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Anti-CD4 therapy for AIDS suggested by mathematical models

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SUMMARY

HIV-1 infection typically involves a long clinical latency stage during which CD4 counts decline slowly. For the later part of the clinical latency stage it was found recently that this is a highly dynamic phase characterized by rapid turnover rates. Clinical latency can therefore be considered as a quasi-equilibrium state in which CD4 and HIV-1 turnover are in almost perfect balance. Here we consider this quasi-equilibrium to be the stable steady state of a simple host–parasite model in which the parasite (HIV-1) level is determined by the availability of infectable hosts (activated $CD4^+$ T cells). Such models adequately account for the clinical data on the evolution of drug resistant mutants appearing after the administration of anti-HIV drugs. The model suggests a novel therapeutic approach for AIDS: reducing the CD4 count slightly will strongly reduce the HIV load. Combining this anti-CD4 treatment with conventional anti-HIV therapy would prevent the outgrowth of drug resistant mutants.

1. INTRODUCTION

Recent clinical data on the decrease of the HIV-1 plasma level, and the increase of the CD4 T cell count, following the treatment with anti-viral drugs have been analysed mathematically to suggest that clinical HIV-1 latency involves high turnover rates of both the virus and the $CD4^+$ T cells (Ho *et al.* 1995; Wei *et al.* 1995; Nowak *et al.* 1995*a*; Perelson *et al.* 1996). Given that the HIV-1 clinical latency stage typically lasts for several years (Fauci 1993; Levy 1993; Weiss 1993), these findings raise the question how virus levels are kept under control for so long (Coffin 1995*a*). One answer to this question is the 'immunological view' stating that the HIV-1 infection is controlled by the immune system. There is good empirical evidence for both a humoral (Fauci 1993; Levy 1993; Weiss 1993) and a cellular (Phillips *et al.* 1991; Nowak *et al.* 1995*b*; Klein *et al.* 1995; Nowak & Bangham 1996; Levy *et al.* 1996) immune response.

An alternative answer is the 'ecological view' stating that the HIV-1 infection is limited by the availability of target cells (McLean *et al.* 1991; McLean & Nowak 1992; Frost & McLean 1994; McLean & Frost 1995; Perelson *et al.* 1993; Essunger & Perelson 1994; Coffin 1995*b*; Phillips 1996). According to this view the immune response may be important, but is assumed to remain fairly constant over the time course of the clinical experiments discussed here. The data supporting the ecological view involve perturbations of the clinical latency equilibrium by therapeutic intervention. Increasing the activation and the numbers of $CD4^+$ T cells by IL2 treatment increases both the viral RNA load and the CD4 cell count (Kovacs *et al.* 1995). Immunization of HIV-1 infected individuals with influenza vaccine has a side effect of increasing $CD4^+$ T cell activation and transiently increases the plasma HIV-1 RNA levels significantly (O'Brien *et al.* 1995; Staprans *et al.* 1995). Conversely, suppressing $CD4^+$ T cell activation by cyclosporin (Andrieu *et al.* 1988; Fauci 1993; Schwarz *et al.* 1993; Weber & Galpin 1995) or prednisolone (Andrieu *et al.* 1995; Corey 1995) can be beneficial by increasing CD4 T cell counts, and, in one instance (Weber & Galpin 1995), decreasing the viral load. Note however, that cyclosporin also has direct anti-viral effects (Billich *et al.* 1995; Steinkasserer *et al.* 1995).

The ecological and immunological views are not mutually exclusive. First, during disease progression the infection could evolve from immunological to ecological control. This is supported by the IL2 treatments where the adverse effects on the viral load are most pronounced in late stage patients (Kovacs *et al.* 1995). Second, even if the control of the virus at the clinical latency equilibrium is dominated by the immune response (Nowak & Bangham 1966), perturbation of this equilibrium by therapeutic interventions can give rises to transients during which the fast expansion of target cells dominates over a more slowly changing immune response. Thus, whenever the immune response adapts sufficiently slow to changes in the virus load, the virus is transiently limited by the available target cells.

