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Influence of follicular dendritic cells on HIV dynamics

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In patients infected with human immunodeficiency virus type 1 (HIV-1), a large amount of virus is associated with follicular dendritic cells (FDCs) in lymphoid tissue. To assess the influence of FDCs on viral dynamics during antiretroviral therapy, we have developed a mathematical model for treatment of HIV-1 infection that includes FDCs. Here, we use this model to analyse measurements of HIV-1 dynamics in the blood and lymphoid tissue of a representative patient, who was treated with a combination of HIV-1 reverse transcriptase and protease inhibitors. We show that loss of virus from FDCs during therapy can make a much larger contribution to plasma virus than production of virus by infected cells. This result challenges the notion that long-lived infected cells are a significant source of HIV-1 during drug therapy. Due to release of FDC-associated virus, we find that it is necessary to revise upward previous estimates of c, the rate at which free virus is cleared, and δ, the rate at which productively infected cells die. Furthermore, we find that potentially infectious virus, present before treatment, is released from FDCs during therapy and that the persistence of this virus can be affected by whether therapy includes reverse transcriptase inhibitors.

Keywords: human immunodeficiency virus type 1; follicular dendritic cells; antiretroviral therapy

1. INTRODUCTION

Follicular dendritic cells (FDCs) in lymphoid tissue (LT) play an important role in the pathogenesis of human immunodeficiency virus type 1 (HIV-1) (Pantaleo *et al*. 1994). The amount of virus associated with these cells exceeds the amount in blood by an order of magnitude or more (Haase *et al*. 1996). A large pool of FDC-associated virus is present even early in infection before seroconversion (Schacker *et al*. 2000). When one considers the amount of virus in the FDC network and the rapid loss of FDC-associated virus during treatment with HIV-1 reverse transcriptase (RT) and protease inhibitors (Cavert *et al*. 1997; Wong *et al*. 1997; Stellbrink *et al*. 1997; Tenner-Racz *et al*. 1998; Orenstein *et al*. 1999), it is conceivable that FDCs have the potential to influence HIV-1 dynamics during antiretroviral therapy. Moreover, the FDC pool of virus may be able to perpetuate infection, as FDC-associated virus is highly infectious (Heath *et al*. 1995) and may remain infectious for long periods (Burton *et al*. 1997).

Because RT and protease inhibitors have no direct effect on viral clearance, Cavert *et al*. (1997) proposed that free and FDC-associated virus are in dynamic equilibrium before treatment and that loss of virus is due to dissociation, with treatment causing a net loss of FDC-associated virus by reducing the pool of free virus available for binding to FDC. Hlavacek *et al*. (1999) developed a physicochemical model for the binding of HIV-1 to receptors on FDCs and showed that reduction of free virus and reversible multivalent ligand–receptor interactions can plausibly account for the dynamics of FDC-associated viral decay during therapy, including the observed pattern of biphasic decay (Cavert *et al*. 1997). In previous analyses of HIV-1 dynamics, release of virus from reservoirs, such as the FDC network, had been considered but not explicitly modelled (Perelson *et al*. 1996, 1997*a*; Perelson & Nelson 1999). Here, by using the model of Hlavacek *et al*. (1999), we begin to assess the influence of FDCs on treatment of HIV-1 infection.

Below, we present a model for HIV-1 dynamics that includes FDCs. It incorporates the model of Hlavacek *et al*. (1999) and ideas from earlier models for HIV-1 dynamics (McLean *et al*. 1991; Nowak & Bangham 1996; Bonhoeffer *et al*. 1997; Perelson *et al*. 1997*b*; Finzi & Siliciano 1998; De Boer & Perelson 1998; Perelson & Nelson 1999). We then use this model to analyse clinical measurements of cellular and viral dynamics in the blood and LT of a representative patient who was treated with a combination of antiretroviral drugs. Measurements include counts of infected cells in LT (Cavert *et al*. 1997), viral RNA on FDC (Cavert *et al*. 1997) and viral RNA in plasma (Notermans *et al*. 1998*b*). Results are compared with those of an earlier analysis for the same patient (Notermans *et al*. 1998*b*), in which neither LT data nor FDCs were considered. This report complements an analysis of Hlavacek *et al*. (2000), which includes more patients who participated in the study of Notermans *et al*. (1998*a*).

