

Effectiveness of a ‘hunter’ virus in controlling human immunodeficiency virus type 1 infection

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Engineered therapeutic viruses provide an alternative method for treating infectious diseases, and mathematical models can clarify the system’s dynamics underlying this type of therapy. In particular, this study developed models to evaluate the potential to contain human immunodeficiency virus type 1 (HIV-1) infection using a genetically engineered ‘hunter’ virus that kills HIV-1-infected cells. First, we constructed a novel model for understanding the progression of HIV infection that predicted the loss of the immune system’s CD4⁺ T cells across time. Subsequently, it determined the effects of introducing hunter viruses in restoring cell population. The model implemented direct and indirect mechanisms by which HIV-1 may cause cell depletion and an immune response. Results suggest that the slow progression of HIV infection may result from a slowly decaying CTL immune response, leading to a limited but constant removal of uninfected CD4 resting cells through apoptosis – and from resting cell proliferation that reduces the rate of cell depletion over time. Importantly, results show that the hunter virus does restrain HIV infection and has the potential to allow major cell recovery to ‘functional’ levels. Further, the hunter virus persisted at a reduced HIV load and was effective either early or late in the infection. This study indicates that hunter viruses may halt the progression of the HIV infection by restoring and sustaining high CD4⁺ T-cell levels.

INTRODUCTION

Current therapies with antiviral drugs reduce the human immunodeficiency virus type 1 (HIV-1) load below detection and increase the viral targeted cells, the immune system’s CD4⁺ T lymphocytes, yet the infection persists because of the presence of a latent cellular reservoir (Haase, 1999; Pierson *et al.*, 2000). Further, this therapeutic approach has significant drawbacks, such as cost, toxicity and evolution of resistance. An alternative therapy is to use genetically engineered hunter viruses capable of infecting and killing HIV-infected cells. This method uses a modified rhabdovirus having an envelope that binds specifically to the proteins gp120/41 of HIV-1 on the surface of infected cells (Schnell *et al.*, 1997). The hunter virus reproduces through a rapid cytopathic infection that kills the cell and terminates HIV-1 replication. *In vitro* studies have shown that this destruction of infected cells decreased HIV-1 load by about 1000-fold (Schnell *et al.*, 1997). Also *in vitro*, a hunter virus designed for killing simian immunodeficiency virus (SIV)-infected cells caused SIV load to fall below the limit of detection. This hunter virus will be used to evaluate this therapy *in vivo* in a non-human primate (Okuma *et al.*, 2006).

Stimulated by these results, we developed a mathematical model for understanding the dynamics and evaluating the effectiveness of this treatment approach. Self-replicating agents have kinetics different from drugs for controlling diseases (Carlton, 1999; Levin & Bull, 1996; Payne & Jansen, 2003), requiring an understanding of interactions among populations of cells and viruses within an individual. The basis for evaluating the impact of this viral therapy is determining how the HIV-1 infection progresses by predicting the loss of CD4 cells. Therefore, this study first addressed HIV infection by considering several mechanisms capable of driving cell depletion; subsequently, the therapeutic virus was introduced. The analysis addressed key questions concerning the efficacy of this therapy. Will a therapeutic virus survive in a system with a depleted HIV load? When should the agent be introduced? Will it affect the immune response? How much reduction in HIV-1 load and recovery in CD4 cells is expected?

Clinical data indicate that after the primary stage, HIV infection commonly progresses with a slow decline in CD4 cells until the onset of AIDS. The asymptomatic progression takes an average of 8–12 years in which CD4 cells decline from about 1000 to 200 cells mm⁻³ (Fauci & Desrosiers, 1997). The disease then emerges due to a weakened immune system that makes individuals highly vulnerable to opportunistic infections. The mechanisms by which HIV-1 infection depletes cells remain a matter of debate (Douek

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et al., 2003; Grossman *et al.*, 2002; McCune, 2001). CD4 can be destroyed directly by the virus – HIV replicates by infecting and killing activated cells – and by an immune response also killing infected cells. Another mechanism is a generalized immune activation and proliferation of CD4 cells (Douek *et al.*, 2003; Grossman *et al.*, 2002; Hazenberg *et al.*, 2000; McCune, 2001). These activated cells may also be infected and killed by HIV or undergo apoptosis (programmed cell death). A third mechanism is apoptosis of uninfected non-specific resting or activated CD4 cells through a number of HIV-mediated mechanisms (Ahr *et al.*, 2004; Finkel *et al.*, 1995; Gougeon, 2003). Additional factors contributing to further cell depletion may appear later in the infection, such as more effective HIV strains for inducing apoptosis or impairment of lymphoid regenerative capacity (Fauci & Desrosiers, 1997). The present study addressed HIV infection from the time of infection through asymptomatic period, under the assumption that these three mechanisms are the main causes of cell depletion in these decisive stages.

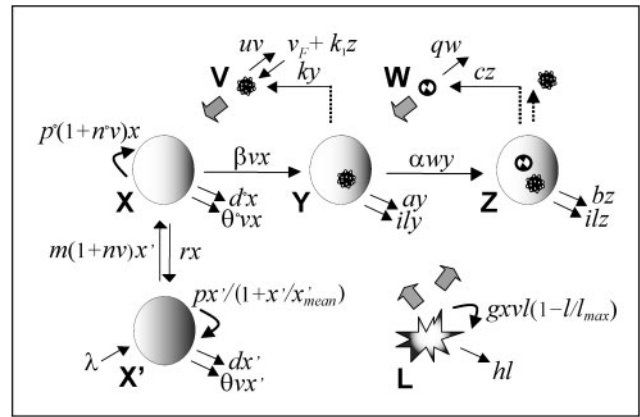
The relative impact of the mechanisms of cell depletion is incompletely understood, but the combination of them may eventually overwhelm the ability of CD4 proliferation to compensate. Immunodeficiency viral infections in other primates have provided insight into these driving forces. Natural hosts of SIV exhibit infections with high levels of viral replication and death of infected cells, but do not develop progressive depletion of cells (Broussard *et al.*, 2001). These infections show limited increase in immune activation and turnover (proliferation and cell death) and no increased apoptosis (Broussard *et al.*, 2001). Increased apoptosis appears to be present only in those non-natural hosts of SIV in which the infection is pathogenic (Davis *et al.*, 1998), suggesting that apoptosis may be a primary mechanism of cell loss.

