



Pre-Existence and Emergence of Drug Resistance in HIV-1 Infection

Sebastian Bonhoeffer, Martin A. Nowak

Proceedings: Biological Sciences, Volume 264, Issue 1382 (May 22, 1997), 631-637.

Your use of the JSTOR database indicates your acceptance of JSTOR's Terms and Conditions of Use. A copy of JSTOR's Terms and Conditions of Use is available at <http://www.jstor.org/about/terms.html>, by contacting JSTOR at jstor-info@umich.edu, or by calling JSTOR at (888)388-3574, (734)998-9101 or (FAX) (734)998-9113. No part of a JSTOR transmission may be copied, downloaded, stored, further transmitted, transferred, distributed, altered, or otherwise used, in any form or by any means, except: (1) one stored electronic and one paper copy of any article solely for your personal, non-commercial use, or (2) with prior written permission of JSTOR and the publisher of the article or other text.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

Proceedings: Biological Sciences is published by The Royal Society. Please contact the publisher for further permissions regarding the use of this work. Publisher contact information may be obtained at <http://www.jstor.org>.

Proceedings: Biological Sciences

©1997 The Royal Society

JSTOR and the JSTOR logo are trademarks of JSTOR, and are Registered in the U.S. Patent and Trademark Office. For more information on JSTOR contact jstor-info@umich.edu.

©2001 JSTOR

Pre-existence and emergence of drug resistance in HIV-1 infection

SEBASTIAN BONHOEFFER* AND MARTIN A. NOWAK

Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, Oxford OX1 3PS, UK (seb@newton.zoo.ox.ac.uk)

SUMMARY

Antiviral treatment of HIV-1 infection often fails because of the rapid emergence of resistant virus within weeks of the start of therapy. This raises the question of whether resistant viruses pre-exist in drug-naive patients or whether it is produced after the start of therapy. Here we compare the likelihood of pre-existence with the likelihood of production of resistant virus during therapy. We show that provided resistant virus pre-exists, then a stronger therapy may lead to a greater initial reduction of virus load, but will also cause a faster rise of resistant virus. In this case the total benefit of treatment is independent of the degree of inhibition of sensitive virus. If, on the other hand, resistant mutants do not pre-exist, then the emergence of resistance during treatment depends on the efficacy of the drug. If the drug is sufficiently potent to eradicate sensitive virus, then the probability that resistant mutants first appear during therapy is smaller than the probability that they existed before therapy. If the drug cannot eradicate the sensitive virus, then after sufficiently long time, resistant mutants will appear. However, mutants that are unlikely to pre-exist may take a long time to appear.

1. INTRODUCTION

In recent years, a number of antiretroviral drugs have been developed that potently inhibit HIV-1 replication *in vivo*. Treatment with such drugs leads to a rapid decline of virus load over several orders of magnitude and a concomitant increase in CD4 cells, the major target cells of HIV-1 (Ho *et al.* 1995; Wei *et al.* 1995). However, the effect of single drug therapy is usually only short lived as the virus readily develops resistance against the drug (Larder & Kemp 1989; Larder *et al.* 1989; Boucher *et al.* 1990; Richman 1990; StClair *et al.* 1991; Ho *et al.* 1994; Richman *et al.* 1994; Markowitz *et al.* 1995; Schuurman *et al.* 1995; Wei *et al.* 1995; Schinazi *et al.* 1996). For some antiviral compounds, single point mutations can confer high-level resistance, and within a few weeks of starting therapy resistant mutants dominate the virus population.

Although multiple resistance and cross-resistance have been observed frequently (Gu *et al.* 1992; Larder *et al.* 1993; Condra & Schleif 1995), certain combinations of drugs can lead to a longer lasting suppression of virus load. For example, clinical trials of AZT-3TC combination therapy have shown a reduction of virus load by a factor of 10–100 for up to one year (Eron *et al.* 1995; Staszewski 1995). Triple drug therapy combining AZT and 3TC with a protease inhibitor can reduce the virus load by four orders of magnitude and can maintain plasma virus below the detection limit for several months (for as long as these studies have been going on).

* Author for correspondence.

Analysis of the decline of plasma virus in drug-treated patients has led to important insights into the dynamics of HIV-1 replication *in vivo* (Ho *et al.* 1995; Nowak *et al.* 1995, 1996; Wei *et al.* 1995; Herz *et al.* 1996; Perelson *et al.* 1996). By fitting theoretically derived functions for plasma virus decline to clinical data, the half-lives of free virus and virus in different cell compartments (actively virus-producing cells, latent cells and PBMCs) were obtained. Central to all these studies is a simple mathematical model for the nonlinear interaction between free virus, virus-infected cells and uninfected cells (McLean & Nowak 1992; Nowak & Bangham 1996).

The goal of this paper is to investigate rigorously the assumptions and predictions of the basic model of virus dynamics with respect to resistance, and to point out where the predictions agree and where they conflict with experimental evidence. We show that according to the model the equilibrium virus load should only be affected strongly by drug treatment if the drug shifts the virus population close to extinction. For less effective drugs the equilibrium virus load before and during long-term treatment is essentially the same, largely independent of the intrinsic growth rates of wild-type and resistant virus. Furthermore, we show that, provided drug-resistant mutants exist before treatment, the total benefit of drug treatment (as measured by the total gain of CD4 cells or the total reduction of virus load compared to baseline) is independent of the degree of inhibition of sensitive virus by the drug. However, the time necessary until a significant fraction of the virus population is resistant is shorter the more effective the drug.

