(q + \frac{uqV}{\alpha+V}\right)P \tag{2.6}$$

$$\frac{dQ}{dt} = \left(q + \frac{uqV}{\alpha+V}\right)P + \frac{bQV}{\alpha+V} - dQ \tag{2.7}$$

Equation (2.1) describes the dynamics of "resting" or as yet unactivated CD4+ cells produced by unedited progenitor cells at a constant rate, a.The parameter w determines the fraction of HIV resistant CD4 cells being produced by gene edited progenitor cells. Setting $w = 0$ and $P(0) = Q(0) = 0$ gives a model for an untreated individual. These cells are activated constantly in response to background virus at rate q. In the presence of HIV, they are activated at a bounded rate in response to the presence of virus, V, with maximal rate uq.

Equation (2.2) describes the activated, and therefore susceptible, CD4+ cells that express the CCR5 protein. In addition to activation, these cells proliferate in response to virus at a rate bounded by b. They also die at constant rate d and become infected with virus at rate kTV. We assume these susceptible immune cells have a negligible effect on reduction of HIV infection as there is no data in the literature supporting the existence of successful immune system.

Equation (2.3) describes the infected CD4+ cell compartment. Infected cells release virus and apoptose at rate e, releasing virions. In addition some of these infected cells become "latent", passing into compartment J at rate mI and returning from that compartment at rate nJ.

Equation (2.4) describes the dynamics of free virus, which is produced at rate epI where e is the death rate of infected cells and p is the number of virions released per cell. Free virions die without a host, at rate cV and enter host cells at rate kTV. In addition the model assumes that a fraction of HIV resistant activated CD4+ cells Q will have the capacity to elicit an immune attack on HIV. Although the process of mounting an immune attack is complex and requires many types of cells, for simplicity we consider it an effect of the presence of Q and model this effect as removal of free virus at rate βQV.

Equation (2.5) describes the linear exchange between infected and latent compartments. The relative size of the two rates mI and nJ can be estimated but the actual rates are not known.

Equation (2.6) describes the production of gene edited HIV resistant cells by a fraction w of genetically altered progenitor cells. We assume that background activation rate q is the same as for unaltered cells and that, in addition, the presence of HIV virus will cause increased activation at the same rate as for unaltered cells (R).

Equation (2.7) describes the dynamics of activated HIV resistant cells, which are exactly the same as for HIV susceptible cells except that no infection term analogous to the kTV in Equation (2.3) is present.

2.3 *Parameterizing the model*

Parametrizing models with many parameters can be approached by reducing the complexity of the task via assumptions and direct biological measurement when possible (Adams et al. (2015)). In this study parameters are derived from biology using laboratory measurement experiment when possible and an approximate fit to the well known disease progression of HIV otherwise. These are given in Table 1.

2.3.1 *Parameters determined from HIV free individuals, a,q,d*

For individuals without disease the model equations are as follows

$$\frac{dR}{dt} = a - qR \tag{2.8}$$

$$\frac{dT}{dt} = qR - dT \tag{2.9}$$

At equilibrium we then have $R^* = a/q$ and $T^* = a/d$. All units will be given in cells per μL, or cells per μL per day as appropriate.

Measurements of HIV free individuals are starred in Table 1. Fleury et al. (2000) reports 10.4 ± 6.5 as the daily production rate of cells, a. They also report the total CD4 count as 967 ($R^* + T^*$) and the fraction of proliferating (activated) cells, ($\frac{T^*}{R^*+T^*}$), as 1.06% in the blood and 0.75% in the lymph node. Hunt et al. (2003) reports 2% of cells activated in a healthy individual. We will take the higher value of 2 %.

A calculation yields $R^* = 0.98(967) = 10.4/q$ and $q = 0.01097$.

Similarly, $T^* = 0.02 * (967) = a/d = 10.4/d$ and $d = 0.5377$.

2.3.2 *Parameters determined from HIV infected individuals without treatment ($w = 0$), $m/n, c, p, e, b$*

In Equations (2.10)-(2.13) we might assume that J arrives at a constant fraction of I in relatively short time and remains at that fraction, which has been loosely estimated at "one in a million" (Chun et al.

(1997)). Assuming that $\frac{dJ}{dt} = 0$ we have the relation ship $J = (m/n)I$, where m/n is approximately 10^{-6}. This relationship also reduces the equations (3.1)-(3.4) to a simpler system:

$$\frac{dR}{dt} = a - \left(q + uq\frac{V}{\alpha + V}\right)R \tag{2.10}$$

$$\frac{dT}{dt} = -dT - kTV + \left(q + uq\frac{V}{\alpha + V}\right)R + bT\frac{V}{\alpha + V} \tag{2.11}$$

$$\frac{dI}{dt} = kTV - eI \tag{2.12}$$

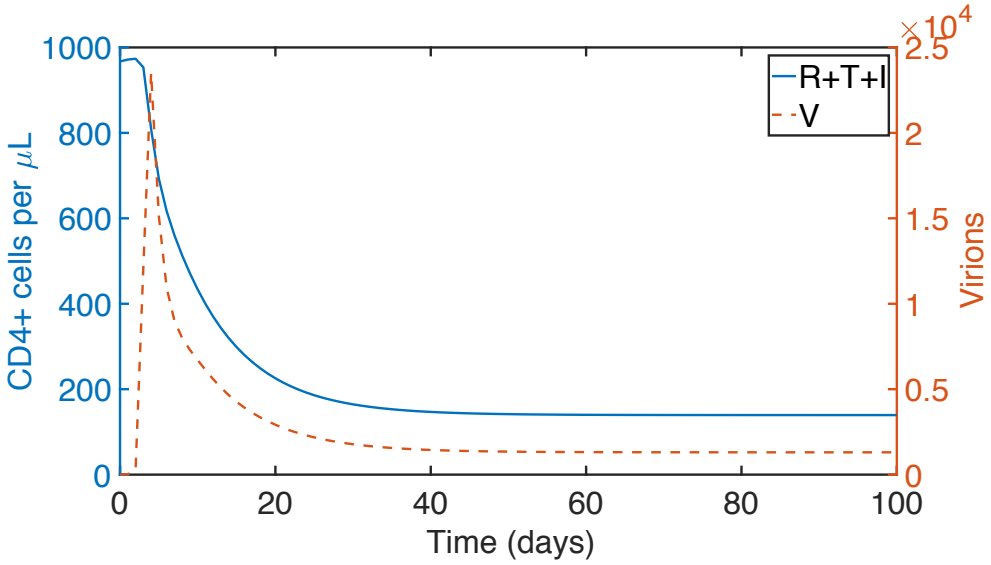
$$\frac{dV}{dt} = epI - cV - kTV \tag{2.13}$$

The virus has a 3-4 minute half life outside of an infected cell (Zhang et al. (1999)). A four minute half life corresponds to 0.00277 days. A 3 minute half life results in a death rate c of 333 percent per day. Virion production rate per cell, p, is estimated at 5×10^4 per cell and the life span of an infected cell is estimated to be about one day (Boer et al. (2010)), giving a virion induced apoptosis rate of 100% per day, or $e = 1$. The doubling time of an activated cell is estimated at 7-10 hours (Jelley-Gibbs et al. (2000)). We will take it as 8 hours, or 0.333 days. This gives a maximum reproduction rate of activated cells of $\ln(2)/0.333 = 2.08$ However only a fraction of CD4+ cells will activate in response to a particular virus, so we take approximately 25% of this and set the maximum rate (b) equal to 0.5.

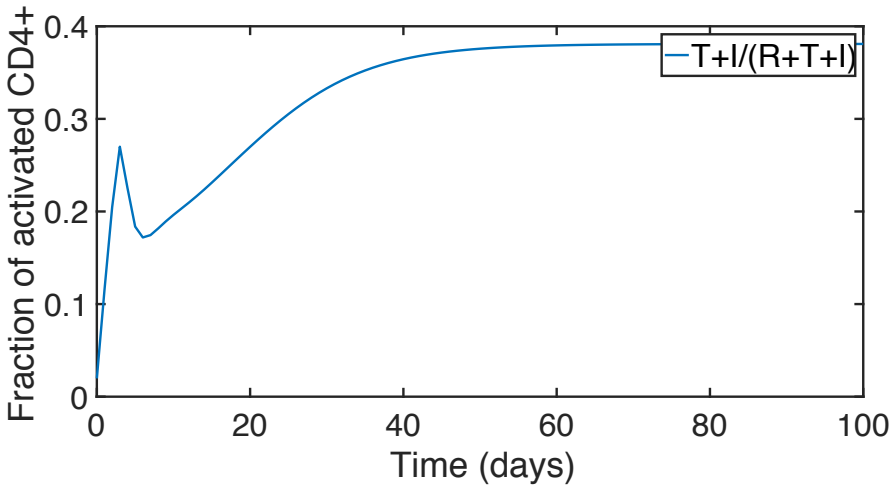
This leaves parameters m, u, α and k, which cannot be observed directly but must be inferred by fitting the model to time series data. The expected development of untreated HIV has been studied extensively, with an often quoted figure in a paper by Perlmutter et al. (1999) that shows peak viremia in early stages at around 10^3 virions per μL of plasma. Our model peaks at a substantially higher level but qualitatively shows the same pattern of viremia.