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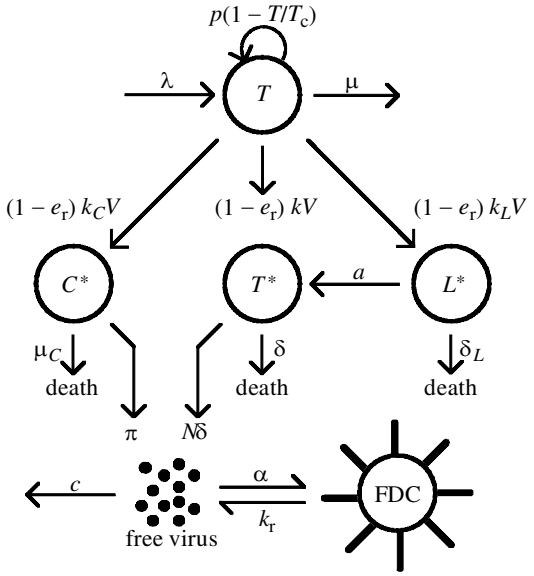

Figure 1. Model for HIV-1 dynamics. The reaction scheme for interaction of HIV-1 with FDC receptors is illustrated elsewhere (Hlavacek *et al*. 1999).

2. MODEL

The model is illustrated in figure 1. We consider four types of cells, the vast majority of which we take to be $CD4^+$ T cells in LT (Haase 1999): uninfected cells (T), short-lived productively infected cells (T^*), long-lived chronically infected cells (C^*) and latently infected cells carrying viral genomes that are replication competent (L^*). As in earlier work (Perelson & Nelson 1999), we characterize cellular dynamics with the following equations:

$$\mathrm{d}T/\mathrm{d}t = \lambda + pT(1 - T/T_c) - \mu T - (1 - e_r)(k + k_C + k_L)VT, \quad (1)$$

$$\mathrm{d}T^*/\mathrm{d}t = (1 - e_r)kVT - \delta T^* + aL^*, \quad (2)$$

$$\mathrm{d}C^*/\mathrm{d}t = (1 - e_r)k_C VT - \mu_C C^*, \quad (3)$$

$$\mathrm{d}L^*/\mathrm{d}t = (1 - e_r)k_L VT - \delta_L L^* - aL^*, \quad (4)$$

where T, T^*, C^* and L^* represent cell numbers, and V represents the number of free, potentially infectious virions. The rates at which uninfected, productively infected, chronically infected, and latently infected cells die are characterized by μ, δ, μ_C, and δ_L, respectively. Uninfected cells are generated at constant rate λ and proliferate according to a logistic growth law (Sachsenberg *et al*. 1998), in which p is the rate constant and T_c is the carrying capacity. Only T appears in the growth law, as we assume infected cells are a small fraction of the total population. The rates at which uninfected cells enter productive, chronic and latent infection are characterized by k, k_C, and k_L, respectively. Latently infected cells are activated and enter the productive state of infection with rate constant a. The efficacy of treatment with RT inhibitors is represented by e_r, with $e_r = 0$ before treatment and $e_r = 1$ during treatment with RT inhibitors that are 100% effective. If $e_r = 1$ during treatment, analytical equations can be found for T, T^*, C^* and L^* as a function of treatment time t; these equations are given in Appendix A.

We consider two types of free virus: potentially infectious virus (V) and virus modified by therapy with protease inhibitors ($\hat{V}$). Therapeutic inhibition of HIV-1 protease prevents formation of functional *gag* and *pol* gene products, so therapy-modified virus is non-infectious. Incorporating the recently developed model for binding of HIV-1 to FDC (Hlavacek *et al*. 1999) into the framework of earlier models for HIV-1 dynamics (Perelson & Nelson 1999), we obtain the following equations for the dynamics of free and FDC-associated virus:

$$\mathrm{d}V/\mathrm{d}t = (1 - e_p)(N\delta T^* + \pi C^*) - cV - (\alpha RV - k_r B_1), \quad (5)$$

$$\mathrm{d}\hat{V}/\mathrm{d}t = e_p(N\delta T^* + \pi C^*) - c\hat{V} - (\alpha R\hat{V} - k_r \hat{B}_1), \quad (6)$$

$$\begin{aligned}
\mathrm{d}B_1/\mathrm{d}t &= \alpha RV - k_r B_1 - (n-1)k_x RB_1 + 2k_{-x}B_2),\\
\mathrm{d}B_i/\mathrm{d}t &= (n - i + 1)k_x RB_{i-1} - ik_{-x}B_i\\
&\quad - (n - i)k_x RB_i + (i + 1)k_{-x}B_{i+1} \quad i = 2,\ldots,n-1,\\
\mathrm{d}B_n/\mathrm{d}t &= k_x RB_{n-1} - nk_{-x}B_n,
\end{aligned} \quad (7)$$