In the second part of the paper, we discuss the likelihoods of pre-existence and emergence of drug-resistant mutants. We show that, as a lower limit, the fraction of cells infected with resistant virus in drug-naïve patients is given by the mutation rate of sensitive to resistant virus. Thus, even if drug-resistant mutants are heavily selected against in the absence of the drug, we expect before treatment, on average, one resistant infected cell in an infected cell population size of the reciprocal of the mutation rate from sensitive to resistant virus. Finally, we calculate the probability that resistant mutants appear after the start of therapy. We show that if the drug can eradicate the sensitive wild-type, then the probability that resistant mutants appear after the start of therapy is smaller than the probability that resistant virus is present before therapy. If the drug cannot eradicate the wild-type then mutants that are unlikely to pre-exist may take a long time to appear after the start of therapy.

2. THE BASIC MODEL

In the basic model of viral dynamics we distinguish three variables: x , for susceptible cells (which are mainly activated CD4 cells); y , for infected cells that produce free virus; and v , for infectious cell-free virus particles. Let us assume that susceptible cells are produced at a constant rate, λ , from a pool of precursor cells, and die at rate dx . This is the simplest possible host cell dynamics. Free virus infects susceptible cells to produce infected cells at a rate βxv . Infected cells die at rate ay . Free virus is produced by infected cells at rate ky and dies at rate uv . These assumptions lead to the following system of ordinary differential equations:

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

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

$$dv/dt = ky - uv. \quad (3)$$

The basic reproductive ratio, R_0 , is defined as the average number of secondary infected cells arising from one infected cell placed into an entirely susceptible cell population (May & Anderson 1979; Anderson & May 1991). Here the basic reproductive ratio is given by $R_0 = \lambda\beta k/(adu)$. Clearly, if $R_0 < 1$, then the virus can neither establish nor maintain an infection. If $R_0 > 1$, then the system converges to the equilibrium $\hat{x} = au/(\beta k)$, $\hat{v} = \lambda k/(au) - d/\beta$, $\hat{y} = \lambda/a - du/(\beta k)$.

This is essentially the model that was fitted to data for the decline of free virus in drug-treated patients to obtain estimates for the rates of viral turnover (Ho *et al.* 1995; Nowak *et al.* 1995; Wei *et al.* 1995; Perelson *et al.* 1996). Implicit in this model are a number of assumptions that are worth pointing out.

First, the model assumes that the contribution of the immune responses to the death of infected cells or free virus (and to reducing the rate of infection of new cells) is either negligible or constant over the time-span of interest. While this assumption may be

justified for the short-term virus dynamics following drug treatment, it need not hold for the long term. Second, the chosen form for the dynamics of the susceptible cell population (e.g. activated CD4 cells) assumes a constant immigration of susceptible cells from a pool of precursors. Alternatively, the dynamics may be governed by proliferation or a disease stage-dependent rate of immigration. We will show that for drugs that potently inhibit infection of susceptible cells, the short-term virus dynamics are largely independent of the dynamics of the susceptible cell population. The long-term behaviour of the model, however, will be affected by the choice of the susceptible cell dynamics. Third, the model neglects the loss of free virus due to the uptake by susceptible cells, assuming that this loss is small compared to the natural decay of free virus (or remains constant). This assumption is crucial insofar as it makes the viral decay independent of the susceptible cell population.

A detailed analysis of the initial decline of free virus in ritonavir-treated patients has shown that the decay rate of free virus, u , is much larger than the decay rate of the virus-producing cell population, a (Perelson *et al.* 1996). We may therefore assume to a good approximation that free virus is in a quasi-steady state (i.e. $dv/dt = 0$), which implies that the free virus load is proportional to the infected cell load. In the following we will therefore eliminate v from equations (1)–(3) by substituting $v = ky/u$.

3. THE RISE OF RESISTANCE DURING DRUG TREATMENT

Assume that a small population of resistant virus pre-exists in the patient before drug treatment. We are interested in the rise of resistant mutants following drug therapy. Extending the basic model to distinguish between cells infected with resistant virus, y_r , and cells infected with sensitive virus, y_s , we get

$$dx/dt = \lambda - dx - (sb_s y_s + b_r y_r)x, \quad (4)$$

$$dy_s/dt = (sb_s x - a)y_s, \quad (5)$$

$$dy_r/dt = (b_r x - a)y_r. \quad (6)$$

The parameters b_s and b_r reflect the infectivity rates of sensitive and resistant virus. Treatment is reflected by the parameter s (between 0 and 1), which describes the inhibition of infection of susceptible cells by sensitive virus. This is an accurate description of the effect of reverse transcriptase inhibitors (like AZT or 3TC), which impair reverse transcription of viral RNA into DNA and thereby prevent infection of susceptible cells. Since the turnover of free virus is fast, it is also a good approximation for protease inhibitors (such as ritonavir), which prevent infected cells from producing infectious virus particles. More accurate models for protease inhibitors incorporate that free virus which is produced before treatment can still undergo one more round of infection (Herz *et al.* 1996; Perelson *et al.* 1996).