This study builds on previous models of HIV infection (De Boer & Perelson, 1998; Murray *et al.*, 1998; Nowak & Bangham, 1996; Perelson *et al.*, 1997; Perelson & Nelson, 1999; Phillips, 1996; Wodarz & Jansen, 2001). It differs by addressing the indirect mechanisms of cell depletion, and implementing an immune response and dynamics of HIV's primary target cell within a single model. Particularly, the therapeutic infection was based on Revilla & García-Ramos (2003), who first modelled hunter viruses against HIV and used a simple framework (i.e. target cell model). Thus, the present study provides a more coherent formulation of HIV infection, allowing an evaluation of the therapeutic approach in a more realistic dynamical system. It makes an important advance by recognizing and evaluating the mechanisms underlying HIV dynamics and their implications in the progression of the infection.

RESULTS

Model

The mathematical model constructed for studying HIV infection and the therapeutic infection is described in Fig. 1,



$$\begin{aligned}
 dx' / dt &= \lambda + px' / (1 + x' / x'_{mean}) + rx - m(1 + nv)x' - \theta vx' - dx' \\
 dx / dt &= p(1 + nv)x + m(1 + nv)x' - rx - \beta vx - \theta' vx - d'x \\
 dy / dt &= \beta vx - ily - \alpha wy - ay \\
 dz / dt &= \alpha wy - ilz - bz \\
 dv / dt &= v_F + ky + k_1 z - uv \\
 dw / dt &= cz - qw \\
 dl / dt &= gxvl(1 - l / l_{max}) - hl
 \end{aligned}$$

X':	resting CD4
X:	activated CD4
Y:	infected CD4
Z:	double-infected CD4
V:	HIV
W:	hunter virus
L:	CTL response

Fig. 1. Model description. The diagram shows the interacting dynamics of CD4 cells (resting x' and activated x), HIV infection (HIV v and HIV-infected cells y), immune response (l) and therapeutic infection (hunter virus w and double-infected cells z). Host cell dynamics: CD4 cells exist as resting or activated cells and activated cells are the targets for HIV replication. In the absence of HIV, resting cells x' are generated at constant rate λ , replicate at rate $px'/(1 + x'/x'_{mean})$, and die at rate dx' . Resting cells x' are transformed into activated x at rate $m(1 + nv)x'$, and return to resting stage at rate rx . Activated cells proliferate at rate $p(1 + nv)x$ and die at rate $d'x$. HIV-infection: HIV v infects activated cells x at rate βvx , producing HIV-infected cells y that die at rate ay . HIV are released from infected cells at rate ky and from a reservoir at rate v_F and are removed at rate uv . In the presence of HIV, the resting cells x' activate at rate $m(1 + nv)x'$ and undergo apoptosis at rate $\theta vx'$; whereas the activated cells x proliferate at rate $p(1 + nv)x$ and undergo apoptosis at rate $\theta' vx$. Therapeutic infection: hunter virus w infects HIV-infected cells y , converting them into double-infected cells z at rate αwy . These cells z produce hunter viruses at rate cz and HIV at rate $k_1 z$ that cause cell death at rate bz . Hunter viruses are removed at rate gw . Immune response: both types of infected cells are killed by CTL response l at rates ily and ilz , respectively. CTL proliferate at rates $gxvl(1 - l/l_{max})$ and die at rate hl . For simulations, CTL and pathogen flux v_F start after HIV reaches 10. The diagram is formalized into equations by considering incoming arrows to be positive terms and outgoing arrows to be negative terms in each equation. Each equation describes the rate of change in the number of cells or viruses $\text{mm}^{-3} \text{day}^{-1}$.

showing the interactions between cells and viruses. The infection system consisted of four interacting dynamic components: (i) the target cell life cycle, addressing the

resting CD4 cells x' and activated-uninfected CD4 cells x ; (ii) the HIV-1 infection, featuring the HIV virus v and activated-infected CD4 cells y ; (iii) the CTL immune response l and (iv) the therapeutic infection, incorporating an engineered-hunter virus w and double-infected CD4 cells z (see Methods).

HIV infection

Numerical simulations generated dynamics of HIV-1 infection along primary and asymptomatic stages comparable to experimental data (Cohen *et al.*, 1999; Douek *et al.*, 2003) (Fig. 2a). Infection initially triggers a rapid change over a few weeks, in which CD4 cells decline, while HIV increases towards a peak. A key element in this pattern is the appearance of a strong CTL response that effectively reduces HIV load to a low level in a few days. This fall was faster than observed data (Lindbäck *et al.*, 2000) due to the

system's simplicity without other HIV compartments. This temporal plummet in HIV coincides with a partial recovery in cells for several months until HIV significantly rebounds. Thereafter, the time-course slows down considerably over several years in which HIV continuously increases and CD4 cells gradually decay until the critical density of immunodeficiency of $200 \text{ cells mm}^{-3}$ is reached. Notice that the model at default values has equilibrium in the region of immunodeficiency; however, the analysis was focused on the infection dynamics above this critical density.

