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ORIGINAL ARTICLE

A Mathematical Model of HIV Infection: Simulating T4, T8, Macrophages, Antibody, and Virus via Specific Anti-HIV Response in the Presence of Adaptation and Tropism

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Abstract A mathematical model of the host's immune response to HIV infection is proposed. The model represents the dynamics of 13 subsets of T cells (HIV-specific and nonspecific, healthy and infected, T4 and T8 cells), infected macrophages, neutralizing antibodies, and virus. The results of simulation are in agreement with published data regarding T4 cell concentration and viral load, and exhibit the typical features of HIV infection, i.e. double viral peaks in the acute stage, sero conversion, inverted T cell ratio, establishment of set points, steady state, and decline into AIDS. This result is achieved by taking into account thymic aging, viral and infected cell stimulation of specific immune cells, background nonspecific antigens, infected cell proliferation, viral production by infected macrophages and T cells, tropism, viral, and immune adaptation. Starting from this paradigm, changes in the parameter values simulate observed differences in individual outcomes, and predict different scenarios, which can suggest new directions in therapy. In particular, large parameter changes highlight the potentially critical role of both very vigorous and extremely damped specific immune response, and of the elimination of virus release by macrophages. Finally, the time courses of virus, antibody and T cells production and removal are systematically investigated, and a comparison of T4 and T8 cell dynamics in a healthy and in a HIV infected host is offered.

Keywords HIV T cell subset dynamics · Infected macrophage dynamics with tropism and mutation

1. Introduction and biological background

1.1. Introduction

HIV infection and disease progression have been carefully studied and well documented. Many successful models investigating fundamental HIV-immune dynamics have been

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developed, among which we may mention the works by Perelson et al. (1993, 1996), Ho et al. (1995), Kirschner and Webb (1996), Perelson and Nelson (1999), Hellerstein et al. (1999), Stafford et al. (2000), and Jones and Perelson (2005) to cite only a few. The mathematical models, in concert with the wealth of experimental data, produced deep understanding of disease dynamics and gave help in devising significant interventions.

This paper presents a multifaceted model of the progression of HIV infection by describing the dynamics of different T cell subpopulations and of infected macrophages, and taking into account immune recognition of infected cells, production of neutralizing specific antibodies, viral adaptation and change in virus tropism, and thymic aging. In particular, the T cell population is subdivided in the model into T4 and T8 cells and into HIV-specific and non-HIV-specific cells and into healthy and infected cells.

The results of model simulation are in agreement with published experimental data regarding virus, T cell subsets, and antibody concentrations and dynamics (Stafford et al., 2000; Pilcher et al., 2004; Scamurra et al., 2000). The results conform to established features peculiar to HIV progression: initial double viral peaks, initial high T cell concentration, sero conversion, inverted T8/T4 ratio, establishment of a long quasi-steady state, with declining T cell concentrations coupled to rising viral concentration, and eventual descent into AIDS. Simulations predict that slight adjustments of single parameter values may account for commonly reported variations of individual responses. An investigation of the model response with large changes in the parameter values has been also performed, revealing unexpected dynamics and suggesting novel possible interventions.

1.2. Biological background

Human immunodeficiency virus infects cells that present the membrane antigen CD4 (CD4+ cells, mainly macrophages and CD4+ lymphocytes), and as any RNA-virus, mutates extensively during its replication. These characteristics are the major factors affecting the success of the immune system and of drug intervention in combating the disease. The immune efforts of host are thought to be indeed critical in disease control, progression, and long-term survival (Greenberg et al., 1997; Walker and Scadden, 2000; Fauci, 2003; Tripathi and Agrawal, 2007).

The HIV inoculate produces extensive infection of host macrophages (Hatzakis et al., 2000), some infection of CD4+ lymphocytes (T4 cells), and rapid exponential viral growth. Pools of infected cells have been documented as early as 10 days after inoculation (Chun et al., 1998; Dimitrov et al., 1998). The viral concentration reaches two peaks, once after several weeks to about 10^6 viral RNA molecules/mL and again, after several months, to about 10^4 viral RNA molecules/mL (Rouzioux, 2001). These extremely high early viral (and infected cells) concentrations stimulate the innate and the specific immune systems, as revealed by seroconversion. Stimulated by the virus, HIV-specific T4 cells proliferate and differentiate into effector helper cells that stimulate specific B cells proliferation and subsequent antibody production (Brander et al., 2005; Tran, 1999). Specific antibodies bind to the virions and neutralize them or target them for removal. Neutralizing antibodies are a promising component of an effective HIV vaccine (Stiegler et al., 2001; Nishimura et al., 2002). Specific CD8+ lymphocytes (T8 cells), stimulated by infected cells, proliferate

and differentiate into cytotoxic effector cells that induce infected cell death (Janeway et al., 1996). After 6 months to 2 years, the viral, antibody, infected macrophages, and T cell concentrations stabilize to set points (Ho, 2000), heralding a quasi-steady state that may last for years, during which the host remains relatively symptom free. During this phase, T cell concentrations slowly decrease, and the viral concentration slowly increases (Pantaleo et al., 1993) and mutate toward greater T-tropism. The system descends into AIDS when the host exhibits severe immune degradation and the viral concentration begins to rise rapidly (Hare, 2006). There is a wide consensus in defining the AIDS as the state in which the T4 concentration in blood is lower than 200 cells/mm^3.

Macrophages are innate cells subject to homeostasis that ingest and remove free virus. When infected, they retain some immune competence and functions and become a viral source, producing new virus at a slow rate, without undergoing cell destruction (Fauci, 2003; Aquaro et al., 2002a, 2002b). T4 cells are also susceptible to infection when, once inside them, the HIV-RNA reversely transcribes into DNA and the HIV-DNA is successfully incorporated into the host cell genome: the T4 cells thus become latently infected. Latently infected cells can remain dormant (Ho, 1997), proliferate generating infected daughter cells (Kim and Perelson, 2006), differentiate, or because of cell stimulation, activate. Actively, or productively infected cells burst in about 2 days producing 500–1000 new virions/cell (Bajaria et al., 2002; Kirschner and Perelson, 1995).

Infected macrophages and latently infected T cells elicit decreased immunogenicity, that results in lower stimulation and replication of specific T8 cells and decreased T8 cytotoxic removal of infected cell (Fujiwara and Takiguchi, 2007; Tripathi and Agrawal, 2007; Swann et al., 2001).

Both viral and antibody adaptation occur during the course of disease progression. HIV is a rather inaccurate and prolific reproducer, and virus mutation can reduce the effective half-life of a new HIV drug to the order of 2–4 weeks (Robertson et al., 1995). Likewise, antibodies adapt over time, following the virus mutation and increasing the strength of the virus binding (affinity maturation and hyperimmunization), with repeated antigen exposure (Richman et al., 2003; Janeway et al., 1999, p. 36; Coleman et al., 1992, p. 126). The initial HIV inoculate seen in all forms of HIV transmission is largely M-tropic, i.e., virus infects macrophages more easily than T4 cells (Poveda et al., 2006; Connor et al., 1997): M-tropic HIV binds to CCR5, a coreceptor on the surface of macrophages, while T-tropic HIV, binds to CXCR4 on the surface of T4 cells (Shankarappa et al., 1999). The virus becomes more T-tropic with time and established tropism data suggests that M-tropism is associated with slow progression and T-tropism with fast progression to AIDS (Azzam et al., 2006; Gorry et al., 2005; Shankarappa et al., 1999; Connor et al., 1997; Crowe et al., 1989).

2. The model of HIV infection

In the simulations, the host is 36 years old with 1300 T4 and 617/mm^3 T8 cells, when infected with 3 virons/mm^3 (6000 RNA/mL). A list of the variables, their initial values, units, and sources are found in Table 1: the parameters, their initial values, units, and sources are found in Table 2. Explicit time varying parameters, time dependent due to age, viral mutation, antibody adaptation, and tropism are discussed in Eqs. (18)–(22).

Table 1 Variables

Variable	Definitions	Initial values	Units (in blood)
$\tilde{T}_{4n}$	Healthy HIV-specific noneffector T4	0.92	Cells/mm^3
$\tilde{T}_{4e}$	Healthy HIV-specific effector T4	0	Cells/mm^3
$\tilde{T}^i_{4n}$	Latently infected HIV specific noneffector T4	0	Cells/mm^3
$\tilde{T}^i_{4e}$	Latently infected HIV specific effector T4	0	Cells/mm^3
T_{4n}	Healthy non-HIV specific non-effector T4	920	Cells/mm^3
T_{4e}	Healthy non-HIV specific effector T4	379	Cells/mm^3
	Total T4	1300	
T^i_{4n}	Latently infected non-HIV specific non-effector T4	0	Cells/mm^3
T^i_{4e}	Latently infected non-HIV specific effector T4	0	Cells/mm^3
T_{ii}	Actively infected T4	0	Cells/mm^3
$\tilde{T}_{8n}$	Healthy HIV-specific noneffector T8	0.47	Cells/mm^3
$\tilde{T}_{8e}$	Healthy HIV-specific effector T8	0	Cells/mm^3
T_{8n}	Healthy non-HIV specific noneffector T8	467	Cells/mm^3
T_{8e}	Healthy non-HIV specific effector T8	150	Cells/mm^3
	Total T8	617	
S	Anti-HIV specific neutralizing antibodies	0	molecules/mm^3 1 molecule/mm^3 $= 1.67 \times 10^{-18}$ M
M_i	Infected macrophages	0	Cells/mm^3
V	Virus	3	Particles/mm^3
	RNA $=$ V $\times$ 2000	6×10^3	RNA/mL

A discussion of the rational for some of the estimated parameters is offered at the end of the model section.

In order to give a detailed description of the immune cell dynamics and their effect on disease progression, T cells are partitioned in T4 cells and T8 cells, HIV-specific (ST4 and ST8) and nonspecific cells, effector cells (i.e., functional CD4+ helper and CD8+ cytotoxic cells) and noneffector cells (i.e., naive and memory cells). Moreover, T cells are subdivided in healthy, i.e., noninfected, and latently infected cells. Only a fraction of T lymphocytes is circulating in blood (about 1/50; Janeway et al., 1996), the most part being concentrated in the lymphatic organs. For simplicity, these two body compartments are not distinguished in the model, implicitly assuming that the exchange between blood and organs is sufficiently fast to guarantee that, at each time, the number of cells of a given subpopulation in blood is a fixed fraction of the number of those cells in the body. Thus, the different cell subpopulation can be traced by their concentrations in blood. The blood concentration of noninfected specific cells in the T4 and T8 cell pools are denoted by $\tilde{T}_{4n}$ and $\tilde{T}_{4e}$, and $\tilde{T}_{8n}$ and $\tilde{T}_{8e}$, respectively, whereas T_4, and T_8 denote nonspecific cell concentrations. The subscripts n and e denote noneffector and effector cells, respectively. The superscript i denote latently infected cells. Actively infected cells are represented by a single subpopulation, whose concentration is T_{ii}. M and M_i denote the concentrations of total and infected macrophages, M is assumed constant. S and V denote the concentra-

Table 2 Parameters

Parameter	Definition	Reference Value	Reference
a_M	Conversion factor for the macrophage compartment	0.03	estimated. See Parameter Estimation
a_T	Conversion factor for the T cell compartment	0.06	estimated. See Parameter Estimation
c_2	Michaelis–Menten half saturation for V in blood	1000 particles /mm^3	estimated. See Parameter Estimation
c_1	Michaelis–Menten half saturation for V in thymus	616.6 particles /mm^3	Appendix B derived
δ	Coefficient of reduced immune recognition of latently infected T4 and infected macrophages.	0.001	Collins et al. (1998); Walker and Scadden (2000); Fujiwara and Takiguchi (2007); Tripathi and Agrawal (2007)
e	Rate of infection of thymic T4 cells	0.064/mm^3-viron-day	Appendix B derived
f	T4/(T4+T8) ratio of neonate T cells	0.524	estimated
k_8	Rate T_{8e} removes infected cells	2.5 mm^3/(cell day)	estimated. See Parameter Estimation
k_m	Rate macrophages remove virus	60 particles/day	estimated. See Parameter Estimation
$\bar{k}_v$	Rate of infection of T4 cells at time of infection	0.089/mm^3-viron-day	Stafford et al. (2000). See Parameter Estimation
$\bar{k}_{vm}$	Rate of infection of healthy macrophages at the time of infection	1.19/day	estimated. See Parameter Estimation
M	Total macrophage (healthy+infected)	360/mm^3	estimated. See Parameter Estimation
μ_{Ab}	Death rate of antibodies	0.023/day	estimated
μ_M	Death rate of healthy and infected macrophages	0.087/day	Yamamoto et al. (2007) (0.1155); Aquaro et al. (2002a, 2002b) Ho (1997) (0.049)
$\mu_{4e} = \mu_{4e}^i$	Death rate of T_{4e}, ST_{4e}, T_{i4e}, and ST_{i4e}	0.015/day	estimated. See Parameter Estimation
$\mu_{4n} = \mu_{4n}^i$	Death rate of T_{4n}, ST_{4n}, T_{i4n}, and ST_{i4n}	0.005/day	estimated. See Parameter Estimation
μ_{8e}	Death rate of T_{8e}, ST_{8e}	0.018/day	estimated. See Parameter Estimation
μ_{8n}	Death rate of T_{8n}, ST_{8n}	0.006/day	estimated. See Parameter Estimation
μ_{ii}	Death rate of actively infected cells	0.47/day	Ho (1997) (0.6); Perelson et al. (1996) (0.5); Kirschner and Webb (1996) (0.24)
μ_v	Death rate of free virus	3.0/day	Ho (1996)
N	Number of virons per bursting T_{ii}	850 particles/cell	Bajaria et al. (2002); Stafford et al. (2000) (used); Kirschner and Perelson (1995)
A	Age at inoculation	36 years	Hellerstein et al. (1999)
p	Fraction of activated latently infected cells that become actively producing cells	0.03	estimated. See Parameter Estimation

Table 2 (*Continued*)

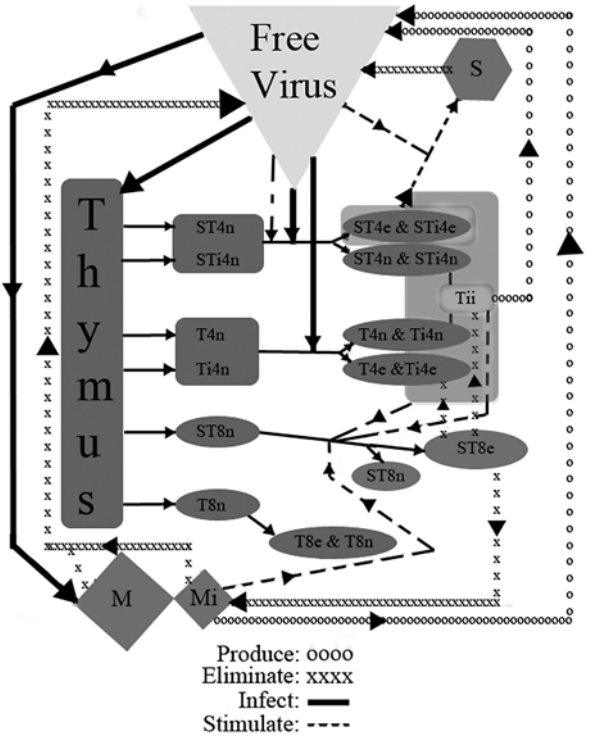
Parameter	Definition	Reference Value	Reference
φ	Fraction of T cells that differentiate	0.64	Appendix C derived
p_{im}	Virus production rate by infected macrophages	34 particles/(cell day)	Tsai et al. (1996)
ρ_{Ab}	Maximal antibody production rate per helper T cell	155 molecules/(cell day)	estimated
ρ_4	Proliferation rate of ST4 in presence of virus	1.98/day	estimated. See Parameter Estimation
ρ_8	Proliferation rate of ST8 in presence of infected cells	0.36 mm^3/(cell day)	estimated. See Parameter Estimation
r_4	Proliferation rate of T4	0.0097/day	Appendix C derived
r_8	Proliferation rate of T8	0.0091/day	Appendix C derived
ν	ST4/total T4 ratio in the thymic output	10^{-3}	Janeway et al. (1999)
$\bar{s}$	T cells flow from thymus to blood	6.09 cells/(mm^3 day)	Appendix C derived
τ	time in thymus T cells are CD4+CD8+	7 days	Janeway et al. (1999); Poveda et al. (2006)
vol	Total volume	15 liter	Janeway et al. (1999)
vblood	Volume of blood	5 liter	Janeway et al. (1999)

To support unambiguous parameter modification, the parameters are not concatenated

tions of specific anti-HIV antibody and free virus, respectively, and their concentrations are assumed the same in blood and in the lymphatic organs.