The recent clinical trials with anti-viral drugs like protease inhibitors (Ho *et al.* 1995; Wei *et al.* 1995; Nowak *et al.* 1995*a*; Perelson *et al.* 1996) and reverse-transcriptase (RT) inhibitors (Schuurman *et al.* 1995; Wei *et al.* 1995; De Jong *et al.* 1996) tend to be unsuccessful because the virus evolves drug-resistant mutants (Larder *et al.* 1989, 1991). These evolutionary data have adequately been described by mathematical models confirming to the ecological view (McLean *et*

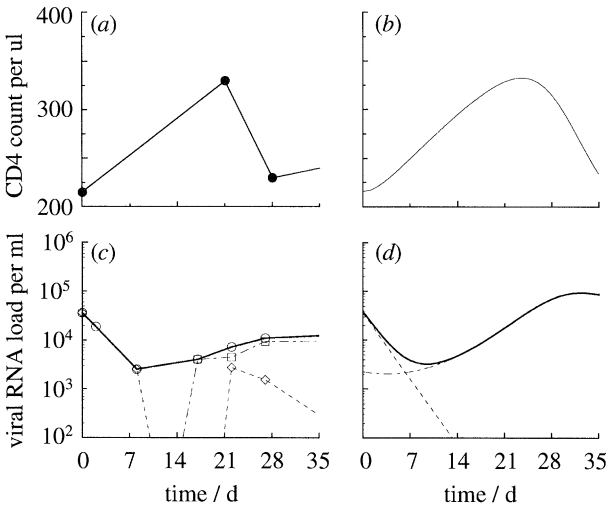

Figure 1. Anti-viral treatment for math-AIDS and for a representative example (i.e. Case number 11) from a group of 20 asymptomatic or mildly symptomatic AIDS patients treated with the RT-inhibitor lamivudine (Schuurman *et al.* 1995). The initial CD4 T cell count and viral RNA load of Case number 11 were 215 cells per μl, and 3.64×10^4 copies per ml, respectively. Assuming this to be an equilibrium we obtain the same initial condition in our model by setting $c = 1136$ and $\gamma = 2.2 \times 10^{-6}$ T cells per scaled virus particle per day. We mimic the lamivudine treatment by administering an RT-inhibitor at day zero, i.e. we set the effective drug concentration $D = 10$. Thus the RT fitness of the wild-type decreases 11-fold (see equation (1*a*)), whereas that of the drug-resistant mutant decreases marginally (i.e. by 2%, see equation (1*b*)). Setting D to higher values hardly changes the math-AIDS results. Figure 1*a*, *b* depict a timeplot of the CD4 T cell count per μl. The data (*a*) and the model (*b*) are in close agreement. Figure 1*c*, *d* depict the total viral RNA load per ml (solid line with circles), and the RNA loads of the wild type (dashed line with diamonds) and the drug-resistant mutant (dash–dotted line with squares). We assume that the drug-resistant mutant is present at low concentration, i.e. $T_r(0) = 0.1$ cell per ml, before the onset of treatment (Najera *et al.* 1995). The model and the data agree closely on the slope with which the total RNA load decreases. In the model and in the data we find that the drug-resistant mutant has completely replaced the wild type after about 2 weeks. In the data we a find a rebound of the wild type after about 3 weeks. For the current parameter values, such a rebound is absent from the model. Decreasing the effective drug concentration and/or the RT fitness e of the mutant we can obtain similar wild-type rebounds in the model (McLean *et al.* 1991; McLean & Frost 1995; De Jong *et al.* 1996; Stilianakis *et al.* 1996). Within the ecological view, the wild type rebound is explained by the recovery of the CD4 count. This provides more 'food' for the virus and thus compensates for the anti-viral treatment. We conclude that our simple host–parasite model provides a reasonable fit for both our own clinical data (i.e. Case number 11), and for a compilation of several independent clinical data sets (Coffin 1995*a*). Parameters: $\sigma = 10$, $d_T = 0.01$, $d_I = 0.5$, $e = 0.99$, $r = 750$ and $\alpha = 0.$, The proportional relation between infected cells and virus stems from a quasi steady state assumption, i.e. from $dV_j/dt = Nd_I T_j - d_V V_j = 0$ for $j \in \{w, r\}$, where N is the number of particles produced per T cell and d_V is the inverse lifetime of the particles. Hence $V_j = cT_j$, where $c = Nd_I/d_V$. Note that the HIV-1 RNA load is $2(V_w + V_r)$.

