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Human Immunodeficiency Virus Drug Therapy and Virus Load

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Analysis of the short-term dynamics of human immunodeficiency virus (HIV) type 1 infection in response to drug therapy has elucidated crucial kinetic properties of viral dynamics in vivo (D. D. Ho et al., Nature 373:123–126, 1995; A. S. Perelson et al., Science 271:1582–1586, 1996; X. Wei et al., Nature 373:117–122, 1995). Here we investigated long-term changes in virus load in patients treated with a combination of lamivudine and zidovudine to identify principal factors responsible for the observed 10- to 100-fold sustained suppression of virus load in vivo. Interestingly, most standard accounts of virus dynamics cannot explain a large sustained reduction without shifting the virus very close to extinction. The effect can be explained by taking into consideration either (i) the immune response against HIV, (ii) the killing of uninfected CD4 cells, or (iii) the differential efficacies of the drugs in different cell populations.

Long-term treatment of human immunodeficiency virus type 1 (HIV-1)-infected patients with a combination of lamivudine and zidovudine results in a 10- to 100-fold reduction of virus load and a 25% increase in CD4 cell count, which is usually sustained for at least 1 year (5) (Fig. 1). Patients receiving this therapy show a rapid decline in virus load over several orders of magnitude in the first 4 weeks after the start of treatment, followed by a resurgence of the virus. After roughly another 4 weeks the virus attains a new steady state approximately 10- to 100-fold lower than the baseline value before treatment (Fig. 1). The resurgence of virus may be caused partly by the concomitant increase in CD4 cells (13) and partly by the emergence of lamivudine-resistant virus, but the long-term suppression of virus is caused by synergistic interactions between the drugs (9); the resulting mutant virus is resistant to lamivudine but inhibited by zidovudine.

The central question of this paper is, what are the principal factors responsible for the sustained reduction of virus load during drug treatment? Both lamivudine and zidovudine are reverse transcriptase inhibitors which impair de novo infection of cells by preventing reverse transcription of viral RNA into DNA. Consequently, these drugs reduce the rate of infection of uninfected cells. It therefore appears obvious that drugs which potently inhibit de novo infection of cells should also have a strong effect on equilibrium virus load, but in the next section we show that most standard models of virus dynamics lead to a scenario where a drug either drives the virus to extinction or has no significant effect on equilibrium virus load at all.

The virus load paradox. Let x denote the density of infectible cells and y denote the density of virus-producing cells. Then the simplest model for virus dynamics (1, 3, 8) is

$$dx/dt = \lambda - dx - \beta xy \quad (1)$$

$$dy/dt = \beta xy - ay \quad (2)$$

where λ is the rate of immigration (or creation) of infectible cells, d is the natural death rate of infectible cells, a is the death rate of virus-producing cells, and β is the rate of infection of uninfected cells. Similar models which include the dynamics of free virus have been used to describe the short-term dynamics of virus load during drug treatment and have helped to estimate virus turnover rates in vivo (4, 6, 7, 16, 17, 19, 22). Here, however, as we were interested in drug-induced changes at steady state, we deliberately omitted a variable for the free virus, assuming that at steady state the free-virus population is proportional to the virus-producing cell population. Note further that x is not simply the total abundance of uninfected CD4 cells, because only a small fraction (i.e., those activated) of CD4 cells are thought to be infectible by HIV. Thus, changes in the CD4 cell count during therapy are not necessarily representative of changes in x.

Figure 2 shows a numerical simulation of a generalized version of the basic model (equations 1 and 2) that includes a drug-resistant mutant which exists at low frequencies before the therapy is initiated. For the purposes of this simulation, the mutant is assumed to infect cells at a 10-fold-lower rate than wild-type virus in the absence of drugs. Following the start of therapy, the cell population infected with drug-sensitive wild-type virus declines exponentially and the drug-resistant mutant grows to a new steady state. At this steady state, however, the virus load is only marginally reduced compared to the baseline before therapy, despite the fact that the infection rate of the drug-resistant mutant is 10-fold lower than that of the wild type in the absence of drugs. The total virus load does not change, because the reduction in the rate of de novo infection is counterbalanced by the gain in infectible cells. We will show that according to the basic model (equations 1 and 2) we do not expect a large reduction of virus load during treatment unless the virus is at the verge of extinction.