For simplicity, we assume that resistant virus is not affected by the drug. (We will point out later

which conclusions are affected by this assumption.) As the resistance mutations are in the genes coding for the reverse transcriptase (RT) or the protease, sensitive and resistant virus may differ in their infectivity rates. In the absence of the drug, the infectivity of the sensitive virus, b_s , is larger than the infectivity of the resistant virus, b_r . Otherwise the resistant virus would dominate the virus population before treatment. The death rate of infected cells should not be affected by the resistance mutation.

(a) *Equilibrium properties*

There are two (non-trivial) stable equilibria depending on the efficiency of drug treatment. If $s > b_r/b_s$, then the system converges to $\hat{x} = a/(sb_s)$, $\hat{y}_s = \lambda/a - d/(sb_s)$, $\hat{y}_r = 0$, and the virus population consists entirely of sensitive virus despite drug treatment. If $s < b_r/b_s$, then the system converges to $\hat{x}' = a/b_r$, $\hat{y}_s' = 0$, $\hat{y}_r' = \lambda/a - d/b_r$, and resistant virus will outcompete sensitive virus. Since we neglect mutation from sensitive to resistant virus (and vice versa), there is no coexistence of sensitive and resistant virus at equilibrium. A model including mutation will be discussed later.

Let us consider a sufficiently strong drug treatment such that $s < b_r/b_s$. Comparing the equilibria in absence and presence of treatment, we obtain for the factor of reduction of virus load

$$\alpha = \frac{1 - 1/R_0}{1 - 1/R'_0}, \tag{7}$$

where $R_0 = \lambda b_s/(ad)$ and $R'_0 = \lambda b_r/(ad)$ are the basic reproductive ratios before and during treatment, respectively. Put another way, to achieve an α -fold reduction in equilibrium virus load the basic reproductive ratio during treatment must be

$$R'_0 = \left(1 - \frac{1}{\alpha} \left(1 - \frac{1}{R_0}\right)\right)^{-1} < \left(1 - \frac{1}{\alpha}\right)^{-1},$$

regardless of the basic reproductive ratio before treatment. Hence, according to this model, we only expect a large reduction in equilibrium virus load if the basic reproductive ratio during treatment is close to 1, which implies that the virus is on the verge of extinction.

This is difficult to reconcile with the strong sustained suppression of virus load observed in several drug trials. For example, patients receiving AZT-3TC combination therapy showed a 10–100-fold reduced equilibrium virus load, but in no patient was the virus ever maintained below the detection limit for a longer period of time (Eron *et al.* 1995). This implies that $R'_0 > 1$ in all patients. Given that R'_0 can in principle be any number smaller than R_0 , it seems unreasonable to assume that R'_0 always falls exactly between 1 and $(1 - 1/\alpha)^{-1} \approx 1.1$. Therefore the basic model of virus dynamics cannot explain a 10–100-fold reduction of virus load as a consequence of drug therapy. For a more detailed discussion and extensions to the basic model that may explain a strong sustained virus load see Bonhoeffer *et al.* (1997).

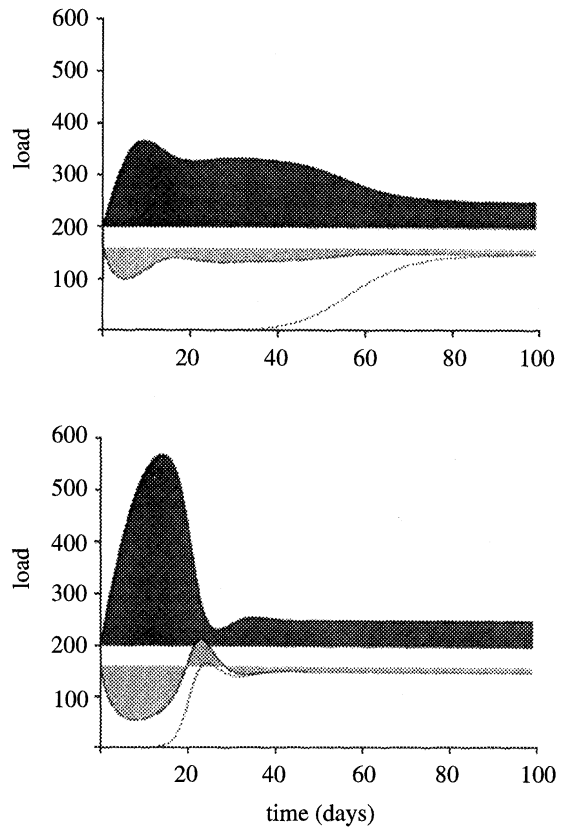


Figure 1. Numerical simulation of drug treatment. The total benefit of treatment, as measured by the total gain of susceptible cells (dark shaded area, solid line) or the total reduction of virus load (light shaded area, dashed line) over a time long enough such that the resistant virus (dotted line) equilibrates during treatment, is independent of the inhibition of sensitive virus by the drug: (a) $s = 0.6$; (b) $s = 0.4$. The shaded areas are of equal size in both plots. We assume that before the start of therapy the virus load is in equilibrium. When therapy is started a small fraction, f , of the total infected cells are assumed to be resistant. The other parameters (in arbitrary units) are: $\lambda = 100$, $d = 0.1$, $a = 0.5$, $b_s = 0.0025$, $b_r = 0.0020$, $f = 0.0001$.