The sources of noneffector cells are the thymus and cell proliferation. The thymus produces both T4 and T8 cells and thymic production decreases as the host ages. In the presence of HIV, thymic T cells can be infected before final selection, after which they are released. The stimulation of proliferation depends on the specificity of the T cell. Replication of specific T4 and T8 cells is stimulated by the recognition of viral epitopes presented by antigen presenting cells and, respectively, by infected cells. Therefore, specific T cell concentrations will be coupled to disease progression. A basal stimulation of nonspecific T4 and T8 cell due to environmental antigens is also assumed. Healthy nonspecific T4 cells provide a background of infectable cells that lack any functional ability to fight HIV: on the contrary, if latently infected T cells are stimulated by ambient antigen, they proliferate infected daughter cells and can be activated to produce virus. The source of effector T cells is the noneffector cells that differentiate into functional cells. The role of specific T4 helper cells and specific cytotoxic T8 cells are the stimulation of anti-HIV antibody production and the cytotoxic removal of HIV infected cells, respectively. Noneffector latently infected cells can be activated to produce free virions, resulting in an accelerated cell death. Sinks from healthy cell compartments are infection and natural cell death, and from infected compartments are cytotoxic removal of infected cells, activation, and bursting, and natural cell death.

The flow chart below depicts the highlights of the model dynamic discussed. The model dynamics involve the virus, T4 and T8 subsets, antibodies, and macrophage and the details are explained with the equations below.



Model Dynamics Flow-chart: The virus infects (e, k_v, k_{vm}) and stimulates T4 cells, is produced $(N,\ p_{\mathrm{im}})$ and is removed (k_m, k_{Ab}). Likewise, the T4 cell subsets can proliferate and differentiate (ρ_4, r_4); ST_{4e} can stimulate antibody production (ρ_{Ab}), whereas infected T4 cells can be removed by cytotoxic T8 cells $(k_8, \delta k_8)$. Infected cells (latently infected, actively infected and M_i), are in turn destroyed by the cytotoxic cells they stimulated at the rates $k_8\delta$ and k_8. Finally, macrophages remove virus (k_m), and, when infected, produce virus (p_{im}).

3. Equations

The equations of the model are grouped into those for specific and nonspecific T4 cells, then for T8 cells. The last group contains the equations for actively infected T cells, HIV-specific antibodies S, infected macrophages M_i, and free virus V. According to the above assumptions, we may write

$$\frac{d\tilde{T}_{4n}}{dt} = vf\left(1 - \frac{eV}{c_1 + V}\right)s + (1-\varphi)\frac{\rho_4 V}{c_2 + V}\tilde{T}_{4n} - k_v\frac{V}{c_2 + V}\tilde{T}_{4n} - \mu_{4n}\tilde{T}_{4n}, \tag{1}$$

$$\frac{d\tilde{T}_{4e}}{dt} = \varphi\frac{\rho_4 V}{c_2 + V}\tilde{T}_{4n} - k_v\frac{V}{c_2 + V}\tilde{T}_{4n} - \mu_{4e}\tilde{T}_{4n}, \tag{2}$$

$$\frac{d\tilde{T}^i_{4n}}{dt} = vf\frac{eV}{c_1 + V}s + \big[(1-\varphi)(1-p) - p\big]\frac{\rho_4 V}{c_2 + V}\tilde{T}^i_{4n}$$

$$+ k_v \frac{V}{c_1 + V} \tilde{T}_{4n} - \left(k_8 \delta \tilde{T}_{8e} + \mu^i_{4n}\right) \tilde{T}^i_{4n}, \tag{3}$$

$$\frac{d\tilde{T}^i_{4e}}{dt} = \varphi(1-p) \frac{\rho_4 V}{c_2 + V} \tilde{T}^i_{4n} + k_v \frac{V}{c_2 + V} \tilde{T}_{4e} - \left(k_8 \delta \tilde{T}_{8e} + \mu^i_{4e}\right) \tilde{T}^i_{4e}, \tag{4}$$

$$\frac{dT_{4n}}{dt} = (1-v) f \left(1 - \frac{eV}{c_1 + V}\right) s + (1-\varphi) r_4 T_{4n} - k_v \frac{V}{c_2 + V} T_{4n} - \mu_{4n} T_{4n}, \tag{5}$$

$$\frac{dT_{4e}}{dt} = \varphi r_4 T_{4n} - k_v \frac{V}{c_2 + V} T_{4e} - \mu_{4e} T_{4e}, \tag{6}$$

$$\frac{dT^i_{4n}}{dt} = (1-v) f \frac{eV}{c_1 + V} s + \left[(1-\varphi)(1-p) - p\right] r_4 T^i_{4n} + k_v \frac{V}{c_2 + V} T_{4n} - \left(k_8 \delta \tilde{T}_{8e} + \mu^i_{4n}\right) T^i_{4n}, \tag{7}$$

$$\frac{dT^i_{4e}}{dt} = \varphi(1-p) r_4 T^i_{4n} + k_v \frac{V}{c_2 + V} T_{4e} - \left(k_8 \delta \tilde{T}_{8e} + \mu^i_{4e}\right) T^i_{4e}. \tag{8}$$

The first and second sets of equations, Eqs. (1)–(4) and Eqs. (5)–(8), represent the dynamics of specific and nonspecific T4 cells. These equations will serve as a template for the T8 cell compartments. In (1) and (3), the first term represents the thymic source. The total production rate (cell/day) is denoted by $s(t)$ and is an assigned function of time because thymic production is dependent on the age of the host. v is the fraction of cells that are specific to HIV epitopes, f is the fraction of T cells that are CD4+. A fraction $eV(t)/(c_1 + V(t))$ of T4 cells released by the thymus is assumed to be infected (see Appendix A for the relationship between e and k_v, and c_1 and c_2). The second term of (1) describes the proliferation of specific T4 cells in the presence of free virus, ρ_4 being the maximal proliferation rate constant. The process of differentiation into effector cells is simply represented assuming that a constant fraction φ of the cells produced by proliferation is differentiated. These cells are moved to (2) and (4) as a source of effector cells. The third term in (1) and (2) simulates the infection of healthy cells by free virus (with a maximal rate k_v). These cells are moved to the infected cell compartments and the corresponding term is found in Eqs. (3) and (4) (Swiggard et al., 2005). The fourth term in (1) is the natural cell death. Proliferation for latently infected cells is the same as uninfected cells but includes the probability of activation p. In particular, we assume that after the stimulation by the specific antigen, a T4 latently infected cell may become actively infected with probability p, whereas with probability $(1-p)$ it may undergo replication. In (3) and (4), HIV-specific cytotoxic T8 effector cells eliminate infected cells by modulating the removal rate; the coefficient k_8 is weakened by the constant $\delta < 1$, due to the lower "visibility" of latently infected cells. The equations for non-specific T4 cells are similar to Eqs. (1)–(4), with a constant proliferation rate, r_4 which takes into account the background average stimulation by their antigens.

$$\frac{d\tilde{T}_{8n}}{dt} = v(1-f)s + (1-\varphi) R \tilde{T}_{8n} - \mu_{8n} \tilde{T}_{8n}, \tag{9}$$

$$\frac{d\tilde{T}_{8e}}{dt} = \varphi R \tilde{T}_{8n} - \mu_{8e} \tilde{T}_{8e}, \tag{10}$$

$$\frac{dT_{8n}}{dt} = (1-v)(1-f)s + (1-\varphi)r_8 T_{8n} - \mu_{8n} T_{8n}, \tag{11}$$

$$\frac{dT_{8e}}{dt} = \varphi r_8 T_{8n} - \mu_{8e} T_{8e}, \tag{12}$$

The quantity R, in Eqs. (9) and (10) is the rate of proliferation of specific T8 cells in the presence of infected cells; R is defined in Eq. (17) below.

The equations for the T8 subsets, Eqs. (9)–(12), are analogous to (1), (2) and (5), (6) with the following differences: specific T8 cell proliferation is stimulated by latently and actively infected cells; the rate constant of proliferation for nonspecific T8 cells is r_8. T8 cells in the main body compartments, since they do not display CD4+ antigen, cannot be infected. T8 cells could be infected in the thymus, since thymocytes are transiently CD4+CD8+ before differentiation into CD4+ and CD8+ cells (Janeway et al., 1999, p. 233; Janeway et al., 1996, p. 6:6–6:9). Thus, a subpopulation of latently infected T8 cells might be present (Saha et al., 2001; Kolchinsky et al., 1999; Kaneko et al., 1997). Its concentration, however, is very low and we disregarded this possibility in the simulations, and derived the suitable equations in Appendix C.

The spontaneous death rates for T4 and T8 cells are different, as are the death rates for noneffector and effector T cells; however, the death rates for the parallel subsets of healthy and latently cells are taken to be the same. Note that nonspecific T8 cells do not influence the progression of infection, thus Eqs. (11) and (12) could be omitted. We include them in the model to describe the time changes of the T4/T8 ratio and the T8 concentration.

$$\frac{dT_{ii}}{dt} = p\left(r_4 T_{4n}^i + \frac{\rho_4 V}{c_2+V}\tilde{T}_{4n}^i\right) - \left(k_8 \tilde{T}_{8e} + \mu_{ii}\right) T_{ii}, \tag{13}$$

$$\frac{dS}{dt} = \rho_{\mathrm{Ab}}\left(\tilde{T}_{4e} + \tilde{T}_{4e}^i\right)\frac{V}{c_2+V} - k_{\mathrm{Ab}} V S - \mu_{\mathrm{Ab}} S, \tag{14}$$

$$\frac{dM_{ii}}{dt} = k_{vm}\frac{V}{c_2+V}(M - M_i) - \left(k_8 \delta \tilde{T}_{8e} + \mu_M\right) M_i, \tag{15}$$

$$\begin{aligned}\frac{dV}{dt} = {} & p_{\mathrm{im}}\frac{M_i}{a_M} + N\mu_{ii}\frac{T_{ii}}{a_T} - k_m \frac{V}{c_2+V}\frac{M}{a_M} - \frac{eV}{c_1+V}\frac{s}{a_T} \\ & - \frac{k_v}{a_T}\frac{V}{c_2+V}\left(\tilde{T}_{4n} + \tilde{T}_{4e} + T_{4n} + T_{4e}\right) - k_{\mathrm{Ab}} S V - \mu_V V.\end{aligned} \tag{16}$$

Equation (13) represents the dynamics of actively infected cells: the source comes from all the latently infected T cell compartments according to the probability of activation p, and the second term, the sink, accounts for the cytotoxic removal and death.

In Eq. (14), HIV-specific neutralizing antibodies are considered. The first term describes antibody production by B-plasma cells, controlled by specific T4 helper cells in the presence of virus, at the rate ρ_{Ab}. Antibodies eliminate free virus by the formation and removal of the antibody-virus complex, and the removal of these complexes implies the removal of antibodies, also. This process is simply depicted in the second term, proportional to the (time dependent) rate k_{Ab}. The third term takes into account the natural antibody loss.

Equation (15) portrays the infected macrophage dynamics. The first term represents the infection of healthy macrophages (whose concentration is $M - M_i$) at the rate k_{vm}.

The loss, described in the second term, is given by the removal by cytotoxic T8 (reduced with respect to the removal of actively infected cells) and by cell death.

The last equation, Eq. (16), describes the dynamics of virus. The first two terms in the equation depict viral production. The first term represents the production by infected macrophages: p_{im} is the number of virions released by an infected macrophage per day. The second term gives the production by actively infected T cells: N is the number of virions released by a bursting actively infected cell, T_{ii}, $N \times \mu_{ii}$ is thus the number of virions produced per cell and per day. The remaining terms represent the viral loss. Virus is removed by macrophages and by antibodies by the formation and removal of antibody-virus complexes (see Eq. (14)). Additional viral losses included in the equation are the loss of virus engaged in the infection of thymic T cells and in the infection of circulating T4 cells, and the spontaneous viral decay.

a_M and a_T in Eq. (16) are correcting coefficients taking into account that the virus is produced (or removed) not only by the macrophages and T cells in blood, but also by the cells residing in other body compartments. If M is the concentration of macrophages in blood, taking into account that the fraction of macrophages circulating is about $1/100$, the total number of macrophages in the body is vblood $M/0.01$, where vblood is the blood volume. Since the total number of virus in the body is V vol, where vol is the volume of body fluids, in writing the differential equation for V the contribution of macrophages will be correctly given dividing M by $a_M = 0.01$ vol/vblood. A similar argument implies that the T cell blood concentrations must be divided by $a_T = 0.02$ vol/vblood, recalling that about $1/50$ of the total T lymphocytes is circulating.

The proliferation rate R in Eqs. (9) and (10) is given by

$$R = \rho_8 \left(T_{ii} + \delta \left(\tilde{T}^i_{4n} + T^i_{4n} + \tilde{T}^i_{4e} + T^i_{4e} + \frac{a_T}{a_M} M_i \right) \right). \tag{17}$$

Where ρ_8 is proportionality constant and δ is the coefficient that expresses the reduced capability of latently infected T4 and infected macrophages in the stimulation of ST8 proliferation. Actively infected cells provide full stimulation.

The parameters $s, k_v, e, k_{vm}, k_{\mathrm{Ab}}$ are time varying according to assigned functions. The thymic source s is decreasing with time following the decline of thymic production with aging. Conversely, k_v, e, k_{vm}, k_{Ab} are increasing functions of t to represent the mutation of virus toward increasingly aggressive strains, related also to the increasing tropism for T cells, and the consequent adaptation of the humoral immune response that results in an increasing rate of the antibody-virus complex removal. We have assumed the following phenomenological expressions:

$$s(t) = \bar{s} \left(\frac{100 - A - t/365}{100} \right)^{1.8}, \tag{18}$$

$$k_v(t) = \bar{k}_v \left(1 + \frac{t}{t_3} \right) u(t), \tag{19a}$$

$$e(t) = e \left(1 + \frac{t}{t_3} \right) u(t), \tag{19b}$$

$$k_{vm}(t) = \bar{k}_{vm} \left(1 + \frac{t}{t_3} \right), \tag{20}$$

$$k_{\mathrm{Ab}}(t) = \bar{k}_{\mathrm{Ab}} \left(1 + \frac{t}{t_3} \right) u(t), \tag{21}$$

where A denotes the age of the host at the inoculation (in years), and

$$u(t) = \frac{t + t_1}{t + t_2}, \tag{22}$$

$u(t)$ simulates the increasing t-tropism of virus for T cells, t_1, t_2, and t_3 are suitable characteristic times with $0 < t_1 < t_2$.

The function $e(t)$ is related to $k_v(t)$ according to Eq. (6) derived in Appendix A.

4. Parameter estimation rationale

The rationale for the choice of the value of some of the parameters is described.