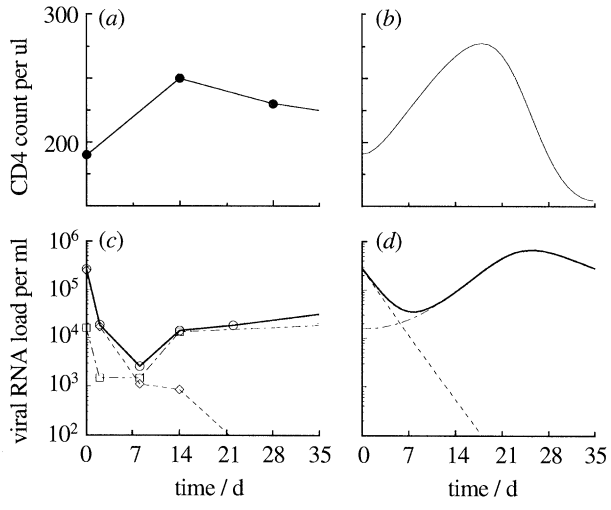

Figure 2. Lamivudine treatment in a second representative example (i.e. Case number 20 (Schuurman *et al.* 1995)) and in our model. The initial CD4 T cell count and viral RNA load of this case were 190 cells per μl, and 2.66×10^5 copies per ml, respectively. We obtain the same initial condition in our model by setting $c = 8046$ and $\gamma = 3.6 \times 10^{-7}$ T cells per scaled virus particle per day. Case number 20 is more typical for the lamivudine data because the total CD4 increase is rather limited (i.e. about 30%). Additionally, the data show that before the onset of treatment 2% of viral quasi species is drug-resistant. We therefore increase the mutant's initial density by setting $T_r = 1$. Thus we obtain an initial viral load of the mutant that is similar to that of the data (cf. panel *c*). All other parameters of the model are kept the same as those in figure 1. The time plots of the CD4 T cell count per μl in the data (*a*) and the model (*b*) agree reasonably. The total CD4 increase in the model is indeed much smaller than that in figure 1 (note difference in scale of the vertical axis). Thus the mild increase of the CD4 counts in the lamivudine data (as compared with the protease data (Ho *et al.* 1995; Wei *et al.* 1995)) can partly be explained by the higher initial frequencies of the drug-resistant mutants (lamivudine-resistant mutants require only one point mutation (Schuurman *et al.* 1995)). The time plots of the viral RNA load per ml of the data (*c*) and the model (*d*) are in qualitative agreement. The total decrease in the viral load in the data is however ten-fold larger than that in the model. Given the simplicity of our model we however consider this fit between model and data to be reasonable.

al. 1991; McLean & Nowak 1992; Frost & McLean 1994; McLean & Frost 1995; De Jong *et al.* 1996; Stilianakis *et al.* 1996). All of these models are conventional host–parasite models for the infection of $CD4^+$ host T cells by the HIV-1 parasite. Recent work (McLean & Frost 1995; De Jong *et al.* 1996; Stilianakis *et al.* 1996) demonstrates that the complex evolutionary timecourse of the various drug-resistant mutants appearing after treatment with zidovudine can be accounted for by such host–parasite models. Like the recent mathematical analysis supporting the notion of a highly dynamic clinical latency phase (Ho *et al.* 1995; Wei *et al.* 1995; Nowak *et al.* 1995*a*; Perelson *et al.* 1996), these host–parasite models assume clinical latency to be a quasi-equilibrium that is perturbed by one of the anti-HIV drugs. Figures 1 and 2 illustrate the close correspondence between an example of such

a host–parasite model and the response of the HIV-1 quasi species to lamivudine treatment (Schuurman *et al.* 1995).

In these host–parasite models HIV-1 production is limited by the availability of target cells, i.e. activated $CD4^+$ T cells, and by the rapid clearance rates which are possibly caused by the immune response. For simplicity we here use the conventional CD4 T cell count as a measure for the target cell density. A natural consequence of our assumption that the target cells are a limiting factor is that, for each particular quasi-equilibrium in the clinical latency phase, the CD4 T cell count represents exactly the critical number of target cells the virus, at that particular time point, requires for its survival (i.e. requires for having a reproduction ratio R larger than one). Thus, one can treat this mathematical form of AIDS (math-AIDS) by deliberately reducing the target cell density, here the CD4 count. This threshold target cell density is determined by the parameters defining the current clinical latency state. Hence the threshold will decrease by disease progression. Thus, clinical examples of patients with normal viral loads, but extremely low CD4 counts (Ho *et al.* 1995; Schuurman *et al.* 1995), need not contradict the ecological view. Even with so few peripheral $CD4^+$ T cells the lymphoid tissue could still have high numbers of activated $CD4^+$ T cells and virus (Embertson *et al.* 1993; Pantaleo *et al.* 1993), the virus quasi species of such cases could have evolved high infection and/or production rates (Tersmette *et al.* 1989), and is likely to have evolved trophisms for other types of target cells (Levy 1993; Weiss 1993).