Most models of HIV infection have assumed that cell depletion results from the killing of CD4-infected cells by HIV (Murray *et al.*, 1998; Nowak & Bangham, 1996; Perelson *et al.*, 1997; Phillips, 1996; Stafford *et al.*, 2000; Wodarz & Jansen, 2001). This study addressed the role of HIV in triggering apoptosis of both activated and resting

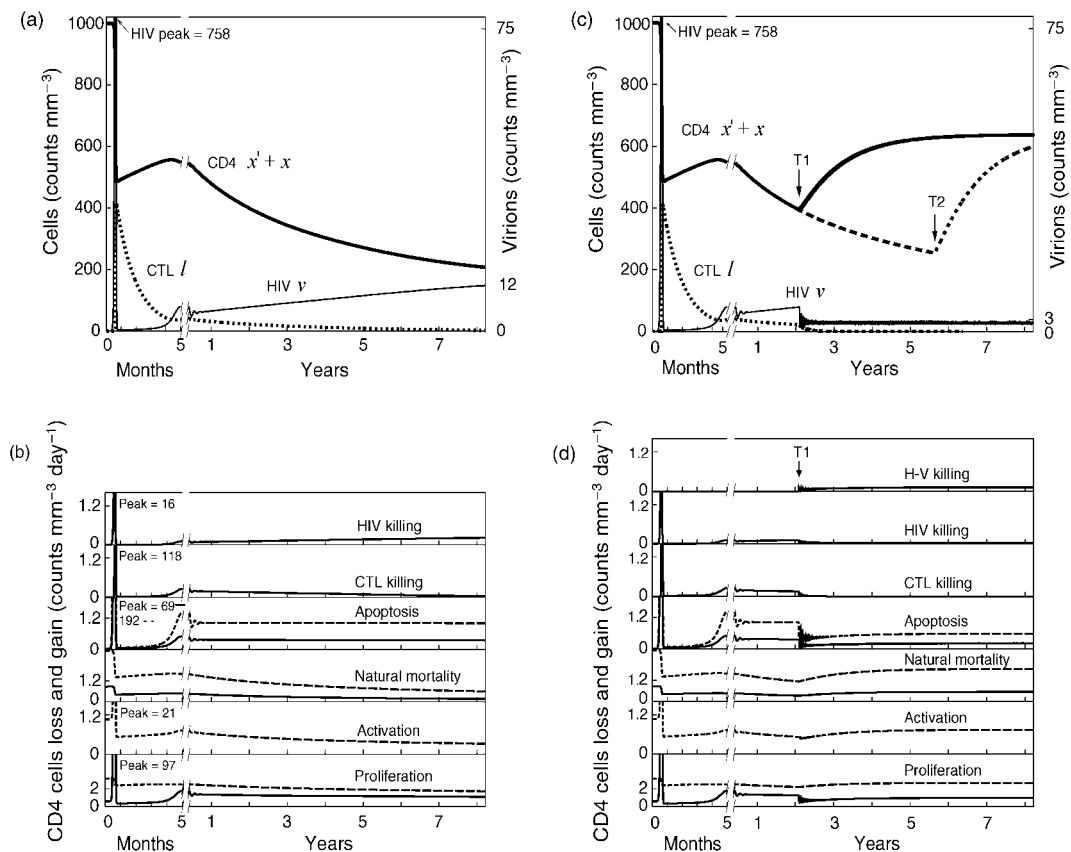


Fig. 2. Temporal dynamics for HIV and therapeutic infection. (a) The progression of HIV infection, indicating the change across time in the number of CD4 cells $x' + x$, HIV v and CTL response l . (b) The dynamics of factors contributing to the number of CD4 cells. Panels show rates of loss and gain in number of CD4 cells due to the following causes: HIV killing infected cell ay ; CTL killing HIV-infected cell ily ; apoptosis $\theta vx'$ (resting), $\theta' vx$ (activated); natural mortality dx' (resting), $d'x$ (activated); activation $m(1 + nv)x'$ (resting); and proliferation $\lambda + px'/(1 + x'/x'_{mean})$ (resting), $p'(1 + n'v)x$ (activated). '-' denotes resting and '-' activated cells. (c) The viral therapeutic infection. T1 indicates an introduction of hunter viruses into HIV infection at 400, and T2 at 250 CD4 total cells mm^{-3} . (d) Detailed dynamics on loss and gain of CD4 cells due to different causes during therapeutic infection. Panel 'H-V killing' indicates loss of HIV-infected cells by hunter virus bz . Initial conditions and parameter values are in Table 1.

uninfected cells. Apoptosis accounts for a deeper cell drop at the primary HIV infection and speeds cell depletion dynamics. Fig. 2(b) shows the dynamics underlying the loss of CD4 cells. Its panels compare the contributions to CD4 depletion caused by HIV and CTL killing cells, apoptosis (resting and activated cells) and natural mortality, together with cell transition by activation and cell gain by proliferation along the progression of the infection (Fig. 2b). Notice that vertical axis dimensions may vary along panels. Results indicate that during the asymptomatic stage the loss of cells by apoptosis is relatively constant (Fig. 2b, apoptosis panel). This constancy results from the balanced product of CD4 decay and HIV rise as time passes ($\theta vx'$, $\theta' vx$). In the model activated cells have greater susceptibility to apoptosis than do resting cells, but since the majority of CD4 cells are resting, the cell loss from both stages was significant. At the asymptomatic stage, apoptosis is the main cause of loss, accounting for far more cell loss than the HIV and CTL killing of infected cells. The panel for activation (Fig. 2b) shows a decay in the number of resting cells activated as time progresses; however, the rate of cell activation $m(1 + nv)x'$ increases with time following viral load v .