(b) *Total gain of CD4 cells and total reduction of virus load are independent of inhibition of sensitive virus*

Before proceeding, we would like to define a criterion to assess the benefit of treatment. One such criterion would be the average time until a given fraction of the virus is resistant. However, it is immediately apparent that this criterion is problematic, since the treatment strategy that maximizes the time for the appearance of resistance is not to treat at all. Therefore, we would like to propose another criterion which balances the value of preserving the drug's effectiveness with the value of reducing virus load or increasing the CD4 cell count. Such a criterion for the evaluation of the benefit of treatment is to measure the total gain of uninfected CD4 cells or the total reduction of virus load over the duration of treatment compared to baseline before treatment (see figure 1 for a graphical illustration).

In our model, the total gain of uninfected CD4

cells and the total reduction of virus load can be derived analytically. Let us assume that at the time $t = 0$ when therapy is started a small population of resistant virus, $y_r(0)$, pre-exists. (Note that, strictly speaking, there is no stable coexistence of sensitive and resistant virus in the current model. This problem can be eliminated by incorporating mutation between sensitive and resistant mutant. See equations (11)–(13).) Integrating over equation (6) we obtain for the total gain of susceptible cells after a time T (which shall be sufficiently long that the resistant virus attains its equilibrium, \hat{y}'_r , during treatment):

$$F(T) = \int_0^T (x(t) - \hat{x}) dt = 1/b_r(\ln(\hat{y}'_r/y_r(0))) + (\hat{x}' - \hat{x})T, \quad (8)$$

where $\hat{x} = a/b_s$ and $\hat{x}' = a/b_r$ are the equilibrium densities of uninfected cells in the absence and presence of treatment, respectively. Note that the derivation of the total gain in susceptible cells is derived using only equation (6). This result is therefore independent of the dynamics of x .

An analogous result can be obtained for the total reduction of virus load, $y = y_s + y_r$. Integration over $dx/dt + dy_s/dt + dy_r/dt$ yields

$$x(T) - x(0) + y(T) - y(0) = \lambda T - d \int_0^T x dt - a \int_0^T y dt, \quad (9)$$

where $x(0)$, $y(0)$, $x(T)$ and $y(T)$ are the uninfected cells and total infected cell load at the start of treatment and after a time T . Using $\hat{x} = a/b_s$ and $\hat{y} = \lambda/a - d/b_s$, we obtain

$$G(T) = \int_0^T (y(t) - \hat{y}) dt = -\frac{d}{a} \int_0^T (x(t) - \hat{x}) dt - \frac{x(T) - x(0) + y(T) - y(0)}{a}, \quad (10)$$

where \hat{y} is the total virus load before therapy. For the derivation of the total reduction of virus load we used equations (4)–(6). Therefore this result depends on the dynamics of x .

Both the total gain of susceptible cells, $F(T)$, and the total reduction of virus load, $G(T)$, depend logarithmically on the initial frequency of resistant virus. Therefore the benefit of treatment only depends weakly on the frequency of resistant virus before treatment. Interestingly, the benefit is independent of the inhibition of wild-type infectivity, s , by the drug (see figure 1). Strictly speaking, b_r represents the infectivity of resistant virus in the presence of the drug. If b_r is not affected by the drug, then, according to this model, the benefit of treatment is independent of the efficacy of treatment. But if we relax this assumption and assume that the infectivity of resistant virus in the presence of a drug is a function $b_r(s)$ of drug concentration, s , then the total benefit of treatment obviously depends on s . However, in any case