2. MODEL

In response to an anti-HIV therapy the virus quasi species typically evolves several drug-resistant mutants (Larder *et al.* 1989, 1991; Wei *et al.* 1995; Coffin 1995*b*; Schuurman *et al.* 1995; De Jong *et al.* 1996). In our data on the resistance to the RT-inhibitor lamivudine we find two drug-resistant mutants that are caused by a mutation in codon 184 of the HIV-1 RT gene, which replaces the wild type methionine residue (ATG) with either an isoleucine (ATA) or a valine (i.e. GTG or GTA) (Schuurman *et al.* 1995). As the drug-resistance of both mutants is 500–1000 fold greater than that of the wild type, and as both mutants appear within a 2–3 week period, we will combine them into one population called the drug-resistant mutant strain V_r. The drug-sensitive wild type is denoted by V_w.

Each of the two strains has a specific RT fitness, i.e. f_w and f_r, which combines the infection rate in the absence of the drug with the resistance in the presence of the drug. In the absence of the drug we scale the RT fitness of the wild type to be one, and we call the RT fitness of the mutant e. Because the RT fitness of mutants in the absence of the drug is usually just a few percent less than that of the wild type (Coffin 1995*b*; C. A. B. Boucher unpublished data) we conservatively set this RT fitness to $e = 0.99$. For the RT fitness in the presence of the drug we employ the conventional notion of the inhibitory concentration, i.e. the IC_{50}, which is the drug concentration at which the effect of the drug is half-maximal. Scaling the IC_{50} of the wild type to one we obtain

$$f_w = \frac{1}{1+D}, \quad \text{and} \quad f_r = \frac{e}{1+D/r}, \tag{1}$$

where D is the effective lamivudine concentration, and $r = 750$ is the IC_{50} of the mutant (Schuurman *et al.* 1995).

We write two differential equations for the infected T cells actively producing either V_w or V_r, i.e.

$$\frac{dT_w}{dt} = \gamma T_4 f_w V_w - d_I T_w, \tag{2}$$

$$\frac{dT_r}{dt} = \gamma T_4 f_r V_r - d_I T_r, \tag{3}$$

where the T_4 variable denotes the infectable $CD4^+$ T cells, γ is an infection rate, and d_I is the turnover rate of productively infected CD4 cells. This turnover rate has recently been estimated to be close to $d_I = 0.5$ per day (Ho *et al.* 1995; Wei *et al.* 1995; Nowak *et al.* 1995*a*; Perelson *et al.* 1996). As the viral particles have a six-fold higher turnover rate than the CD4 cells (Perelson *et al.* 1996) it is reasonable to assume that the number of virus particles is proportional to the corresponding number of infected cells, i.e. we assume $V_w = cT_w$ and $V_r = cT_r$ (see legend to figure 1).

The reproduction ratio is classically defined as the number of target cells that become productively infected over the entire lifespan of a productively infected cell. From equation (2) we derive that, before treatment (i.e. when $f_w = 1$), the reproduction ratio of the wild type virus is $R = c\gamma T_4/d_I$. Similarly, we derive from (2) that its equilibrium is at $T_4 = d_I/(c\gamma)$. Thus we see (i) that the virus determines the equilibrium CD4 count, and (ii) that at equilibrium $R = 1$, i.e. every virus particle is on average replaced by exactly one other particle. We can therefore 'starve' the HIV-1 infection by reducing the target cells to a level below that of the clinical latency equilibrium of $T_4 = d_I/(c\gamma)$. This is the main result of this paper.