$$\begin{aligned}
\mathrm{d}\hat{B}_1/\mathrm{d}t &= \alpha R\hat{V} - k_r \hat{B}_1 - (n-1)k_x R\hat{B}_1 + 2k_{-x}\hat{B}_2,\\
\mathrm{d}\hat{B}_i/\mathrm{d}t &= (n - i + 1)k_x R\hat{B}_{i-1} - ik_{-x}\hat{B}_i\\
&\quad - (n - i)k_x R\hat{B}_i + (i + 1)k_{-x}\hat{B}_{i+1} \quad i = 2,\ldots,n-1,\\
\mathrm{d}\hat{B}_n/\mathrm{d}t &= k_x R\hat{B}_{n-1} - nk_{-x}\hat{B}_n,
\end{aligned} \quad (8)$$

$$R_T = R + \sum_{i=1}^{n} i(B_i + \hat{B}_i). \quad (9)$$

In these equations, V and $\hat{V}$ represent virion numbers, R represents the number of free receptors on FDCs, and B_i and $\hat{B}_i$ represent the numbers of potentially infectious and non-infectious virions on FDC that are bound to i receptors (a virion can bind up to n receptors). Equation (9) is a conservation law, in which R_T represents the total number of receptors in the FDC network. We assume that R_T is constant, because antigens, including HIV-1, are retained on the surface of FDCs without significant internalization (Mandel *et al*. 1980; Joling *et al*. 1993; Schmitz *et al*. 1994), which suggests that bound receptors on FDCs are not targeted for internalization and that receptor trafficking is limited. Parameters in these equations include N, the number of virions released on death of a productively infected cell, π, the rate constant for production of virions by long-lived chronically infected cells, c, the rate constant for clearance of free virus and e_p, the efficacy of protease inhibitors. Before treatment, $e_p = 0$; during treatment with protease inhibitors that are 100% effective, $e_p = 1$. The remaining parameters, α, k_r, k_x, and k_{-x}, are rate constants that characterize interactions between FDC receptors and virions, which are taken to have n binding sites: α characterizes initial attachment of virions to FDCs, k_r characterizes release of singly bound virions from FDCs, and k_x and k_{-x} characterize binding reactions on the cell surface that involve the addition or removal of a receptor (Hlavacek *et al*. 1999).

Table 1. *Parameter estimates and parameter sensitivity*

(The values of δ, c and K_xR_T are determined in a nonlinear least-squares fitting procedure. The values of $\mathcal{N}$, R_T and kT_0 are derived from relationships that hold, according to the model, in the pre-treatment steady state. In the nominal case, the following (fixed) values are assigned to parameters before starting the fitting procedure: $V_0 = 4.1 \times 10^8$, $F_0 = 4.6 \times 10^{10}$, $T_0^* = 1.1 \times 10^8$, $a = \pi = 0$, $e_r = e_p = 1$, $n = 20$, $\alpha = 1.5 \times 10^{-10}\,\mathrm{day}^{-1}$ and $k_{-x} = k_r = 8600\,\mathrm{day}^{-1}$. In cases (1)–(6), these values are also specified with the following exceptions: (1) $\alpha = 1.5 \times 10^{-11}\,\mathrm{day}^{-1}$, (2) $\alpha = 1.5 \times 10^{-9}\,\mathrm{day}^{-1}$, (3) $k_{-x} = k_r = 860\,\mathrm{day}^{-1}$, (4) $k_{-x} = k_r = 86\,000\,\mathrm{day}^{-1}$, (5) $n = 10$, and (6) $n = 40$. For each case, the quality of the fit is the same as that illustrated in figure 2.)

case	$\delta\,(\mathrm{day}^{-1})$	$c\,(\mathrm{day}^{-1})$	$\mathcal{N}$	K_xR_T	$R_T/10^{12}$	$kT_0\,(\mathrm{day}^{-1})$
nominal	0.78	39	200	0.97	1.7	0.20
1	0.55	49	360	1.1	2.7	0.14
2	0.80	37	180	0.77	1.2	0.20
3	0.73	40	220	0.76	1.3	0.18
4	0.75	39	210	1.2	2.2	0.19
5	0.69	42	240	3.2	1.2	0.17
6	0.78	38	190	0.39	2.1	0.20

The limitations of this model deserve comment. Equations (7) and (8) are derived by treating virions as multivalent ligands, with (mean) effective valence n, that interact with monovalent receptors (Perelson 1981, 1984). As discussed elsewhere (Hlavacek *et al*. 1999), the receptors are considered to be type 2 complement receptors (CR2), and the binding sites on virions that interact with these receptors are considered to be terminal fragments of complement component C3. Equations of this type have been used previously to model viral attachment to cell surfaces (Wickham *et al*. 1990), but equations (7) and (8) should be understood to idealize the complicated chemistry that governs binding of HIV-1 to FDCs, which involves random deposition of C3 on viral surfaces (Stoiber *et al*. 1997), resulting in a distribution of valences, multiple receptor populations on FDCs (Reynes *et al*. 1985) that interact differentially with C3 fragments (Ross & Medof 1985) and enzymatic modification of C3 (Liszewski *et al*. 1996). The simple model that we consider here is appropriate if creation of CR2 binding sites on virions is fast. We consider this a reasonable first approximation, because factor I, which cleaves C3 to generate terminal C3 fragments (Liszewski *et al*. 1996), is closely associated with FDCs (Yamakawa & Imai 1992). Also, complement receptor type 1 (CR1), a cofactor for factor I (Liszewski *et al*. 1996), is abundant on FDCs (Reynes *et al*. 1985).