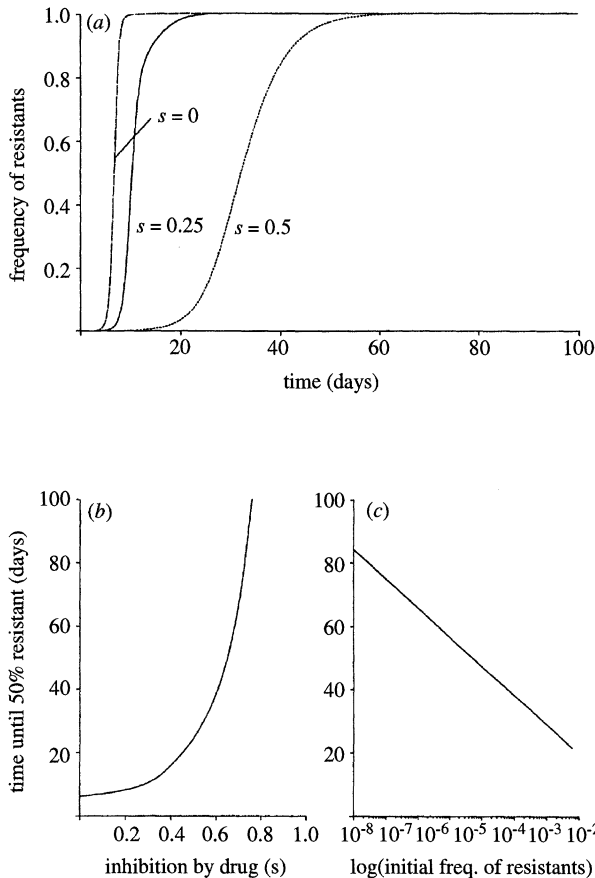


Figure 2. The stronger the treatment, the faster the evolution of resistance. (a) shows the frequency of resistant virus for three different values for the inhibition of sensitive virus by the drug. (b) and (c) show the time, $T_{1/2}$, necessary until 50% of the total cells are infected with resistant virus as a function of the inhibition of sensitive virus, and the initial frequency of resistant virus before treatment. Parameters (in arbitrary units): $b_s = 0.005$, $b_r = 0.004$, $f = 0.0001$ (plots A and B) and $s = 0.6$ (plot C); all other parameters as in figure 1.

the total gain of CD4 cells and the total reduction only depends on the effect of the drug on resistant, but not sensitive, virus.

The frequency of resistant mutants before treatment and their infectivity are likely to vary for different drugs. Hence, the model does not predict that the total gain in CD4 cells or the reduction of virus load should be the same for different drugs.

(c) A stronger drug selects for faster emergence of resistance

The time necessary for a significant fraction of the virus to become resistant depends on the inhibition of sensitive virus by the drug. Figure 2a shows that the stronger the inhibition, the faster the emergence of resistance. An approximate solution for the time necessary for 50% of the total infected cell load to be infected by resistant virus is $T_{1/2} = (sb_s)/(ab_r) \ln((1 - b_r/b_s)b_r y_r(0)/d)$. (For the range of validity of this approximation see Nowak *et al.* (1996).) Numerical simulation shows that $T_{1/2}$ depends strongly on s , but depends only logarithmically

mically on the initial frequency of resistant infected cells before treatment (see figure 2*b, c*). The effect that resistant virus emerges faster the stronger the inhibition by the drug is based on the pre-existence of resistant virus before treatment. We will show that if resistant virus does not pre-exist, then the time until resistant virus appears is longer the stronger the drug.

4. THE FREQUENCY OF RESISTANT VIRUS IN UNTREATED PATIENTS

Next we determine the likelihood that resistant mutants pre-exist in drug-naive patients. We assume that following infection of a cell, resistant mutants are generated by the sensitive wild-type with a probability μ due to erroneous reverse transcription. Incorporating mutation into the model we obtain

$$dx/dt = \lambda - dx - (sb_s y_s + b_r y_r)x, \tag{11}$$

$$dy_s/dt = (s(1 - \mu)b_s x - a)y_s, \tag{12}$$

$$dy_r/dt = (b_r x - a)y_r + \mu sb_s x y_s. \tag{13}$$

We neglect back mutation from resistant to sensitive virus, because we assume that in the absence of a drug ($s = 1$) the population is dominated by sensitive virus (i.e. $b_r < (1 - \mu)b_s$). In the absence of a drug the equilibrium fraction of cells infected with resistant virus is given by $f = \mu / (1 - b_r/b_s)$.

Hence, the equilibrium frequency of drug-resistant mutants in untreated hosts depends on the mutation rate and the ratio of the infectivities of resistant versus sensitive virus. If the infectivity of resistant virus is much smaller than that of sensitive virus, then the relative frequency of resistant infected cells is equal to the mutation rate from sensitive to resistant virus, μ . Only if the infectivities of resistant and sensitive virus are of the same order will the frequency of resistant infected cells be markedly larger than μ . (Note that if b_s and b_r are very similar, then the description of virus dynamics using ordinary differential equations becomes problematic, since the frequencies of wild-type and resistant virus will be strongly influenced by stochastic events.)

The nucleotide misincorporation rate of HIV (per nucleotide and per replication cycle) is about 10^{-3} – 10^{-5} (Preston *et al.* 1988; Roberts *et al.* 1988; Mansky 1996). If wild-type and mutant differ only in a single base, then $\mu \approx 10^{-3}$ – 10^{-5} . Thus, as a lower limit, about 1 in 10^3 – 10^5 infected cells should contain mutant virus before therapy. If wild-type and mutant differ in two bases, then about 1 in 10^6 – 10^{10} cells should contain the resistant mutant before therapy. For a three-error mutant it would be less than 1 in 10^9 cells. Therefore, as a rule of thumb, most one-error mutants, many two-error mutants, but only a few three-error mutants will pre-exist.