We now proceed by adding an equation for the uninfected T cells to illustrate that these models are in agreement with data, and to explore an immuno-suppressive anti-CD4 treatment. The T cell repertoire is maintained by self-renewal and by a source of virgin T cells from the thymus. Most previous models for studying drug-resistance rely on the thymus for maintaining the T cell repertoire (McLean *et al.* 1991; McLean & Nowak 1992; Frost & McLean 1994; McLean & Frost 1995). The process of T cell self-renewal on the other hand can be modelled by simple logistic growth or by distinguishing resting and cycling T cells (Perelson *et al.* 1993; Essunger & Perelson 1994; Stilianakis *et al.* 1996). For the results presented here it makes no difference what model we choose. Thus we opt for mathematical convenience and write that the T cell repertoire is maintained by the thymus, i.e.

$$\frac{dT_4}{dt} = \sigma - d_T T_4 - \gamma T_4 (f_w V_w + f_r V_r), \tag{4}$$

where σ is the source of newborn cells from the thymus,

d_T is the death rate of uninfected CD4 cells, and the γ term describes the infection of T_4 cells by either wild type or by resistant virus. As the model combines resting and cycling T cells the lifetime of the T cells is set to an intermediate length of 100 d (cf. McLean & Mitchie 1995), i.e. we set $d_T = 0.01$ per day. Thus we obtain a normal CD4 count of a 1000 cells per µl when we set $\sigma = 10$ cells per µl per day (which is in full agreement with recent estimates (Ho *et al.* 1995; Wei *et al.* 1995)).

3. RESULTS

We first compare the behaviour of our model with our data on the response of the plasma HIV-1 RNA load and the CD4 T cell count to treatment with lamivudine (Schuurman *et al.* 1995). This study involved 20 asymptomatic or mildly symptomatic HIV-1 infected men with an average CD4 T cell count of about 200 cells per µl and HIV-1 RNA loads ranging between 4×10^3 to 9×10^5 RNA copies per ml. Following treatment with lamivudine, HIV-1 RNA loads dropped to about 95 % below baseline in about a week. Thus, the typical exponential slope of the RNA decline is comparable to that reported recently (Ho *et al.* 1995; Wei *et al.* 1995; Nowak *et al.* 1995*a*; Perelson *et al.* 1996), i.e. $\ln[0.05]/7 \simeq 0.4$ per day. An unexpected finding in the lamivudine data is that the CD4 cell counts decrease in about one third of the patients. Thus the average CD4 increases is about one cell per day only (Schuurman *et al.* 1995), which is small compared with the slope of eight CD4 cells per µl per day reported recently (Ho *et al.* 1995; Wei *et al.* 1995).

For a generic comparison of our model with the several clinical data sets we therefore pick two cases from our lamivudine study with significant increases in the CD4 cell counts (Schuurman *et al.* 1995). The parameters c and γ are set such that the equilibrium of the model corresponds to the initial CD4 count and the initial viral load of each patient. Lamivudine treatment is simulated by setting $D = 10$ (see equation 1). In figures 1 and 2 we plot the CD4 counts, the total RNA load, and the RNA load of the wild type and the drug-resistant mutant as they are obtained from the data and from the model. The model behaviour and the lamivudine data correspond closely. Additionally, the model behaviour is in close correspondence with the earlier clinical data on both protease inhibitors and RT-inhibitors (Ho *et al.* 1995; Wei *et al.* 1995; Coffin 1995*b*). As the virus quasi species contains, or rapidly evolves, mutants with both a high drug-resistance and with a RT fitness comparable to that of the wild type, drug therapies like this tend to be unsuccessful.

By considering the pre-treatment situation more closely we now develop our immuno-suppressive therapy for math-AIDS. Because the mutant strain is typically small in the pre-treatment situation we can ignore equation (3). Thus, the model simplifies into a simple epidemic of one parasite T_w infecting a host population T_4 with a reproduction ratio of $R = c\gamma T_4/d_I$. In epidemiology it is well known that a parasite requires a critical density of infectable hosts for its survival (Kermack & McKendrick 1927), i.e. in our case the virus will only survive when $R \geqslant 1$. At the clinical latency equilibrium $R = 1$ because the CD4 level, i.e. $T_4 = d_I/(c\gamma)$ (see equation 2), is exactly at the threshold.