Besides these limitations, we also emphasize that our model only considers the effects of ligand–receptor interactions on the dynamics of FDC-associated virus. The model does not include other processes that might influence these dynamics, such as turnover of FDCs, slow growth of the FDC network during therapy (Zhang *et al*. 1999*b*), shedding of iccosomes that bear HIV-1 (Szakal *et al*. 1988) and structural breakdown of virions.

3. PARAMETER ESTIMATES

We estimate parameters for a representative patient who received triple therapy with a protease inhibitor (ritonavir) and two RT inhibitors (lamivudine and zidovudine) in the study of Notermans *et al*. (1998*a*). This patient is identified as patient 10 and 20 485 in the substudies of Cavert *et al*. (1997) and Notermans *et al*. (1998*b*), respectively. Estimates are summarized in table 1.

The densities of HIV-1 RNA in plasma (Notermans *et al*. 1998*b*), FDC-associated HIV-1 RNA in LT and infected mononuclear cells in LT (Cavert *et al*. 1997) were monitored during treatment. We convert these densities to total body numbers on the basis of 700 g of LT (Haase *et al*. 1996; Cavert *et al*. 1997; Zhang *et al*. 1998), 15 litres of extracellular fluid (Niyongabo *et al*. 1999; Kim *et al*. 1999) and two copies of HIV-1 RNA per virion. Thus, we estimate $V_0 = 4.1 \times 10^8$, $F_0 = 4.6 \times 10^{10}$ and $T_0^* = 1.1 \times 10^8$, the total body numbers of free virions, FDC-associated virions and productively infected cells at the start of therapy, from baseline measurements (Cavert *et al*. 1997; Notermans *et al*. 1998*b*). To estimate T_0^*, we assume that the baseline density of productively infected cells in LT reflects mainly the density of short-lived cells.

Several simplifying assumptions aid the analysis. We assume that drugs are 100% effective, i.e., $e_r = e_p = 1$ during treatment. Under this assumption, we need not consider infection, which is characterized by the rate constants k, k_C and k_L, and we can compare our results with those of an earlier analysis (Notermans *et al*. 1998*b*), in which the same assumption was made. We assume that parameter values are such that $L^*(t) \approx 0$ throughout the treatment period of interest. Measurements of latently infected cells with replication competent viral genomes indicate that this population is extremely small (Chun *et al*. 1997). We examine the case $\pi = 0$; setting $\pi = 0$ prevents long-lived cells from contributing to viral dynamics. This allows us to demonstrate that these cells, though they may exist, are not required to explain the decay of HIV-1 during therapy.

Hlavacek *et al*. (1999) determined that the following values are reasonable for parameters that characterize HIV-1 binding to FDC: $n = 20$, $\alpha = 1.5 \times 10^{-10}\ \mathrm{day}^{-1}$ and $k_{-x} = k_r = 8600\ \mathrm{day}^{-1}$. We use these estimates here, but in a sensitivity analysis, we also determine how variation of these parameter values affects our qualitative results.

Estimates of some parameters are obtained from steady-state relationships. The assumption of a pre-treatment steady state is supported by a stable plasma viral load before treatment (Notermans *et al*. 1998*b*). To find $\mathcal{N}$, we use the steady-state forms of equations (5) and (7), which yield the following equation

$$\mathcal{N} = cV_0/(\delta T_0^*). \tag{10}$$

To find R_T and to characterize the pre-treatment state of FDC receptors and virions on FDCs, we take the following approach. We determine the pre-treatment fraction of receptors that are free, R/R_T, by numerically solving

$$1 = (R/R_T)[1 + (\alpha V_0/k_r)(1 + (R/R_T)(K_xR_T))^{n-1}], \tag{11}$$

which is derived from equation (9) and the steady-state forms of equations (5) and (7). The equilibrium cross-linking constant K_x is defined as k_x/k_{-x}. We calculate the

pre-treatment steady-state value of B_i/R_T for $i = 1, \ldots, n$ by using

$$B_i/R_T = \frac{1}{n}\binom{n}{i}(\alpha V_0/k_r)(K_x R_T)^{i-1}(R/R_T)^i, \quad (12)$$

which is also derived from equation (9) and the steady-state forms of equations (5) and (7). We find R_T by using

$$R_T = F_0 \Big/ \left(\sum_{i=1}^{n} B_i/R_T\right), \quad (13)$$

which is derived from the identity $F_0 = \sum_{i=1}^{n} B_i$. Equations (11)–(13) can be combined to determine the baseline values of R and each B_i. To find the value of kT_0, we use

$$kT_0 = \delta T_0^*/V_0, \quad (14)$$

which is derived from the steady-state form of equation (2) and our assumption that $L^*(t) \approx 0$. The value of kT_0 is used only in a hypothetical comparision of treatment strategies, in which we assume $T(t) \approx T_0$ to simplify the analysis.