A feature of this model is the addition of a small flux of HIV, v_r . It represents HIV released from cells not represented in the model, such as macrophages and infected-resting CD4 cells (Perelson *et al.*, 1997). In the absence of this flux, HIV plummets to extinction at the acute infection (below 10^{-8} virions mm^{-3}). A trace flux results in strong oscillations in CTL and HIV along the transition to asymptomatic infection. A small flux (0.05–1 virions $\text{mm}^{-3} \text{day}^{-1}$) reduces or eliminates these oscillations with negligible change in the subsequent dynamics. Notice that damped oscillations in HIV load also result from spatially explicit models, which, similar to our flux will supply virions but from other localities in the body (Funk *et al.*, 2001). Experimental data indicated that HIV load after the rapid decline remains detectable (Lindbäck *et al.*, 2000), consistent with a low level flux.

Results showed that CTL did not disappear after primary infection, but slowly decayed towards immunodeficiency (Fig. 2a). Without CTL the system reaches equilibrium within several weeks. Simulations also showed how CTL strength affects the transient length and number of CD4 at equilibrium. A slow-growth CTL (small g) led rapidly to immunodeficiency. Instead, a fast-growth CTL (g larger than default) delays immunodeficiency or lifts the equilibrium above the immunodeficiency threshold (and may eliminate the sharp cell drop at primary infection). In these cases, CTL decayed very slowly or stabilized at a large number. These results suggest that a slow decay in CTL strength may delay immunodeficiency by partially controlling HIV viral load, thereby slowing the rate of CD4 cell depletion.

Since both CTL and resting cells change slowly during the asymptomatic stage, inferring cause and effect is not simple. To investigate if the slow dynamics of resting cells may also account for the speed of infection progression, we increased

the removal of resting cells. An increase in activation rate did not lead to cell depletion; however, a rise in death rate or apoptosis speeds cell loss in comparison to default. Assuming that natural mortality does not change with infection, we further investigated apoptosis. An infection with no resting cell apoptosis led to 93 % normal cell count, which indicates that the default CTL is capable of a major control of non-apoptotic HIV strains. Afterward, cell counts at equilibrium decreased in proportion to the severity of apoptosis (i.e. 63 to 44 % normal levels for half to three-quarters default apoptosis, respectively).

Since CD4 cell proliferation does not balance losses in HIV infection, a daily deficit accumulates over time until reaching immunodeficiency. By early asymptomatic infection this deficit is only a fraction of the resting cells removed by apoptosis, indicating the role of proliferation in ameliorating the cell loss and slowing the dynamics to the infection equilibrium. Also at early asymptomatic stage, the CTL strength (i.e. number of CTL cells) sets the HIV load and thus, the intensity of damage through apoptosis. At that time, CTL was partially effective and further deteriorated over time according to resting cell count (since help from activated cells was proportional to resting cell count). As CTL decayed, the HIV load increased; however, apoptosis remained approximately constant and became the major removal of cells at immunodeficiency. Consequently, these results suggest that the slow progression of HIV infection may result from a slowly decaying CTL, leading to a limited but constant removal of resting cells through apoptosis, as well as from the resting cell proliferation that ameliorates the cell depletion rate over time.

Parameter values are subject to some degree of uncertainty and variability (Supplementary Table S1, available in JGV Online). In this respect, simulations showed that parameter values differing $\leq 6\%$ from defaults yielded qualitatively similar results in leading to immunodeficiency. This relatively narrow consistency range indicated sensitive parameters and suggested the presence of non-linear stabilizing mechanisms *in vivo* that the current model did not include. We further explored a broader parameter space that simultaneously sampled all the parameter values within plausible ranges (Supplementary Table S2, available in JGV Online). This analysis showed a wider range of progression varying from minor cell losses through the asymptomatic period to immunodeficiency by year 3, which corresponds to the interquartile range around the progression for median values (Supplementary Fig. S1, available in JGV Online). Thus, the model predicts substantial deviation in the progression when both host and pathogen exhibit variation. Notice that high variation in infection progression has been observed within populations (Concerted Action on SeroConversion to AIDS and Death in Europe, 2000).

Hunter virus therapeutic infection

The analysis evaluated therapeutic introductions of hunter viruses early or late in HIV infection (at 400 or 250 cells

mm^{-3} , respectively). In both cases, the effect of delivering a hunter virus into the HIV infection is to halt CD4 cell depletion and generate a rebound over 2/3 of healthy levels (Fig. 2c). For the default parameter values, the recovery is 64 % of the CD4 cell normal level. The rebound trajectory follows a saturation curve with initial speeds proportional to the magnitude of CD4 reduction. After the first year of therapy, more than 50 % of the expected recovery is reached. The speeds of recovery were comparable to rises after highly active antiretroviral therapy (HAART) treatment where cell count typically increases rapidly for a few months, followed by more gradual rises until a normal value may be reached (Geng & Deeks, 2009; Hunt *et al.*, 2003; Vriskoop *et al.*, 2008). These clinical data indicate that the immune system is capable of producing substantial numbers of cells even after advanced immune depletion. This supports the model's assumption that the parameters stay constant during disease progression and that the variables are capable of capturing the dynamic process. Thus, infection progression constitutes a slow transient from the uninfected to the infected equilibrium, which could be substantially reversed with hunter viruses. Cases of severe depletion with compromised regenerative capacity may change parameter values (i.e. resting cell growth rate) and may thus lead to a reduced therapy efficiency.

The HIV load that coexists with the hunter virus is comparable to the load at the setpoint – at the time of the largest recovery of CD4 from acute infection (Fig. 2c). The CTL response rapidly decays after the hunter virus is delivered (Fig. 2c), suggesting that this immune response will not persist with the hunter virus under this therapy. Panels in Fig. 2(d) show that the hunter virus caused a reduction in the number of CD4 killed by HIV and apoptosis and in proliferation of activated cells. The hunter virus allowed mortality and proliferation of resting cells to approach normal values.

