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Immune responses and the emergence of drug-resistant virus strains *in vivo*

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The treatment of viral infections using antiviral drugs has had a significant public health benefit in the setting of human immunodeficiency virus (HIV) infection, and newly developed drugs offer potential benefits in the management of other viral infections, including acute self-limiting infections such as influenza and picornaviruses (including the rhinoviruses that are responsible for a large proportion of 'common colds'). A serious concern with such treatments is that they may lead to the selection of drug-resistant strains. This has been a significant problem in the case of HIV infection. Existing mathematical-modelling studies of drug resistance have focused on the interactions between virus, target cells and infected cells, ignoring the impact of immune responses. Here, we present a model that explores the role of immune responses in the rise of drug-resistant mutants *in vivo*. We find that drug resistance is unlikely to be a problem if immune responses are maintained above a threshold level during therapy. Alternatively, if immune responses decline at a fast rate and fall below a threshold level during treatment (indicating impaired immunity), the rise of drug-resistant mutants is more likely. This indicates an important difference between HIV, which impairs immunity and for which immune responses have been observed to vanish during treatment, and viral infections such as influenza and rhinoviruses, for which such immune impairment is not present. Drug resistance is much more likely to be a problem in HIV than in acute and self-limiting infections.

Keywords: mathematical models; drug resistance; acute infection; HIV; rhinoviruses; common cold

1. INTRODUCTION

Drug resistance is a major concern in the treatment of some human infectious diseases. The most prominent example is human immunodeficiency virus (HIV). Strains that are resistant to one or several antiviral drugs have increased in frequency, and patients can become infected with resistant virus, rendering therapy ineffective (Richman 1994, 1996; Coffin 1995, 1996). A patient's resistant virus must have arisen in one of two ways: either they were infected with a drug-resistant strain or a resistant strain was generated during the course of their infection. In the former case, resistant virus must have been present (and at a sufficient level to allow for transmission) in another infectious individual. Understanding the transmission dynamics of a drug-resistant virus therefore requires knowledge of two processes: the generation and emergence of resistant viral strains over the course of an infection within an individual and the transmission of resistant strains between individuals. This study will address only the first of these questions; we shall not address epidemiological issues here.

For resistance to emerge over the course of an infection, drug-resistant strains can either have been generated before the onset of treatment or appear during therapy. Mathematical models of HIV infection have shown that the probability of generating a resistant variant before the onset of therapy is higher than the probability of generation once therapy has been initiated (Bonhoeffer & Nowak 1997; Ribeiro & Bonhoeffer 2000). This is because the chance of resistant virus variants being generated and growing is significantly reduced if the drugs efficiently inhibit viral replication. This argues for treating HIV infection early and aggressively. The probability of the emergence of drug-resistant variants during therapy is, however, greatly increased if the patient does not comply with the prescribed therapy regime (Wahl & Nowak 2000; Turner 2002; Volberding 2002).

Drugs are now also becoming available against other viruses, most notably the influenza virus (amantadine, rimantadine, zanamivir, oseltamivir) and rhinovirus (pleconaril; Hayden *et al.* 2003). While these diseases cause considerable morbidity and in some cases mortality, they are generally self-limiting: the immune response leads to resolution of the infection within one to two weeks. Drug therapy could, however, reduce the period of time during which the patient experiences symptoms and reduce the probability of secondary transmission. Drug treatment might be especially beneficial for those, such as the elderly or the immunocompromised, who are at high risk of developing the serious complications that often result from the infection. Alternatively, in view of the difficulties experienced with HIV antiviral therapy, there is concern that treating acute viral infections might lead to the growth and selection of resistant virus variants, and that this might become a public-health concern.

The evolutionary dynamics of drug resistance *in vivo* have been studied in the context of HIV infection by

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mathematical models (Frost & McLean 1994; Bonhoeffer *et al.* 1997; Bonhoeffer & Nowak 1997; Kepler & Perelson 1998; Ribeiro & Bonhoeffer 2000; Wahl & Nowak 2000). These studies have, however, taken into account only the viral dynamics (i.e. the interactions between virus, target cells and infected cells). Here, we study the effect of immune responses on the evolution of drug-resistant virus variants. This is important because HIV is characterized by specific impairment of immunity, whereas this is not the case for acute self-limiting viral infections such as those caused by the influenza virus or rhinovirus. We show that, in the presence of sustained immune responses, the *in vivo* growth of drug-resistant virus variants upon therapy is less likely. Alternatively, the model suggests that resistant virus readily rises if immune responses are not sustained above a threshold during treatment, as in the case of HIV infection.