The Perlmutter study reports less than 10 per μL during a latent period starting sometime after 12 weeks, although subsequent studies show high variability among individuals with long term virion levels as high as a 1000 per μL (Fraser et al. (2007)). In addition, CD4+ counts drop from an initial 10^3 cells per μL to latent levels between 200 and 600 cells per μL. The Perlmutter data also show resurgence of disease after some years, which is not modeled here. In addition, it is known that the percent of activated cells rises to as much as 8% (Hunt et al. (2003)). With the parameters listed in Table 1, Figure 2 shows a maximum viral load of about 25,000 per μL in less than 10 days, which declines to around 2000 per μL at equilibrium. The CD4+ cells decline to less than 200 per μL and remain at equilibrium there. The percent of activated cells rises to over 30% after an initial peak.

Note that parameters for HIV resistant cells are the same as for unaltered cells except for β which describes the unknown efficiency with which HIV resistant cells reduce viral load, and parameter w which describes the percent of HIV resistant cells produced by CRISPR modified stem cells. Figure 2 shows the progress of HIV in an untreated individual given by the model with default parameters in Table 1.



(a)



(b)

FIG. 2. Model without P and Q: **(a)** CD4+ count drops from normal to under 200 per μL and initial viremia rises to 2.5×10^4 within 2 weeks and drops to a steady state higher than reported. **(b)** Progression of percent activated CD4+ cells over time. Parameter values are given in Table 1.

3. Model Analysis

In this section we determine existence and stability properties of the model. We also compute the basic reproductive ratios R_0 and R_0^P for the model without HIV resistant cells and with HIV resistant cells respectively. Detailed proofs of the results are given in the Appendix.

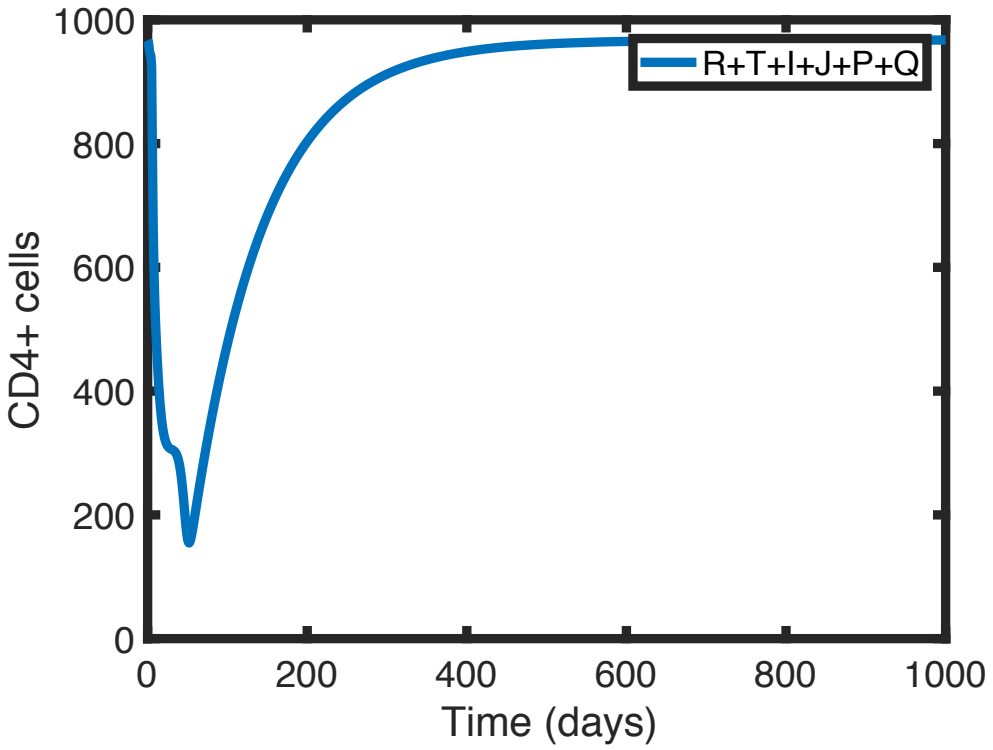


FIG. 3. With 50% of new CD4+ cells HIV resistant, and an immune response to HIV, the model predicts a fully recovered immune system in the presence of HIV resistant cells. ($w = .5, \beta = 10$, remaining parameters as in Table 1) (Figure produced by Matlab. MATLAB (2016))

3.1 *Submodel without HIV resistant cells*

Model equations in the absence of HIV resistant cells are as follows:

$$\frac{dR}{dt} = a - \left(q + uq\frac{V}{\alpha + V}\right)R \tag{3.1}$$

$$\frac{dT}{dt} = -dT - kTV + \left(q + uq\frac{V}{\alpha + V}\right)R + bT\frac{V}{\alpha + V} \tag{3.2}$$

$$\frac{dI}{dt} = kTV - eI - mI + nJ \tag{3.3}$$

$$\frac{dV}{dt} = epI - cV - kTV \tag{3.4}$$

$$\frac{dJ}{dt} = mI - nJ \tag{3.5}$$

THEOREM 3.1 The equilibrium values for the system described by Equations (3.1)-(3.5) are given as roots of a cubic polynomial in J:

$$J(J^2 + a_1 J + a_0) = 0 \tag{3.6}$$

where:

$$\begin{aligned} a_1 &= \frac{m[k\alpha c - ka(p-1) + c(d-b)]}{ken(p-1)} \\ a_0 &= \frac{cm^2\alpha(cd - ka(p-1))}{ke^2n^2(p-1)^2} \end{aligned}$$

COROLLARY 3.1 (Disease-free equilibrium). The model equations (3.1) - (3.5) have a disease-free equilibrium $E_0 = (a/q, a/d, 0, 0, 0)$. Further the disease-free equilibrium is the only equilibrium if $p = 1 + \frac{cd}{ka}$ and $b = \alpha k$.

LEMMA 3.1 If $p < 1$ there is no endemic equilibrium.

THEOREM 3.2 (Endemic equilibrium) The model has either one or two endemic equilibrium points, determined by parameter values as follows:

1. Let $p = 1 + \frac{cd}{ka}$ and $b > \alpha k$. Then there exists one endemic equilibrium.
2. Let $p > 1 + \frac{cd}{ka}$. Then there exists one endemic equilibrium.
3. Let $1 < p < 1 + \frac{cd}{ka}$ and $\alpha k < b < ak + d + \alpha k$. Then there exists two endemic equilibria E_3^1 and E_3^2.
4. Above mentioned cases are the only ones giving positive equilibria.

THEOREM 3.3 (Stability: disease-free equilibrium) The disease-free equilibrium $E_0 = (a/q, a/d, 0, 0, 0)$ is unstable if $p > 1 + \frac{cd}{ka}$.

THEOREM 3.4 The basic reproduction number, R_0 for the model (3.1)-(3.5) is given by:

$$R_0 = \frac{m}{2(e+m)} + \sqrt{\left(\frac{m}{2(e+m)}\right)^2 + \frac{kaep}{(ka+cd)(e+m)}}. \tag{3.7}$$

3.2 *Model with HIV-resistant cells*

The full system with HIV resistant cells has equilibria defined by a quartic polynomial. We begin the analysis by verifying the root corresponding to the disease-free equilibrium.

THEOREM 3.5 (Disease-free equilibrium.) The model with HIV resistant cells (equations (2.1)-(2.7)), has a disease-free equilibrium given by $E_0' = ((1-w)a/q, (1-w)a/d, 0, 0, 0, wa/q, wa/d)$.

THEOREM 3.6 (Stability: disease-free equilibrium). The disease-free equilibrium E_0' is unstable if $p > 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$.

THEOREM 3.7 The basic reproduction number for the model with HIV resistant cells is given by

$$R_0^P = \frac{m}{2(e+m)} + \sqrt{\left(\frac{m}{2(e+m)}\right)^2 + \frac{kaep(1-w)}{(cd + ka(1-w) + \beta wa)(e+m)}}. \tag{3.8}$$

An algebra calculation leads to the following observation.

COROLLARY 3.2 $R_0^P < 1$ if and only if $p < 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$.

THEOREM 3.8 The remaining equilibrium values for the system described by Equations (2.1)-(2.7) are given as roots of a cubic polynomial in the state space variable Q as follows:

$$0 = C_3Q^3 + C_2Q^2 + C_1Q^1 + C_0$$

where

$$C_3 = (p-1)^{-1}((-d-\alpha k+b)\alpha d\beta(b-d) - d\alpha\beta(b-d)^2 - k\alpha^2 d^2\beta) \tag{3.9}$$

$$\begin{aligned} C_2 = {} & \alpha(1-w)ak(b-d)^2 + (p-1)^{-1}((-d-\alpha k+b)\alpha(d\beta wa + dc(b-d) - wa\beta(b-d)) \\ & - d\alpha(p-1)^{-1}(2\beta wa(b-d) + c(b-d)^2) \\ & + (1-w)ak\alpha d(b-d) - k\alpha^2(p-1)^{-1}(-2dwa\beta + d^2c) \end{aligned} \tag{3.10}$$

$$\begin{aligned} C_1 = {} & \alpha(1-w)ak2(b-d)wa + (p-1)^{-1}((-d-\alpha k+b)\alpha(dcwa - w^2a^2\beta - wac(b-d)) \\ & - (p-1)^{-1}d\alpha(\beta w^2a^2 + c2wa(b-d)) \\ & + (1-w)ak\alpha(dwa - wa(b-d)) - (p-1)^{-1}k\alpha^2(w^2a^2\beta - 2dwac) \end{aligned} \tag{3.11}$$

$$C_0 = (p-1)^{-1}((-d-\alpha k+b)\alpha(-w^2a^2c) - d\alpha cw^2a^2 - k\alpha^2w^2a^2c) \tag{3.12}$$

3.3 *Simulations of the analytical results*

This simulation experiment (represented by Figure 4) mimics the results of the stability analysis of the model with and without HIV resistant cells (P and Q). Panels 4 (a) and 4 (b) correspond to the model without HIV resistant cells. The model was run without disease until it reached a steady state. HIV was introduced into the system by using positive values for the initial I and V population. This experiment was done for $p = 10000$ (such that $p < 1 + \frac{cd}{ka}$ is satisfied) and then for $p = 50000$(such that $p > 1 + \frac{cd}{ka}$is satisfied). The two values for p were chosen to verify analytical results using simulation experiments. Remaining parameters are given in the legend of Figure 4. The parameters also satisfy $\alpha k < b < ak + d + \alpha k$ (cf Theorem 4). For $p < 1 + \frac{cd}{ka}$, the virion population immediately drops down from 300 to 0 and stays there. For $p > 1 + \frac{cd}{ka}$, the virion population increases and reaches a maximum of 10,0000 virions and after a few oscillations then stabilizes at 9000. Figure 4 (b) represents the phase portrait (T vs V) for $p < 1 + \frac{cd}{ka}$ and $p > 1 + \frac{cd}{ka}$.