The basic reproductive ratio, R_0, is defined as the average number of secondary infected cells generated by a single infected cell placed in an uninfected cell population (2, 3, 12). For the above-described model we have $R_0 = \lambda\beta/da$. (An intuitive understanding of R_0 can be obtained by noting that λ/d is the density of uninfected cells in the absence of virus, $1/a$ is the average life span of infected cells, and β is the rate of infection per unit of time.) Clearly, if R_0 is <1, then on average each cell will produce less than one newly infected cell. In this case the virus is unable to maintain the infection and will go extinct (the uninfected cell population will converge to the equilibrium $x_0 = \lambda/d$). If on the other hand R_0 is >1, then the

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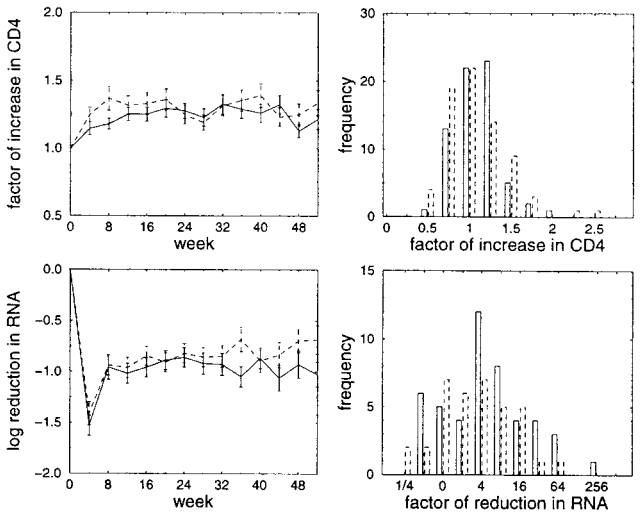

FIG. 1. Mean change in CD4 cell count and log mean change of HIV-1 RNA load compared to baseline in patients treated with a low-dose (dashed line) and high-dose (solid line) combination of lamivudine and zidovudine. In each treatment arm there were about 90 patients at the beginning of the trial. In both treatment arms about 25% of the patients either were lost to follow-up or discontinued drug treatment because of adverse effects of the drug (for a detailed description of the drug trial see reference 5). The two left plots show the changes in CD4 cell count and RNA load compared to the baseline over the 52-week-long trial. The error bars indicate the standard errors. In the first 4 weeks after the start of therapy the RNA load declines rapidly over several orders of magnitude. Then the virus starts to resurge, and after roughly another 4 weeks the virus load equilibrates at a new steady state 10-fold lower than the baseline before the start of treatment. Concomitant with the equilibration of the virus load, the CD4 cell population attains a new steady state 25% above baseline over the entire duration of the trial. The two right plots show the factor of increase of CD4 cell count and the factor of reduction in RNA load between the baseline and the average of measurements taken between weeks 32 and 52 for each patient. The maximal factor of decline in RNA load was 300-fold, but in no patient did the virus population remain below the detection limit.