5. THE LIKELIHOOD OF PRODUCING A RESISTANT MUTANT AFTER THERAPY

Let us now consider a situation where no resistant mutant is present before therapy. What is the like-

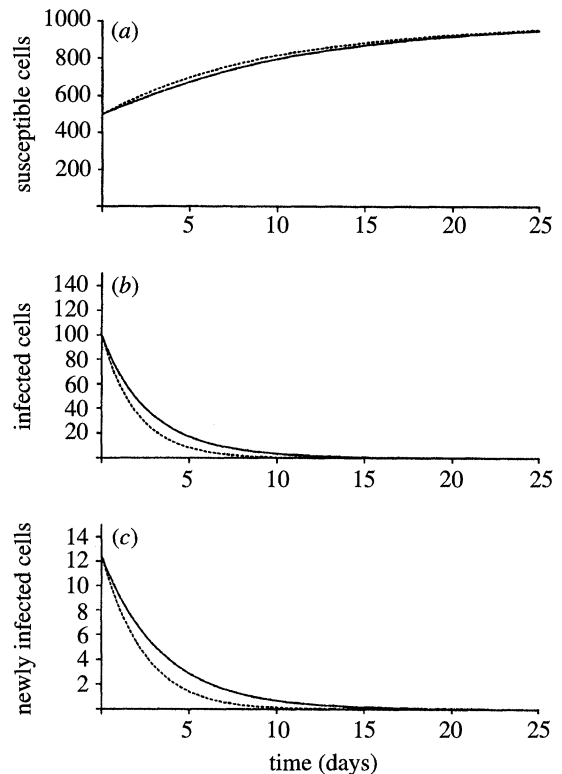


Figure 3. Approximation of the new infections after therapy is started. The solid line represents the numerical solution of the full model (equations (14)–(15)) and the dotted line is the approximation described in the main text. (a) and (b) show the dynamics of the susceptible and infected cell population, respectively. (c) shows the total production rate of infected cells during treatment given by $sb_s y_s x$. Parameters: $s = 0.25$, $b_s = 0.001$, all other parameters as in figure 1.

lihood that a resistant mutant appears during therapy?

In the absence of resistant virus the dynamics of the virus population during drug treatment are

$$dx/dt = \lambda - dx - sb_s x y_s, \tag{14}$$

$$dy_s/dt = sb_s x y_s - a y_s. \tag{15}$$

The total number of cells infected after the start of therapy is given by $sb_s \int_0^\infty x y_s$. Unfortunately, we do not have exact analytical solutions for $x(t)$ and $y_s(t)$, but we can approximate the integral (see figure 3).

We distinguish between two scenarios: (i) the drug treatment is sufficiently potent to eradicate the virus (provided no resistant mutant emerges); and (ii) the drug treatment cannot eradicate the sensitive virus but reduces the infection of susceptible cells by sensitive virus.

The first scenario implies that the basic reproductive ratio during treatment, $R'_0 = s\lambda b_s / (ad)$, is smaller than unity. If we assume that the basic reproductive ratio before treatment is distinctly larger than unity, then the total natural death rate of infectable cells, dx , far outweighs the total rate of infection of infectable cells, $sb_s x y_s$. Hence, we get for the infectable cell population $x(t) \approx \lambda/d - (\lambda/d - x_0)e^{-dt}$, where $x_0 (= a/b_s)$ is the initial load of infectable cells

when treatment is started. We can also neglect sb_sxy_s in comparison to ay_s , and we get for the dynamics of the infected cell population $y_s(t) \approx y_0e^{-at}$, where y_0 is the infected cell load when therapy is started. Integrating over sb_sxy_s we get for the total number of infected cells produced after the start of therapy

$$N \approx R'_0 \left[1 - \left(1 - \frac{1}{R_0} \right) \frac{a}{a+d} \right] y_0.$$

Obviously, when the inhibition is complete ($R'_0 = 0$), then we have $N = 0$. For incomplete inhibition ($1 > R'_0 > 0$) we find that the number of infected cells produced during treatment, N , is less than the number of infected cells, y_0 , before treatment (since $R'_0 < 1$ and $R_0 > 1$). Thus, if a resistant mutant is unlikely to pre-exist in untreated patients, it is also unlikely to appear during drug treatment, provided that the treatment can eradicate the sensitive wild-type virus.

If the drug cannot eradicate the sensitive virus, the resistant mutant will eventually appear, given enough time. Neglecting the transients before the infected cell load reaches its reduced equilibrium, $\hat{y}'_s = \lambda/a - d/(sb_s)$, during treatment, we can obtain a very rough estimate for the time until the first resistant mutant is produced. In equilibrium, the total rate of infection of susceptible cells equals the total death rate of infected cells. Thus, the total daily production of infected cells is given by $a\hat{y}'_s$. We expect one resistant mutant to be produced in $1/\mu$ infections. Hence, we get $T = 1/(\mu a\hat{y}'_s)$ for the average time until a resistant mutant is produced. If the equilibrium virus load during treatment is reduced by a factor α compared to baseline (i.e. $\hat{y}_s = \alpha\hat{y}'_s$), we get $T \approx 2\alpha/(\mu\hat{y}_s)$, where we used that $a \approx 0.5$ (Ho *et al.* 1995; Nowak *et al.* 1995; Wei *et al.* 1995; Perelson *et al.* 1996). Hence, if a mutant is unlikely to pre-exist (i.e. $\mu\hat{y}_s \ll 1$), then it may take a long time until the first resistant mutant is produced.