Panels 4 (c) and 4 (d) correspond to the model with HIV resistant cells. We ran the simulations for the parameters for which the disease-free equilibrium of the model without HIV resistant cells is unstable (i.e. $p > 1 + \frac{cd}{ka}$). We then introduced HIV resistant cells into the system for two choices of parameters (i) $p = 10000$ such that $p < 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$ is satisfied and (ii) $p = 50000$ such that $p < 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$ is satisfied. Remaining parameters are given in the figure legend. Temporal dynamics of virion population (in Figure 4 (c)) suggests that for $p < 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$, the virion population drops down from 8950 to 0 and stays there. However for $p > 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$, the virion population reaches a steady state of 2100 virions after 1000 days. Panel 4 (d) represents the phase portrait of T and V population. It is observed that for $p > 1 + (cd + \beta w)(ka(1-w))^{-1}$, the activated CD4+ cells and virions reaches an endemic equilibrium and for $p < 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$ the activated CD4+ population increases to 820 cells per μL but the virion population dies off.

Note that the survival and die off of the virions and CD4+ population takes place under the same conditions as predicted by the model analysis.

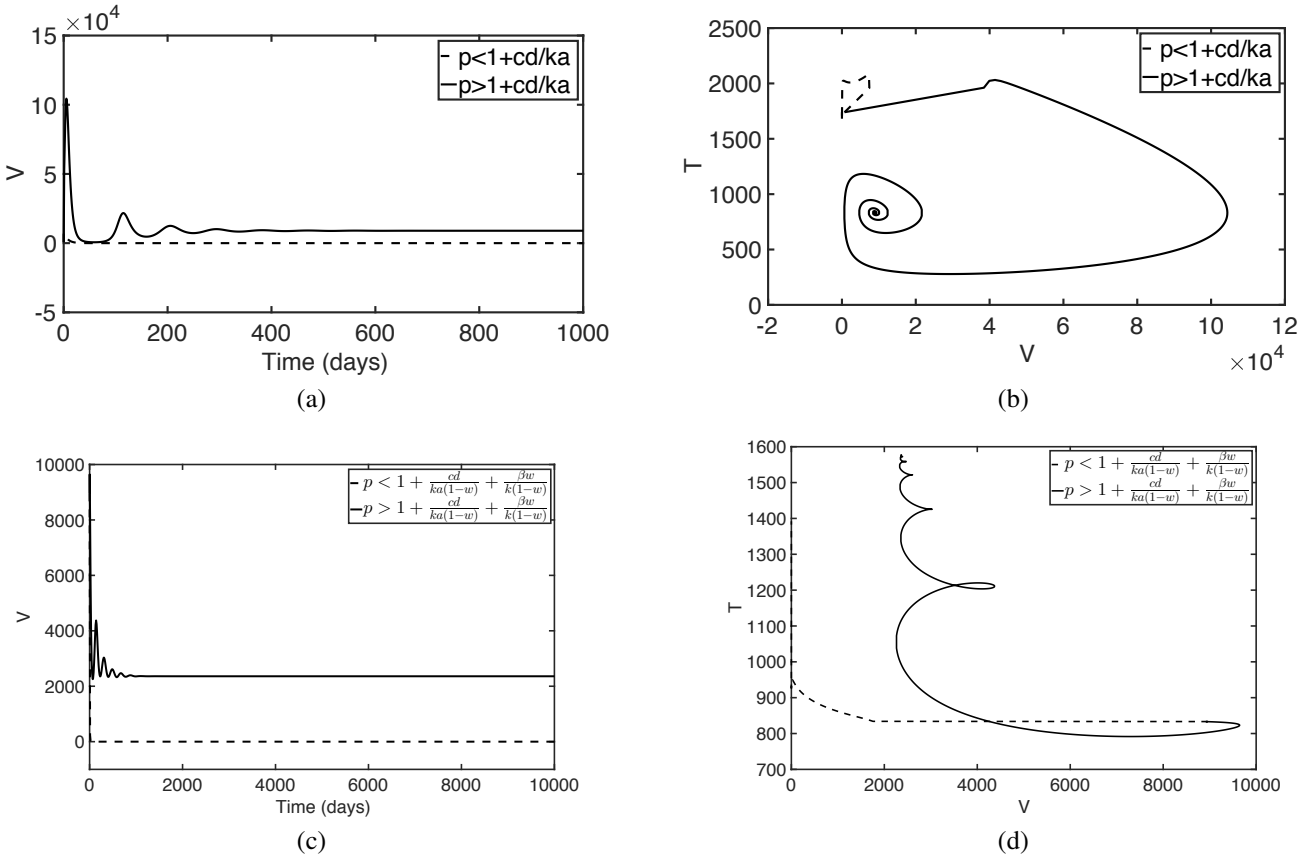


FIG. 4. Panels (a) and (b) correspond to the model without HIV resistant cells. Panel (a) shows the temporal dynamics of the virions and panel (b) shows the phase portrait (T vs V). Panels (c) and (d) correspond to the model with HIV resistant cells, with $\beta = 0.1, w = 0.2$. Each panel represents two different scenarios based on the values of p. Parameters used are not biologically derived but are chosen to illustrate the theorems. ($a = 20, q = 0.05625, u = 200, d = 1/87, b = 0.01, k = 2.4 * 10^{-6}, \alpha = 1000, e = 0.25, m = 10^{-6}, n = 1, c = 100$). Initial conditions for (i) panels (a) and (b) are $[355, 1740, 300, 700, 10]$ (ii) panels (c) and (d) are $[1.95, 833, 8958, 71, 10]$. (Figure produced by Matlab MATLAB (2016)).

4. Numerical experiments

4.1 *Sensitivity of the Model*

To investigate the effect of our ranges of parameters on model outcomes, a sensitivity analysis is performed on all parameters and initial conditions of the model. Values for each parameter are taken from a uniform distribution that is supported on the intervals with mean values given by Table 1 unless otherwise stated in the figure caption, and 70% variation. A Latin Hypercube sampling method is used to randomly select vectors of parameter-values to be used for each run. Details of the Latin Hypercube Sampling procedure can be found in Stein (1987). Partially ranked correlation coefficients (PRCC) are calculated from these 5000 simulations where ranked parameter values are correlated with the infected CD4+ cells (I population) and the virions (V population).

Figures 5 and 6 represent the correlation (negative and positive) with the outcome. The experiment is run for 100 days (Figure 5) and 500 days (Figure 6) to see whether or not the parameters that are significant in a short span of time are significant in the longer span as well. In both figures, panel (a) represents the model without HIV resistant cells and panel (b) represents the model with

HIV resistant cells. In panel (a) of both figures, the mean initial conditions for R,T,I,V and J are 500, 500, 10000, 100, and 1 respectively. Similarly, in panel (b) of both figures, the mean initial conditions for R,T,I,V,J,P and Q are 500, 500, 10000, 100, 1, 50 and 50 respectively.

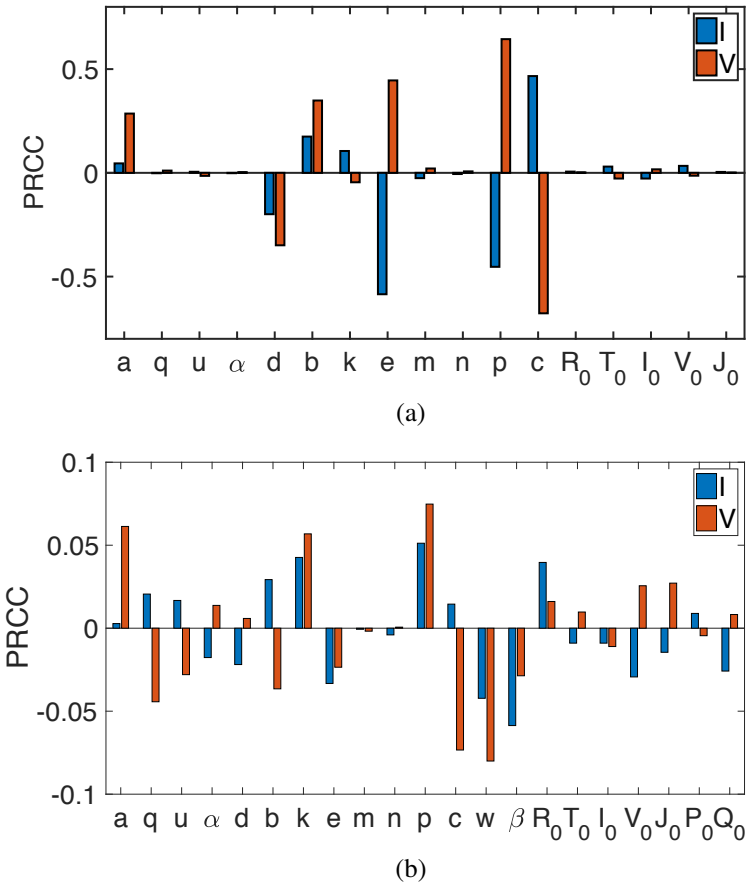


FIG. 5. Sensitivity analysis of the **(a)** Model without HIV resistant cells, **(b)** Model with HIV resistant cells with respect to the infected cells (I) and virions (V). Mean parameter values for w and β are $w = 0.5, \beta = 10$. The remaining mean parameter values for both panels are given in Table 1 and the variation is 70%. The final time for which simulations are run is 100 days.