The estimation of k_8, the rate of cytotoxic removal of infected cells, was performed following a derivation found in Edelstein-Keshet's (1988) book (pp. 409–413) of the time for a macrophage to find a bacterium in two dimensions. In three dimensions, using the reported values, if the macrophage goes straight for the target driven by efficient chemotaxis, the time to reach it in a sphere of volume equal to 1 mm^3 can be predicted to be 3.4 hours. We assume a similar scenario for cytotoxic ST8 cells reaching infected cells. Taking into account that a cytotoxic T8 cells spends 40–60 minutes with the target cell to induce apoptosis, disengages, and moves on to kill other targets (Janeway et al., 1999, p. 279; Tizard, 1992, p. 264), a cytotoxic T8 cell spends ~4.5 hours for every induced killing, and hence can kill more than five infected cells in a day. Thus, the conservative value $k_8 = 2.5$ (mm^3/cell)/day has been chosen.

The time thymic T cells are CD+4 (double positive CD+4 and CD+8) and can be HIV infected, τ is used to derive the values of e and cv_1 in Appendix A. The total thymic T cell residence is ~3 weeks (Poveda et al., 2006; Janeway et al., 1999, pp. 233, 242). The cells are CD+4 during 2 of the 4 developmental stages: double negative, double positive with positive selection, double positive with negative selection, and either CD+4 or CD+8 (single positive) for final selection. The double positive cells first proliferate and then, by the 16 or 17 day of residence, express α and $\alpha\beta$ chains, leading to the final selection and to the single positive thymocytes release into the periphery (Kuby, 1997, Chap. 12). A conservative estimate for τ of 1 week is used.

For p, the probability for a latently infected cells activation, the value used by Bajaria et al. (2002), 0.03 is chosen. The death rate of the actively (productively) infected T4 cells, $\mu_{ii} = 0.47$/day, is slightly larger than the average of the following three reference values: Ho (1997) $\mu_{ii} = 0.6$, Perelson et al. (1996) $\mu_{ii} = 0.5$, and Kirschner and Webb (1996) $\mu_{ii} = 0.24$/day. The parameters a_T and a_M are estimated using the volume of extracellular fluids (vol) which is 15 liter and the volume of blood (vblood) which is 5 liter. Hence, $a_T = (0.02/\text{vblood}) \times \text{vol} = 0.06$ and $a_M = (0.01/\text{vblood}) \times \text{vol} = 0.03$.

k_v, the intrinsic rate of infection of T4 cells, is chosen to be 0.065/day according to Stafford et al. (2000). However, the actual value of the rate of infection is an increasing function of t, to represent the mutation of virus toward increasing aggressivity and increasing tropism for T cells, given by Eq. (19) and Eq. (22). The values of t_1 and t_2 are 7 and 42 days, respectively; these values are chosen to reproduce a reasonable delay of tropism adaptation, so that $k_v(0) = 0.065 \times 7/42 = 0.0108$/day. The value of t_3 is chosen equal to 40,000, so reproducing a very mild increase of the basic infectiveness.

k_{vm} and k_m, the rate of infection of macrophages and the rate of macrophage removal of free virus, respectively, have been adjusted to give simulation results that agree with the reported values for virus concentration (Pantaleo et al., 1993). The rate of infection of macrophages should, however, be much greater than the initial rate of infection of T4 cells since the initial inoculate is M-tropic. Moreover, as the virus is "looking" for CD+4 cells to infect, the macrophages are "looking" for virus to engulf, so that the dynamics of infection may be ambiguous. All the macrophages that are not infected are considered here to be susceptible to infection. Because of this assumption that also includes immature macrophages (monocytes), the concentration of infectable macrophages in blood is taken to be 360 cells/mm^3, about 10 times greater than the value used by Kirschner and Perelson (1995), while the number of virions released per infected macrophage is assumed to be 34, about 1/10 the value they used.

Concerning the death rates of T4 and T8 cells, different estimated have been reported in the literature, although there is little information about the subsets, T_{4n}, T_{4e}, T_{8n}, and T_{8e}. Hellerstein et al. (1999) report the values of 0.008 and 0.009/day for T4 and T8 death rates, respectively. Stafford et al. (2000) gives a range of T4 death rates between 0.0043–0.02/day. Harrington et al. (2008) report that the half-lives of CD4 and CD8 T-cell effectors are not significantly different, and they estimate the effector half-life to be around 15 days, i.e., death rate ∼0.046/day, which is more than twice the highest value found by Stafford. Using these values as guidelines, we select the following values: $\mu_{4n} = 0.005$, $\mu_{4e} = 0.015$, $\mu_{8n} = 0.006$, and $\mu_{8e} = 0.018$/day.

The value of parameter c_2, the Michaelis–Menten half saturation for V, is chosen to be of the order of the viral blood concentration at first peak (Pantaleo et al., 1993).

5. Global response of the model and model validation

The validity of the model is assessed by considering the global variables, T4, T8, and virus over the course of infection, and then looking at the specific cell and the infected cell dynamics during illness.

5.1. General results

The agreement of the model response with established features of the HIV infection is presented in this section, to show the competence of the model to be a valid simulator of HIV dynamics and progression. The host, a 36-year-old subject with 1300 T4 and 617 T8 cells/mm^3 in plasma at the time of infection, is inoculated with a viral concentration of 3 virions/mm^3 (i.e., 6000 viral RNA molecules/mL). The simulated time course of T4 and T8 cells and virus, of the specific immune response, and finally of infected cells, are described. Simulations of T4, T8 and viral RNA concentrations in plasma are seen in Fig. 1.

These results simulate initial inoculation, double viral peaks, and progression through the acute stage when T4 and T8 cells reach a maximum, all features widely described in the literature (Roos et al., 1994; Koup et al., 1994).

With the establishment of the set points, first for T4 after 8–9.6 months at 1020 cells/mm^3, next for viral RNA decreasing to 4.2×10^4 molecules/mL in 1.4–1.8 years, and then for T8 reaching 1130 cells/mm^3 in 1.3–2.3 years, the system stabilizes

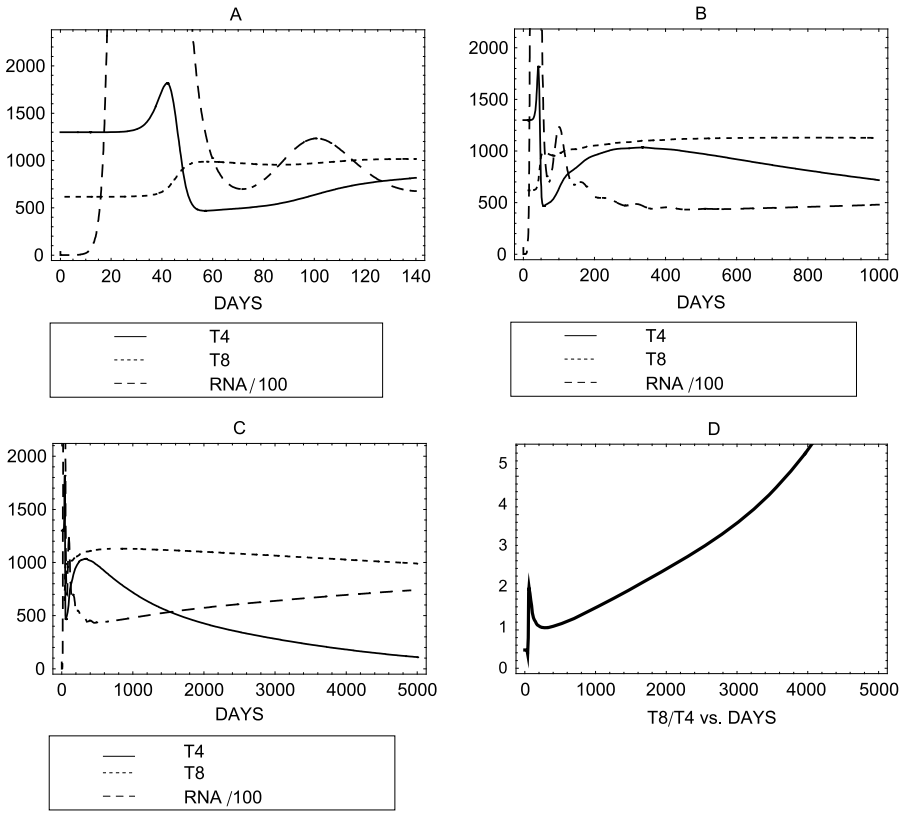


Fig. 1 Global HIV infection dynamics; T4, T8, and viral RNA concentrations (panels A, B, C); T8/T4 ratio (panel D). The viral concentration rises after 10 days, reaching an initial viral peak of 1.84×10^6 RNA molecules/mL in 30 days, followed by the rising of T4 and T8 cells to 1750 and 1000 cells/mm^3 respectively. These concentrations subsequently decrease, T4 concentration to below 500. Viral RNA reaches a second smaller peak of 1.23×10^5 molecules/mL in 100 days (see Daar et al., 1991). At set points, T4 concentration is 1020 cells/mm^3, T8 concentration is 1120 cells/mm^3, below and above their initial respective values, and viral RNA is 4.2×10^4 molecules/mL. AIDS occurs in 10 years.

and the variables begin their final changes (panel B). Steady state is indeed in progress, heralding a long "quasi-stable" period, during which the virus increases and T cells decrease. AIDS occurs after 10 years when T4 falls to 200 cells/mm^3 (Bacchetti and Moss, 1989; Piatak et al., 1993), viral RNA reaches 6.6×10^4 molecules/mL and T8 drops to 980 cells/mm^3 (panel C).

In panel B, as well as in the perturbations, a third viral peak, reaching 7×10^4, a value very close the concentration at AIDS, is observed. This third peak occurs after about 5 months, as establishment of the set points begins, and because of the timing, since the stage for progression is set early in disease, this peak may possibly inform the set points and the course of further disease.

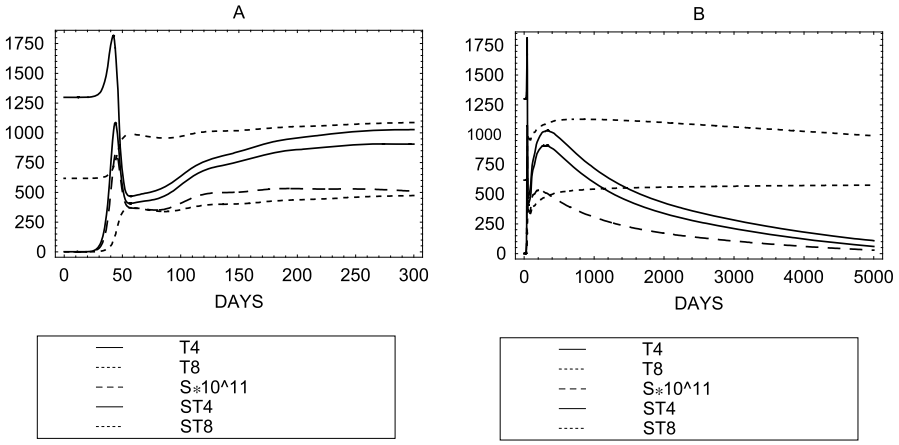


Fig. 2 HIV-specific response: specific T cell (ST4 and ST8) concentrations and neutralizing antibody concentration (S). ST4 concentration reaches a peak of 1010 cells/mm^3 in 40 days, decreases and rises again; ST8 concentration rises to 370 cells/mm^3 in 50 days, decreases slightly and continues rising. S, correlated to the ST_{4e} concentration, increases to 8.6×10^{-9} M, drops to 3.7×10^{-9} and rebounds with ST4 (panel A). ST4 reaches a second peak at 950 cells/mm^3 and drops to below 200 cells/mm^3 more than a year before the total T4 concentration does (panel B). ST8 concentration reaches 550 cells/mm^3 and decreases slightly: the second rise in ST8 is very small and the subsequent ST8 decrease is nominal and probably due to thymic aging. The antibody concentration S reaches a second peak at 5.4×10^{-9} M and then drops as does ST4, seemingly in opposition to the second viral decrease and subsequent rise (see Fig. 1B).

The T8/T4 ratio, initially at 0.475, after a short slight decrease rises and becomes inverted (i.e., >1) in 50 days, reaching 2 after the second viral peak, decreases for a short time to 1.1, and then increases through steady state and beyond AIDS (panel D).

5.2. The specific immune response to HIV infection

Activation of the specific immune system depends on virus and infected cells to stimulate the proliferation and differentiation of specific T4 and T8 cells, and on specific T4 helper cells and virus for inducing antibody production. Figure 2 shows the simulated non-specific and HIV-specific T4 and T8 cell concentrations (cell/mm^3) and the antibody concentration (M).

T4, ST4, T8, ST8 cell concentrations, and antibody concentration all peak and fall, after the first and second viral humps, at 30 and 100 days. The specific immune response begins after 30 days. ST4 cells begin to rise first than antibodies and ST8 cells after 40 days. This is a very strong early response, since after the first and second viral peak most to the T4 and 1/3 of the T8 cells are HIV specific (panel A and B). Specific T cells and antibodies have individual set points (panel B), in fact, specific T cells inform the total T4, and to a lesser degree, total T8 set points.

According to the simulation, sero-conversion may be considered to occur after 3 weeks, when ST4 cells and antibodies begin to rise, or with the decline of the first viral peak after 8–9 weeks, or with the second peak, depending on its definition (Janeway et

al., 1999). The specific immune system appears to mount a vigorous response throughout disease progression.

5.3. Infected cells dynamics

The third groups of major agents are the infected cell populations: latently and actively infected T4 cells, and infected macrophages.

Infected cell concentrations achieved during the first weeks after infection, seen in Table 3, depict the rapid course of systemic HIV infection; however time delay is not included so the actual times are approximate.

The initial inoculate of free virus of 9×10^7 RNA molecules, after a very sharp drop, rebounds quickly as infected CD+4 cells begin producing virus. Viral production by infected macrophages is at least of two orders of magnitude greater than the production by actively infected T4 cells. Figure 3 depicts the global course of HIV infected cells.