We use a nonlinear least-squares fitting procedure to determine the values of δ, c, and $K_x R_T$ (recall that $K_x = k_x/k_{-x}$). With the values of these parameters free to vary, and other parameter values fixed or derived from steady-state relationships, the model is fit simultaneously to the measured time-courses of decay for plasma virus (Notermans *et al.* 1998*b*) and FDC-associated virus (Cavert *et al.* 1997). In this procedure, we numerically integrate the model equations.

4. RESULTS AND DISCUSSION

(a) *Release of virus from FDCs and infected cells*

HIV-1 is released from FDCs during therapy. To estimate the magnitude of this release, we compare the measured loss of virus from FDC in a triple-therapy patient (Cavert *et al.* 1997) with the predicted release of virus from infected cells in this patient, which can be determined from the analysis of Notermans *et al.* (1998*b*).

Notermans *et al.* (1998*b*) used a conventional model for HIV-1 dynamics, without FDCs, to analyse plasma viral decay in patients who received combination therapy. In our nomenclature, this model, a special case of the one presented here, can be rewritten as follows:

$$T^*(t) = T_0^* e^{-\delta t}, \quad (15)$$

$$C^*(t) = C_0^* e^{-\mu_C t}, \quad (16)$$

$$V(t) + \hat{V}(t) = V_0[A e^{-\delta t} + B e^{-\mu_C t} + (1 - A - B)e^{-ct}], \quad (17)$$

where

$$A = \frac{\mathcal{N}\delta T_0^*}{(c - \delta)V_0}, \quad (18)$$

$$B = \frac{\pi C_0^*}{(c - \mu_C)V_0}. \quad (19)$$

The parameter C_0^* is the number of long-lived infected cells at the start of treatment. For the patient of interest, the following parameter values were estimated by Notermans *et al.* (1998*b*): $c = 3\,\text{day}^{-1}$, $\delta = 0.36\,\text{day}^{-1}$, $\mu_C = 0.031\,\text{day}^{-1}$, $A = 1.1$, and $B = 0.051$.

In the model of Notermans *et al.* (1998*b*) and here also, as can be seen by inspecting equations (5) and (6), the rate of virus production by short- and long-lived infected cells is given by $\mathcal{N}\delta T^*$ and πC^*, respectively. Thus, the amount of virus produced by infected cells during therapy at treatment time t can be found from

$$\nu_s(t) = \int_0^t \mathcal{N}\delta T^*(\tau)\mathrm{d}\tau = \mathcal{N}T_0^*(1 - e^{-\delta t}), \quad (20)$$

and

$$\nu_l(t) = \int_0^t \pi C^*(\tau)\mathrm{d}\tau = \left(\frac{\pi}{\mu_C}\right)C_0^*(1 - e^{-\mu_C t}), \quad (21)$$

where $\nu_s(t)$ is the amount produced by short-lived infected cells and $\nu_l(t)$ is the amount produced by long-lived infected cells. Equations (20) and (21) determine the following limits on viral production by infected cells: $\lim_{t\to\infty} \nu_s(t) = \mathcal{N}T_0^*$ and $\lim_{t\to\infty} \nu_l(t) = (\pi/\mu_C)C_0^*$.

Based on these results, the parameter estimates of Notermans *et al.* (1998*b*), and estimates of V_0, T_0^*, and C_0^*, which can be obtained from baseline measurements, we can place an upper bound on the amount of virus produced by infected cells during therapy and we can compare this number with the measured release of virus from FDCs (Cavert *et al.* 1997). We have estimated the total numbers of free virions, V_0, and short-lived infected cells, T_0^*: $V_0 = 4.1 \times 10^8$ and $T_0^* = 1.1 \times 10^8$. To estimate the number of long-lived infected cells C_0^*, we use equation (16), the estimated value of μ_C (Notermans *et al.* 1998*b*), and the measured density of infected cells in LT at $t = 21$ days (Cavert *et al.* 1997), a time at which short-lived infected cells can be expected to have largely disappeared. Thus, $C_0^* = 5.4 \times 10^6$. We can now determine $\mathcal{N}$ and π by using the estimated values of A and B (Notermans *et al.* 1998*b*) and equations (18) and (19): $\mathcal{N} = 31$ and $\pi = 12\ \text{day}^{-1}$. It follows that ν_s is less than 3.3×10^9 virions and that ν_l is less than 2.0×10^9 virions. Furthermore, total production of virus by infected cells is no more than 5.3×10^9 virions. In comparison, our estimate for the baseline number of virions on FDCs, F_0, is 4.6×10^{10} virions, an order of magnitude larger; Cavert *et al.* (1997) observed that approximately 90% of this virus is lost from FDCs after three weeks of treatment. It appears that release of HIV-1 from FDC is a significant process, one that must be considered in models of HIV-1 dynamics. Earlier estimates of parameters that characterize HIV-1 dynamics (e.g. δ and c) should probably be reconsidered, as many of these estimates have been determined on the basis of models without FDCs.