4.2 *Cessation of antiretroviral treatment with and without HIV resistant cells*

It is thought that the reason antiretroviral (ART) therapy must be continued indefinitely may be the presence of a population of latently infected cells, represented in the model by J. At interruption of antiretroviral therapy few cells are found in circulation and the viral load is undetectable, yet the disease rebounds, hypothetically due to this latent compartment. The existence of latent cells has been verified (Chun et al. (1997)), and to check that the model exhibits the correct behavior we ran it with no infected cells I or virus V but a small concentration of latent cells J. We omit a figure of this experiment because it cannot be distinguished visually from the default run, and looks exactly like Figure 2.

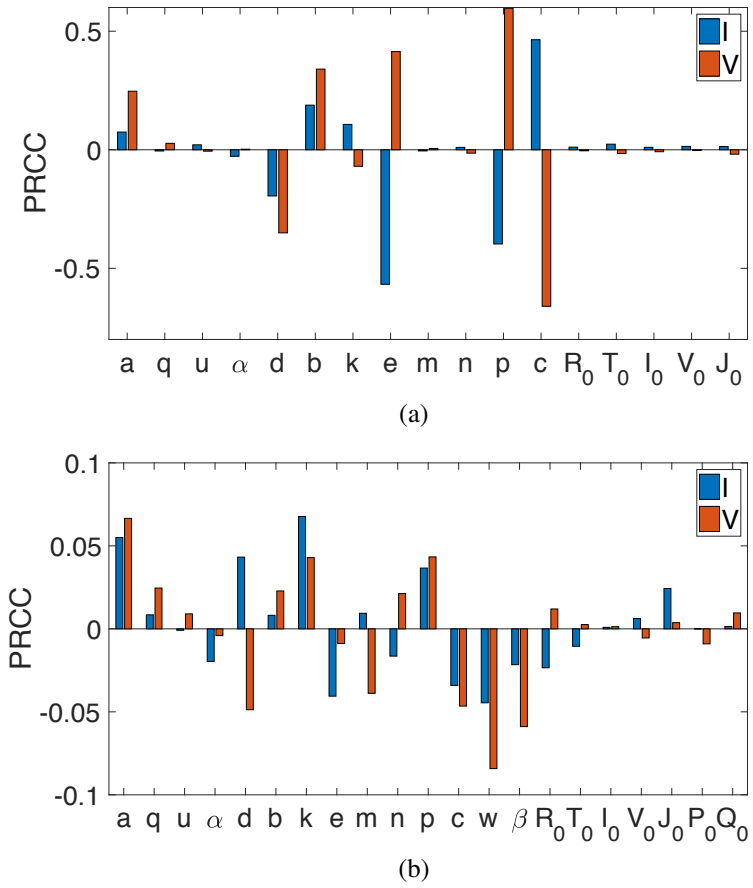


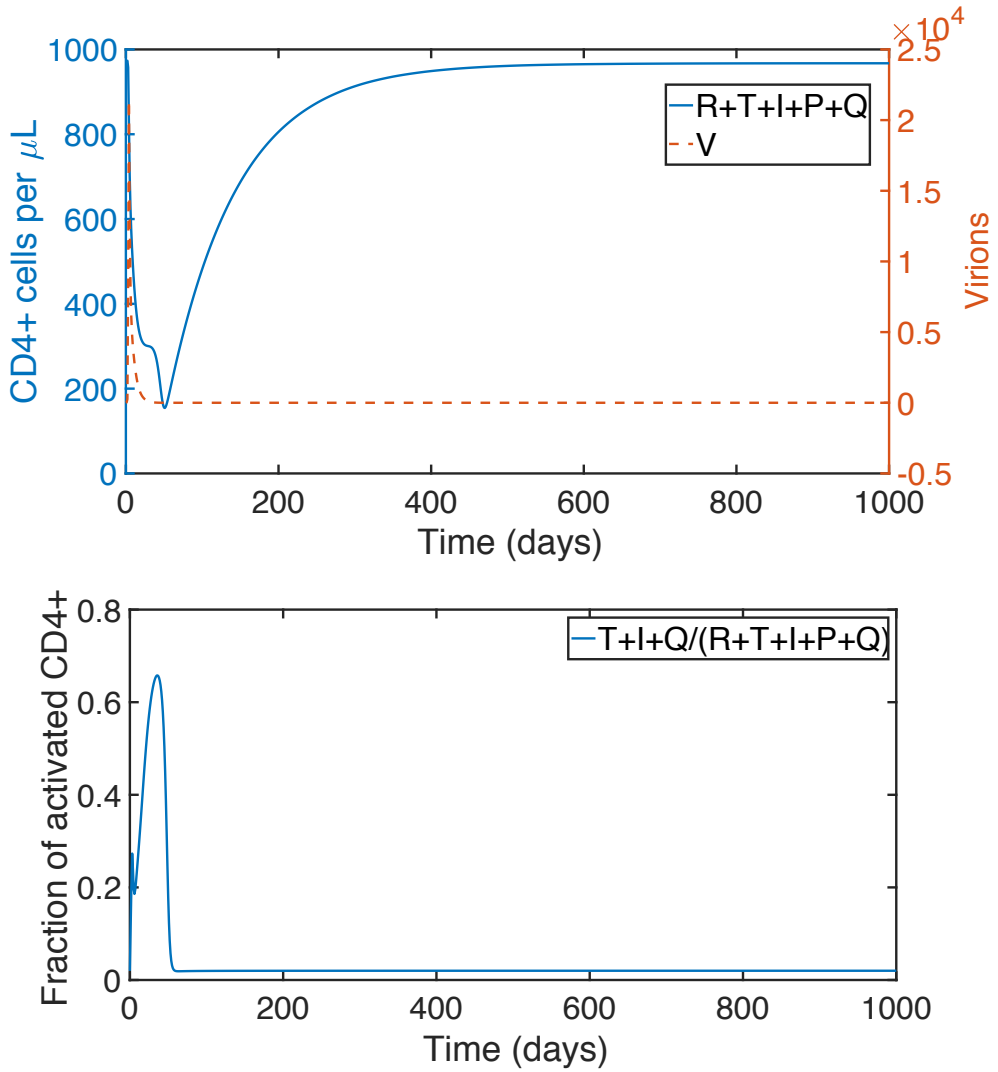
FIG. 6. Sensitivity analysis **(a)** Model without HIV resistant cells. **(b)** Model with HIV resistant cells with respect to the infected cells (I) and virions (V). Mean parameter values for w and β are $w = 0.5, \beta = 10$. The remaining mean parameter values for both panels are given in Table 1 and the variation is 70%. The final time for which simulations are run is 500 days.

Although the observed ratio of I to J determines the ratio of parameter m to n, there is still an unmeasured rate to be determined. In Table 1 we set $n = 10^6 m$ but the value of m was arbitrary. We found that varying m by many orders of magnitude did not appreciably alter the timing or intensity of the rebound.

4.3 *Dependence of therapeutic response on the percent of HIV resistant CD4+ cells produced (w) and the effectiveness of their immune response to HIV (β).*

The proposed therapy involves the successful introduction of gene edited progenitor cells into the immune system, which will continuously produce HIV resistant CD4+ cells at the usual rate. The parameter w is the fraction of progenitor cells that have been edited. A numerical experiment shows the result of infection after 50% of the CD4+ cells being produced are HIV resistant , under the assumption that β is not zero and ART therapy is discontinued. Figure 7 shows a return to healthy CD4+ levels when sufficiently many HIV resistant cells are being produced.

A nonzero value of β reflects the assumption that a fraction of HIV resistant CD4+ cells will



(a)
(b)

FIG. 7. Model with P and Q: **(a)** CD4+ count drops from normal to under 200 per μL and then rebounds to normal levels. Initial viremia rises to 2.5×10^4 within 2 weeks, and drops to a set point close to zero. **(b)** Progression of percent activated CD4+ cells over time. In these runs, $w = 0.5, \beta = 10$. Remaining parameter values are given in Table 1.

have a successful immune response to the HIV virion and remove it from the system. However, it is not known if this will be the case, or to what extent these HIV resistant cells could remove virus effectively. Clearly the effectiveness of treatment must also depend on the fraction of HIV resistant cells being produced as well as the effectiveness of their immune response to HIV. With parameters and initial conditions as in Table 1, Figure 8 shows a heat map of the equilibrium CD4+ count for various choices of w and β. There is a clear threshold effect, with few intermediate values.

To gain some insight as to what is happening to CD4+ T cell subpopulations as the parameters vary, Figure 9 shows the populations of HIV resistant cells as treatment parameters vary. Infected

and latent cell populations are shown in Figure 10.