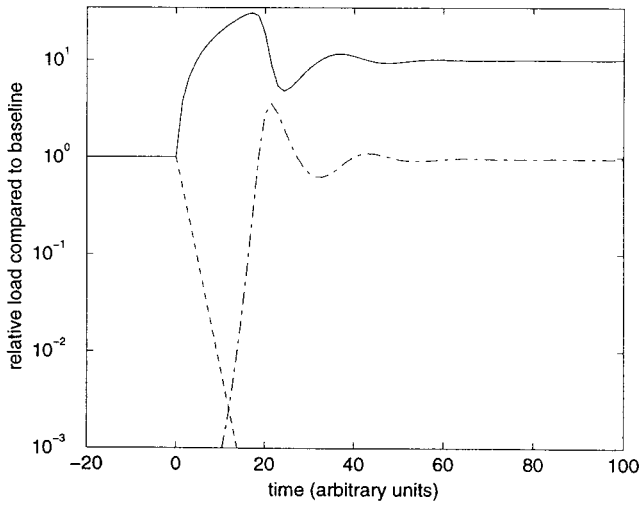

FIG. 2. Numerical simulation of drug therapy. The model used here [$dx/dt = \lambda - dx - x(\beta_w y_w + \beta_m y_m)$; $dy_w/dt = \beta_w x y_w - a y_w + q(y_m - y_w)$; $dy_m/dt = \beta_m x y_m - a y_m + q(y_w - y_m)$] is a generalization of equations 1 and 2 that includes a drug-sensitive wild-type virus (subscript w) and a drug-resistant mutant virus (subscript m). The parameter q reflects the forward and backward mutation rate between the wild type and mutant. Before therapy was started, the infection rate of the wild type, β_w, was set at 0.1 and the infection rate of the mutant, β_m was set at 0.01. At time zero, drug therapy was started and the infection rate of the wild type, β_w, was set at 0. The wild type declined exponentially, and the mutant grew to a new steady state. Interestingly, this new steady state was only marginally reduced compared to the baseline before the start of therapy, despite the fact that the mutant has a 10-fold-lower infection rate than the wild type in the absence of drugs. —, infectible cells; ····, drug-sensitive wild type; — · —, drug-resistant mutant.

virus can establish an infection and equations 1 and 2 lead to a stable equilibrium between infected and uninfected cells [given by: $x^* = x_0/R_0$ and $y^* = \lambda/a(1 - 1/R_0)$].

The effect of reverse transcriptase inhibitors on virus replication corresponds to a reduction of the infection rate parameter, β, and consequently of the basic reproductive ratio. Let R_0 be the basic reproductive ratio of virus before drug therapy and R_1 be the (reduced) basic reproductive ratio of the virus population that replicates during treatment. This population can consist of the drug-impaired wild-type virus or drug-resistant mutants. If R_1 is <1, then treatment is sufficiently potent to eradicate the virus. If R_1 is >1, then treatment will not eradicate the virus [and the new equilibrium is determined as follows: $\hat{x} = x_0/R_1$ and $\hat{y} = \lambda/a(1 - 1/R_1)$]. Hence, the factor of reduction in equilibrium virus load due to drug therapy is determined as follows: $\alpha = y^*/\hat{y} = (1 - 1/R_0)/(1 - 1/R_1)$. Put another way, the basic reproductive ratio during treatment has to be determined as follows: $R_1 = [1 - 1/\alpha(1 - 1/R_0)]^{-1} < (1 - 1/\alpha)^{-1} \approx 1 + 1/\alpha$, where the approximation is valid for $\alpha \gg 1$. Hence, in order to achieve a 10 (or 100)-fold sustained reduction in the virus load, the basic reproductive ratio during drug treatment must be smaller than 1.1 (or 1.01), regardless of the basic reproductive ratio before the start of treatment. However, in no patient receiving lamivudine-zidovudine combination therapy was the virus load maintained below the detection limit for a long time, which suggests that treatment does not drive the virus to extinction and therefore implies that R_1 is >1. Consequently, if the basic model of viral dynamics (7, 19, 22) given by equations 1 and 2 is used to explain a 10-fold reduction in virus load, one would have to postulate that the drug leads to a basic reproductive ratio during therapy such that $1 < R_1 < 1.1$. Given that R_1 can in principle be any number smaller than R_0, it seems unreasonable to assume that R_1 falls exactly between 1 and 1.1. Therefore, the basic model of virus dynamics does not explain a strong suppression of equilibrium virus load during long-term drug therapy except for a very narrow, artificial parameter range. We will now discuss various extensions of the model that may help to explain the observed reduction of virus load.

Immune responses. The assumption implicit in the basic model is that the effects of immune responses are either negligible or remain constant over the time period of interest. While the assumption of constancy may be justified for the short-term dynamics (7, 19, 22), it might not hold for the long term. We therefore include another variable, z, for the density of the cytotoxic-T-lymphocyte (CTL) responses against virus-infected cells. The expanded model (14, 18) is

$$dx/dt = \lambda - dx - \beta xy \quad (3)$$

$$dy/dt = \beta xy - ay - pyz \quad (4)$$

$$dz/dt = ky - bz \quad (5)$$

where p is the killing rate of virus-producing cells by CTL, k is the rate of stimulation of CTL, and b is the death rate of CTL. The equilibrium density of virus-producing cells is determined as follows: $y^* = 1/2\ [-(1 + \rho/R_0) + \sqrt{(1 + \rho/R_0)^2 + 4\rho(1 - 1/R_0)}]$, where $\rho = \lambda pk/(ba^2)$.