6. CONCLUSIONS

If resistant virus exists before treatment, we find that the larger the inhibition of sensitive virus by the drug, and hence the larger the difference in basic reproductive ratios of sensitive and resistant virus during treatment, the shorter the time until a significant fraction of the virus is resistant. However, the total benefit of treatment (as measured by the total gain of susceptible cells or the total reduction of virus load compared to baseline before treatment) is independent of the degree of inhibition of the sensitive virus by the drug. Provided that the resistant virus is not affected by the drug, the basic model of virus dynamics predicts that the total benefit of treatment is independent of drug dosage. However, the total benefit depends on the frequency of resistant virus before treatment, which in turn is likely to be specific for each drug. Therefore the total gain of CD4 cells and the reduction of virus load should vary between different drugs. It should also vary for

different drug dosages, if the infectivity of resistant virus is strongly affected by drug dosage.

In the absence of a drug, the frequency of resistant mutants is, as a lower limit, equal to the mutation rate of wild-type to resistant virus. Thus, in a cell population of the size of the reciprocal of the mutation rate from sensitive to resistant virus, we expect to find at least one cell infected with resistant virus. For some antiviral compounds a single point mutation can confer a significant degree of resistance (Larder *et al.* 1989; StClair *et al.* 1991; Mellors *et al.* 1993; Schinazi *et al.* 1993; Tisdale *et al.* 1993; Richman *et al.* 1994; Foli *et al.* 1996). Assuming a point mutation rate of 10^{-3} – 10^{-5} , such mutants are very likely to pre-exist in drug-naïve patients. However, if three or more point mutations are required for a resistant mutant to be able to maintain infection ($R'_0 > 1$) in the presence of a drug, then the likelihood that such mutants pre-exist is small (provided their basic reproductive ratio is significantly smaller than that of the sensitive virus in the absence of a drug).

If resistant virus does not pre-exist, we distinguish between two cases. If the drug is sufficiently potent to eradicate the sensitive virus, then a mutant that is unlikely to exist before therapy is also unlikely to be produced during therapy. If the drug cannot eradicate the wild-type, then, after sufficient time, resistant mutants will appear. However, mutants that are highly unlikely to pre-exist will take a long time to appear.

Taken together, these results suggest that viral resistance is a consequence of (i) either the drug not being sufficiently potent to eradicate the virus, or (ii) the presence of resistant mutants prior to treatment. In the latter case we expect resistance to appear rapidly after the start of therapy. In the former case resistance may only appear long after therapy is started.

S.B. and M.A.N. acknowledge support from the Wellcome Trust.

REFERENCES

- Anderson, R. M. & May, R. M. 1991 *Infectious diseases of humans*. Oxford University Press.
- Bonhoeffer, S., Coffin, J. M. & Nowak, M. A. 1997 HIV-drug therapy and virus load. *J. Virol.* **71**, 3275–3278.
- Boucher, C. A. B., Tersmette, M., Lange, J. M. A., Kellam, P., Degode, R. E. Y., Mulder, J. W., Darby, G., Goudsmit, J. & Larder, B. A. 1990 Zidovudine sensitivity of human immunodeficiency viruses from high-risk, symptom-free individuals during therapy. *Lancet* **336**, 585–590.
- Condra, J. H. & Schleif, W. A. 1995 *In vivo* emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature, Lond.* **374**, 569–571.
- Eron, J. J., Benoit, S. L., MacArthur, R. D., Santana, J., Quinn, J. B., Kuritzkes, D. R., Fallon, M. A. & Rubin, M. 1995 Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200–500 CD4+ cells per cubic millimeter. *N. Engl. J. Med.* **333**, 1662–1669.