The paper is structured as follows. We start by reviewing the basic model of viral dynamics and extend the model to include both wild-type virus and drug-resistant virus variants. We first analyse the evolution of drug-resistant strains during chronic infection since it allows us to gain analytical insights. This helps in the subsequent analysis of acute virus infection dynamics. We discuss our model with respect to HIV and rhinovirus infections.

2. DRUG THERAPY AND THE BASIC MODEL OF VIRAL DYNAMICS

The dynamics of drug therapy and resistance have been considered in the context of the basic model of viral dynamics (Frost & McLean 1994; Nowak & Bangham 1996; Bonhoeffer *et al.* 1997). Here, we add a general immune response, the exact identity of which is left open. It is assumed that the immune response expands in the presence of antigenic stimulation and inhibits viral growth. Thus, it could correspond to CD8 T-cell-mediated activity, B-cell or antibody responses, or CD4 T-cell responses leading indirectly to inhibition of viral replication. The model is given by the following set of differential equations:

$$\dot{x} = \lambda - dx - \beta xy;$$

$$\dot{y} = \beta xy - ay - pyz;$$

and

$$\dot{z} = F(y,z) - bz.$$

The variable x denotes the population of uninfected cells, y the population of infected cells and z the immune response. We make the standard assumption that viral turnover is fast relative to the turnover of infected cells, which implies that the viral population is in a quasi steady state with the number of infected cells. Uninfected cells are produced at rate λ, die at rate dx and become infected by virus at rate βxy. The parameter β is a compound parameter, reflecting the rate at which target cells are infected by free virus particles, the rate at which infected cells produce free virus and the lifespan of free virus particles. For simplicity, we call this parameter the replication rate of the virus. Infected cells die at rate ay and are further reduced by the immune response at rate pyz. The immune response expands in the presence of antigenic stimulation. This expansion term is described by the function $F(y,z)$, which can take a variety of forms (De Boer & Perelson 1998; Wodarz *et al.* 2001). The most appropriate way to describe immune expansion is currently unclear, and might also differ between different branches of the immune response. In the absence of this detailed information, we instead employ a simple description of immune expansion, namely straightforward proliferation of the immune cells, i.e. $F(y,z) = cyz$. The detailed form of this term is relatively unimportant for the qualitative nature of the results derived in the following text. Finally, in the absence of antigenic stimulation, the immune response decays at a rate bz.

Note that most of the parameter values that appear in our model are currently unknown. Even for HIV infection, which has received considerable attention, only a few parameters have been measured. No parameter values have been measured in the context of most other viral infections, including the rhinovirus and influenza virus settings that are of interest here. This does not, however, compromise the insights that are gained by analytical approaches.

The behaviour of the system depends on the basic reproductive number of the virus, R_0, which gives the average number of newly infected cells produced by one infected cell at the beginning of the infection. In terms of the parameters of the model, R_0 equals $\beta\lambda/da$. If R_0 is less than 1, then the viral population cannot grow within the host because one infected cell gives rise, on average, to less than one newly infected cell. If, on the other hand, R_0 is greater than 1, then the viral population grows within the host and can establish an infection. In the absence of an immune response the system converges to equilibrium EQ1: $x^{(1)} = a/\beta$; $y^{(1)} = \lambda/a - d/\beta$; $z^{(1)} = 0$. If $cy^{(1)} > b$, the immune response reacts and reduces the viral population. The system then converges to equilibrium EQ2: $x^{(2)} = \lambda c/(dc + b\beta)$; $y^{(2)} = b/c$; $z^{(2)} = (\beta x^{(2)} - a)/p$.

Drug therapy is included in the model by reducing the replication rate of the virus to β', and therefore the basic reproductive ratio of the virus to $R_0' = \beta'\lambda/da$. If the drugs are potent enough, the replication rate of the virus will be sufficiently reduced that R_0' falls below the threshold value of 1, leading to eradication of the virus. If the drugs are less potent, so that R_0' remains greater than 1, the viral population will be suppressed to a certain degree but will not be driven to extinction.