Table 3 The early effects of the initial inoculate on infected CD+4 cells and their subsequent viral production

Day	Viral RNA molecules viral inoculate = 9×10^7 RNA molecules	Infected [M_i] macrophages (cells): M_i RNA production (molecules/day)	Latently infected T4 (cells)	Activated [T_{ii}] actively infected (cells): T_{ii} RNA production (molecules/day)
1/2	1.15×10^5	1.15×10^6	1.4×10^5	15
		8×10^7		1.2×10^3
1	1.5×10^5	2×10^6	2.5×10^5	30
		1×10^8		2.5×10^3
2	2.5×10^5	2.7×10^6	5×10^5	76
		1.85×10^8		6.3×10^3
3	5×10^5	5×10^6	9×10^5	170
		3.6×10^8		1.4×10^4
6	26×10^5	2.7×10^7	7×10^6	1.3×10^5
		1.8×10^9		1×10^6
10	2.7×10^7	2.7×10^8	8.5×10^7	1.6×10^4
		1.8×10^{10}		1.4×10^7
16	80×10^7	7.5×10^9	3×10^9	7.5×10^5
		5.3×10^{11}		2.7×10^8
20	7.2×10^9	1.6×10^{10}	2.5×10^{10}	5×10^6
		3.4×10^{12}		3.8×10^9
25	2.4×10^{10}	1.3×10^{11}	1.22×10^{11}	1.75×10^7
		9×10^{12}		4×10^{10}
30	2.65×10^{10}	1.45×10^{11}	2.2×10^{11}	1.8×10^7
		9.65×10^{12}		1.42×10^{10}
35	2.5×10^{10}	1.39×10^{11}	2.8×10^{11}	1.9×10^7
		9.5×10^{12}		1.6×10^{10}
40	2×10^{10}	4.35×10^{10}	3.4×10^{11}	1.7×10^7
		8.9×10^{13}		1.33×10^9

Initial values: infected cells equal to zero, macrophages $= 1.66 \times 10^{11}$ (cells)
The virus initially decreases to due to macrophage removal and infection of CD+4 cells

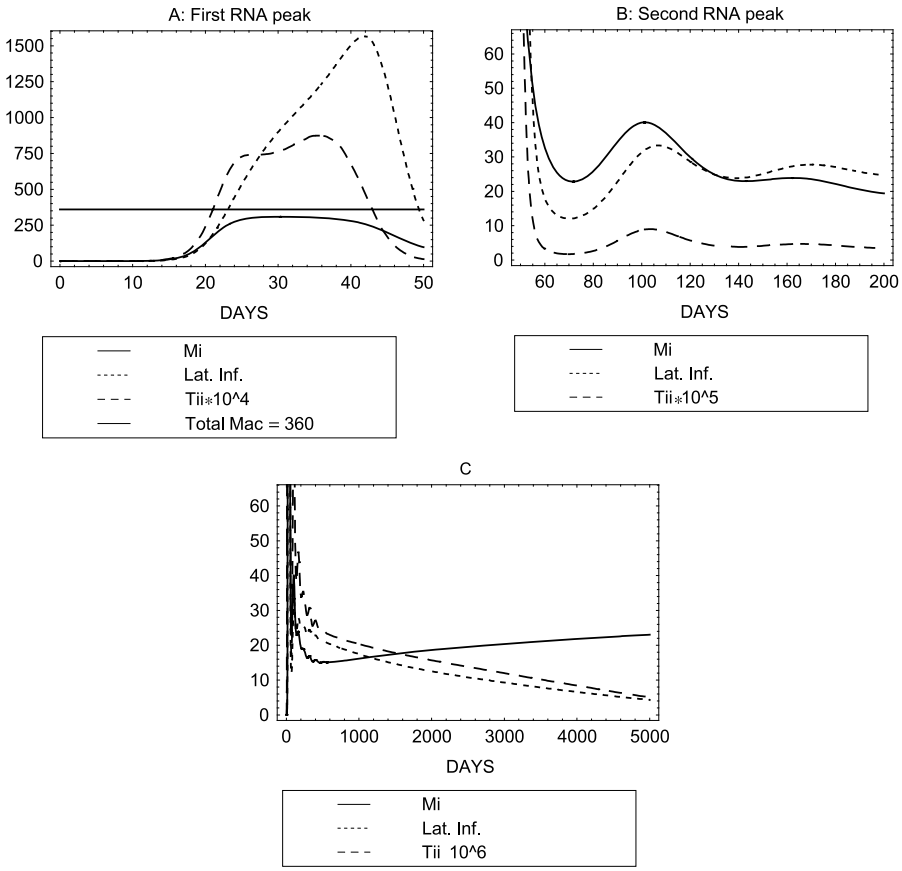


Fig. 3 Infected cell dynamics: latently and actively infected T4 cells and infected macrophages. The infected cell populations dramatically rise and fall with the first and less with the second viral peak (panels A and B): infected macrophage concentration (M_i) is 1/10 of its first peak value, latently infected cell concentration is 1/100 and actively infected cell concentration (T_{ii}) is less than 1/100 of their first peak values (panel C). During steady state and beyond, M_i rises from 15 to 22 cells/mm^3 at AIDS, the latently infected cell concentration decreases from over 30 to 7 cells/mm^3 at AIDS, and T_{ii} drops from 10^{-4} to 10^{-5} cells/mm^3.

Much of the striking infected cell activity occurs in conjunction with the first viral peak (panel A). M_i reaches the maximum of 309 cells/mm^3, 85% of the total macrophages (360 cells/mm^3) are infected, whereas when the concentration of latently infected cells peaks at 42 days, over 80% of total T4 population is latently infected. After a very significant decrease, M_i rises slightly during steady state and at AIDS represents only 6% of the total macrophage population. During steady state, the ratios of infected T4 cells are T_{ii}/Latently Infected T4 $\sim 10^{-6}$; T_{ii}/T4 about 4×10^{-8}; and Latently Infected T4/T4 about 3×10^{-2}.

The simulations capture the course of HIV infection, describing the documented behavior of HIV infection, milestones, dynamics, and progression. In the simulations, the

initial viral peak, leads to a strong specific immune response set in motion after 30 days, together with early acute infection and probably sero-conversion.

The T8/T4 ratio inverts, decreases slightly, and then rises throughout progression. A second viral peak in 3 months precedes the establishment of the set points and the establishment of a quasi-steady state with rising virus and decreasing antibody and T cell concentrations. The system reaches AIDS in 10 years after the initial infection.

The soundness of the global response suggests that the model is adequately conceived and potentially useful for exploring other aspects of HIV disease.

The above results do not depend on T4 cell exhaustion, excessive replication, decreased latently infected cell life span, or virulent viral destruction of critical life sustaining cells, or other aberrations. Yet the simulation suggests that dynamics alone can generate the events associated with HIV infection and progression. HIV-specific T cell subsets exhibit vigorous immune involvement throughout disease progression. Most of T4 and 30% of the T8 cells are HIV specific during steady state, demonstrating an active enduring specific response.

Decreasing T4 concentration during steady state is the effect of lower viral concentration that stimulates less specific T4 replication than during the early stage, in addition to robust infected cell removal by cytotoxic ST8 cells, some cell loss due to host aging and the small loss due to activated cell bursting. It appears that the long-lived concentration of latently infected cells, although decreasing, coupled to the slight rise in M_i, maintains the activation of cytotoxic specific T8 cells, resulting in a ST8 concentration of $\sim$500 cells/mm^3 throughout steady state.

T cell depletion and significant antibody decline occur as the consequences of the responses of the virus and the host to each other, before and during the quasi-maintenance of a steady state of infection. "Increasing evidence suggests ... that infection-induced immune activation drives both viral replication and CD4+ cell depletion" (Grossman et al., 2002).

Before closing this section on the verification of model, we will speculate on the in vivo dynamics underlying the simulation results. The growth of the first peak, due to profuse viral production, mainly by infected macrophages, may be controlled by healthy macrophage target scarcity that limits the increase of M_i viral production. After the first viral peak, the highly excited specific immune system begins to exert its influence, as antibodies assist macrophages in viral removal, and cytotoxic ST8 removal of infected cells begins (see Fig. 2), followed by the precipitous decrease of infected macrophages and T cells. However, the rapid decline of virus and infected cells curtails specific T cell stimulation and antibody activation. Hence, specific cell concentrations drop quickly (Fig. 2A), and renewed viral and infected cell growth occurs, leading to the second viral peak. In response, a second weaker specific immune response emerges, curbing and removing the HIV excesses of the second peak. As the dynamics between infection and the immune system adjust, even fine-tuning each other, set points are established and a quasi-steady state is achieved.

6. Parameter values variation

Changes in the parameter values mimic a wide range of individual responses to infection and display some processes involved in disease progression. Small changes offer

reasonable results, while large modification, although sometimes unrealistic, can imply important new dynamics that might be promising in view of therapy.

6.1. ±10% single parameter perturbations

A wide range of individual host responses can be simulated by ±10% single parameter perturbations. Experiments on a small parameter subset, unique to the host and to the infecting HIV, are tabulated in Table 4. Time for progression to AIDS varies from 4.1 to over 20 years, T4 set points range from 650 to 1390 cells/mm^3, and second viral peaks vary from 1.4×10^5 to 6×10^4 viral RNA molecules/mL.

The results conform to individual host differences commonly observed in disease development and suggest that interventions inducing small immune and/or viral parameter changes could favorably affect individual disease outcomes.

Increased T cell replication rates or diminished viral T4 infection rates extends progression to AIDS from 10 to 18.4 and 20.2 years, respectively. In both of these cases, the specific immune response is strong, ST4 set points are over 1000, and the antibody set point is high. It may be, however, that high ST4 and lower antibody concentrations extend progression more successfully than low ST4 and high S values. While increasing and decreasing the rate of infection of macrophages, or the number of virions released by an infected macrophage per day has a much smaller effect on AIDS progression. However, rather surprisingly, by increasing these parameters, the time to AIDS is somewhat augmented. The time to AIDS is also slightly augmented by decreasing the value of delta, i.e., by decreasing the immune recognition of latently infected T cells and infected macrophages. These results suggest that an appropriate presence of virus can sustain the T4 cell pool by stimulating the HIV-specific subpopulation.

Perturbations of the rate of antibody removal of virus, k_{Ab} and of the specific antibody adaptation, show only trivial effect on disease outcome, and are not displayed.

A possible explanation of this last finding is that the role of macrophages is so dominant with respect to the role of antibodies that the outcome is resistant to small perturbations in antibody parameters. The results suggest that drug intervention altering the specific T cell replication and the infection of T4 cells could be more effective than interventions of similar extent effecting macrophages or antibodies.

The correlation between 10% parameter changes and the AIDS progression time may suggest choices for research directions. Using the perturbation results in Table 4, the Pearson correlation coefficient between time of progression to AIDS and the set-point concentrations of ST4 is 0.94, between time and antibody concentration is 0.93, and between time and latently infected cell concentration is 0.87, so that all these set-point values are potentially good predictors of progression. The correlation between set-points values of latently infected cells and ST4 cells is $r = 0.84$, between antibody concentration and latently infected cells is 0.81, and as expected, between ST4 cells and antibody concentration is 0.99. Although these correlations are strong, it must be recalled that the sample size is small, only 15 single perturbation points.

6.2. Large modifications of parameter values

Looking for dynamics not necessarily evident in the simulations with small parameter changes, dramatic perturbations on T cell proliferation (Table 5), viral production by infected macrophages (Table 6), cytotoxic removal of infected macrophages (Table 7), infection rates and viral adaptation (Table 8), and tropism (Table 9) are tested. Because the

Table 4 Effect of parameter modification of ±10%. +10% is the first entry and −10% the second entry in each cell

Parameter ±10%	Years to AIDS	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to AIDS $\times 10^{-9}$ M	Latently infected cell set-points cells/mm^3	Immune concentrations
No modification	10	1.84 0.123	1020 950	1130 550	5.4–0.6	32.8–7.5	ST4 = 950: ST8 = 550 ST8 > 500 at AIDS $S = 5.4–0.6 \times 10^{-9}$ Lat Inf. = 32.8–7.5
r_4, r_8, ρ_4, ρ_8: $\bar{s} = 6.0899$	18.4	1.76 0.125	1250 1100	1200 510	7–0.96	40–8.5	ST4 = 1100: ST8 = 51. Lat Inf. = 40–8.5 st= 4.9
	4.4	1.8 0.14	680 580 ST4 < 200 in 3 years:	1040 530	4 –0.7	28–8	$S = 7 - 0.96 \times 10^{-9}$ ST4 = 580 < 200 in 3 years ST8 = 530: Lat Inf.=28-8 $\bar{s} = 7.35$ $S = 4 - 0.7 \times 10^{-9}$
p_{im}	12.3	2.2 0.07	1200 1150	1150	7–0.75	40–9.5	ST4 = 1150 $S = 7 - 0.75 \times 10^{-9}$ ST4=630
	7.7	1.4 0.06	750 630	1050	4–0.7	27–5	$S = 4 > 0.7 \times 10^{-9}$
δ	7.8	1.8 0.145	850 700	1100 460	4.7–0.98	26–6.9	ST4 = 700 ST8 = 460 $S = 4.7 - 0.98 \times 10^{-9}$ Lat Inf. = 26–6.9
	11.8	0.76 0.12	1150 1205	1200 570	6.5–0.8	40–8.9	ST4 = 1025: ST8 = 570 $S = 6.5 - 0.8 \times 10^{-9}$ Lat Inf. = 40–8.9

Table 4 (*Continued*)

Parameter ±10%	Years to AIDS	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to AIDS $\times 10^{-9}$ M	Latently infected cell set-points cells/mm^3	Immune concentrations
$\bar{k}_v$, e, $\bar{k}_{vm}$	4.7	1.76 0.125	700 700	1200 600 600 at AIDS	4.5–1	27–7	ST4 = 700: ST8 = 600 ST8 = 600 at AIDS Lat.Inf = 27–7 $S = 4.5–1 \times 10^{-9}$
	19.5	1.76 0.125	1200 1050	1050 451	6.5–1.5	40–9	ST4 = 1050: ST8 = 451 Lat Inf. = 40–9 S 6.5-1.5 $\times 10^{-9}$
$\bar{k}_v$, e	4.3	1.8 0.065	690 580	1130 538	4 –0.7	26–8	ST4 ~ 580: ST8 = 538 Lat Inf. = 26–8 S 4–0.7 $\times 10^{-9}$
	20.2	1.82 0.115	1390 1250	1096 500	7.8–0.77	39–8.1	ST4 = 1250: ST8 = 500 Lat Inf. = 39–8.1 S 7.8–0.77 $\times 10^{-9}$
$\bar{k}_{vm}$	10.1	1.9 0.12	1120 1000	1170 580	6.5–0.78	34–6.8	ST4 = 1000: ST8 = 580 Lat Inf. = 34–6.8 $S = 6.5–0.78 \times 10^{-9}$
	9.1	1.75 0.14	850 720	1064 467	4.5–0.73	33–7	ST4 = 720: ST8 =: 467 Lat Inf. = 33–7 $S = 4.5–0.73 \times 10^{-9}$
$\bar{k}_v \times 0.9$, $e \times 0.9$ and $\bar{k}_{vm} \times 1.1$	21	1.76 0.13	1550 1400	1100 850	9–0.7	36–7.8	ST4 ~ 1400: ST8 = 560 > 500 $S = 9–0.7 \times 10^{-9}$ Lat Inf. 36–7.8

ST4 set points ≥1000 are underlined

model does not constrain T cells and antibody values, some simulations show unreasonable concentrations but can still be revealing. When interpreting the results, it is important to realize that for healthy hosts, according to our assumptions, the T4 concentration falls below 200 cells/mm^3 at the age of 79.8 years, solely because of the decay of thymic output with age. Since all simulations begin with a 36 years old host, when the T4 concentration falls below 200 after 43.8 years from inoculum, this impairment is not HIV disease related. An asterisk will accompany data when T4 drops below 200 and the host's age is close to 79.8 years.

In Table 5, the results of simulations with significant perturbations of the proliferation rates, r_4 and r_8 for nonspecific cells, and ρ_4, and ρ_8 for specific T cells, are tabulated. Increasing the rate of specific T cells proliferation by 20% allows for high immune cell set points and a very well-defended steady state that extends progression to 27 years, with the ratio of healthy to infected cells being 25/1: a very strong specific immune system. Perturbation of $\pm 20\%$ of nonspecific proliferation rates give results very close to the unmodified runs since in the simulation the majority of T4 cells are HIV-specific and not affected by the modification. Decreasing the rate of specific cell proliferation decreases both progression time and depresses the specific immune response: cutting the rates to one-half dramatically increases the ratio of infected to healthy cells. AIDS occurs in about 3 months and the T4 cell concentration continues to fall.

However, the dynamics transform with a further decrease of the specific cell proliferation rates. With severely damped specific T cell concentrations, the second viral peak is not induced; M_i sets at higher concentrations and the progression time to AIDS increases. At the extreme, when ρ_4, and ρ_8 are zero, there is no sero conversion, T8/T4 ratio is less than 0.6, M_i remains at its initial peak, and no real AIDS occurs, and after 39.7 years T4 cells are below 200 cells/mm^3 substantially because of thymic aging. With the innate system responding and the specific system blind to HIV, progression could be very significantly altered. Shutting down all the specific responses would be devastating to the hosts' health, but intermittent turn off at appropriate intervals may be of use.

Modifications of p_{im} and of cytotoxic removal of infected macrophages, depicted in Tables 6 and 7, produce interesting and, seemingly, conflicting dynamics.