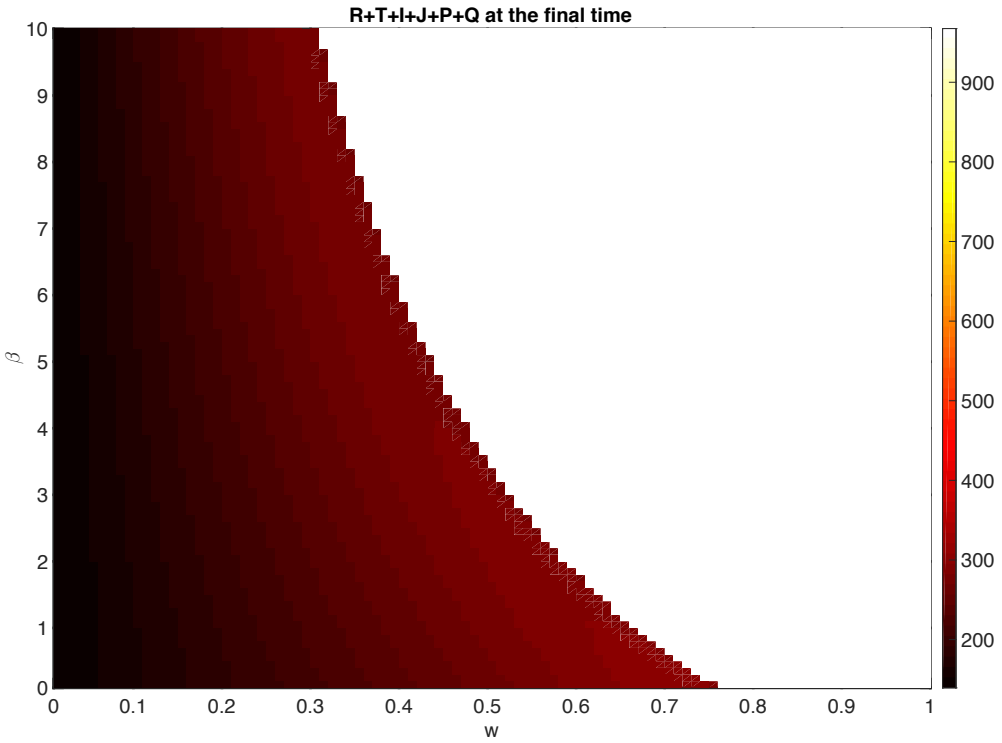


FIG. 8. Figure shows the heat map of w and β on the total CD4+ population $(R+T+I+J+P+Q)$ at the final time.

5. Results and Discussion

A mathematical model for a new gene-therapy (CRISPR/CAS) approach for treating HIV in a hypothetical patient is introduced and analyzed theoretically and using numerical simulations. The model for an untreated HIV infection takes into account relevant features of several models reviewed. The full model includes the production from gene edited hematopoietic progenitor cells of a class of CD4+ T cells that are HIV resistant, but otherwise immunologically competent. This model corresponds to one proposed therapeutic approach. It describes a patient whose hematopoietic progenitor cells were extracted from the body and treated via CRISPR/Cas to block production of the CCR5 protein that enables HIV virus to enter CD4+ T cells. Progeny of gene-edited progenitor cells would be also be resistant to HIV infection and form a fraction of the total resting CD4+ T cell population produced. A larger proportion of these HIV resistant cells results in fewer CD4+ T Cells available to HIV to replicate in, resulting in higher virus death in the body. An additional benefit would arise from the immune response of HIV resistant CD4+ T cells to the HIV infection itself. The model describes a treatment that is under development in the Howell laboratory.

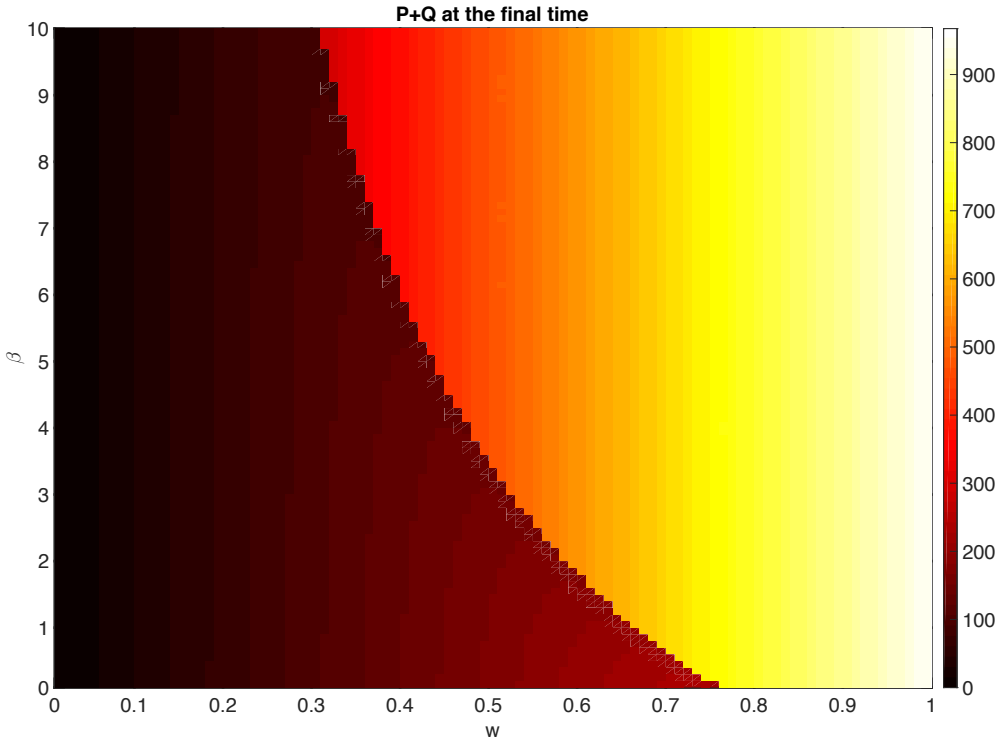


FIG. 9. Figure shows the heat map of w and β for the total HIV resistant cell population $(P+Q)$ at the final time.

5.1 *Mathematical analysis*

Equilibrium analysis tells us that there is one healthy equilibrium in both models (with and without compartments for HIV resistant cells). Stability analysis provides parameter thresholds for a disease-free state. Our calculation of the reproduction number (using Next Generation Matrix), confirms our findings in the stability analysis. and gives a threshold condition on the virion production rate p, for endemic equilibrium to exist. The threshold value of p for the model without therapy is smaller than when gene-therapy is introduced.

The equilibrium and stability analysis shows that there is a region of parameter space in which both the disease-free and the diseased equilibria exist and are stable. Our biologically derived parameters fall into this region. Therefore, in our model, the development of disease depends on initial conditions. Insufficient initial viremia will not result in HIV infection. The tendency of an organism (in this case the HIV virus) to become extinct when its numbers fall below a certain amount is called the Allee effect, and we observe it in this model. It is known that not every encounter between a healthy and contagious individual results in disease. Our model is consistent with these observations.

The expression for R_0^P makes it clear that if w is sufficiently close to 1 or if β is sufficiently large, the disease free equilibrium will be stable no matter what the other parameters may do. However, this is not enough information to determine at what level of treatment the nontrivial stable equilibrium values for infected cells and free virus will be pushed to negligible levels or disappear. Nor does it

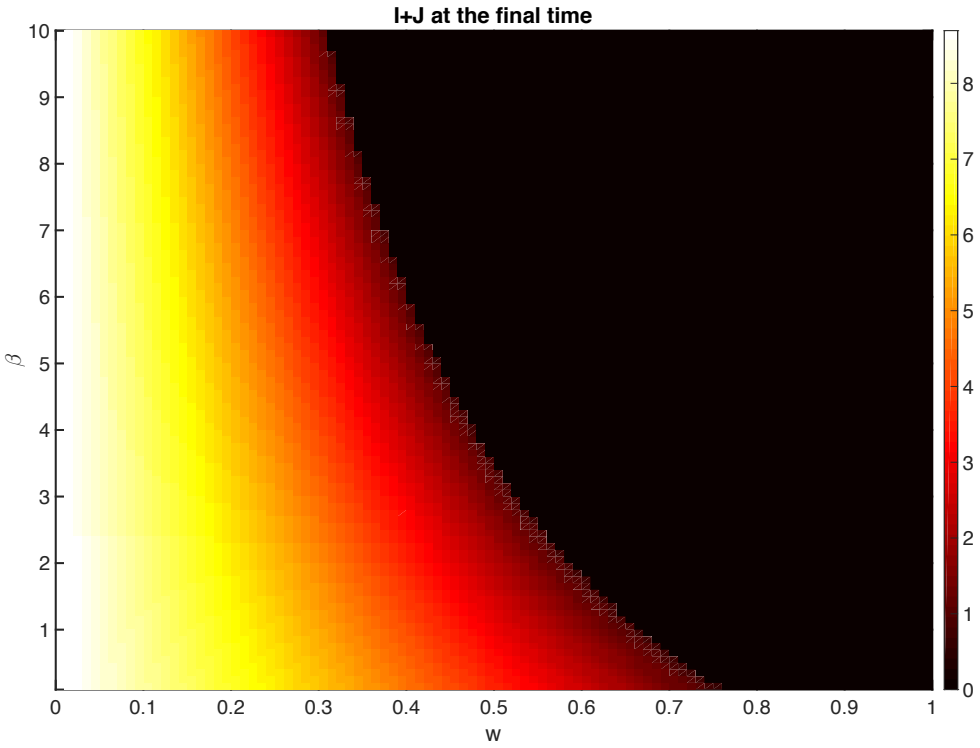


FIG. 10. Figure shows the heat map of w and β for the infected and latent cell population $(I+J)$ at the final time.

give information about the return of CD4+ T cells to normal levels.