3. WILD-TYPE VERSUS DRUG-RESISTANT VIRUS

To study the dynamics of resistance in the absence and presence of drug therapy, we expand the model in § 2 to include two viral populations: the drug-susceptible or wild-type viral population, y_w; and the drug-resistant viral population y_r. We define drug resistance by the relative replication rates of the two strains during therapy. In the presence of the antiviral drug, we assume that the replication rate of the resistant strain, β_r', is greater than that of the wild-type strain, β_w', i.e. $\beta_r' > \beta_w'$. Furthermore, we assume that the changes required for resistance carry a fitness cost, so that, in the absence of therapy, the replication rate of the resistant strain, β_r, is less than the replication rate of the wild-type virus, β_w. Notice that the basic reproductive numbers of the wild-type and resistant

viruses in the absence and presence of therapy can be written in terms of these replication rates: these quantities are denoted by $R_0^{(w)}$, $R_0^{(r)}$, $R_0^{(w)\prime}$ and $R_0^{(r)\prime}$.

We assume that both viral strains are recognized by the same immune responses, i.e. immunity is cross-reactive between the two strains. Assuming that, during the course of normal wild-type viral replication, virus variants that are resistant to the drug arise at a rate μ, and ignoring back mutation from the resistant strain to the wild-type strain, the model is given by the following set of differential equations:

$$\frac{dx}{dt} = \lambda - dx - x(\beta_w y_w + \beta_r y_r);$$

$$\frac{dy_w}{dt} = \beta_w x y_w(1 - \mu) - a y_w - p y_w z;$$

$$\frac{dy_r}{dt} = \beta_r x y_r - a y_r - p y_r z + \mu \beta_w x y_w;$$

and

$$\frac{dz}{dt} = cz(y_w + y_r) - bz.$$

In §§ 4–6 we analyse this model. We first investigate the conditions under which drug-resistant strains can grow to significant levels, assuming that they have already been generated. We first focus on the dynamics of the system during the chronic phase of infection as this enables analytical insights to be gained. We then apply these results to acute infections. Finally, we consider the probability of a resistant mutant being generated, examining the importance of the time at which treatment is started and the strength of the immune responses present at that time.

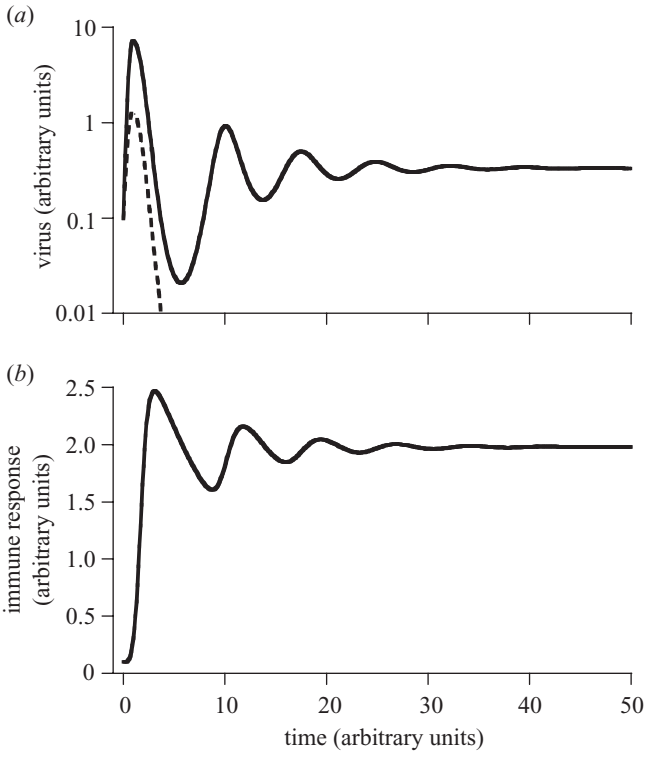

Figure 1. Simulation showing the infection dynamics of wild-type and resistant virus in the absence of antiviral drugs. (*a*) The wild-type virus outcompetes the resistant strain and elicits an immune response. (*b*) The immune response suppresses viral load and settles at a memory level. Parameters were chosen as follows: $\lambda = 1$; $d = 0.1$; $a = 0.2$; $p = 1$; $c = 0.3$; $b = 0.1$; $\beta_w = 0.8$; $\beta_r = 0.5$. Solid line, wild-type; dashed line, drug-resistant mutant.