Thus, bringing the target cell level below that of the clinical latency quasi-equilibrium should eradicate the virus in our model. This means that a therapeutic approach for math-AIDS is the administration of an immuno-suppressive drug reducing $CD4^+$ T cell activation or the CD4 T cell counts (e.g. cyclosporin, or an anti-CD4 antibody (Wofsy & Carteron 1990)). In our simple model we implement the latter by adding to equation (4) a term increasing the removal of $CD4^+$ T cells, i.e.

$$\frac{dT_4}{dt} = \sigma - d_T T_4 - \gamma T_4(f_w V_w + f_r V_r) - \alpha T_4, \qquad (5)$$

where α is the rate at which CD4 T cells are removed by the drug. Similar results would be obtained if we were to reduce the source of target cells σ as a model for blocking CD4 T cell activation (not shown). In the absence of the virus the equilibrium of equation (5) is at $T_4 = \sigma/(d_T + \alpha)$. Hence we find the critical anti-CD4 treatment at which $R = 1$ by setting this non-infected equilibrium equal to the clinical latency equilibrium, i.e. by setting $\sigma/(d_T + \alpha) = d_I(c\gamma)$.

Solving for α, we obtain $\alpha = c\gamma\sigma/d_I - d_T$ d^{-1} for the critical anti-CD4 treatment beyond which the virus is eradicated. One can easily check from our model that this rate α corresponds to the equilibrium rate at which a target cell is expected to be infected by virus. Thus, by suppressing as many T cells as the virus is infecting, the critical anti-CD4 treatment yields a CD4 T count that is identical to that of the clinical latency equilibrium. Because the virus is infecting large numbers of T cells per day (Ho *et al.* 1995; Perelson *et al.* 1996), the anti-CD4 treatment has to be strong. The surprising mathematical result is however that, despite the strong treatment, the CD4 T cell count of the math-AIDS patient need only by reduced marginally.

This is illustrated in figure 3 where we introduce the anti-CD4 therapy into the clinical latency state of the math-AIDS case depicted in figure 1. The effects of a minimal anti-CD4 treatment is studied by setting $\alpha = 0.041$ in figure 3*a*. We observe that HIV RNA levels decline steadily, that CD4 levels decline about 25 % until day 15, and then recover to about 10 % below baseline. The CD4 recovery is caused by the reduction of the virus load. The crucial new phenomenon is that the virus never rebounds (as long as the anti-CD4 therapy is continued). Slowly increasing the dosage of the anti-CD4 therapy until one observes a rapid drop of the HIV-1 RNA load, allows one to determine *in vivo* the critical anti-CD4 treatment. In figure 3*b* we linearly increase the anti-CD4 treatment from $\alpha = 0$ at day zero to $\alpha = 0/041$ at day 41, from then on the treatment remains at $\alpha = 0.041$.

Our immuno-suppressive therapy should have a highly synergistic effect on any of the conventional anti-viral drugs. In Figure 3*c* we do a combination of the anti-CD4 and the anti-HIV treatment in the math-AIDS patient depicted in figure 1. Importantly, the drug-resistant mutants do not appear (cf. figure 1), and