Delivery time. The delivery time for a successful therapeutic introduction was predicted with the 'basic reproductive ratio' R_0^w (Nowak & May, 2000). R_0^w describes the number of secondary infections produced by a single infection, and when this ratio is larger than one ($R_0^w > 1$), the hunter virus is capable of invading the HIV infection ($R_0^w = \frac{\alpha y}{q} \frac{c}{(b+il)}$; see symbols in Table 1 and derivation in Supplementary Material, available in JGV Online). This ratio is commonly evaluated when the system to invade is at equilibrium (i.e. equations set to zero in Fig. 1), requiring the number of HIV-infected cells y and CTL l in that situation. However, this calculation is of limited use for therapy since the equilibrium is located within the region of immunodeficiency (i.e. below 200 cells mm^{-3}). The basic reproductive ratio, indicating the invasion suitability throughout the course of the infection is shown in Fig. 3. In Fig. 3, R_0^w was estimated by replacing the values of $y(t)$ and $l(t)$ through time. Fig. 3 shows two shaded areas for successful invasion concerning the establishment and persistence of the therapeutic

infection: (i) a brief interval during primary infection, and (ii) shortly before the setpoint and thereafter. Introductions at the primary infection reached the same equilibrium as introductions in asymptomatic infection but in a smooth fashion with a small HIV peak and CD4 fall. These results indicate that the hunter virus can be introduced anytime after reaching a threshold of infected cells before the peak of infection or within a few months of infection, indicating its capability of surviving at limited and severe CD4 depletion.

Efficiency. The number of recovered CD4 cells measured the efficiency of the therapy, and it depended on the hunter virus vital rates. Recovery increased with increasing infectivity α and production rate c , and with reducing hunter virus removal rate q . Fig. 4(a) shows that cell recovery follows a saturating curve as the product of infectivity α and hunter virus production c increases – relative to the corresponding product for HIV (i.e. $\alpha c/\beta k$: infectiveness amplification). For example, an efficient recovery over 600 CD4 cells (i.e. 60 % healthy level) required that infectiveness of the hunter virus be amplified 155 times with respect to HIV (Fig. 4a). This requires, for example, a hunter virus infectivity of $\alpha=0.02$ and production $c=1550$; or $\alpha=0.004$ and $c=7750$ with other parameters at defaults. Notice in Fig. 4(a) that cell recovery is very sensitive to infectiveness amplification increments for values below 300, suggesting a range of high profitability. Another factor that proportionally increases recovery is a lowering hunter virus removal rate q in relation to HIV u (q/u). The default values assumed a quotient $q/u=2/3$, though, smaller quotients lessen infectiveness amplification for a cell recovery level. For instance, an order of magnitude reduction in default removal rate q increased the efficiency of cell recovery from 64 to 89 %. On the other hand, the HIV population is affected inversely to CD4 cells (Fig. 4b), and the curve shifts downwards, increasing infectivity α and production of hunter virus per cell c . In Fig. 4(b), the lines bifurcate at lower values of c , indicating a region of values where the therapy leads to oscillations in HIV. These oscillations belong to a periodic attractor with one fundamental frequency (for default values at 45 days), indicating that HIV periodically fluctuates over time with maxima amplitude given by bottom and top line values (Fig. 4b) (Liu, 2003). This dynamical behaviour is also observed for the hunter virus (Fig. 4c) but is absent in CD4. One cost of this viral therapy is measured in terms of the population size of hunter virus maintained at equilibrium; and it ranged from few dozens (default) to few hundreds. This cost decreases with larger infectivity and lower production rate of hunter viruses (Fig. 4c). Notice that the viral flux, as well as HIV released from double-infected cells promotes stability of the therapeutic infection by reducing the amplitude of oscillations in both viruses. The therapeutic efficacy was evaluated on a typical progression of HIV-1 infection. Results were qualitatively similar to default therapeutic infection when parameter values for HIV