4. CHRONIC-INFECTION DYNAMICS

We consider the competition dynamics between wild-type virus and resistant strains during chronic infection. We first assume that resistant strains already exist, and so ignore the generation of new virus variants for now (this corresponds to setting $\mu = 0$). In the absence of drugs, the replication rate of the wild-type virus is faster than that of the resistant strain ($\beta_w > \beta_r$). This means that the wild-type virus is the superior competitor and will eventually take over the population. Assuming the presence of an immune response, the infection will converge to equilibrium EQ3: $x^{(3)} = \lambda c/(dc + b\beta_w)$; $y_{(w)}^{(3)} = b/c$; $y_{(r)}^{(3)} = 0$; $z^{(3)} = (\beta_w x^{(3)} - a)/p$. These dynamics are shown in figure 1.

When $\mu > 0$, mutation will lead to the creation of resistant virus, but, as these are outcompeted by the wild-type strain, the resistant virus will only ever be found at low levels (mutation–selection balance).

When therapy is applied, the resistant virus has, by definition, a significant replicative advantage and so can outcompete the drug-sensitive virus. In the absence of an immune response, the prevalence of the resistant strain can increase after the start of therapy if its basic reproductive ratio is greater than 1 ($R_0^{(r)\prime} > 1$). Note that this assumes that, upon suppression of the wild-type virus, the number of susceptible target cells, x, rebounds to pre-infection levels. This should be the case for most infections; a delay of this rebound can lead to a delay in the rise of the drug-resistant virus.

The situation is, however, different in the presence of an immune response. Before therapy starts, the immune response is at an equilibrium level. This immune response can counter the growth of the resistant strain during treatment. First, consider the immediate dynamics after the onset of treatment. The resistant strain can grow if $\beta_r'\lambda/d - a - pz^{(3)} > 0$, where $z^{(3)}$ is the equilibrium level of the immune response before therapy. (Note that this assumes that the level of the immune response remains constant for some time during therapy and, as before, that the number of susceptible host cells returns to pre-infection levels at a relatively fast rate upon therapy.) This condition can be rewritten as $R_0^{(r)\prime} - 1 - pz^{(3)}/a > 0$, showing that the condition for growth of the resistant strain is more stringent than in the absence of the immune response.

Substituting the expression for $z^{(3)}$, the condition for the growth of resistant virus becomes $\beta_r' > \beta_w/(1 + b\beta_w/dc)$. This means that the replication rate of the resistant strain during therapy, β_r', must lie above a threshold, which is given by the product of the wild-type replicative rate, β_w, and the term $F = 1/(1 + b\beta_w/dc)$. This threshold condition depends on a number of parameters, but we shall see that a simplification occurs in a limiting case, which we shall examine in detail.

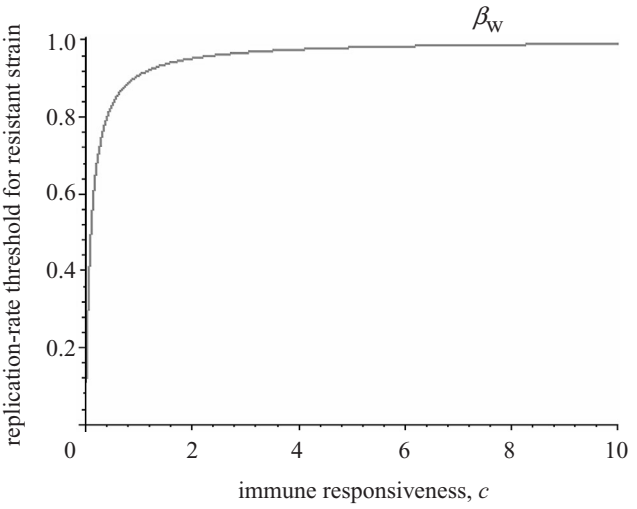

Figure 2. The replication-rate threshold for the resistant virus, beyond which it can emerge, as a function of the immune responsiveness, c. The threshold is an asymptotic function of the parameter c, and approaches the replication rate of the wild-type virus in the absence of antiviral drugs, β_w. Parameters were chosen as follows: $\lambda = 1$; $d = 0.1$; $b = 0.01$; $\beta_w = 1$.