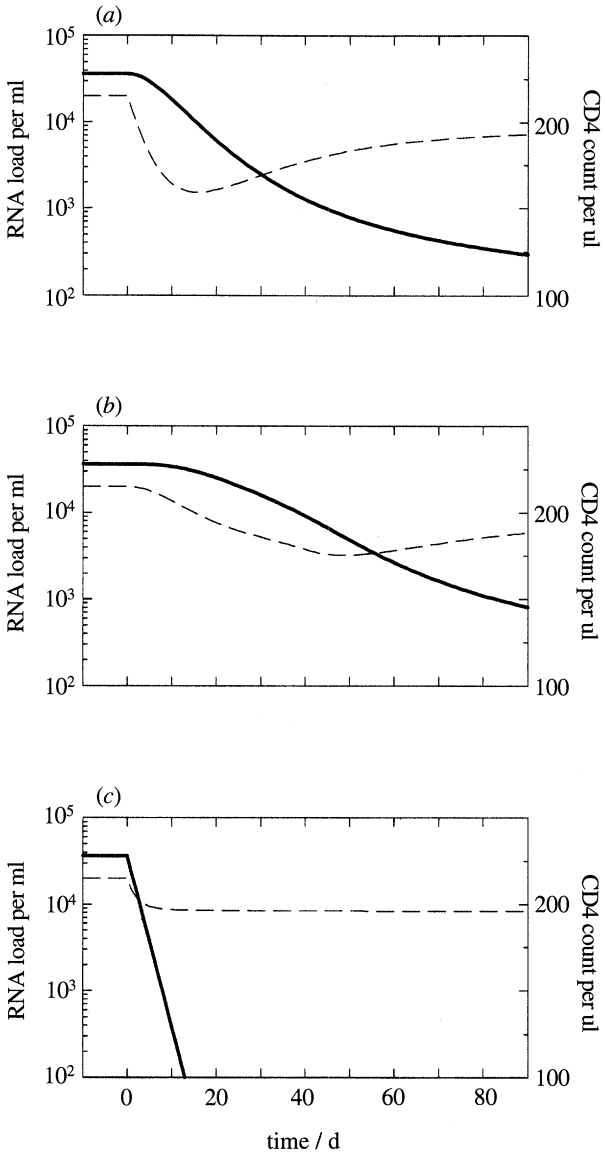

Figure 3. Three examples of anti-CD4 therapies. The CD4 T cell counts per μl (dashed lines) are depicted on a linear scale; the viral RNA loads per ml (heavy solid lines) are depicted on a logarithmic scale. At day zero in panel (*a*) we give an anti-CD4 treatment to the math-AIDS case number 11 (see figure 1) by setting $\alpha = 0.041$ (which is slightly above the critical level $\alpha = c\gamma\sigma/d_I - d_T = 0.0402$ for this case). The treatment cures math-AIDS: the total virus load declines monotonically, no mutant ever appears, and CD4 levels first decrease (by approximately 25%) and then increase to approximately 10% below baseline. This anti-CD4 therapy thus suffices for markedly reducing the virus load; it is however associated with some reduction of the CD4 T cell count. To clinically assess the critical anti-CD4 treatment, we gradually build up the anti-CD4 therapy in panel (*b*). At day zero $\alpha = 0$, it increases linearly to $\alpha = 0.041$ at day 41, then the treatment remains at $\alpha = 0.041$. One can thus increase the anti-CD4 dosage, monitoring the CD4 counts and the HIV-1 RNA loads, until one sees a rapid drop of the HIV-1 RNA load. In panel (*c*) we combine the anti-HIV therapy of figure 1 (i.e. $D = 10$) with the critical anti-CD4 therapy of Figure 3*a* (i.e. $\alpha = 0.041$). The virus now drops much faster, the CD4 levels never decrease more than 10%, and the drug-resistant mutant never appears. (The decrease of the CD4 count is largely caused by the disappearance of the infected CD4 cells.) Thus one can prevent the evolution of drug-resistant mutants by preventing the increase of the CD4 count evoked by the anti-HIV treatment.

the CD4 levels never decrease more than 10%. Thus, by careful tuning the anti-CD4 therapy one can prevent the outgrowth of the drug-resistant mutants while keeping the CD4 count at reasonable levels.

4. CAVEATS

Decreasing activated $CD4^+$ T cells is here proven to be an efficient therapy for math-AIDS, but the strong suppressive treatment that we require could of course be dangerous in AIDS patients. Here we spell out a number of caveats. First, AIDS patients typically suffer from various opportunistic infections, which become problematic, and eventually lethal, when CD4 levels drop further by ongoing HIV infection. Suppressing the $CD4^+$ T cells any further may therefore have counter-productive effects. This would suggest testing the anti-CD4 therapy early in clinical latency so that the CD4 T cell counts can be kept above the clinical level below which the opportunistic infections become problematic (see however the discussion below). Second, reducing the target cell density only marginally (or slowly, figure 3*b*) may invite the virus to evolve mutants which survive on lower target cell densities, i.e. it may invite the virus to increase the number of virus particles per productively infected T cell (c), and/or the infection rate (γ). Thus the anti-CD4 therapy needs to be strong and might be dangerous. This suggests that employing the synergy between anti-HIV with anti-CD4 therapy could indeed be more successful because the mutants selected by an anti-HIV therapy tend to have somewhat lower infection rates (Coffin 1995*b*; C. A. B. Boucher, unpublished data). Third, we expect the virus to evolve novel trophisms. However, if one could develop an anti-CD4 approach reducing not only $CD4^+$ T cells, but also all other $CD4^+$ cell types, the virus would have to evolve an entirely new route of cellular infection.

5. DISCUSSION

Our hypothesis is extremely controversial. Most previous therapies were aimed at increasing the CD4 count. Here we propose to prevent the CD4 count from increasing, or even to lower it. It seems likely however that the increase of the CD4 levels following an anti-HIV therapy reflects an increase in the availability of target cells and increases the spread of the (pre-existing) drug-resistant mutants (see the data in figure 1, and McLean & Frost (1995) and De Jong *et al.* (1996)). As the drug-resistant mutants make the anti-HIV therapy quite unsuccessful, the possibility that the appearance of drug-resistant variants can be prevented by an anti-CD4 treatment is not only extremely controversial but also extremely intriguing.