(b) *Estimates of δ and c when FDCs are considered*

Using our model, which includes FDCs, we fit the measured decay of plasma and FDC-associated virus. Theoretical decay curves and clinical measurements are shown in figure 2; best-fitting parameter values are given in table 1. We find that our best estimates of δ ($0.78\,\text{day}^{-1}$) and c ($39\,\text{day}^{-1}$) are higher than previous estimates. Recall that Notermans *et al.* (1998*b*) estimated

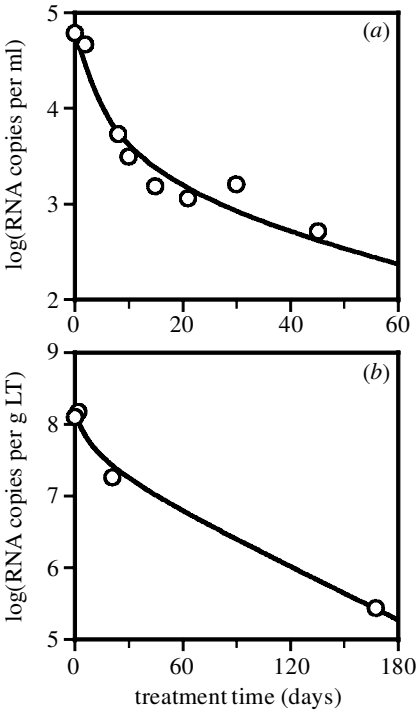

Figure 2. Decay of free and FDC-associated virus in a triple-therapy patient. (*a*) Measured HIV-1 RNA ml^{-1} of plasma (points) and the best-fit theoretical curve, which is found by calculating $V(t) + \hat{V}(t)$. (*b*) Measured FDC-associated HIV-1 RNA g^{-1} of LT (points) and the best-fit theoretical curve, which is found by calculating $\sum_{i=1}^{n} B_i(t) + \hat{B}_i(t)$. Calculations are based on equations (1)–(9) with the parameter values for the 'nominal' case in table 1 (e.g. $\delta = 0.78\,day^{-1}$, $c = 39\,day^{-1}$ and $K_x R_T = 0.97$).

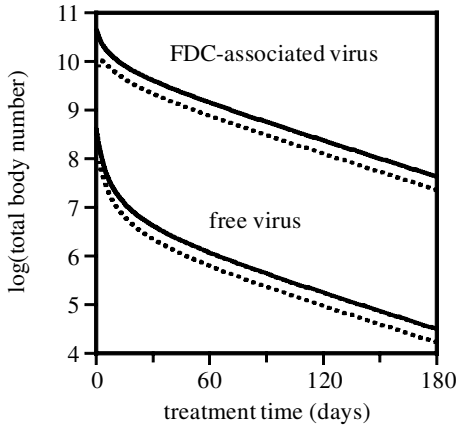

Figure 3. Persistence of potentially infectious virus during therapy and parallel decay of free and FDC-associated virus. The theoretical decay curves of figure 2 are replotted to show total body numbers of potentially infectious (solid lines) and therapy-modified non-infectious (dotted lines) virions that are associated with FDCs in LT (top) and free in extracellular fluid (bottom).

$\delta = 0.36\,day^{-1}$ and $c = 3\,day^{-1}$, with the latter value based on the analysis of Perelson *et al.* (1996). Higher estimates are obtained because cell death and viral clearance are offset by release of virus from FDCs. Recent direct measurements of viral clearance (Zhang *et al.* 1999*a*; Ramratnam *et al.* 1999; Igarashi *et al.* 1999) are consistent with higher estimates of c.