Figure 3 shows the virus load and the percentage of virus-producing cells killed by the immune responses as a function of the basic reproductive ratio. Provided that the fraction of virus-

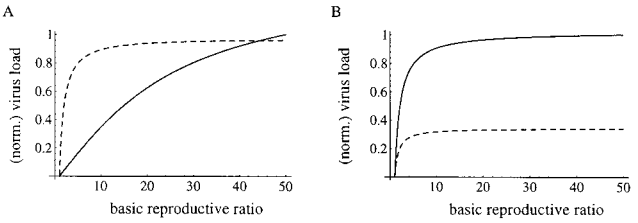

FIG. 3. Equilibrium virus load (solid line) and percentage of cells killed by immune responses in equilibrium (dashed line) as a function of the basic reproductive ratio (see equation in the text). The equilibrium virus load is normalized to 1 for an R_0 of 50. The total death rate of virus-producing cells is determined as follows: $a + pz = a + pk/by$ (see equation 4). For both plots, the total death rate of virus-producing cells is set at 0.5 for an R_0 of 50. This corresponds to a turnover rate of the virus-producing cell population of 1.5 days (7, 17, 19, 22). For an R_0 of 50, the percentages of virus-producing cells killed by the immune responses are ≈95% (A) and ≈30% (B). If a large part of the virus-producing cells are removed by the immune responses (as in plot A), then the equilibrium virus load is affected by changes in the basic reproductive ratio, even if the wild-type virus does not have a basic reproductive ratio close to 1 before the start of therapy. (A) $\lambda = 10$, $a = 0.02$, $d = 0.01$, and $pk/b = 0.048$; (B) $\lambda = 10$, $a = 0.33$, $d = 0.01$, and $pk/b = 0.009$.

producing cells removed by the immune response is large compared to the fraction killed by the virus, changes in the basic reproductive ratio, R_0, can have a strong effect on virus load even if the virus is not close to extinction. This result can be explained as follows. A strong immune response results in a low virus load. If the virus load (y) is sufficiently small, then the total rate of de novo infection of infectible cells (βxy) is small compared to the total loss of infectible cells (dx), and therefore the equilibrium density of infectible cells is only weakly affected by the virus. Consequently, under these circumstances a reduction in the infection rate is not counterbalanced by a gain in infectible-cell density, and the virus load decreases in response to drug therapy. According to this model, patients with weak CTL responses should have a smaller reduction in equilibrium virus load than those with strong CTL responses. The model predicts a positive correlation between the strength of the CTL response against immunodominant epitopes in drug-naive patients before treatment and the magnitude of reduction in equilibrium virus load in response to drug therapy.

If the density of infectible cells, x, is regulated to a constant level, $\hat{x}$, by a homeostatic process, then the virus dynamics are given by:

$$dy/dt = \beta\hat{x}y - ay - pyz \quad (6)$$

$$dz/dt = ky - bz \quad (7)$$

The equilibrium virus load is given by: $y^* = b/(pk)(R_0 - 1)$, where $R_0 = \beta\hat{x}/a$. Hence, the equilibrium virus load depends linearly on the rate of infection: a strong reduction of the infection rate will cause a strong reduction in equilibrium virus load.

These simple models predict that the density (or strength) of the specific CTL response should decline during drug therapy. It is conceivable that in models that include CD4 help the CTL response would increase with decreasing virus load. If, however, the long-term changes in the CTL responses are sufficiently small (such that z can be treated as a constant in equation 5), then the CTL responses cannot be responsible for the reduction in virus load (because equations 4 through 7 reduce to the basic model in equations 1 and 2).

Virus-induced killing of uninfected cells. An alternative possibility to explain the observed reduction in virus load without invoking control by the immune responses is to incorporate a term for the virus-induced killing of uninfected cells that is not related to their infection. Examples of such infection-independent killing mechanisms are the killing of uninfected cells due to the shedding of gp120 or to syncytium formation (11, 21, 23; reviewed in reference 10). This can be incorporated in the basic model by adding a term to equation 1, qxy, where q reflects the rate of virus-induced killing of infectible cells that is not related to infection. We assume that the drugs affect only the infection rate, β, and not the parameter q.