The main controversy remains our assumption that the virus can be target-cell-limited. Not denying that

the immune response may dominate in the control of the virus in the clinical latency equilibrium, we would argue that several clinical data sets do support the notion that the virus becomes target-cell-limited when the clinical latency equilibrium is perturbed by therapeutic intervention. Perturbations of the clinical latency equilibrium with anti-viral drugs inhibiting HIV-1 RT or HIV-1 protease (Ho *et al.* 1995; Wei *et al.* 1995; Nowak *et al.* 1995*a*; Perelson *et al.* 1996), with the immuno-stimulatory drug IL2 (Kovacs *et al.* 1995), with influenza vaccination (Staprans *et al.* 1995), and with immuno-suppressive drugs like cyclosporin or prednisolone (Andrieu *et al.* 1988, 1995; Fauci 1993; Schwarz *et al.* 1993; Weber & Galpin 1995; Corey 1995), are all reported to affect target cell numbers (as measured by the CD4 cell count). The fact that immuno-stimulation by either influenza vaccination (Staprans *et al.* 1995; O'Brien *et al.* 1995) or IL2 treatment tends to increase the viral load (Kovacs *et al.* 1995), and that immuno-suppression can have beneficial effects (Andrieu *et al.* 1988, 1995; Fauci 1993; Schwarz *et al.* 1993; Weber & Galpin 1995; Corey 1995), is most readily explained by target-cell-limitation, i.e. by the ecological view.

The issue is complicated however because the ecological and immunological views are not mutually exclusive, e.g. during disease progression the infection could evolve from immunological to ecological control. If this is so, anti-CD4 treatment will be detrimental during the early stages and beneficial during the late stages of disease. Furthermore, in models where the specific immune response changes rather slowly (e.g. because of immune memory effects) the more rapid ecological effects dominate transiently such that anti-CD4 treatment is transiently beneficial (manuscript in preparation).

The fact that in some of the data on the immuno-suppressive therapies the CD4 count increases but the viral load hardly responds (Andrieu *et al.* 1988, 1995; Corey 1995), is in agreement with a more detailed model of ours in which HIV-1 only infects activated T cells (De Jong *et al.* 1996; Stilianakis *et al.* 1996). The same model also allows us to model disease progression as the hyperactivation of the immune system (Fauci 1993). Thus, modelling progression by increasing the T cell activation rate, our ecological model accounts for the conventional increase in the viral load accompanied by a degree of the CD4 levels (De Jong *et al.* 1996; Stilianakis *et al.* 1996).

The main problem to expect with an anti-CD4 therapy is that the virus will not really be eradicated. HIV-1 can persist in long-lived cells (e.g. wild-type HIV-1 DNA remains present for months after anti-HIV therapy (Wei *et al.* 1995; C. A. B. Boucher, unpublished data). Thus one expects the virus to re-appear from such 'reservoirs' as soon as the anti-CD4 treatment is interrupted. Clinical data (Weber & Galpin 1995) from an AIDS patient treated with cyclosporin are in agreement with this. Over a 30-week-period the patient's CD4 counts fell from 960 to 530, and the viral load dropped 84%. When the cyclosporin treatment was interrupted, viral load and CD4 counts rose back to baseline. Similar data have hitherto been interpreted in the light of chronic hyperactivation (Ascher & Sheppard 1988; Fauci 1993) or auto-immune effects (Habeshaw *et al.* 1992; Weiss 1993; Andrieu *et al.* 1995) involved in HIV-1 infection.

Given the results presented here we prefer to interpret these data as support for the ecological view, and as its natural consequence, as support for an anti-CD4 therapy. Moreover, an anti-CD4 therapy killing $CD4^+$ cells should be much more effective than cyclosporin because killing $CD4^+$ cells should also affect virus hiding in the resting T cell (Bukrinsky *et al.* 1991; Zack *et al.* 1991) and macrophage reservoirs (Weiss 1993). Finally, we stress again that our hypothesis depends strongly on the ecological view that we have adopted here, and that anti-CD4 treatment should be detrimental if this view turns out to be wrong. As a critical test distinguishing the ecological from the immunological view one should begin to design experiments measuring the effects of anti-CD4 treatments in animal models.

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