5.2 *Parameters*

We begin by giving a description of default parameters based on several studies. Five studies found in the literature (specifically Fleury et al. (2000), Hunt et al. (2003), Boer et al. (2010), Jelley-Gibbs et al. (2000), Chun et al. (1997)) gave direct experimental results that allowed us to calculate specific parameters. A few remaining parameters were obtained by matching to a well known description of disease progression in Perlmutter et al. (1999). Some parameters are unknown, in particular the rate, m, at which infected cells become latent. The treatment parameters, w and β, are the quantities that must be determined experimentally (in the case of β) or manipulated (in the case of w) to depress HIV sufficiently to give a functional cure. Finding direct experimental measurements corresponding to particular constants was difficult, and it is possible that if more studies were found perhaps a known range could be placed around some of the parameters in this study, in particular those parameters to which the model is highly sensitive.

For the default parameters in this study, $R_0 < 1$ and there is also a stable equilibrium with disease present. The initial conditions and default parameters in this study yield solutions that tend to the equilibrium with disease present in the case where there is no treatment, as shown in Figure 2. The CD4+ T cell count behaves as described in Perlmutter et al. (1999), The timing of the viral load behaves correctly. However both at peak and at equilibrium the model predicts a substantially higher

concentration of free virus than was observed in Perlmutter et al. (1999), although subsequent studies found much higher viral loads in some individuals, as high as 1000 per μL with substantial variation among individuals (Fraser et al. (2007)). The model here gives about twice that at equilibrium. The percent of CD4+ T cells that are activated is known to rise from around 2% to as much as 8% as reported in Hunt et al. (2003). With the default parameters the model produces a rise from about 2% to over 30%, much higher than the observed range. This observation indicates that more data and a better model is needed.

5.3 *Sensitivity of the model*

The sensitivity of the model outcomes (load of infected cells and virions) to changes in parameters is quantified in Figures 5 and 6. We find 5 parameters (p,c,d,k,a) in the model for an untreated individual that are most significant with regard to disease burden. These parallel the findings of the equilibrium analysis which shows that, for an untreated individual, the reproduction number, $R_0 < 1$ if and only if $p - cd(ka)^{-1} < 1$. However, the sensitivity analysis is describing the equilibrium values at the nontrivial stable equilibrium. The same five parameters that control the stability of the disease free equilibrium are also controlling the equilibrium with disease present in an untreated individual. In addition to the parameters appearing in the expression for R_0, the parameters e and b are also observed to have a strong effect on the outcome.

When treatment is present the reproduction number, $R_0^P < 1$ if and only if $k(p-1)(1-w) - cda^{-1} < \beta w$. The important parameters for this criterion are $p,c,d,k,a,w\beta$. In parallel to that result, the sensitivity analysis shows that these are also the most important parameters for the equilibrium with disease present in a treated individual.

5.4 *Treatment simulations and threshold phenomena*

The model exhibits clear threshold effects for the proposed treatment. The immune system of the patient either remains severely depressed or enjoys full recovery, as seen in Figure 8. If the HIV resistant CD4+ T cells contribute no immune response against HIV ($\beta = 0$), Figure 8 indicates that over 75% of CD4+ production must be HIV resistant to achieve normal levels of these cells. However, no estimate for β yet exists. As β increases, the percent of HIV resistant cells produced (w) does not need to be as large.

Because CD4+ T cells are activated in response to virus, and then proliferate in response to virus, the input of gene edited cells (w) is not the sole determinant of final population values. The presence of virus also plays a role because activated cells have a relatively short life span. In fact, in this model we have ignored death rates for resting T cells entirely. If no virus were present and a fraction w of gene edited CD4+ T cells were produced, Equations 2.8 and 2.9 would guarantee that counts would arrive at normal levels with w of the cells of the gene edited type and the remaining $1-w$ of the susceptible type. This is not what happens in the presence of virus. The number of gene edited cells (P and Q) at equilibrium for various choices of w and β are shown in Figure 9. There is a clear jump in population as w rises, and how quickly this jump occurs depends on β.

Note that, above the visible threshold, the level of gene edited cells shown in Figure 9 can still be far below 100% of the total CD4+ T cell count, which rises to normal levels as seen in Figure 8. The remaining CD4+ T cells must be those that are not gene altered. These could be healthy or infected. But Figure 10 shows that they are healthy. Figure 10 shows the level of infected cells dropping to near zero (or possibly zero) in the region where Figure 8 indicates normal CD4+ T cell counts. So, in the region that is white in Figure 8 where CD4+ counts are normal, not all of the CD4+ cells need be HIV resistant. A fair percentage of them, in some cases, are susceptible uninfected cells.

The effectiveness with which HIV resistant CD4+ cells can clear virus from the body, parameterized by β, is not yet known. The choice of this beta affects the percentage of HIV resistant cells

that must be produced to achieve a functional cure of HIV, with CD4+ counts returning to normal. Figure 8 shows that, in the absence of any such effect ($\beta = 0$) over 75% of CD4+ cells that are produced must be HIV resistant to give a functional cure. If the effectiveness of virion removal is better, a far smaller fraction of HIV resistant cells need be produced.

5.5 *Future Work*

The results of this study inform the experiments that are needed to determine whether the proposed HIV treatment will work. The threshold dependence of the model on parameters w and β indicate that these are key questions that must be answered by experiment:

1. Under what conditions is it possible for gene altered hematopoietic progenitor cells to supplant a fraction of the existing progenitor cell population? The model assumes this can be done for any desired fraction, w.

2. Will gene edited hematopoietic progenitor cells produce HIV resistant CD4+ cells at the same rate as unedited cells? The model currently assumes these rates are the same.

3. What effect will HIV resistant CD4+ cells will have on the existing HIV infection? Will they function as an immune protection by assisting the removal of virus from the body, and to what extent?

In addition, mathematical questions remain. One question concerns the role of latent cells, which biologists believe are a major factor in the failure of antiretroviral therapy (Sebastian & Collins (2014), Persaud et al. (2000)). For both treated and untreated versions of this model, R_0 depends on m, the rate at which infected cells become latent. However, the dependency is not strong enough to determine whether $R_0 > 1$, a criterion that is shown not to depend on m. With the default parameters for this model, the disease free equilibrium is always stable and $R_0 < 1$. But whether $R_0 > 1$, taken alone does not determine the size of the basin of attraction for the disease free equilibrium. A successful therapy would push the state variables into this basin, the size of which may very well depend upon m. To what extent m determines the success of therapy in this way will be part of future research.

6. Appendix 1

THEOREM 6.1 The equilibrium values for the system described by Equations (3.1)-(3.5) are given as roots of a cubic polynomial in J:

$$J(J^2 + a_1 J + a_0) = 0 \tag{6.1}$$

where:

$$\begin{aligned} a_1 &= \frac{m[k\alpha c - ka(p-1) + c(d-b)]}{ken(p-1)} \\ a_0 &= \frac{cm^2\alpha(cd - ka(p-1))}{ke^2n^2(p-1)^2} \end{aligned}$$

COROLLARY 6.1 (Disease-free equilibrium). The model equations (3.1) - (3.5) have a disease-free equilibrium $E_0 = (a/q, a/d, 0, 0, 0)$. Further the disease-free equilibrium is the only equilibrium if $p = 1 + \frac{cd}{ka}$ and $b = \alpha k$.

Proof. Since $J = 0$ is a solution of equation (6.1), it is a straight forward calculation to get associated values: $R = \frac{a}{q},\ T = \frac{a}{d},\ I = 0$ and $V = 0$. We call this disease-free equilibrium E_0 and write it in vector form $E_0 = (a/q, a/d, 0, 0, 0)$. In equation (6.1) if $a_0 = 0, (p = 1 + \frac{cd}{ka})$ and $a_1 = 0 (b = \alpha k)$ then E_0 is the only equilibrium point.

□

LEMMA 6.1 If $p < 1$ there is no endemic equilibrium.

Proof. At equilibrium, Equation (3.5) implies that $J = mn^{-1}I$. Substituting this into Equation (3.3) gives that $kTV = eI$. Substituting this relation into Equation (3.4) gives $V = ec^{-1}(p-1)^{-1}I$. Thus if $p < 1$, one of V, I must be negative or else both are zero. □

THEOREM 6.2 (Endemic equilibrium)

The model has either one or two endemic equilibrium points, determined by parameter values as follows:

1. Let $p = 1 + \frac{cd}{ka}$ and $b > \alpha k$. Then there exists one endemic equilibrium.
2. Let $p > 1 + \frac{cd}{ka}$. Then there exists one endemic equilibrium.
3. Let $1 < p < 1 + \frac{cd}{ka}$ and $\alpha k < b < ak + d + \alpha k$. Then there exists two endemic equilibria E_3^1 and E_3^2.
4. Above mentioned cases are the only ones giving positive equilibria.

Proof. The proof follows by examining the roots of the quadratic term in equation (6.1). Based on the signs of a_0 and a_1, we have the following:

1. If $a_0 = 0,\ (p = 1 + \frac{cd}{ka})$ and $a_1 \neq 0\ (b \neq \alpha k)$, there is one endemic equilibrium. If $b > \alpha k$ we get $J = a_1 > 0$. Corresponding to this J, call it J^*, we get equilibrium values for other populations which we denote by R^*, T^*, I^*, V^* and hence $E_1 = (R^*, T^*, I^*, V^*, J^*)$ is the endemic equilibrium. If $b < \alpha k$ we get $J = a_1 < 0$ which is not biologically feasible. Additionally we get the disease-free equilibrium E_0 for these $a_0,\ a_1$ values already dealt with in Theorem 6.1.