The equilibrium virus load as a function of the infection rate is then given by: $y^* = (\lambda\beta/a - d)/(\beta + q)$. Hence, if the infection-independent rate of killing, q, is large compared to the rate of infection, β, before treatment, then the virus load decreases linearly with decreasing β. In this case a drug-induced reduction of β has a strong effect on the equilibrium virus load.

Differential effects of drugs on various cell types. A further possibility to account for the observed reduction in virus load is to assume that a drug has differential effects on cells in different tissues. Assuming that a drug has only a small effect on replication in some tissues but can drive the virus to extinction in other tissues, then of course drug treatment may significantly reduce the virus load, provided that those tissues which are not strongly affected by the drug contribute only little to the plasma virus load before treatment is started.

Model extensions that do not work. Surprisingly, many other extensions to the basic model cannot explain a sustained strong suppression of equilibrium virus load unless the virus is assumed to be arbitrarily close to extinction. We have tested models that incorporate (i) alternative forms for the growth of the infectible cell population [i.e., $dx/dt = \lambda x - dx^2$ or $dx/dt = \lambda x/(1 + \varepsilon x) - dx$ in the absence of infected cells], (ii) different forms of latency (in which cells either become latently infected immediately upon infection or convert between latent and active stages), (iii) activated and resting CD4 cells (such as those discussed in reference 20), (iv) replication of the provirus through T-cell proliferation, and (v) immune responses that are activated according to classical predator-prey dynamics (i.e., $dz/dt = kyz - uz$, such as those discussed in reference 15). In all these models virus load either does not change at all (case v) or changes only when the virus is very close to extinction (cases i to iv).

We have also investigated a model with a distribution of infectibility of target cells rather than an average rate of infection. Imagine we have a collection of infections going on in several cell types with different infectibilities. As drugs are administered, the basic reproductive rate in the least susceptible cell types may fall below unity, and the infection is unable to maintain itself in these cell types. However, the total basic reproductive rate in these cells may still be larger than 1 if there is an influx of free virus produced in the more susceptible cell compartments. Therefore, if there is a considerable flux between cell compartments, then drug treatment should not have a strong effect on equilibrium virus load. However, if there is no exchange of virus between different cell compartments, then drug treatment may have a significant effect on equilibrium virus load.

Conclusion. The central point of this paper is that the strong sustained suppression of virus load observed in patients treated with both lamivudine and zidovudine is not a trivial consequence of a drug-induced reduction of virus infectivity. Most standard models cannot explain this observation without the unrealistic assumption that the drugs always shift the virus population very close to but never beyond the verge of extinction. According to these models, the total virus load should not change significantly, because the drug-induced reduction of the

rate of de novo infection is counterbalanced by the increased availability of infectible cells.

In all models we have made the assumption that free virus is proportional to the virus-producing cell population at equilibrium before and during therapy. This implies that the burst size of the virus is not affected by treatment. The reasoning behind this assumption is that reverse transcriptase inhibitors reduce the rate of infection of infectible cells but should not affect the rate of production of virus particles from infected cells. However, we cannot exclude the possibility that an evolutionary adaptation in the reverse transcriptase gene has also had an indirect effect on the burst size of the virus mutant. If resistant mutants have a reduced burst size, then the basic model would predict that the level of virus-infected cells during therapy will be essentially unchanged, while the free-virus load will be reduced during therapy. This prediction is experimentally testable.

We have proposed three hypotheses that can account for a strong drug-induced reduction of equilibrium virus load without postulating that the virus is at the verge of extinction during drug treatment. These models make testable predictions for correlations between the strength of the immune response before the start of therapy and the magnitude of reduction in the equilibrium virus load in response to drug treatment. If the virus is controlled by the immune responses (as in equations 3 to 5), then the prediction is that the magnitude of reduction in equilibrium virus load should correlate with the strength of the CTL response before the start of drug therapy. If host cell availability limits virus load (as in the models of infection-independent CD4 cell depletion or tissue-dependent drug efficacy), then we do not expect such a correlation. Therefore, future drug trials providing quantitative data on immune responses along with virus load and CD4 cell count may help to identify the principal factors controlling virus load in vivo.

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