According to the model considered by Notermans *et al.* (1998*b*), as can be seen from equation (17), the first phase of viral decay should match the death rate of productively infected cells (if $c \gg \delta$), and the second phase of viral decay should match the death rate of long-lived infected cells (if $\delta \gg \mu_C$). When we consider FDCs, we find, in contrast, that both phases of viral decay may be significantly influenced by interactions of HIV-1 with FDCs. The theoretical curves shown in figure 2 are based on $\pi = 0$, no production of virus by long-lived infected cells. Estimates of δ and c are not significantly different nor are fits significantly improved if, instead of setting $\pi = 0$, we allow the value of π to vary during the fitting procedure. This suggests that long-lived infected cells may play a smaller role than previously expected in determining viral kinetics during therapy. In our analysis, the rate of second-phase decay matches the net rate at which virus is lost from FDCs, as the FDC reservoir is the only source of virus when $\pi = 0$ and t is much larger than the half-life of short-lived infected cells.

(c) *Parameter sensitivity*

Estimates of c and δ depend on estimates of other parameters, including the rate constants α, k_r, and k_{-x} and the valence n. However, when we vary the values of these parameters, we consistently estimate values for c and δ that are higher than previous estimates (table 1). For example, the nominal value of α can be increased or decreased by an order of magnitude without changing the qualitative result that the values of δ and c are higher than those previously estimated by Notermans *et al.* (1998*b*).

(d) *Persistence of potentially infectious virus*

Another important result of including FDCs in our analysis is the observation that potentially infectious virus can persist during therapy (figure 3). This result is obtained even though we assume drugs are 100% effective. The source of the potentially infectious virus is FDCs. Virus present before therapy, a large amount of which is associated with FDCs, is unaffected by RT and protease inhibitors. This potentially infectious virus is released from FDCs and is present as a significant fraction of the viral load throughout treatment, even during the second phase of viral decay. This is opposite of what would be expected from earlier models of HIV-1 dynamics, which suggest that second-phase virus is largely non-infectious if drugs are 100% effective. Although some experiments suggest that HIV-1 retained on FDCs remains infectious for long periods (Burton *et al.* 1997), additional experiments are required to determine the properties of FDC-associated virus. If this virus is proven to be infectious for long periods, retention of HIV-1 on FDCs may affect the long-term outcome of therapy with currently available drugs, and elimination of FDC-associated virus may be as important as ridding the body of latently infected cells (Chun & Fauci 1999). In addition to the persistence of potentially infectious virus, figure 3 also illustrates the influence of FDCs on

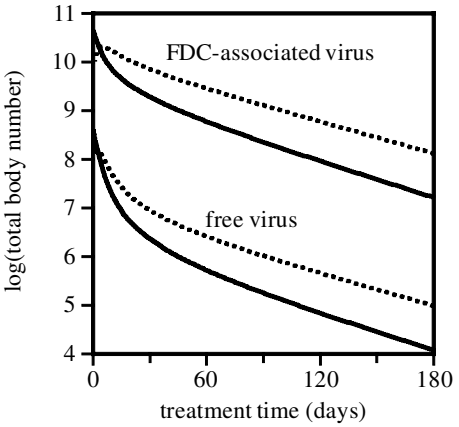

Figure 4. Effect of drug regimen on persistence of potentially infectious virus during therapy. The curves of figure 3 are recalculated for a 100% effective drug regimen that includes protease inhibitors but not RT inhibitors. The solid lines represent total body numbers of potentially infectious virions, whereas the dotted lines represent total body numbers of therapy-modified non-infectious virions. Curves for FDC-associated virus are shown at the top, and curves for free virus are shown at the bottom. Calculations are based on equations (2)–(9) and the approximation $T(t) \approx T_0$. The parameter values are the same as for the 'nominal' case in table 1 (e.g. $kT_0 = 0.20\ \text{day}^{-1}$), except $e_r = 0$.

viral decay: decay of free virus can be seen to parallel the loss of virus from FDCs.

(e) *Comparison of drug regimens*

Exchange of free and FDC-associated virus is rapid according to our model. This suggests that therapy with only protease inhibitors, such as ritonavir–saquinavir therapy (Cameron *et al*. 1999), might have an advantage over drug regimens containing both RT and protease inhibitors in the following respect. Virus produced during protease inhibitor therapy, which will largely lack *gag* and *pol* gene products, might compete with potentially infectious virus, present before therapy, for receptors on FDCs. Thus, protease inhibitor therapy might be more effective than RT and protease inhibitor combinations at reducing the infectious titre of virus. A test of this idea is illustrated in figure 4. The decay curves shown in this figure are based on the parameter values used to calculate those in figure 3, but now we have set $e_r = 0$ (no RT inhibitors). As can be seen by comparing figures 3 and 4, 100% effective therapy with protease inhibitors reduces the level of potentially infectious virus present on FDCs and free in extracellular fluid throughout treatment, though the total viral load (potentially infectious virus plus therapy-modified non-infectious virus) is higher than with a RT and protease inhibitor combination. Despite this relative improvement, a significant fraction of virus remains potentially infectious. The advantage of protease inhibitor therapy over RT and protease inhibitor combinations that we have shown here is hypothetical and the level of therapy-modified virus has yet to be measured in patients, but our results suggest that such measurements might be seriously considered.