Table 1. Basic parameter values and initial conditions used in simulations

	Parameter (c, cells mm ⁻³ ; v, virions mm ⁻³ ; d, day)	Value	Reference(s)
λ	Production rate of resting cell (cd ⁻¹)	1	Di Mascio <i>et al.</i> (2006); Kaufmann <i>et al.</i> (2001)
p	Replication rate of resting cell (d ⁻¹)	0.0045	Kaufmann <i>et al.</i> (2001)
p°	Replication rate of activated cell healthy condition (d ⁻¹)	0.02	Ribeiro <i>et al.</i> (2002)
$p^\circ n^\circ$	Replication rate of activated cell from infection (v ⁻¹ d ⁻¹)	0.007	Ribeiro <i>et al.</i> (2002)
d	Death rate of resting cell (d ⁻¹)	0.003	Kaufmann <i>et al.</i> (2001); Michie <i>et al.</i> (1992); Richman (2000)
d°	Death rate of activated cell (d ⁻¹)	0.03	Kaufmann <i>et al.</i> (2001); Stafford <i>et al.</i> (2000)
a	Death rate of HIV-infected cell (d ⁻¹)	0.33	Davenport <i>et al.</i> (2004); Perelson <i>et al.</i> (1996); Phillips (1996)
b	Death rate of double-infected cell (d ⁻¹)	2	Schnell <i>et al.</i> (1997)
m	Activation rate healthy condition (d ⁻¹)	0.0011	Kaufmann <i>et al.</i> (2001)
mn	Activation rate from infection (v ⁻¹ d ⁻¹)	4.4×10^{-5}	Kaufmann <i>et al.</i> (2001); Phillips (1996)
r	Deactivation rate (d ⁻¹)	0.026	Kaufmann <i>et al.</i> (2001)
β	HIV infection rate (v ⁻¹ d ⁻¹)	0.004	Phillips (1996); Layne <i>et al.</i> (1989); Murray <i>et al.</i> (1998)
θ	Apoptotic rate of resting cell* (v ⁻¹ d ⁻¹)	0.00044	
θ	Apoptotic rate of activated cell* (v ⁻¹ d ⁻¹)	0.005	
k	HIV production rate by HIV-infected cell (vc ⁻¹ d ⁻¹)	50	Dimitrov <i>et al.</i> (1993); Haase (1999)
k_1	HIV production rate by double-infected cell (vc ⁻¹ d ⁻¹)	5	Schnell <i>et al.</i> (1997)
u	HIV removal rate (d ⁻¹)	3	Perelson <i>et al.</i> (1996); Markowitz <i>et al.</i> (2003); Ramratnam <i>et al.</i> (1999); Perelson <i>et al.</i> (1997)
v_F	HIV flux rate (vd ⁻¹)	0.84	
α	Hunter virus infection rate† (v ⁻¹ d ⁻¹)	0.02	
c	Hunter virus production rate (vc ⁻¹ d ⁻¹)	1800	Schnell <i>et al.</i> (1997)
q	Hunter virus removal rate† (d ⁻¹)	2	
g	CTL proliferation rate (v ⁻¹ c ⁻¹ d ⁻¹)	0.00038	Kaufmann <i>et al.</i> (2001); Davenport <i>et al.</i> (2004)
h	CTL death rate (d ⁻¹)	0.025	Ogg <i>et al.</i> (1999)
i	CTL killing rate (c ⁻¹ d ⁻¹)	0.02	Altes <i>et al.</i> (2002); Appay <i>et al.</i> (2002); Klenerman <i>et al.</i> (1996); Mandl <i>et al.</i> (2007)
l_{max}	Maximum CTL number (c)	500	Appay <i>et al.</i> (2002); Betts <i>et al.</i> (2001); Lindbäck <i>et al.</i> (2000)
x'_{mean}	Average resting CD4 cell number (c)	970	Hellerstein <i>et al.</i> (2003); Hunt <i>et al.</i> (2008)
	Variable initial value		
x'	CD4 resting cell number (c)	970	
x	CD4 activated cell number (c)	30	
y	HIV-infected cell number (c)	0	
z	Double-infected cell number (c)	0	
v	HIV number (v)	5×10^{-5}	
w	Hunter virus number (v)	0.1	
l	CTL number (c)	0.03	

*Inferred.

†Inferred in relation to HIV. Supplementary Table S1 compares and justifies parameter values.

infection (i.e. default rates of apoptosis θ , activation mn , HIV proliferation k and CTL proliferation g) were changed at least within a factor of 0.5–1.5. When all the model parameter values varied simultaneously within ranges around defaults (Supplementary Material), the mean CD4 recovery was about 46 % of the total healthy level (95 % CI, 44–47 %) (measured in resting cells at day 3000; 86 % of simulations rebound from introductions at 300

cells; and HIV load dropped to 7.6 % [95 % (CI, 6.9–8.2 %) upon delivery]. This recovery was smaller than default therapeutic infection (64 % total, 62 % resting), suggesting that maximum recovery may not be reached for all of the simulations, as well as asymmetrical effects of varying parameters values [i.e. recovery followed a saturating curve more sensitive towards smaller infectiveness-amplification values (Fig. 4a)].

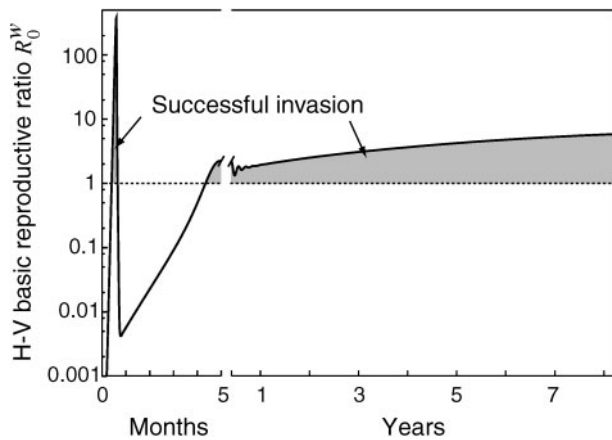


Fig. 3. Basic reproductive ratio R_0^w for the therapeutic infection throughout the HIV infection. For R_0^w larger than one, the hunter virus can successfully invade an HIV infection (shaded areas).

DISCUSSION

This study satisfactorily predicted the typical course of HIV infection from infection through asymptomatic stage. It was able to capture from the initial viraemia peak and drop in CD4 cells to the strong CTL response and temporary recovery of cells and HIV rebound. It also described the asymptomatic stage, with viraemia slowly rising and CD4 slowly decaying towards immunodeficiency. Most models of HIV infection address short-term dynamics, though, long-term progression has been studied (Bajaria *et al.*, 2002; Duffin & Tullis, 2002; Nowak *et al.*, 1990; Stilianakis *et al.*, 1997; Stilianakis & Schenzle, 2006). These models generally have considered simplistic assumptions about the infection system and earlier mechanisms of cell depletion. The present study analysed the impact of HIV on CD4 cells through recently proposed mechanisms (i.e. generalized activation and increased apoptosis), as well as an immune response. Our analysis indicated that an efficient CTL accounts for most of the loss of infected cells in the acute infection, and that CTL killing and virus cytopathicity contribute similarly to the loss of cells during the asymptomatic phase. These results are consistent with a comparison for the loss of infected cells by McMichael & Phillips (1997).