Severely reducing p_{im}, the number of virons produced by an infected macrophage per day leads to longer progression time. Lower p_{im} values should imply lower virus, less infection, and decreased removal of infected cells: thus, the T4 concentration remains above 200 cells/mm^3 for a longer time. When $p_{\text{im}} = 0$, the initial inoculate virtually disappears in a few days, after 22 years virus concentration rises to the negligible value of 0.2 RNA molecules/mL, there are no viral peaks and no set points. T4 and T8 cell concentration do not rise, and only 0.25 latently infected cells/mm^3 are present after 22 years. The T4 concentration falls below 200 in 43.8 years, when the host is 79.8 years old. Similar dynamics are found for $p_{\text{im}} = 3.4$. If it were medically possible to prevent virus bleeping by infected macrophages, or keep it as low as a few virions/cell/day, according the present model HIV, illness could be virtually prevented.

On the other hand, rising p_{im} induces a vigorous specific immune response and leads to longer progression with high T4 cell counts due to the sustained ST4 stimulation and proliferation (however, some unrealistic values of cell concentrations are reached). Parameter changes inducing strong damping of the specific immune responses were found to be associated with better disease prognosis (see Table 5). We see that as p_{im} increases the damping of the specific response is less, T cell set points rise and progression time

Table 5 Modification of proliferation rates of T cells

Parameter rate of proliferation	Years to AIDS	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to AIDS $\times 10^{-9}$ M	Peak M_i concentration
$\rho_4, \rho_8 \times 1.2$ and $r_4, r_8 \times 1.2$	27.4	1.68	1400 1150	1350 500	6.5–1	M_i peak and low
Only $(\rho_4, \rho_8) \times 1.2$		0.115	1400 1150	1350 500	6.5–1	M_i peak and low
Only $r_4 \times 1.2, r_8 \times 1.2$	10	1.71 0.12	900 820	1350 520	5.4 –0.6	M_i peak level < 20
Only $(r_4, r_8)/1.2$	10	1.8 0.135	950 800	980 500	5.4–0.6	M_i peak level < 20
$\rho_4, \rho_8/1.2$ and $r_4, r_8/1.2$	2.9	1.79 0.16	440 380	1000 550	2.5–1	M_i peak, level at 25
Only $(\rho_4, \rho_8)/1.2$	2.5 yrs.	1.8 0.165	480 380	1130 550	2.5–1	M_i peak, level at 30
Only $(\rho_4, \rho_8)/2$	80 days	1.9 no 2 peak	@ 300	1980 500	2.4–0	M_i peak, level at 60
Only $(\rho_4, \rho_8)/4$	135 days	1.82 no 2 peak	@ 150	980 425	8×10^{-10}–0 in 200 days	M_i peak, level at 60
Only $(\rho_4, \rho_8)/10$	320 days	1.84 no 2 peak	@ T4 = 300 in 50 days 100	650 100	3.5×10^{-10} in 300 days– $S \sim 0600$ days	M_i peak, level at 60
Only $(\rho_4, \rho_8)/100$ T8/T4 remains < 0.7	39.2 yrs.[a]	1.86×10^6 no 2 peak: 1.55×10^6 at AIDS	@ 1.2	@ 0.6	10^{-12}–0+	M_i peaks 312.5 stays high

@ The set point is not established, the variable is continuously decreasing
[a]T4 drop mainly due to thymic aging

Table 6 Effect of modification of the parameter p_{im}: viral production by infected macrophages (particles/(cell day))

Parameter p_{im} viral production rate by M_i	Years to AIDS	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to AIDS $\times 10^{-9}$ M	Latently infected cell set-pt. cells/mm^3
0	43.8[a]	<1	@ @	@ @	10^{-21}	0.02
34/4	19.2	0.1 in 1/2 yr. 0.014	500 1.5 @	700 71	5×10^{-12} -6.8×10^{-13}	18
34	10.3	1.84 0.123	1020 950	1130 550	5.4–0.6	33
42	13.7	3.1×10^6 1.6×10^5	1500 1400	1200 648	9–0. 9	50
34×2 68	23.1	3.8×10^7 2.8×10^5	3100[b] 2900 #	15800 1000	21–0.92	125
34×4 136	30.9	4.8×10^{8}[b] 1.2×10^5	6600 # 6450 #	2900[b] 28 00 #	50–0.87	200
34×6 204	34.2	9.5×10^{8}[b] 1.2×10^5	9500 # 9300 #	3800 # 2600 #	75–0.5	280
34×8 272	>35	1.4×10^{9}[b] 1.2×10^5	12000 # 12000 #	3500 # 3000 #	100–0.75	300

[a] due to thymic decline not AIDS

[b] Unrealistic variable concentration

@ The set point is not established, the variable is continuously decreasing

decreases, until p_{im} is large enough to induce a strong specific immune response with ST4 concentration >1000 cells/mm^3, when the time for progression to AIDS increases. Recall that the Pearson correlation coefficient between time to AIDS and ST4 set point is 0.94. Significant intervention might be accomplished by either strengthening the specific immune response or by radically decreasing viral production by macrophages, with the depressed specific immune system being the response to the much decreased viral presence and subsequent viral stimulation.

As the presence of higher virus concentrations appears to support strong immune system response and good disease outcome, it brings into question the soundness of blanket virus removal when total removal is not feasible. In addition, the data suggests that when the antibody concentration falls below $10^{-9}M$ the system approaches AIDS.

Using Table 6 data, without $p_{\text{im}} = 0$, Pearson correlation coefficients for disease progression and the S set point is 0.92, progression and the ST4 set point is 0.90, and with the latently infected cells set point is 0.94 (not shown). Again, a very small sample is used.

Infected macrophages are the main virus producers, so the efficacy of infected macrophage removal as an effective intervention is here considered. The cytotoxic removal term, $(k_8 \tilde{T}_{8e} \delta M_i)$ in Eq. (15) is multiplied by 0.01, 0.5, and 2: while effector ST8 cell production and the cytotoxic removal rate k_8 are not altered. The concentration M_i

Table 7 Effect of modification of the rate of cytotoxic removal of infected macrophages

Parameter rate of cytotoxic removal of infected macrophages	Years to AIDS	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to AIDS $\times 10^{-9}$ M	T8/T4 ratio	Viral concentration at AIDS $\times 10^6$/mL
removal/10	16.4	1.86	>5000 #	>5000[a]	38–0.65 #	>1 in 60 days, then decreases to 0.5:	0.77
		0.65	5000 #	4700[a] Set points reached at AIDS		>1 in 2 yrs.	
removal/2	12.6	1.85	1750	1500	10–0.8	>1 in 45 days, then <1 in 3 months:	0.71
		0.82	1650	1000		>1 after 2 yrs.	
unchanged	10.3	1.84	1020	1130	5.4–0.6	>1 in 47 days, decreases to ∼1 in 10 months, then rises.	0.67
		0.123	950	550			
removal*2	8	1.80	550	950	2.5–0.47	>1 in 50 days	0.58
		0.24	420	280			

[a]unrealistic concentration

remains at about 20–30 cells/mm^3 during steady state and the resulting data are reported in Table 7.

The tiny sets of results suggest that a decreased cytotoxic removal of infected macrophages leads to better disease prognosis, although with possible very high concentrations of ST4. As cytotoxic removal of infected macrophages increases from 1/10 to two, the progression time to AIDS decreases by more than half, the second viral peak rises, and ST4 and ST8 concentrations decrease. The cause of the inverse relationship between removal and time to AIDS could be that by decreasing their removal, the greater number of infected macrophages enhances the stimulation of specific T4 and specific T8 cells delaying the AIDS onset, or some other mechanism, not yet recognized.

All this might argue against interventions enhancing cytotoxic removal of infected macrophages.

Perturbations of δ, of the rates of infection, of viral adaptation, and of the ST4/total T4 ratio in thymic output ν, are assessed in Table 8.

With stronger immune ability to see infected cells, $\delta = 10^{-2.2}$, progression increases to over 20 years, while the comparable decreased recognition, $\delta = 10^{-3.8}$, raises progression to over 30 years (although with unrealistic cell concentrations). As already seen with perturbed specific T cell proliferation rates in Table 5, and with p_{im} modifications in Table 6, it appears that both strengthening the specific immune response and severely damping it, increase the progression time. A 30% decrease in the viral ability to infect CD+4 cells as

Table 8 Effect of modification of δ, of the rates of CD+4 cellular infection, of adaptation, and of the ST4/total T4 ratio in the thymic output

Parameter	Years to AIDS	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to AIDS $\times 10^{-9}$ M	T8/T4 ratio
$\delta = 10$–2.2	20.8	1.2 0.9	1300 8	900 740	0.09– 0.0004	oscillates for first 3 years, then >1
$\delta = 10$–3.8	32.9	1.9	180000[c] 10000[c]	2100 1500	100–1	>1 in 3 months and decreases below 1: >1 ~19 years and continues rising
$\bar{k}_{vm} \times 15$	16.4	2.55 1.1	10100[c] 10000 #	& &	81–1.8	<1 in 2 yrs.
$\bar{k}_{vm} \times 10$	16.4	2.55 1.1	8100 # 8000 #	& &	65–1	>1 in 2 yrs.
$\bar{k}_{vm} \times 2$	12.88	2.15 0.8	2800 2300	1600 1250	14.70–1	=1 in 50 days, then decreases: >1 in 2 yrs
$\bar{k}_{vm}/2$	5.9	1.2 0.15	340 220	840 240	1.15–0.05	>1 in 77 days
$\bar{k}_{vm}/5$	4.9	0.135 in 0.7 yrs 0.026 in 1.37 yrs	400 1.4	650 50	0.6–0.4	>1 in 256 days
$\bar{k}_{vm}/10$	42.5[a]	690 in 3.8 yrs 360 in 7.7 yrs 690 in 42 yrs	@ <1	@ <2	$<10^{-14}$	<0.5
$e, \bar{k}_v \times 1.5$ $\bar{k}_{vm}/1.5$	50 days	1.475 one peak	1450 drops [does not rise] 700	950 350	5–0.5	>1 in 46 days : 8 at AIDS.

Table 8 (*Continued*)

Parameter	Years to AIDS	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to AIDS $\times 10^{-9}$ M	T8/T4 ratio
$\bar{k}_v, e, \bar{k}_{vm} \times 1.5$	260 days	2 one peak	300 200	1000 350	1.8–0.12	>1 in 40 days
$\bar{k}_v, e, \bar{k}_{vm} \times 1.3$	1.66 yrs.	2 0.15 slight	500 400	1300 700 Rising at AIDS	3–0.5	>1 in 45 days
$\bar{k}_v, e, \bar{k}_{vm} \times 0.7$	No AIDS 48 yrs[a]	1.53 0.13	1700 1450	950 320	7.3–0.8	>1 and decreases <1: then >1 in 35 years.
NO viral and antibody adaptation $\bar{k}_v, e, \rho_{\text{Ab}}$ and $\bar{k}_{vm}$ are constant	No AIDS T4 = 400 in 48 yrs[b]	1.8 0.12	1050 950	930 320	6.25–2[c]	>1 and decreases <1, then = 2 in 11 yrs and decreases ~1.5 after 48 years
NO viral adaptation $\bar{k}_v, e, \bar{k}_{vm}$ constant, antibody adaptation	No AIDS T4 = 400 in 48 yrs[b]	1.8 0.115	1050 950	1100 550	5.4–1.5[c]	>1 and decreases <1, then = 2 in 11 yrs and decreases. ~ 1.5 after 48 years
viral adaptation: $\bar{k}_v, e, \bar{k}_{vm}$ time varying	10.3	1.84 0.123	1020 950	1130 550	5.4–0.6	>1 in 47 days, = 1 in 10 months, and then rises
NO antibody adaptation viral adaptation	10.3	1.86	1200	1160	300–38[c]	>1 in 45 days and decreases <1
$k_{\text{Ab}} = 0$		0.15	1060	570		in 6 months: >1 after 1.2 yrs
$\nu = 10^{-3}$ ST4/total T4 ratio in the thymic output	10.3	1.84 0.123	1020 950	1130 550	5.4–0.6	>1 in 47 days, up to 2.1: decreases to 1.6 and rises

Table 8 (*Continued*)

Parameter	Years to AIDS	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to AIDS $\times 10^{-9}$ M	T8/T4 ratio
$\nu = 10^{-4}$	10	1.93 0.15	780 620	1130 530	3.7–0.6	>1 in 55 days, up to 5: decreases to 1.6 and rises
$\nu = 10^{-5}$	9.86	1.94 double first peak 0.165	590 450	1130 530	2.4–0.8	>1 in 70 days, up to 15: decreases to ~2 and rises
$\nu = 10^{-6}$	9.7	1.96 double first peak 0.18	420 340	1130 530	1.75–0.68	>1 in 80 days, up to 22: decreases to 3 and rises

[a] Due to thymic decline not AIDS

[b] T4 = 400, T4 = 200 in healthy host after 48 years

[c] Unrealistic concentration

@ The set point is not established, the variable is continuously decreasing

& The set point is not established, the variable is continuously increasing

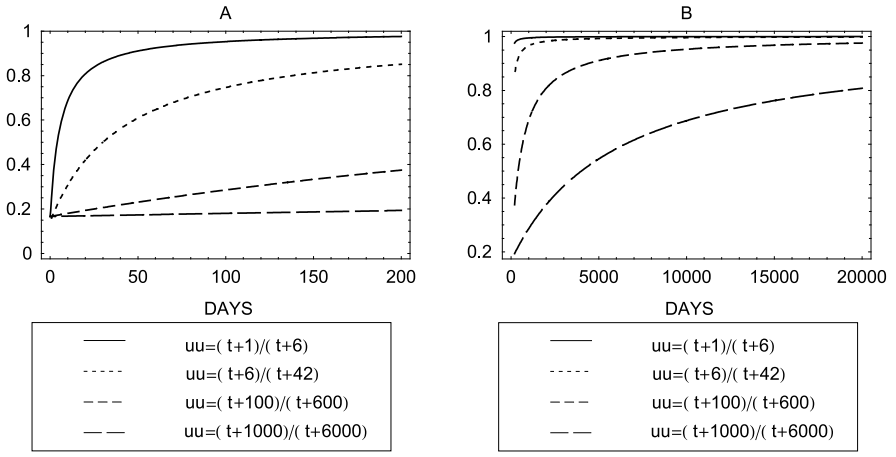


Fig. 4 Behavior of 4 different functions $u(t)$ describing the rise of T-tropism with different rates, during the first half year after infection (panel A), and for over 13 years (panel B).

well as the elimination of viral adaptation result in strong specific immune response and no AIDS. Simulations with the removal of antibody adaptation or the removal of antibody function do not significantly differ from the unperturbed case, suggesting that viral adaptation is a key factor in the progression of HIV infection while neither antibody adaptation nor antibody function is.

ν, the ST4/total T4 ratio in the thymic output, in the unperturbed model is $\nu = 10^{-3}$. As ν is changed from 10^{-3} to 10^{-6}, although the T4 set-point concentration decreases by more than half and the antibody set point to one quarter of its unperturbed value, the time for progression to AIDS decreases by a nominal 4 months. In addition, T8 and ST8 set points are unaltered by the perturbations, and the viral peak values change very little.

Current interest in tropism suggests consideration of different rates of increase of T-tropism. In Fig. 4, four different functions $u(t)$ (Eq. (22)) are shown, representing different velocities of T-tropism increase. The chosen functional form yields the same initial value for u, and the system goes from M to T-tropism earlier during disease progression as u rises faster.

AIDS progression is inversely correlated to the rate of increase of u, rising from 7.7 to 18 years as u grows more slowly (see Table 9). For the slowest increase rate of u, real AIDS is not reached (T4 cells under 200 cell/mm^3 in 42 years). This result is an additional support of model adequacy, as the correlation between progression to AIDS and tropic adaptation is established in the literature (Mugwagwa and Witten, 2006).