5. CONCLUSION

When we include FDCs in a model for treatment of HIV-1 infection (figure 1), we find that parameters characterizing HIV-1 dynamics, δ and c, must be revised upwards (table 1). This is because decay of infected cells, characterized by δ, and clearance of free virus, characterized by c, are buffered by release of virus from FDCs. Release of virus from FDCs also suggests an alternative explanation for biphasic decay of virus during therapy, as we have been able to fit the viral decay in a triple-therapy patient without including viral production by long-lived infected cells (figure 2). The importance of long-lived infected cells in viral dynamics is also challenged by our demonstration that release of virus from FDCs may be an order of magnitude larger than the amount of virus expected to be produced by infected cells during therapy, which we have calculated using equations (20) and (21). These results, which suggest that FDCs play an important role in HIV-1 dynamics, are obtained largely for two reasons: the large amount of virus on FDCs in untreated patients relative to the amount in cells (Cavert *et al*. 1997), most of which have a short half-life, and the potential for biphasic release of virus from FDCs during treatment if binding of virus to FDC receptors is reversible and multivalent (Hlavacek *et al*. 1999).

We also find that FDCs may be a source of potentially infectious virus during therapy (figure 3), even if drugs are 100% effective. Persistence of virus due to antigen trapping by FDCs suggests that, if virus on FDCs remains infectious (Burton *et al*. 1997), therapies should be developed to interfere with the interaction of HIV-1 and FDCs. Such therapies, because second-phase decay of virus parallels loss of virus from FDCs (figure 3), may also have the potential to dramatically accelerate viral decay; this potential kinetic effect alone may be sufficient to motivate the development of therapeutics to block HIV-1 binding to FDCs. Experience suggests that a monovalent ligand that binds to receptors on FDCs may be an effective means for displacing FDC-associated HIV-1 (Goldstein & Wofsy 1996). The design of such a drug may be feasible, because CR2 is believed to be the principal receptor on FDCs that interacts with HIV-1 (Hlavacek *et al*. 1999). Although many cells express CR2, a distinct isoform of this receptor, the long form, is expressed on FDCs (Liu *et al*. 1997).

Finally, analysis of the model suggests that treatment with a combination of RT and protease inhibitors may be less effective than treatment with only protease inhibitors, in that the latter strategy allows non-infectious virus produced during treatment to displace potentially infectious virus on FDCs (figure 4). Of course, combinations of RT and protease inhibitors may still be more desirable than therapy with protease inhibitors alone for other reasons, such as suppression of drug resistance and the side-effects of potent protease inhibitor regimens.

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APPENDIX A

Under the assumption of 100% effective therapy (i.e. $e_r = 1$ for $t \geqslant 0$), analytical expressions can be derived from equations (1)–(4) for $T(t)$, $C^*(t)$, $L^*(t)$ and $T^*(t)$. From equation (1), if $p = 0$,

$$T(t) = T_0 e^{-\mu t} + (\lambda/\mu)(1 - e^{-\mu t}), \tag{A1}$$

where $T_0 = T(0)$. From equation (1), if $\lambda = 0$,

$$T(t) = \frac{(1 - \mu/p)\,T_c}{1 - [1 - (1 - \mu/p)\,T_c/T_0]e^{-(p-\mu)t}}. \tag{A2}$$

From equation (1), if $p \neq 0$ and $\lambda \neq 0$:

$$T(t) = r_2 T_c + \frac{(r_1 - r_2)\,T_c}{1 - [1 - (r_1 - r_2)/(T_0/T_c - r_2)]e^{-p(r_1 - r_2)t}}, \tag{A3}$$

where

$$r_{1,2} = \frac{1}{2}\left(\frac{p - \mu}{p}\right) \pm \frac{1}{2}\left[\left(\frac{p - \mu}{p}\right)^2 + \frac{4\lambda}{pT_c}\right]^{1/2}. \tag{A4}$$

From equation (3),

$$C^*(t) = C_0^* e^{-\mu_C t}, \tag{A5}$$

where $C_0^* = C^*(0)$. From equation (4),

$$L^*(t) = L_0^* e^{-\mu_L t}, \tag{A6}$$

where $\mu_L = a + \delta_L$ and $L_0^* = L^*(0)$. From equation (2) and the analytical expression for $L^*(t)$,

$$T^*(t) = T_0^* e^{-\delta t} + \left(\frac{aL_0^*}{\delta - \mu_L}\right)\left(e^{-\mu_L t} - e^{-\delta t}\right), \tag{A7}$$

provided $\delta \neq \mu_L$, which is likely. Here, $T_0^* = T^*(0)$.

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