Our model also found that the loss of uninfected cells (either resting or activated) by apoptosis is a major cause of cell loss during acute infection. This important effect persisted through the asymptomatic stage and was several times larger than other causes of cell loss. Moreover, the cell loss by apoptosis in the asymptomatic stage followed a relatively constant pattern independent of the depletion level. A similar steady pattern for apoptosis at asymptomatic infection is suggested by Gougeon (2003). In our model, activated-uninfected cells had a single term for apoptosis, and thus, the two indirect mechanisms by which HIV may

lead to CD4 depletion (i.e. generalized activation and enhanced apoptosis) overlapped. Nonetheless, apoptosis of resting cells alone may account for more of half of the cell loss, pointing out the importance of increased apoptosis. Furthermore, this study suggests that the slow progression of HIV infection may result from a slowly decaying CTL response, leading to a limited but constant removal of uninfected resting cells through apoptosis, and from resting cell proliferation that ameliorates the rate of cell depletion over time.

The proposed therapeutic hunter virus consisted of a highly prolific, short life cycle, cytopathic virus for targeting HIV-infected cells (Schnell *et al.*, 1997). Results indicated that it was efficient in controlling HIV load and allowing the recovery of the immune system's CD4 cells to high levels (64% at default values) without eliminating HIV-1. The therapeutic virus establishes readily, since it survives in a system with a depleted HIV load. It can persist if introduced either at an early stage or an advanced stage of CD4 depletion. This therapy stabilizes HIV at low levels, resulting in no further depletion of CD4 associated with the progression of HIV infection. The CTL rapidly decays after the hunter virus is delivered (Fig. 2c), suggesting that this immune response will not coexist with the hunter virus under this therapy. This indicates that the hunter virus and CTL are strong competitors for the same resource (HIV-infected cells); and the elimination of the less efficient competitor follows the competitive exclusion principle. By selecting a fast-growth CTL ($g > 1.5 \times$ default), the model generated behaviour where the CTL dominated and the hunter virus did not invade.

Our results are consistent with those of Revilla & García-Ramos (2003). There are structural differences between the 2003 and the present model; further the current model evaluated a more efficient hunter virus with adjustments in parameter values for better fit to clinical data. Nevertheless, considering identical values when it was possible and using equations 3 and 5 from Revilla & García-Ramos (2003), the current model showed a moderate enhancement of cell recovery and a lower reduction in HIV after therapy. The target cell (2003) model produced higher HIV loads, suggesting a greater conversion of cell number into viruses, which may result from an entire pool of cells that was activated and without apoptosis.

Therefore, the results reported here indicate that the beneficial effects of hunter viruses are sustained under a more realistic and general HIV infection dynamics. The present study incorporated resting cells for quantifying the cycling pattern between resting and activated stages over time, as well as the mortality of uninfected-resting cells due to HIV. In particular, the implementation of a CTL response predicted long-term progression of the HIV infection that was not observed by Revilla & García-Ramos (2003), where the dynamics reached equilibrium rapidly. This indicated that the therapeutic agent can establish along the progression of HIV infection, besides the equilibrium at immuno-