Slower adaptation to T-tropism results in higher T4, T8, and latently infected cell set points. In addition, with slower adaptation, the T8 set point rises later and not as high, but on average decreases less per year. The later T8 rises, the later the T8/T4 ratio will invert. Because T8/T4 becomes inverted later with slower tropic switching, this result, added to the T8/T4 ratios in Tables 8 and 9, suggest that the T8/T4 "set-point" inversion time may be a prognostic indicator of progression. Correlation between progression time and the T8/T4 ratio inversion is 0.814, using the 27 data points in Tables 7–9. The data comes from diverse large-scale perturbations; correlations for the smaller data sets associated

Table 9 Effect of change in the velocity of T-tropism increase

$u(t) = \frac{t+t_1}{t+t_2}$,	Years to aids	First & second viral peaks $\times 10^6$/mL	T4 & ST4 set points cells/mm^3	T8 & ST8 set points cells/mm^3	S from set point to aids $\times 10^{-9}$ M	T8/T4 ratio
$u = (t+1)/(t+6)$	7.7	1.80 0.16	1350 600	950 350	7–0.3	>1 in 45 days, rises to 12; falls to 4 in 2 months and rises slowly
$u = (t+7)/(t+42)$	10.3	1.84	1020	1130	5.4–0.6	>1 after 47 days, decreases close to 1, and rises
		0.123	950	550		
$u = (t+100)/(t+600)$	18	1.82 0.17	3900[b] 300	1000 400	75–0.75	>1 after 8½ years
$u = (t+1000)/(t+6000)$	43[a]	1.85 0.185	4500[b] 3600[b]	1000 400	120– 102.5[b]	<0.50 for 20 years >1 after 32.8 years

[a]T4 drop due mainly to thymic aging not AIDS

[b]Unusual results

with the each perturbation are over 0.9. The time of the ratio inversion is measurable and could be a useful prognosticator of progression if the approximate date of infection is known.

The single parameter perturbation results described here, demonstrate the possible range of individual outcomes, and may offer preliminary directions for intervention. The disadvantages of infected macrophages removal, as well as some new possible prognosis indicators of disease progression are discussed. Moreover, according to these simulations, the inhibition of viral production by infected macrophages could block the progression of infection.

Finally, the perturbations illustrate that both a very strong specific immune response as well as a strongly damped specific immune response are associated with better progression outcomes. Removal of only the HIV-specific immune response (keeping active the non-HIV specific stimulation and the innate immunity), appears paradoxically to offer good disease prognosis.

7. Virus, antibody, and subset T4 dynamics

An interesting use of the model is to unravel the dynamics governing the production and removal of virus, antibodies, and T4 cells as disease progresses. Simulations of the model with the reference parameter values, whose global behavior has been previously described, have performed a detailed investigation of these total body production and removal per day.

7.1. Virus production and removal

After inoculation, virus is produced by infected macrophages and by actively infected T4 cells; virus is removed by macrophages, in the infection of CD4+ lymphocytes, as antibody-virus complex, and by natural death.

At the first viral peak, the magnitude of the macrophage production of virus in the body (per day) and the macrophage removal of virus is about the same, while the removal of virus by antibodies is ∼100 times greater than the production of virus by actively infected cells. At the second peak, the removal by antibodies is more than 4×10^4 times the viral production by actively infected cells, and macrophage removal is slightly greater than production. Additional viral sinks are the infection of T cells, 4.5×10^{10} and 7×10^9 RNA molecules/day, and removal by natural death, 8×10^{10} and 5×10^9 RNA molecules/day, at the first and second peak, respectively. The sum of all the productions, about 5–6 $\times$ 10^{11} RNA molecules/day, is aligned to published production data (Ho, 1997), and total production and removal are very close. During steady state, viral production and removal by infected macrophages increases, while production from actively infected T cells and removal by antibodies decrease.

The simulations indicate that macrophages play a major role in orchestrating virus production and removal throughout the course of HIV infection. Virus production is largely controlled by M_i concentration, the removal by the homeostatic macrophage activity. Experiments on virus production with SHIV strains, performed by M.A. Martin, M.D., chief of NIAID's Laboratory of Molecular Microbiology "found that 95% of the virus-producing cells were macrophages and only 1 to 2% were T cells" (Igarshi et al., 2001).

7.2. Antibody production and removal

Anti-HIV antibodies are produced by B plasma cells that are activated by specific T4 helper cells (ST4e), and their removal is due to the elimination of the viral complex, and to the natural loss.

Discussing DNA vaccines, Siliciano and Siliciano (2005) points out that "the general experience in this field is that it has been hard to induce high levels of neutralizing antibodies to HIV" (Seppa, 2000). Simulation of the production and removal of antibodies is an attempt to consider this issue. The production and removal of neutralizing anti-HIV antibodies, and their resulting decrease, are simulated in Fig. 6. It appears that the combination of a large decrease in ST4e concentration that results in a decreased antibody production, and the removal of antibody-virus complexes, may cause low antibody levels.

In Fig. 6, panel A1, the variables responsible for antibody production, specific T4 helper cells and virus, are tracked over time. From set point to AIDS, ST4e cells decrease by ∼1/8 and antibody by 1/10. The lack of sustained vigorous production of neutralizing anti-HIV antibody may well be due to the lowered viral stimulation of ST4 cells when compared to the critical peak concentrations. These would result in reduced activation of specific B plasma cells: decreased antibody concentration has been associated with a low T-cell-dependent immune response, rather than to a lack of available B cells (Hattori et al., 1998). Antibody production and antibody removal by virus complexes are very close, but when natural elimination is included, removal is greater than production (panels A2 and A3) during most of the steady state, thus inducing the concentration decline.

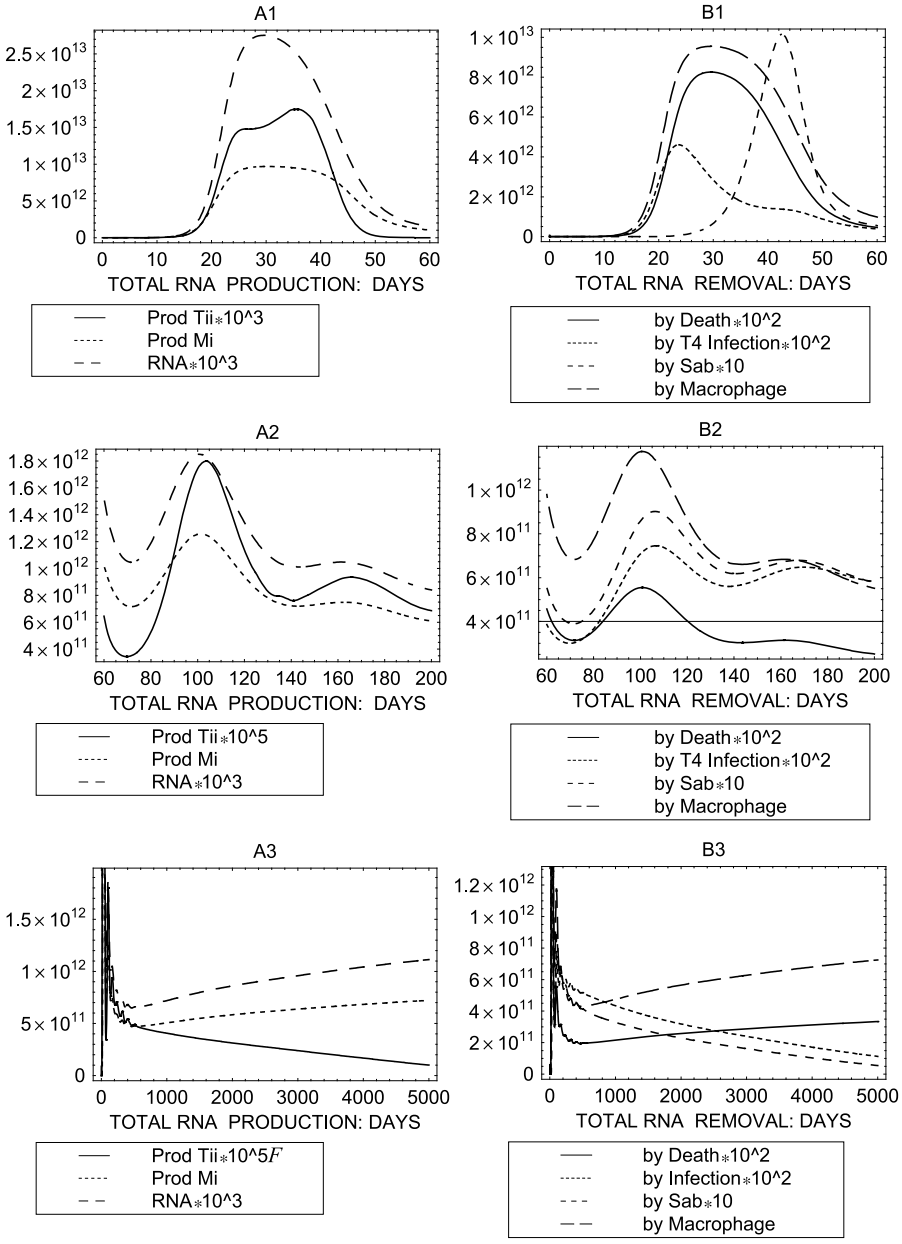


Fig. 5 Total viral RNA production (molecules/day) from infected macrophages and from actively infected T cells (panels A1 to A3) and total viral RNA removal (molecules/day) by natural death, infection, antibody, and macrophages (panels B1 to B3).

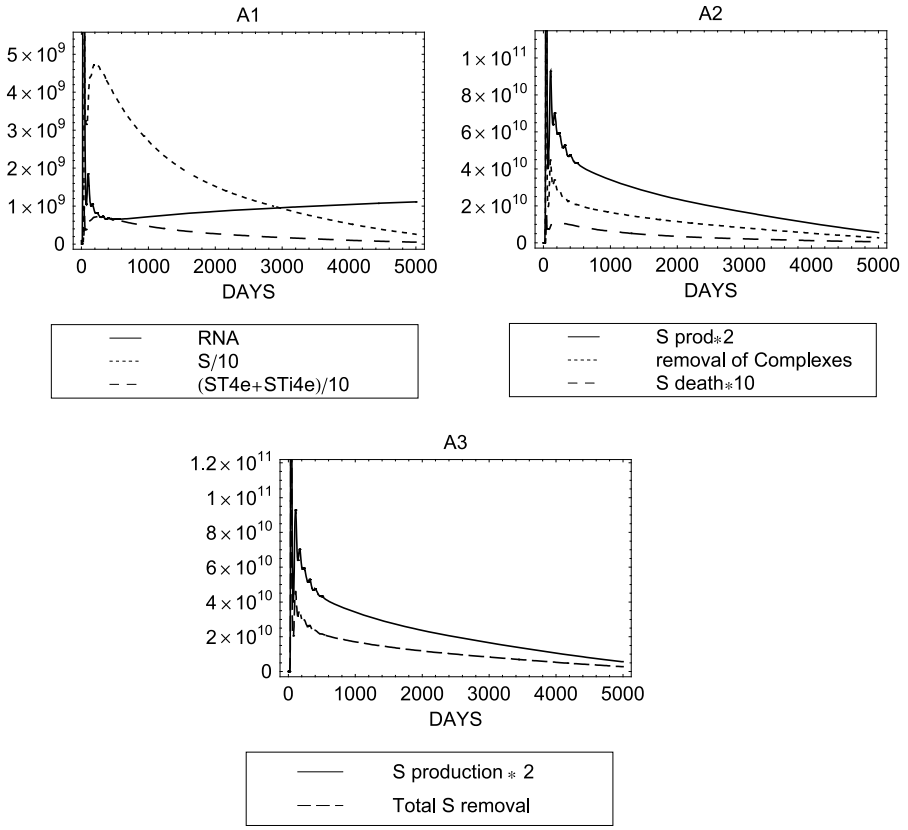


Fig. 6 Total amounts of virus (as viral RNA molecules) and of ST4 cells responsible for specific antibody production (panel A1). Antibody production and removal (molecules/day) by the formation and removal of viral complexes and by natural loss (panel A2) Total antibody production and removal (panel A3).

7.3. T4 production and removal

Production and removal of T4 cells from set points to AIDS are shown in Fig. 7. T4 cell production is regulated by thymic output (implicitly dependent on host age), and proliferation, with HIV-specific cells being activated by the virus. Removal is caused by natural death and, for infected cells, by cytotoxic removal (dependent on the capability of host infected cell recognition), and activation of latently infected cells.

T4 cell dynamics are studied by partitioning the T4 cells in nonspecific (healthy and infected) and specific (healthy and infected) cells, with infected cells being latently or actively infected. Production and removal of these subsets is computed from 3 to 13 years; after initial inoculation and after partial disease stabilization is achieved.

As Fig. 7 shows, both production and removal are decreasing in all the cases, with removal slightly greater than production as obviously expected since cell concentrations are declining. The imbalance is larger for nonspecific T cells where removal is 33% higher. Most of the T4 cells are healthy HIV-specific cells (panel F).

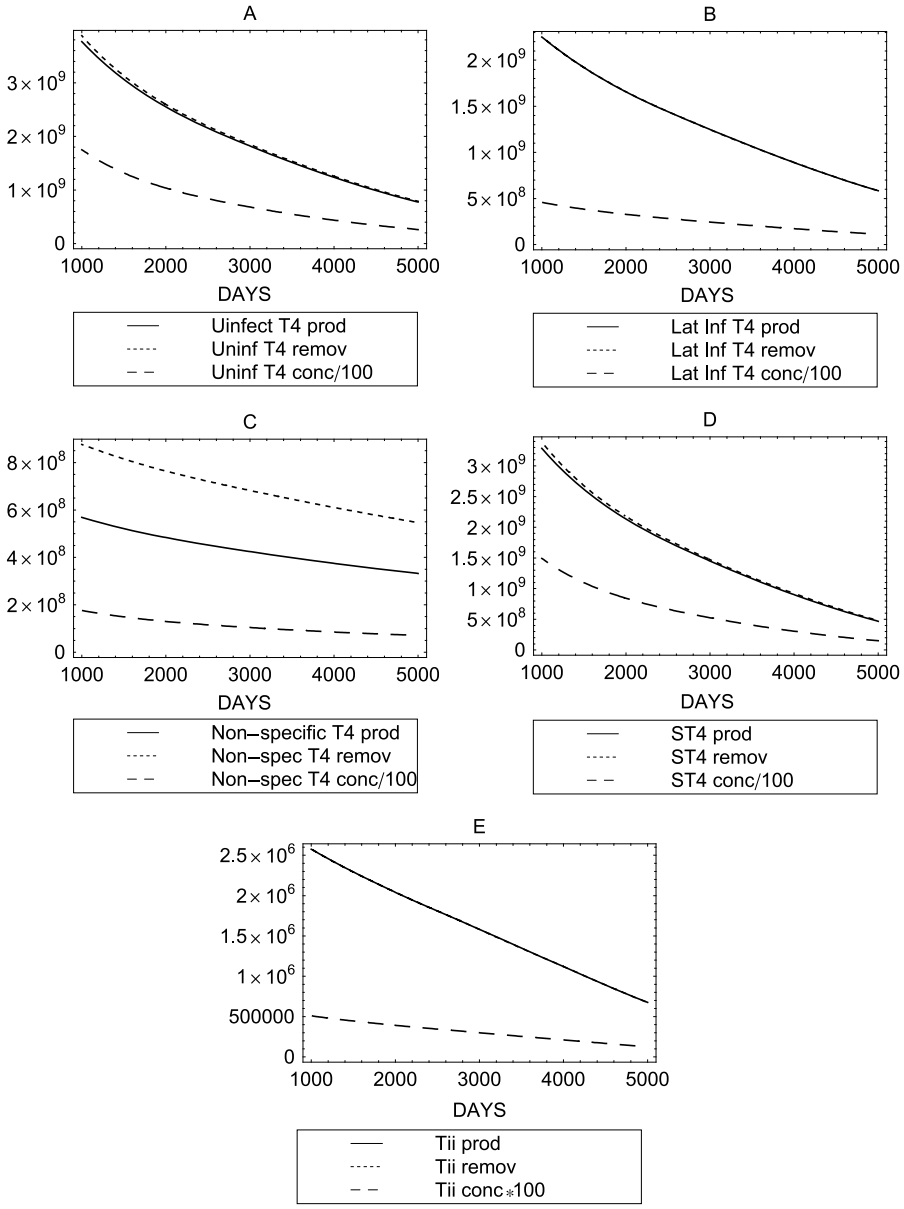


Fig. 7 Total production (cells/day), total removal (cells/day), and the distribution of the number of T4 cell in subsets during progression from 3 to 13 years. Uninfected cells (panel A), latently infected cells (panel B), nonspecific cells (both healthy and infected) (panel C), specific cells (both healthy and infected) (panel D), actively infected cells (panel E) and the total number of T4 cell in each sub-set is shown in (panels F). Panel G depicts total T8 and ST8 production.