   If $a_0 < 0$ then $p > 1 + \frac{cd}{ka}$. Solving for the roots of the quadratic expression in (6.1), we get two values of opposite signs for J. Call the positive root J_2^* and evaluating the other populations we get an endemic equilibrium point $(R_2^*, T_2^*, V_2^*, I_2^*, J_2^*)$ which is different from the previous case.

2. If $1 < p < 1 + \frac{cd}{ka}$ and $a_1 < 0$ (which happens when $\alpha k < b < \frac{ak}{c} + d + k\alpha$), the quadratic expression in equation (6.1) gives two positive roots J_3^1, J_3^2, which lead to positive values of other populations. We denote these two endemic equilibrium points $E_3^1 = (R_3^1, T_3^1, V_3^1, I_3^1, J_3^1)$ and $E_3^2 = (R_3^2, T_3^2, V_3^2, I_3^2, J_3^2)$ in addition to E_0. The upper bound for b ensures that the virion release rate $p \geqslant 0$.

3. Note that all other combination of values of a_1 and a_0 will lead to either negative or complex population values which are not biologically feasible.

□

THEOREM 6.3 (Stability: disease-free equilibrium) The disease-free equilibrium $E_0 = (a/q, a/d, 0, 0, 0)$ is unstable if $p > 1 + \frac{cd}{ka}$.

Proof.

The Jacobian of the system at any equilibrium point$(R^*, T^*, I^*, V^*, J^*)$ is given by:

$$J_e = \begin{pmatrix} -(q + \frac{uqV^*}{\alpha+V^*}) & 0 & 0 & -\frac{uqR^*\alpha}{(\alpha+V^*)^2} & 0 \\ q + \frac{uqV^*}{\alpha+V^*} & -d - kV^* + \frac{bV^*}{\alpha+V^*} & 0 & -kT^* + \frac{uqR^*\alpha}{(\alpha+V^*)^2} + \frac{bT^*\alpha}{(\alpha+V^*)^2} & 0 \\ 0 & kV^* & -e-m & kT^* & n \\ 0 & -kV^* & ep & -c - kT^* & 0 \\ 0 & 0 & m & 0 & -n \end{pmatrix}$$

At the disease-free equilibrium $R^* = a/q$ and $T^* = a/d$ with all other quantities equal to 0, yielding the following:

$$J_0 = \begin{pmatrix} -q & 0 & 0 & -ua\alpha^{-1} & 0 \\ q & -d & 0 & -kad^{-1} + ua\alpha^{-1} + \frac{ab}{\alpha d} & 0 \\ 0 & 0 & -e-m & kad^{-1} & n \\ 0 & 0 & ep & -c - kad^{-1} & 0 \\ 0 & 0 & m & 0 & -n \end{pmatrix}$$

Clearly two eigenvalues of J_0 are $-q, -d$. The remaining three eigenvalues are given by the roots of the cubic polynomial:

$$b_3\lambda^3 + b_2\lambda^2 + b_1\lambda + b_0 = 0, \tag{6.2}$$

where

$$\begin{aligned} b_3 &= 1 > 0, \\ b_2 &= n + c + e + m + \frac{ka}{d} > 0, \\ b_1 &= n(c + e + m + \frac{ka}{d}) + (e+m)(c + \frac{ka}{d}) - ep\frac{ka}{d} - mn \\ &= c(n+m+e) + ne + \frac{ka}{d}(n+m) + \frac{ka}{d}e(1-p) \\ b_0 &= n(e+m)(c + \frac{ka}{d}) - nm(c + \frac{ka}{d}) - ep\frac{ka}{d}n \\ &= ne(c + \frac{ka}{d}(1-p)) \end{aligned}$$

By the Routh-Hurwitz criterion Murray (2002) all the roots of (6.2) are negative or have negative real part if and only if $b_0 > 0, b_2 > 0$ and $b_2 b_1 > b_0$. If $p > 1 + \frac{cd}{ka}$ we have $b_0 < 0$ implying that there exists an eigenvalue of J_0 with a positive real part. Hence E_0 is unstable.

□

THEOREM 6.4 The basic reproduction number, R_0 for the model (3.1)-(3.5) is given by:

$$R_0 = \frac{m}{2(e+m)} + \sqrt{\left(\frac{m}{2(e+m)}\right)^2 + \frac{kaep}{(ka+cd)(e+m)}}. \tag{6.3}$$

Proof. R_0 is obtained as the spectral radius of the next generation matrix. The first step in obtaining this matrix is to determine the infected subsystem in our model, that is those equations that produce new infections, leading to the transmission matrix F, and those that cause changes in state among infected individuals, leading to the transition matrix S. These equations are (3.2) - (3.5). We linearize this subsystem about the disease-free equilibrium. Details about this method may be viewed in Diekmann et al. (2009).

The transmission matrix F and transition matrix S:

$$F = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{ka}{d} & n \\ 0 & ep & 0 & 0 \\ 0 & m & 0 & 0 \end{pmatrix}, \quad S = \begin{pmatrix} -d & 0 & \frac{-ka}{d} + \frac{ua}{\alpha} + \frac{ab}{\alpha d} & 0 \\ 0 & -e-m & 0 & 0 \\ 0 & 0 & -c - \frac{ka}{d} & 0 \\ 0 & 0 & 0 & -n \end{pmatrix}.$$

The next generation matrix is $K = -FS^{-1}$ and is given by

$$K = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{ka}{cd+ka} & -1 \\ 0 & \frac{-ep}{e+m} & 0 & 0 \\ 0 & \frac{-m}{e+m} & 0 & 0 \end{pmatrix}.$$

The largest eigenvalue of K gives the basic reproduction number:

$$R_0 = \frac{m}{2(e+m)} + \sqrt{\left(\frac{m}{2(e+m)}\right)^2 + \frac{kaep}{(ka+cd)(e+m)}}. \tag{6.4}$$

The main theorem in Diekmann *et al* guarantees that the disease-free equilibrium E_0 is locally asymptotically stable if $R_0 < 1$, and unstable if $R_0 > 1$ Diekmann et al. (2009). □

6.1 *Model with HIV-resistant cells*

The full system with HIV resistant cells has equilibria defined by a quartic polynomial. We begin the analysis by verifying the root corresponding to the disease-free equilibrium.

THEOREM 6.5 (Disease-free equilibrium.) The model with HIV resistant cells (equations (2.1)-(2.7)), has a disease-free equilibrium given by $E_0' = ((1-w)a/q, (1-w)a/d, 0, 0, 0, wa/q, wa/d)$.

Proof. Assuming that a disease-free equilibrium exists, set $I = 0$. The remaining values follow by simple calculation.

□

THEOREM 6.6 (Stability: disease-free equilibrium). The disease-free equilibrium E_0' is unstable if $p > 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$.

Proof.

The Jacobian of the system at equilibrium $(R^*, T^*, I^*, V^*, J^*, P^*, Q^*)$ is given by:

$$\begin{pmatrix} -(q+\frac{uqV^*}{\alpha+V^*}) & 0 & 0 & -\frac{uqR^*\alpha}{(\alpha+V^*)^2} & 0 & 0 & 0 \\ q+\frac{uqV^*}{\alpha+V^*} & -d-kV^*+\frac{bV^*}{\alpha+V^*} & 0 & -kT^*+\frac{uqR^*\alpha}{(\alpha+V^*)^2}+\frac{bT^*\alpha}{(\alpha+V^*)^2} & 0 & 0 & 0 \\ 0 & kV^* & -e-m & kT^* & n & 0 & 0 \\ 0 & -kV^* & ep & -c-kT^*-\beta Q^* & 0 & 0 & -\beta V^* \\ 0 & 0 & m & 0 & -n & 0 & 0 \\ 0 & 0 & 0 & -\frac{P^*\alpha uq}{(\alpha+V^*)^2} & 0 & -\left(q+\frac{uqV}{\alpha+V}\right) & 0 \\ 0 & 0 & 0 & \frac{P^*\alpha uq}{(\alpha+V^*)^2}+\frac{bQ^*\alpha}{(\alpha+V^*)^2} & 0 & q+\frac{uqV}{\alpha+V} & \frac{bV^*}{\alpha+V^*}-d \end{pmatrix}$$

Linearising the system of equations (2.1)-(2.7)) about E_0' we get the Jacobian:

$$J_0' = \begin{pmatrix} -q & 0 & 0 & -\frac{u(1-w)a}{\alpha} & 0 & 0 & 0 \\ q & -d & 0 & -(1-w)a(\frac{k}{d}+\frac{u}{\alpha}+\frac{b}{\alpha d}) & 0 & 0 & 0 \\ 0 & 0 & -e-m & \frac{k(1-w)a}{d} & n & 0 & 0 \\ 0 & 0 & ep & -c-\frac{k(1-w)a}{d}-\frac{\beta wa}{d} & 0 & 0 & 0 \\ 0 & 0 & m & 0 & -n & 0 & 0 \\ 0 & 0 & 0 & -\frac{uwa}{\alpha} & 0 & -q & 0 \\ 0 & 0 & 0 & wa(\frac{u}{\alpha}+\frac{b}{\alpha d}) & 0 & q & -d \end{pmatrix}$$

Clearly four eigenvalues of J_0' are $-q,-q,-d,-d$. The remaining eigenvalues are given by zeros of:

$$c_3\lambda^3 + c_2\lambda^2 + c_1\lambda + c_0 = 0, \tag{6.5}$$

where

$$\begin{aligned} c_3 &= 1 > 0, \\ c_2 &= n+e+m+c+\frac{ka}{d}(1-w)+\frac{\beta wa}{d}, \\ c_1 &= ne+(n+e+m)\left(c+\frac{ka}{d}(1-w)+\frac{\beta wa}{d}\right)-\frac{epka}{d}(1-w), \\ c_0 &= nec+\frac{neka(1-p)(1-w)}{d}+\frac{ne\beta wa}{d} \end{aligned}$$

Again by Routh-Hurwitz all the roots of (6.5) are negative or have negative real part if and only if $c_0 > 0, c_2 > 0$ and $c_2c_1 > c_0$. We note that if $p > 1+\frac{cd}{ka(1-w)}+\frac{\beta w}{k(1-w)}$ then $c_0 < 0$. This implies that there is one eigenvalue of J_0' has a positive real part. Thus the disease-free equilibrium E_0' is not stable.