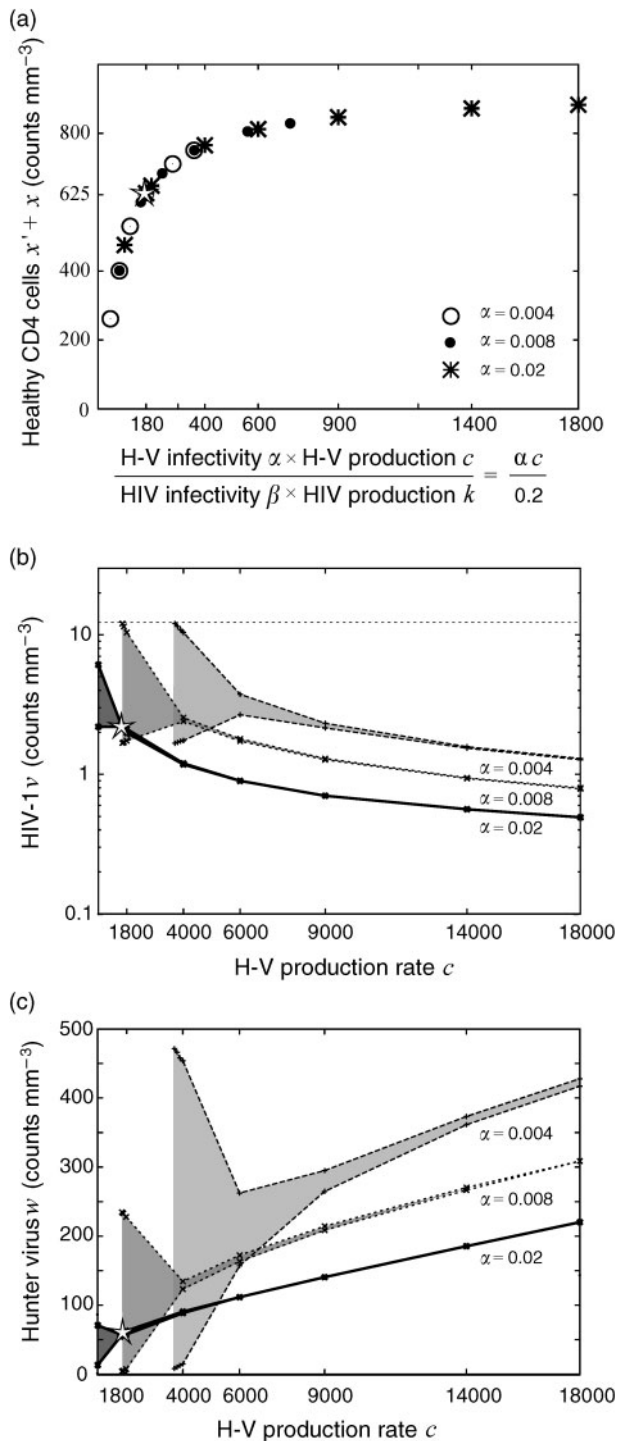


Fig. 4. Therapy effects on number of CD4 cells, HIV and hunter viruses at equilibrium with varying hunter virus vital rates. (a) Efficiency in recovering CD4 cells in terms of an amplification ratio relating therapeutic and HIV infection parameters. This ratio relates the product of infectivity and production for hunter viruses to the corresponding product for HIV ($\alpha c/\beta k$). (b) HIV load after therapy. Shaded areas describe maximum amplitude oscillations. Stars correspond to HIV load after therapy depicted in Fig. 2(b). Horizontal line indicates HIV load at equilibrium in absence of hunter virus. (c) Cost of therapy in terms of population size of hunter virus. Default values are indicated with stars.

hunter viruses display human proteins that may lead to very low removal rate).

Alternative strategies using viruses for controlling HIV replication consider the defective interfering viruses (DIVs) and conditional replicating anti-HIV gene therapy viruses (Morris *et al.*, 2004; Westerhout *et al.*, 2006). DIVs and gene therapy viruses to treat viral infections have been studied theoretically, and particularly interesting are the analyses by Nelson & Perelson (1995) targeting HIV-infected cells and Weinberger *et al.* (2003) for uninfected cells using ‘crHIV’ viruses (discussed in Supplementary Material). These studies relied on different assumptions (i.e. mechanisms by which HIV causes cell death, target cells and dynamics and parameter values) and therefore, only rough comparisons are possible. These studies suggest that replicate-competent modified viruses may have long-term therapeutic potential. The efficiencies of these strategies depended on the underlying dynamics of pathogen and therapeutic infections and thus, on the amplification of the therapeutic infection relative to HIV. In particular, the hunter viruses have advantages over using DIV- and HIV-derived vector viruses, since the hunter virus could be selected from highly prolific viruses without the restrictions of using a related virus (i.e. vesicular stomatitis virus may release 10^5 virions per cell; Rose & Whitt, 2001). Moreover, a hunter virus may be advantageous over crHIV, since it produces numerous crHIV-infected cells ($\sim 3/4$ cells, Fig. 2 in Weinberger *et al.*, 2003) that may generate a CTL response. The hunter virus approach has the additional advantage of the relevant type of hunter virus may occur in the cell cytoplasm, eliminating the issue of insertion into the nucleus.

A matter of concern of this hunter virus therapy is the permanent population of virions thus created. Hunter viruses are expected to replicate only in HIV-infected cells, and because their envelopes carry human proteins, they are expected to generate a minimal immune response. Notice that the alternative strategies discussed also involved significant populations of therapeutic agents. Nonetheless, inserted or circulating viruses are not necessarily an issue of concern. For instance, many species of primates carry high viral load of SIV without apparently affecting individual survival (Holzammer *et al.*, 2001). Moreover, a conditionally replicating anti-HIV-1 gene therapy virus

deficiency. When compared with other therapies, the hunter viruses recovered 60% cell healthy level with a 155 ratio for the production by infection rate of the hunter virus in relation to the same product for HIV ($\alpha c/\beta k$, Fig. 4a). However, this ratio decreased with lower hunter virus removal rates with respect to HIV (i.e. the ratio fell one order of magnitude when hunter virus removal rates are one order of magnitude lower than in HIV. The envelopes of the

(HIV-derived) has been clinically evaluated and declared safe regarding mutagenesis and recombination (Levine *et al.*, 2006). Viruses offer major new opportunities to treat diseases, and the development of efficacious therapies requires a full understanding of dynamics and efficiencies, as well as safety.

This kind of modelling analysis is an essential step in the development of biologically dynamic therapy whenever the constraints on experimentation are severe, as for HIV. This analysis presents a number of predictions for experimental examination. The key role of apoptosis and resting cell proliferation in the gradual loss of CD4 in the simulations argues strongly for empirical documentation of these features (i.e. quantification with sufficient precision and throughout infection). Complementary SIV studies *in vitro* and *in vivo* will allow more extensive experimental manipulation. For example, the SIV-system can be used to test whether the hunter viruses would replace the CTL response, as our model predicts. As well, it could assess if the therapeutic effect is largely independent of the time since infection. This system could also test the impact of quantitative features of a hunter virus on disease development, such as R_0^w and infectiveness amplification, while addressing the basic question of whether the hunter virus can sustain high CD4 cell levels *in vivo* and halt disease progression. In particular, the results highlight the importance of raising the infectiveness amplification ratio, and our findings predict that this ratio could be better improved by increasing the infection rate of hunter viruses via lowering their viral load. Future advances in the uses of dynamic biological therapies will surely hinge on our ability to pursue experimentation and empirical observations informed by (and informing) further development of realistic simulation models.

METHODS

The model was formulated in differential equations (Fig. 1) and analysed numerically using MATLAB. Parameters were time independent and are described in Table 1. The rationale used for representing the infections is explained in the Supplementary Material. The model assumed that HIV causes CD4 death through the killing of activated-infected cells (Haase, 1999), and indirectly by inducing a generalized immune activation [i.e. HIV increases activation, cell division and activation-induced cell death (Grossman *et al.*, 2002)], and enhanced apoptosis [i.e. HIV-mediated mechanisms that induce death of uninfected CD4 either activated or resting (Gougeon, 2003)]. The indirect mechanisms were implemented by considering that activation, activated cell expansion, and apoptosis of uninfected resting and activated cells were dependent on HIV load.

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