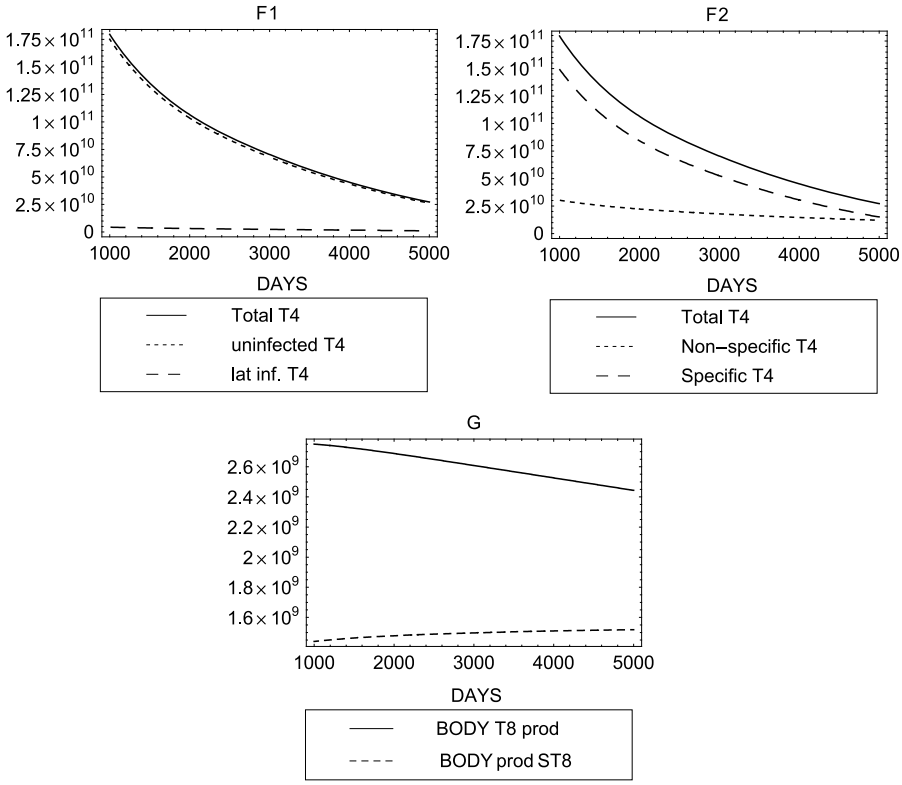


Fig. 7 (*Continued*)

Continuing the description of T4 cell dynamics, simulations gave also some elucidation of "sub" dynamics. For uninfected cell removal, natural death accounts for 45% of the loss, and infection for the rest. Latently infected cell removal is mainly due to cytotoxic T8 cells, 10^9 cells/day, followed by natural death at 10^7 and activation at 10^6 cells/day.

Removal of actively infected cells is mainly cytotoxic, at 2.4×10^6 cells/day, and natural death that is negligible (10^3 cells/day). As the system approaches AIDS, infected cell production equals, and then rises above removal, but their difference is rather small. Macrophages emerge as the dominant producer and remover of virus. Viral production by infected macrophages is 10^5 times greater than the production by actively infected cells, and macrophage removal is 10^4 times greater than removal by antibodies. This phenomenon, which model simulations evidences, is now recognized in the literature and has influenced research imperatives (Aquaro et al., 2002a, 2002b). Interventions enhancing macrophage removal of virus with the "most potent macrophage activating factor termed GcMAF)" has successfully maintained healthy T4 cell concentrations and "eradicated" HIV concentrations for 7 years (Yamamoto et al., 2009). This is an example of how understanding the dynamics can lead to important new therapies.

For completeness, panel G is included to shows T8 production. ST8n production is about 1/3 of the total T8n production analogous to their respective concentrations seen in Fig. 2A and B.

8. T4 and T8 dynamics of HIV-infected host vs. matched healthy host

An in-depth comparison between the immune status of a HIV-infected host and a healthy subject is here simulated. Healthy and infected host outcomes are matched using the same parameter values to observe simultaneous progression. Without the virus and infected cells, stimulation of the HIV specific immune system does not occur in the healthy host, whereas all the other equations remain unchanged. Both the subjects are 36 years old with 1300 T4 and 617.2 T8 cells/mm^3, at time zero. Although the ground work for progression is set early in disease, the period of observation, as in the previous section, begins 3 years after infection, when viral oscillations begins to stabilize. These comparisons are shown in Figs. 8 and 9.

8.1. T4 dynamics in infected and healthy hosts

Production and removal of T4 cells seen in Fig. 8, panels A, almost overlap with production slightly less than removal in both hosts. Three years after infection, production is higher in the infected host because of the intense HIV-specific cell stimulation; however, this difference decreases and after 7 years infected host production and removal is below the levels of healthy subject. At AIDS, the healthy subject T4 production and removal is about 30% higher. We recall that the decrease with time of T4 cells in the healthy subject is only due to the decrease of the thymic output with age.

Three years after HIV infection, removal of T4 cells in the infected host (2/3 of which is due to cytotoxic removal), is greater than healthy host removal, and decreases below the healthy host value after about 7 years. At AIDS, T4 removal is more than 50% cytotoxic, yet healthy host removal is $\sim$1.4 times greater. In fact, loss due to natural death, the only T4 sink for the healthy host, obviously varies with concentration, and T4 concentration is consistently higher in the healthy subject.

The infected host T4 turnover rate is in agreement with Ho et al. (1995), the healthy host turnover is slightly above Sachsenberg et al. (1998) mean turnover of 1.1% (panels D), and the turnover is 4 times greater in the HIV infected host. Proliferation rates (panels E) are higher than Hellerstein et al. (1999) data, and proliferation for the healthy host (displayed in panel F2) is lower than that found by Hellerstein, although proliferation for both hosts is of the same order as Hellerstein. We note that Hellerstein et al., reported 324 cells/mm^3 as the concentration of T4 cells in the infected host, and 1300 cells/mm^3 in the healthy host; the simulated T4 concentrations after 3 years are 680 in the infected host and 1000 cells/mm^3 in the healthy subject.

8.2. T8 dynamics in infected and healthy hosts

T8 production and removal, see Fig. 9, in both hosts evolve in parallel; production is less than removal and drops a little more than removal, as time progresses. However, production, removal, and the total number of T8 cells is about 2.5 times greater in the infected

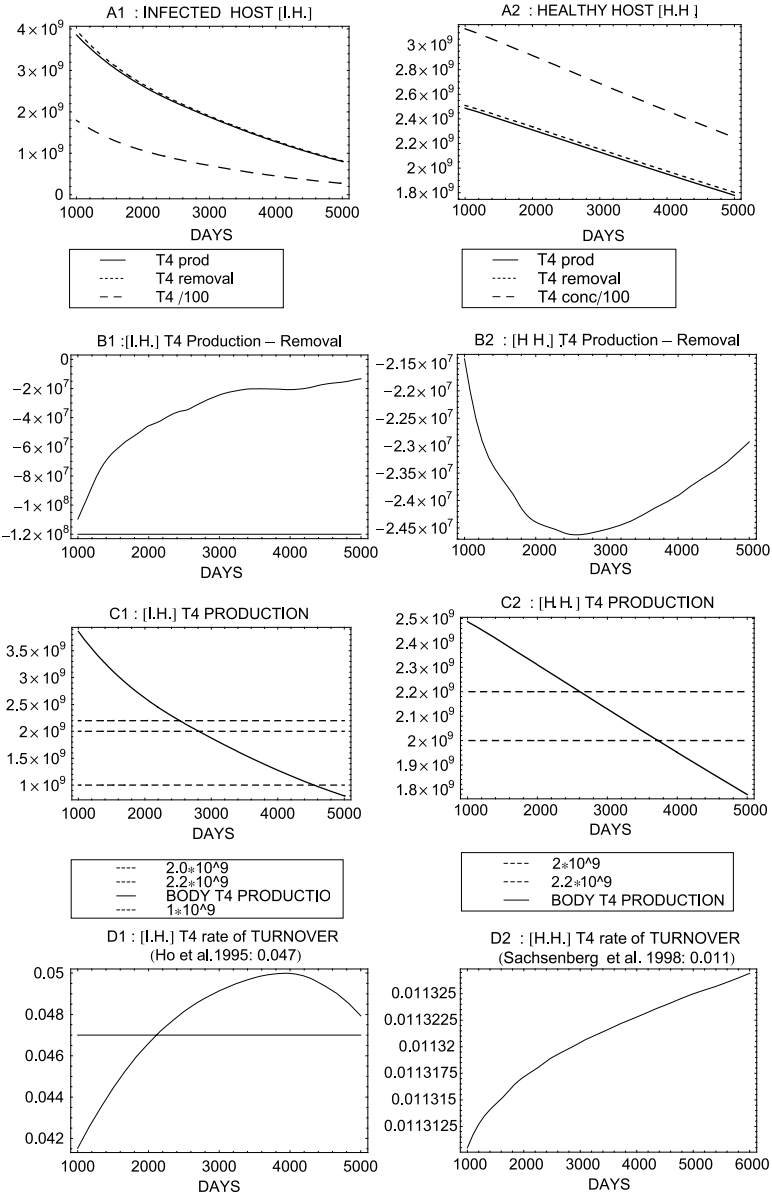


Fig. 8 Healthy and HIV infected hosts: comparison between their T4 cell dynamics. Infected host data (panels from A1 to F1) are parallel to healthy host data (panels from A2 to F2). Panels A, B, and C depict T4 production and removal (cells/day). Panels D, E, and F depict T4 turnover and proliferation rates. Available experimental values and references are cited under the relevant panels. Turnover, in panels D is calculated by T4 sinks (cytotoxic removal of infected cells and natural death of all T4) divided by total T4 concentration. The rate of proliferation, in panels E, is equal to the total proliferation of T4 cells (specific and non-specific, infected and healthy cells) divided by the total T4 concentration, and panels F is the T4 proliferation measured in (cells/mm^3/day).

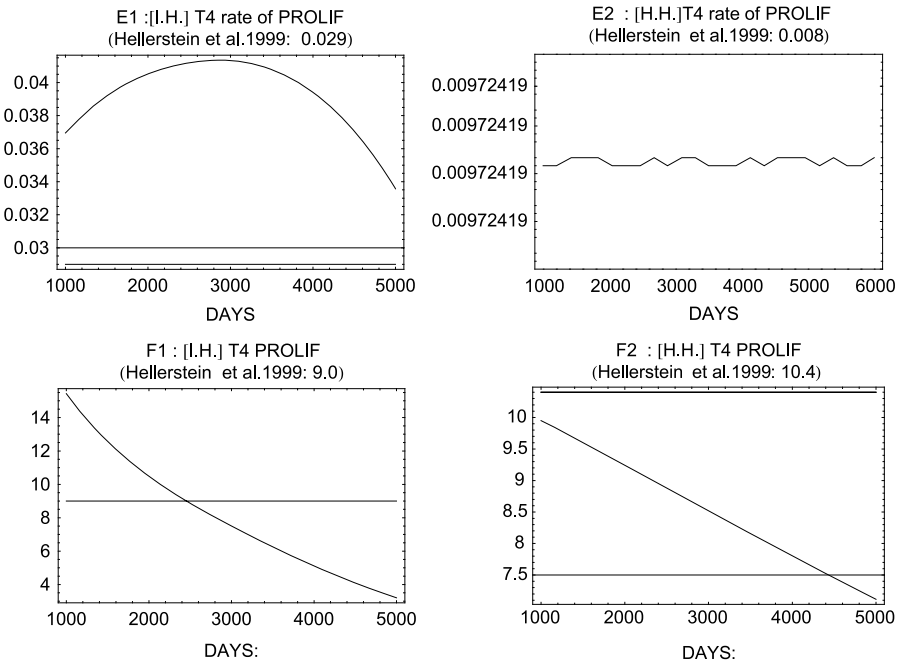


Fig. 8 (*Continued*)

host. This is due to infected cells stimulation of ST8 cells, not found in the healthy host: T8 proliferation in both hosts is lower than that reported by Hellerstein et al. (panel D).

The infected hosts' T8 proliferation is less than $1/2$ that predicted by Hellerstein et al. (1999) and twice the healthy rate (panels D). T8 turnover rates in the infected host is higher than the healthy host and both rise slightly and almost linearly (panels B).

Comparing T4 and T8 cells in the infected host; T4 rate of turnover is four times that of T8 cells and the rate of T4 proliferation is more than twice that of T8, whereas for the healthy host, T8 turnover and proliferation are slightly higher than that of T4 cells (Figs. 8 and 9). This suggests that only the T4 dynamics are unusual, however, not as unusual as this author expected.

9. Discussion and conclusions

The simulation results appear to soundly describe the documented course of HIV infection, in terms of variable concentrations, milestones, dynamics, and disease progression.