□

THEOREM 6.7 The basic reproduction number for the model with HIV resistant cells is given by

$$R_0^P = \frac{m}{2(e+m)} + \sqrt{\left(\frac{m}{2(e+m)}\right)^2 + \frac{kaep(1-w)}{(cd+ka(1-w)+\beta wa)(e+m)}} \tag{6.6}$$

Proof. As in Theorem 6.4 we use the next generation matrix, using the following relevant subsystem:

$$\begin{aligned}
\dot{I} &= (-e-m)I + k(1-w)ad^{-1}V + nJ \\
\dot{V} &= epI + (-c-\beta wad^{-1} - k(1-w)ad^{-1})V \\
\dot{J} &= mI - nJ \\
\dot{Q} &= (uwa\alpha^{-1} + bwa(\alpha d)^{-1})V - dQ
\end{aligned}$$

The transmission matrix F_P and transition matrix S_P for this system are

$$F_P = \begin{pmatrix} 0 & \frac{k(1-w)a}{d} & 0 & 0 \\ ep & 0 & 0 & 0 \\ m & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}, \quad S_P = \begin{pmatrix} -e-m & 0 & n & 0 \\ 0 & c-\beta\frac{wa}{d} - \frac{ka(1-w)}{d} & 0 & 0 \\ 0 & 0 & -n & 0 \\ 0 & \frac{uwa}{d} + \frac{bwa}{\alpha d} & 0 & -d \end{pmatrix}$$

The next generation matrix is defined by $K_P = -F_P S_P^{-1}$ (inverse of the matrix S_P exists) and is given by

$$K_P = \begin{pmatrix} 0 & \frac{ka}{d}(c + \frac{ka(1-w)}{d} + \frac{\beta wa}{d})^{-1} & 0 & 0 \\ \frac{ep}{e+m} & 0 & \frac{ep}{e+m} & 0 \\ \frac{m}{e+m} & 0 & \frac{m}{e+m} & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}$$

The largest eigenvalue of K_P is the basic reproduction number R_0 and is calculated as

$$R_0^P = \frac{m}{2(e+m)} + \sqrt{\left(\frac{m}{2(e+m)}\right)^2 + \frac{kaep(1-w)}{(cd+ka(1-w)+\beta wa)(e+m)}} \tag{6.7}$$

The disease-free equilibrium E_0 is locally asymptotically stable if $R_0^P < 1$, but unstable if $R_0^P > 1$ Diekmann et al. (2009). □

An algebra calculation leads to the following observation.

COROLLARY 6.2 $R_0^P < 1$ if and only if $p < 1 + \frac{cd}{ka(1-w)} + \frac{\beta w}{k(1-w)}$.

THEOREM 6.8 The remaining equilibrium values for the system described by Equations (2.1)-(2.7) are given as roots of a cubic polynomial in the state space variable Q as follows:

$$0 = C_3Q^3 + C_2Q^2 + C_1Q^1 + C_0$$

where

$$C_3 = (p-1)^{-1}((-d-\alpha k+b)\alpha d\beta(b-d) - d\alpha\beta(b-d)^2 - k\alpha^2d^2\beta) \tag{6.8}$$

$$\begin{aligned}
C_2 = {} & \alpha(1-w)ak(b-d)^2 + (p-1)^{-1}((-d-\alpha k+b)\alpha(d\beta wa + dc(b-d) - wa\beta(b-d)) \\
& - d\alpha(p-1)^{-1}(2\beta wa(b-d) + c(b-d)^2) \\
& + (1-w)ak\alpha d(b-d) - k\alpha^2(p-1)^{-1}(-2dwa\beta + d^2c)
\end{aligned} \tag{6.9}$$

$$\begin{aligned}
C_1 = {} & \alpha(1-w)ak2(b-d)wa + (p-1)^{-1}((-d-\alpha k+b)\alpha(dcwa - w^2a^2\beta - wac(b-d)) \\
& - (p-1)^{-1}d\alpha(\beta w^2a^2 + c2wa(b-d)) \\
& + (1-w)ak\alpha(dwa - wa(b-d)) - (p-1)^{-1}k\alpha^2(w^2a^2\beta - 2dwac)
\end{aligned} \tag{6.10}$$

$$C_0 = (p-1)^{-1}((-d-\alpha k+b)\alpha(-w^2a^2c) - d\alpha cw^2a^2 - k\alpha^2w^2a^2c) \tag{6.11}$$

561 *Proof.* As the proof is an algebra exercise, we give only a sketch based on equations (2.1)-(2.7)
562 taken at equilibrium. Equation 5 expresses J in terms of I. Substituting this into equation (2.3) gives

$$TV = ek^{-1}I \tag{6.12}$$

563 Summing equations (2.1) and (2.2), replacing TV with $ek^{-1}I$ and clearing the denominator gives

$$0 = \alpha(1-w)ak + Ie(-d\alpha - \alpha k + b) - d\alpha kT + (1-w)akV - ekIV \tag{6.13}$$

564 Replacing TV with $ek^{-1}I$ in equation (2.4) gives an expression for V in terms of I and Q

$$V = e(p-1)I(c+\beta Q)^{-1} \tag{6.14}$$

565 Summing equations (2.6) and (2.7) and solving for V gives

$$V = (dQ - wa)\alpha(wa + (b-d)Q)^{-1} \tag{6.15}$$

566 Comparing the last two equations gives an expression for I in terms of Q, thus of T in terms of Q
567 using the equation (6.12). Substituting all of this into equation (6.13) gives an expression in Q alone
568 which, when denominators are cleared, leads to the following cubic polynomial with the coefficients
569 given.

$$\begin{aligned} 0 = {} & \alpha(1-w)ak(wa+(b-d)Q)^2 + (-de - e\alpha k + eb)\alpha e^{-1}(p-1)^{-1}((dQ-wa)(c+\beta Q)(wa+(b-d)Q) \\ & - dk\alpha k^{-1}(p-1)^{-1}(c+\beta Q)(wa+(b-d)Q)^2 + (1-w)ak\alpha(dQ-wa)(wa+(b-d)Q) \\ & - ek\alpha^2 e^{-1}(p-1)^{-1}(dQ-wa)^2(c+\beta Q) \end{aligned} \tag{6.16}$$

570 □

571 7. Appendix 2

Table 1: Default model parameter values as described in text.

Parameter	Description	Units	Values	Source
a	Source for uninfected resting CD4$^+$	cells/μ L/day	10.4($\pm$4.8)	Fleury et al. (2000)
q	Activation rate of resting CD4$^+$	day^{-1}	0.01097	calculated Hunt et al. (2003)
d	Death rate of activated CD4$^+$	day^{-1}	0.5377	calculated Hunt et al. (2003)
c	Virus death rate	day^{-1}	333 (5-500)	Boer et al. (2010)
p	Number of virions produced by an infected cells	integer	5×10^4	Boer et al. (2010)
e	Death rate of infected cells	day^{-1}	1	Boer et al. (2010)

Continued on next page

Table 1 – continued from previous page

Parameter	Description	Units	Values	Source
b	Activation of $CD4^+$ from activated $CD4^+$ in virus presence	day^{-1}	0.5	Jelley-Gibbs et al. (2000)
m	Daily rate of infected cells that become latent	day^{-1}	.000001	arbitrary
n	Daily rate of latent cells converting to infected population	day^{-1}	$10^6 m$	Chun et al. (1997)
u	Activation of $CD4^+$ from uninfected resting in virus presence	constant	10	matched to dataPerlmutter et al. (1999)
α	Saturation speed	cells/μL^3	1.8	matched to data Perlmutter et al. (1999)
k	Rate constant for $CD4^+$ becoming infected by free virus	(virions/μL).$^{-1}$ $(day)^{-1}$	0.00015	matched to data Perlmutter et al. (1999)
β	Rate constant for HIV resistant $CD4^+$ reducing free virus	(virions/μL).$^{-1}$ $(day)^{-1}$	0	unknown
w	percent of HIV resistant cells produced	none	0	variable
$R(0)$	Initial concentration of unactivated CD4+ cells	number per μL	948	Fleury et al. (2000)
$T(0)$	Initial concentration of activated CD4+ cells	number per μL	19	Hunt et al. (2003)
$I(0)$	Initial concentration of infected CD4+ cells	number per μL	0.01	arbitrary
$V(0)$	Initial concentration of virions	number per μL	0	estimated
$J(0)$	Initial concentration of latent CD4+ cells	number per μL	0	estimated
$P(0)$	Initial concentration of HIV resistant unactivated CD4+ cells	number per μL	0	estimated
$Q(0)$	Initial concentration of HIV resistant activated CD4+ cells	number per μL	0	estimated

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