- Foli, A., Sogocio, K. M., Anderson, B., Kavlick, M., Saville, M. W., Wainberg, M. A., Gu, Z. X., Cherrington, J. M., Mitsuya, H. & Yarchoan, R. 1996 *In vitro* selection and molecular characterization of human-immunodeficiency-virus type-1 with reduced sensitivity to 9-[2-(phosphonomethoxy)ethyl]adenine (pmea). *Antiviral Res.* **32**, 91–98.
- Gu, Z. X., Qing, G., Li, X. G., Parniak, M. A. & Wainberg, M. A. 1992 Novel mutation in the human-immunodeficiency-virus type-1 reverse-transcriptase gene that encodes cross-resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine. *J. Virol.* **66**, 7128–7135.
- Herz, A. V. M., Bonhoeffer, S., Anderson, R. M., May, R. M. & Nowak, M. A. 1996 Viral dynamics *in vivo*: limitations on estimates of intracellular delay and virus decay. *Proc. Nat. Acad. Sci. USA* **93**, 7247–7251.
- Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M. & Markowitz, M. 1995 Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature, Lond.* **373**, 123–126.
- Ho, D. D., Toyoshima, T., Mo, H. M., Kempf, D. J., Norbeck, D., Chen, C. M., Wideburg, N. E., Burt, S. K., Erickson, J. W. & Singh, M. K. 1994 Characterization of human-immunodeficiency-virus type-1 variants with increased resistance to a c-2-symmetrical protease inhibitor. *J. Virol.* **68**, 2016–2020.
- Larder, B. A. & Kemp, S. D. 1989 Multiple mutations in HIV-1 reverse-transcriptase confer high-level resistance to zidovudine (AZT). *Science* **246**, 1155–1158.
- Larder, B. A., Darby, G. & Richman, D. D. 1989 HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **243**, 1731–1734.
- Larder, B. A., Kellam, P. & Kemp, S. D. 1993 Convergent combination therapy can select viable multidrug-resistant HIV-1 *in vitro*. *Nature, Lond.* **365**, 451–453.
- Mansky, L. M. 1996 Forward mutation-rate of human-immunodeficiency-virus type-1 in a T-lymphoid-cell line. *Aids Res. Human Retroviruses* **12**, 307–314.
- Markowitz, M., Mo, H. M., Kempf, D. J., Norbeck, D. W., Bhat, T. N., Erickson, J. W. & Ho, D. D. 1995 Selection and analysis of human-immunodeficiency-virus type-I variants with increased resistance to ABT-538, a novel protease inhibitor. *J. Virol.* **69**, 701–706.
- May, R. M. & Anderson, R. M. 1979 Population biology of infectious diseases II. *Nature, Lond.* **280**, 455.
- McLean, A. & Nowak, M. 1992 Competition between zidovudine-sensitive and zidovudine-resistant strains of HIV. *AIDS* **6**, 71–79.
- Mellors, J. W., Im, G. J., Winkler, S. R., Medina, D. J., Dutschman, G. E., Bazmi, H. Z., Piras, G., Gonzalez, C. J. & Cheng, Y. C. 1993 A single conservative amino acid substitution in the reverse transcriptase of HIV-1 confers resistance to TIBO R82150. *Mol. Pharmacol.* **43**, 11–16.
- Nowak, M. A. & Bangham, C. R. M. 1996 Population-dynamics of immune-responses to persistent viruses. *Science* **272**, 74–79.
- Nowak, M. A., Bonhoeffer, S., Loveday, C., Balfe, P., Semple, M., Kaye, S., Tenant-Flowers, M. & Tedder, R. 1995 HIV-1 dynamics: results confirmed. *Nature, Lond.* **376**, 193.
- Nowak, M. A., Bonhoeffer, S., Shaw, G. S. & May, R. M. 1997 Anti-viral drug treatment: dynamics of resistance in free virus and infected cell populations. *J. Theor. Biol.* (In the press.)
- Perelson, A. S., Neumann, A. U., Markowitz, M., Leonard, J. M. & Ho, D. D. 1996 HIV-1 dynamics *in vivo*: virion clearance rate, infected cell life-span, and viral generation time. *Science* **271**, 1582–1586.
- Preston, B. D., Poesz, B. J. & Loeb, L. A. 1988 Fidelity of HIV-1 reverse transcriptase. *Science* **242**, 1168–1171.
- Richman, D. D. 1990 Zidovudine resistance of human-immunodeficiency-virus. *Rev. Infect. Dis.* **12**, S507–S512.
- Richman, D. D., Havlir, D., Corbeil, J., Looney, D., Ignacio, C., Spector, S. A., Sullivan, J., Cheeseman, S., Barringer, K., Pauletti, D., Shih, C. K., Myers, M. & Griffin, J. 1994 Nevirapine resistance mutations of human-immunodeficiency-virus type-1 selected during therapy. *J. Virol.* **68**, 1660–1666.
- Roberts, J. D., Bebenek, K. & Kunkel, T. A. 1988 The accuracy of reverse transcriptase from HIV-1. *Science* **242**, 1171–1173.
- Schinazi, R. F., Larder, B. A. & Mellors, J. W. 1996 Mutations in retroviral genes associated with drug resistance. *Int. Antiviral News* **4**, 95–107.
- Schinazi, R. F., Lloyd, R. M., Nguyen, M. H., Cannon, D. L., Mcmillan, A., Ilksoy, N., Chu, C. K., Liotta, D. C., Bazmi, H. Z. & Mellors, J. W. 1993 Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. *Antimicrobial Agents Chemotherapy* **37**, 875–881.
- Schuurman, R., Nijhuis, M., Vanleeuwen, R., Schipper, P., Dejong, D., Collis, P., Danner, S. A., Mulder, J., Loveday, C., Christopherson, C., Kwok, S., Sninsky, J. & Boucher, C. A. B. 1995 Rapid changes in human-immunodeficiency-virus type-1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). *J. Infect. Dis.* **171**, 1411–1419.
- Staszewski, S. 1995 Zidovudine and lamivudine – results of phase-III studies. *J. AIDS Human Retrovirol.* **10**, S57.
- StClair, M. H., Martin, J. L., Tudorwilliams, G., Bach, M. C., Vavro, C. L., King, D. M., Kellam, P., Kemp, S. D. & Larder, B. A. 1991 Resistance to DDI and sensitivity to AZT induced by a mutation in HIV-1 reverse-transcriptase. *Science* **253**, 1557–1559.
- Tisdale, M., Kemp, S. D., Parry, N. R. & Larder, B. A. 1993 Rapid *in vitro* selection of HIV-1 resistant to 3' thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc. Nat. Acad. Sci. USA* **90**, 5653–5656.
- Wei, X., Gosh, S. K., Taylor, M. E., Johnson, V. A., Emini, E. A., Deutsch, P., Lifson, J., Bonhoeffer, S., Nowak, M. A., Hahn, B. H., Saag, M. S. & Shaw, G. M. 1995 Viral dynamics in human immunodeficiency virus type-1 infection. *Nature, Lond.* **373**, 117–122.

Received 20 December 1996; accepted 8 January 1997