The simulations of the global response with a reasonable set of parameter values capture the double viral humps with further viral oscillation, early peaks in ST4 and ST8 suggestive of acute infection, seroconversion, and the establishment of local maxima and minima, for each of the variables (set points). The T8/T4 ratio inverts in 2 months, drops slightly, and rises throughout progression. A quasi-steady state follows the establishment

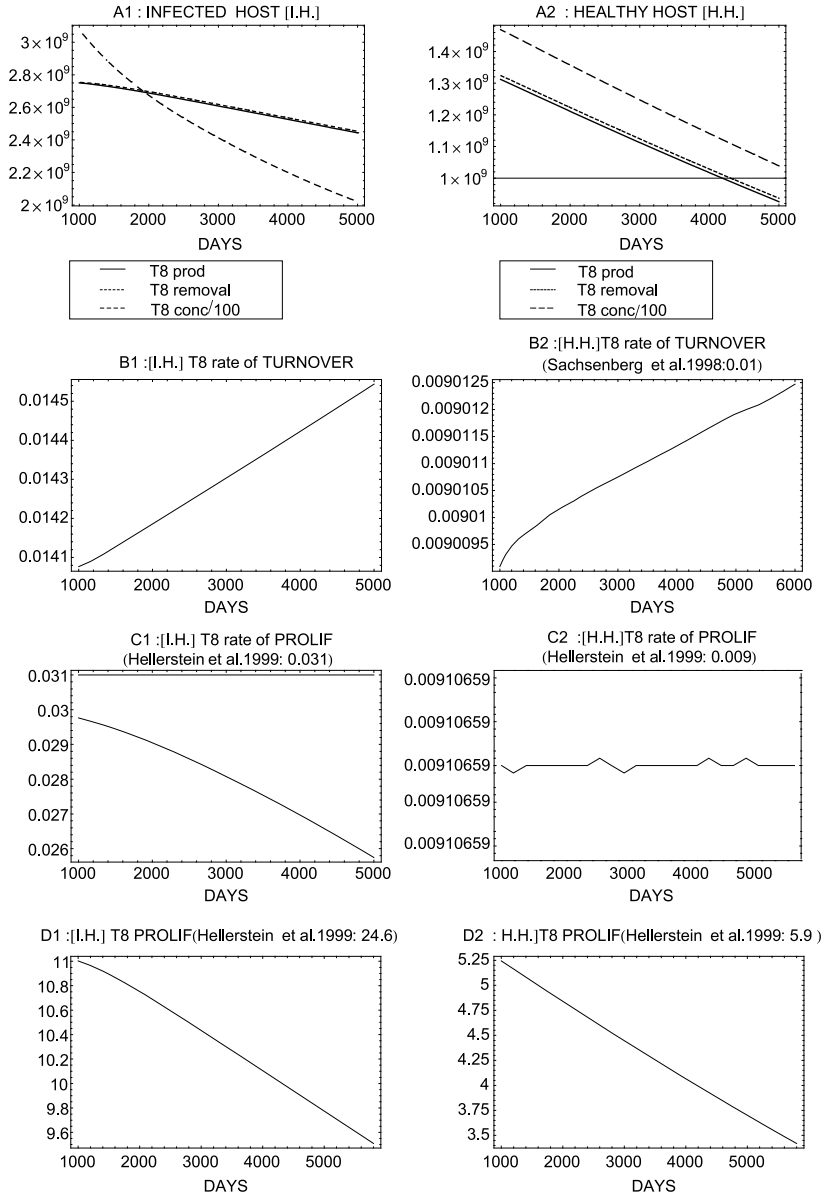


Fig. 9 Healthy and HIV infected hosts: comparison between their T8 cell dynamics. Infected host data (panels from A1 to C1) are parallel to healthy host data (panels from A2 to C2). Panels A depict T8 production and removal (cells/day). Panels B, and C depict T8 turnover and proliferation rates and panels D depict T8 proliferation. Available experimental values and references are cited under the relevant panels. Turnover, in panels B is calculated by total T8 sinks (natural death of all T8 cells) divided by total T8 concentration, the rate of proliferation, in panels C, is equal to the total proliferation of T8 cells (non specific and specific) divided by the total T8 concentration, and panels D represents the T8 proliferation (cells/mm^3/day).

of the set points, with rising virus and decreasing antibody and T cell concentrations and thereafter, the system descends into AIDS (after 10 years). Infected cells appear early in disease, suggesting that total elimination of virus is currently not feasible after infection.

The dynamics of the specific T4 subsets show a vigorous immune involvement throughout disease progression. Experiments suggest that the lack of sustained production of neutralizing anti-HIV antibodies is due to lowered viral stimulation of ST4 cells. Artificial stimulation of available naive specific B cells might thus be considered as potentially positive intervention.

Macrophage dynamics exhibit interesting features: simulations shows over 80% of the macrophages are infected at the first viral peak, whereas after that infected macrophages fall to <20 cells/mm^3, suggesting a significant contribution of this population to the first peak. In addition, the evolution of actively infected T cells reveal only modest viral production by these cells, much less than the total virus produced by infected macrophages.

The infection dynamics that emerge from the model simulations do not depend on T4 cell exhaustion, excessive replication, decreased latently infected cell life span, nor virulent viral destruction of critical life sustaining cells or other aberrations. The capacity of the model to simulate HIV disease progression, suggests that the included dynamics alone, can lead to the T4 decline. The decrease of T4 concentration, after the initial peaks, seems be the effect of lower specific T4 replication and differentiation due to insufficient viral stimulation, coupled to robust ST8 cytotoxic infected cell removal and to some loss due to host aging, and less to activated cell bursting: all these processes without any T cell exhaustion. T cell depletion and significant antibody decline occur as the natural consequences of the immune-viral interaction, of adaptation, and of the quasi-maintenance of a steady state of infection. "Increasing evidence suggests ... that infection-induced immune activation drives both viral replication and CD4+ cell depletion" (Grossman et al., 2002).

Simple single $\pm 10\%$ parameter modification experiments mimic individual host differences in T cell concentrations, antibody, and viral set points and in disease progression: the length of progression to AIDS was found to vary from 4 to 21 years. The correlation between progression to AIDS and particular response characteristics suggest possible progression predictors: for example, the correlation between progression time and the time of inversion of T8/T4 ratio is 0.814 in our simulations.

From the large parameter perturbation experiments, we find two "opposing dynamics" that might be exploited to alter significantly, disease progression. When a vigorous excitation of the HIV-specific immune system is present, generating high levels of ST4 helper cells and cytotoxic ST8 cells, progression to AIDS is extended over many years. The opposite dynamic is damping of the specific immune system to such an extent that very few ST4 helper and ST8 cytotoxic cells generated. In simulations of extreme damping, the T4 concentration drops below 200 after $\sim$40 years in some scenarios. Both increasing and decreasing immune recognition of infected cells extend progression to AIDS to 20 and more than 30 years, respectively. Elimination of viral adaptation, keeps the T4 concentration greater than T8 for 48 years, and there is no progression to AIDS, although virus and infected cells are present. This is surprising since the time dependent adaptation term used is quite conservative.

Macrophages emerge as the as the dominant producer and the dominant remover of virus: elimination of viral production by infected macrophages eliminates AIDS in the simulations. In general, all the substantial changes in the values of the parameters that

control the concentration and the functions of infected macrophages appear to have quite a significant effect on disease progression.

The behavior of the production and removal of the T4 subsets, of virus and antibodies clarify the contributions of each of these actors to disease evolution.

Simulations of T cell production and removal comparing infected and healthy hosts illustrate that a billion or more T4 cells are produced, removed, and replaced, in both hosts daily. T4 cell production and removal for matched HIV-infected and healthy hosts appear to be comparable: the range of healthy host production and removal data falls within the infected host's range, and for both hosts removal is greater than production. T8 production and proliferation in infected hosts is about 2 times greater than for healthy subjects, and they exhibit parallel dynamics.

In the future, additional simulations on the dynamics of substantial stimulation and damping of the immune system could be conducted, as well as a study of the ramifications of double parameter modifications. Simulated timed drug interventions should be informative and potentially useful.

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Appendix A: Thymic infection: derivation of e and cv_1

Thymocytes are CD4+ cells for about the last week of their residence in the thymus (Janeway et al., 1996, p. 6:6–6:9), and thus during this period they can be infected. The ratio between the outflow of mature T4 cells and the number of CD4+ thymocytes, is approximately given by the inverse of the thymic residence time of CD4+ thymocytes, τ.

Furthermore, the viral concentration in the thymus is equal to the viral concentration in blood and the system is in a quasi-steady state at each time.

Cells from bone marrow are assumed to be healthy; their parameter values are found in Table 2.

Definitions:

X_u Number of uninfected CD4+ cells in thymus
X_i Number of infected cells CD4+ cells in thymus
X Number of CD4+ cells in thymus, $X = X_u + X_i$
Y Number of precursor cells that become CD4+ per unit time
S_u Outflow of uninfected mature T4 cells to blood (cells per unit time)
S_i Outflow of infected mature T4 cells to blood (cells per unit time), $S_i = e \times V/(cv_1 + V)S_4$

$\bar{s}$ Outflow of mature T cells to blood (cells per unit time) $= S_4 + S_8$
S_4 Outflow of mature T4 cells to blood $= f \times \bar{s}$ in the model equations
S_8 Outflow of mature T8 cells to blood $= (1 - f) \times \bar{s}$ in the model equations
τ Residence time of CD+4 cells in thymus

Equations:

$$X_u' = Y - k_v V/(cv + V)X_u - S_u = 0 \quad \text{in steady state,}$$
$$S_u = Y - k_v V/(cv + V)X_u, \tag{A.1}$$

$$X_i' = k_v V/(cv + V)X_u - S_i = 0 \quad \text{in steady state,}$$
$$S_i = k_v V/(cv + V)X_u \equiv eV/(c_1 + V)S_4, \tag{A.2}$$

$$S_u = (X_u/X)S_4 \quad \text{and} \quad S_i = (X_i/X)S_4 \quad \text{in steady state } S_4 = Y,$$
$$S_4 = k_v V/(cv + V)X_u + (X_u/X)S_4, \tag{A.3}$$

$$S_4/X_u = k_v V/(cv + V) + 1/\boldsymbol{\tau}. \tag{A.4}$$

Equations (2) and (4) and the definition of S_4, after some algebra:

$$S_i = k_v/(k_v + 1/\boldsymbol{\tau}) \times V/\big(cv/(kv + 1)(\boldsymbol{\tau}) + V\big)S_4, \tag{A.5}$$

$$e = k_v/(k_v + 1/\boldsymbol{\tau}), \tag{A.6}$$

$$cv_1 = cv/\big(kv\boldsymbol{\tau} + 1\big). \tag{A.7}$$

Appendix B: Derivation of T cell flow from the thymus, $\bar{s}$, and $\boldsymbol{\Phi}$, the rate of differentiation of proliferating cells, T_{4n}, T_{4e}, T_{8n}, T_{8e}, and the proliferation parameters, r_4 and r_8

In the absence of RNA, the system is in steady state. Total concentration at time $t = 0$ is T4 $= 1300 = T_{4n} + T_{4e}$: T8 $= 617.2 = T_{8n} + T_{8e}$ [cells/mm^3].

1–1.1% of the T4 cells and 0.9–1% of the T8 cells are proliferating (in cycle) in a healthy host (Sachsenberg et al., 1998). Assuming the time for a new cell to proliferate is 1 day, the number of new cells/day is # in cycle $\times$ 0.69. f and $1 - f$ are respectively the proportions of T4 and T8 cells released from the thymus; these and other parameter values can be found in Table 2.

Equations:

T4 $= 1300$ cells/mm^3 $\Rightarrow$ T4 $\times$ 0.0105 $= 13$ cells/mm^3 are in the proliferating cycle.

$$13 \times 0.69 = 8.977 \text{ new T4 cells/mm}^3\text{/day}, \tag{B.1}$$

T8 $= 617.8$ cells/mm^3 $\Rightarrow$ T8 $\times$ 0.001 $= 6.178$ cells/mm^3 are in the proliferation cycle.

$$6.178 \times 0.69 = 4.26 \text{ new T8 cells/mm}^3\text{/day}. \tag{B.2}$$

Using the equations for T_{4n}' and T_{8n}' in the text and the above equations (B.1) and (B.2),

$$r_4 T_{4n} = 8.977, \tag{B.3}$$

$$r_8 T_{8n} = 4.26. \tag{B.4}$$

Using the equations for T'_{4n} and T'_{4e} and T'_{8n} and T'_{8e} in the text, in steady state with $V = 0$:

$$0 = (1-\nu)\bar{s}\,f + r_4 T_{4n} - \mu_{4n} T_{4n} - \mu_{4e} T_{4e}, \tag{B.5}$$

$$0 = (1-\nu)\bar{s}(1-f) + r_8 T_{8n} - \mu_{8n} T_{8n} - \mu_{8e} T_{8e}. \tag{B.6}$$

The equations in the text [Eqs. (6) and (12)] for T'_{4e} and T'_{8e} imply:

$$T_{4e} = \Phi r_4 T_{4n}/\mu_{4e}, \tag{B.7}$$

$$T_{8e} = \Phi r_8 T_{8n}/\mu_{8e}, \tag{B.8}$$

$$T_{4n} + T_{4e} = 1300, \tag{B.9}$$

$$T_{8n} + T_{8e} = 617.2. \tag{B.10}$$

Using Mathematica 5.0, Eqs. (B.3) to (B.10) are used to solve for $\bar{s}$, Φ, T_{4n}, T_{4e}, T_{8n}, T_{8e}, r_4 and r_8.

The values obtained are:

$$\begin{aligned} &\bar{s} = 6.089, \quad \Phi = 0.64, \quad T_{4n} = 921, \quad T_{4e} = 379, \\ &T_{8n} = 467, \quad T_{8e} = 150, \quad r_4 = 0.0097, \quad r_8 = 0.0091. \end{aligned} \tag{B.11}$$

Appendix C: Equations for T8 subsets when T8 cells are subject to thymic HIV infection

During the later part of their natal thymic production, thymocytes fated to become T4 or T8 cells are both CD+4 and CD+8 (double positive CD+4+8), and "they comprise the vast majority of thymocytes" (Janeway et al., 1999, p. 233; Janeway et al., 1996, p. 6:6–6:9). HIV infection can occur during this time (Kitchen et al., 1997; Lee et al., 1997). Those cells that survive the subsequent thymic selection process, will lose one of the two co receptors and become CD+4 or CD+8, entering the body as either T4 or T8 cells. Infected T8 cells can proliferate and activate. Activated T4 cells are moved to the T_{ii} subset. T8 cells cannot be infected after leaving the thymus.

$\bar{s}$ is the outflow of mature T cells to blood. Thymic infected T8 cells are included in the thymic source $\bar{s}$ released into the body [see derivation in Appendix B]. The thymic outflow of mature uninfected T8 cells to blood $= \bar{s}(1-f)$ in the model equations and the outflow of mature latently infected T8 cells to blood $= \bar{s}(1-f)e \times V/(cv_1 + V)$.

To first order, the fraction of latently infected T8 cells that become actively producing cells is p.

Equations:
Modeling thymic infection of T8 cells necessitates 4 additional equations for the latently infected specific and non-specific non-effector and effector partitions: Eqs. (C.2) to (C.5) below. R defined in Eq. (17), becomes

$$\mathfrak{R} = R + R^*, \quad \text{where } R^* = \rho_8 \delta [ST_{i8n} + T_{i8n} + ST_{i8e} + T_{i8e}]. \tag{C.1}$$

$$\begin{aligned} ST'_{i8n} = {} & (\nu)\bar{s}(1-f)eV/(cv_1 + V) + [(1-\Phi)(1-p) - p]\mathfrak{R} ST_{i8n} \\ & - \big(k_8 \delta (ST_{8e} + ST_{i8e}) + \mu_{8n}\big) ST_{i8n}, \end{aligned} \tag{C.2}$$

$$ST'_{i8e} = \Phi(1-p)\Re ST_{i8n} - \big(k_8\delta(ST_{8e}+ST_{i8e})+\mu_{8e}\big)ST_{i8e}, \tag{C.3}$$

$$T'_{i8n} = (1-\nu)\bar{s}(1-f)eV/(cv_1+V) + \big[(1-\Phi)(1-p)-p\big]r_8T_{i8n} - \big(k_8\delta(ST_{8e}+ST_{i8e})+\mu_{8n}\big)T_{i8n}, \tag{C.4}$$

$$T'_{i8e} = \Phi(1-p)r_8T_{i8n} - \big(k_8\delta(ST_{8e}+ST_{i8e})+\mu_{8e}\big)T_{i8e}. \tag{C.5}$$

Equations (9) to (13) in the text are modified to include T8 infection dynamics and are below Eqs. (C.6) to (C.10).

$$\tilde{T}'_{8n} = \nu\bar{s}(1-f)\big(1-eV/(cv_1+V)\big) + (1-\Phi)\Re\tilde{T}_{8n} - \mu_{8n}\tilde{T}_{8n}, \tag{C.6}$$

$$\tilde{T}'_{8e} = \Phi\Re\tilde{T}_{8n} - \mu_{8e}\tilde{T}_{8e}, \tag{C.7}$$

$$T'_{8n} = (1-\nu)\bar{s}(1-f)\big(1-eV/(cv_1+V)\big) + (1-\Phi)r_8T_{8n} - \mu_{8n}T_{8n}, \tag{C.8}$$

$$T'_{8e} = \Phi T_{8n}r_8T_{8n} - \mu_{8e}T_{8e}, \tag{C.9}$$

$$T'_{ii} = p\big(T_{i4n}r_4 + ST_{i4n}\rho_4\times V/(cv_2+V) + T_{i8n}r_8 + ST_{i8n}\Re\big) - \big(k_8(ST_{8e}+ST_{i8e})+\mu_{ii}\big)T_{ii}. \tag{C.10}$$

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