We notice that the threshold for the replication rate of the resistant strain during therapy, β_r', is an asymptotic function of the immune responsiveness, c (figure 2), and at the asymptote, the value of this threshold is β_w. At this limit, in the presence of an immune response, the condition for the rise of resistance becomes $\beta_r' > \beta_w$. In other words, the replication rate of the resistant virus variant in the presence of therapy needs to be higher than the replication rate of the wild-type virus in the absence of therapy. This is most unlikely to be the case since the changes necessary for drug resistance typically carry a cost (Back *et al.* 1996) and hence $\beta_r < \beta_w$. Growth of resistant strains during this initial phase of therapy, therefore, requires that $\beta_r' > \beta_r$: the replicative ability of the resistant virus in the presence of therapy would have to be larger than its replicative ability in the absence of therapy, which seems quite unlikely. The substantial inhibition of the growth of the resistant strain results from the immune response, the level of which is strongly determined by the replication rate of the wild-type virus.

As we do not know the value of the parameter c, we cannot be sure that this limiting case is applicable to a given infection. Given the form of figure 2, we notice that, provided that the immune responsiveness, c, is not within a narrow parameter region where immunity is close to extinction, the simplified expression of the previous paragraph provides a reasonable estimate (although always an overestimate) of the threshold condition. The condition for the growth of resistant virus in this case is, therefore, slightly less stringent than that just discussed, and so growth of the resistant strain is possible if its replicative ability during therapy is close to that of the wild-type in the absence of therapy. Growth of resistance in this case is therefore possible but can happen only if the cost of resistance is very small. When the value of c lies on the left-most part of the curve in figure 2, resistant virus will grow more easily, but we suggest that this corresponds to a situation in which the immune response is relatively inefficient in controlling the level of virus. The exact value of the immune responsiveness, c, is likely to be important only in a region where the above simplification does not hold, i.e. if viral replication is much faster than immune reactivity.

The form of figure 2 suggests that the growth of resistant virus during therapy is not strongly dependent on the strength of the immune response over wide parameter regions. Hence, even if the immune response is not so strong and a persistent infection is established, the above conditions suggest that resistance will be of little consequence when therapy is started. Therefore, the wild-type virus can suppress the resistant strain by two mechanisms. In the absence of therapy, the wild-type virus outcompetes the resistant strain. In the presence of therapy, the resistant strain can be suppressed by the immune response, which was developed against the wild-type virus. In ecology, such indirect exclusion, mediated through a shared enemy, is referred to as apparent competition (Holt 1977).

As pointed out already, this argument holds for the time immediately following the start of treatment. The long-term dynamics depend on the kinetics of the immune responses during therapy. An effect of therapy is the reduction of viral load and thus antigenic stimulation. It is thought that immune responses can persist in the long term and decline at only a very slow rate in the absence or at low levels of antigenic stimulation. This can be referred to as memory (Slifka & Ahmed 1998; Slifka *et al.* 1998; Murali-Krishna *et al.* 1999). In terms of our model, it is represented by a low value of the parameter b. If the value of b is higher, the level of immune response declines faster after a reduction of antigenic stimulation. This can correspond to impaired memory. A discussion of the role of memory in antigen persistence versus clearance is given in Wodarz (2001).

We can distinguish two scenarios as follows.

(i) If immune responses decline at only a very slow rate upon reduction of antigenic stimulation, they will not decline significantly during the phase of treatment and will remain above a threshold level. Hence, resistant mutants will not be able to grow during this time, even if the relevant mutants have been generated. (Note that, even if the value of b is very low, the level of immunity will eventually fall to low levels as long as $b > 0$; this, however, will take a very long time and is thus unlikely to occur during the phase of treatment).

(ii) If immune responses decline at a faster rate upon reduction of antigenic stimulation, then resistant virus variants will be able to grow once immunity falls below a threshold value, given by $z_{\text{threshold}} = (1/ap)(R_0^{(r)\prime} - 1)$. The higher the fitness cost to the resistant virus variant relative to the wild-type virus, the lower this threshold level of immunity below which the resistant strain will grow. The faster immunity declines during therapy, the faster the drug-resistant virus variants rise after the start of treatment (figure 3). A fast decline of immunity at low levels of antigenic stimulation might indicate that the immune responses are somehow impaired, leading to defective generation of immunological memory (e.g. owing to a deficiency in CD4-cell help). These notions have also been explored before (Wodarz 2001).

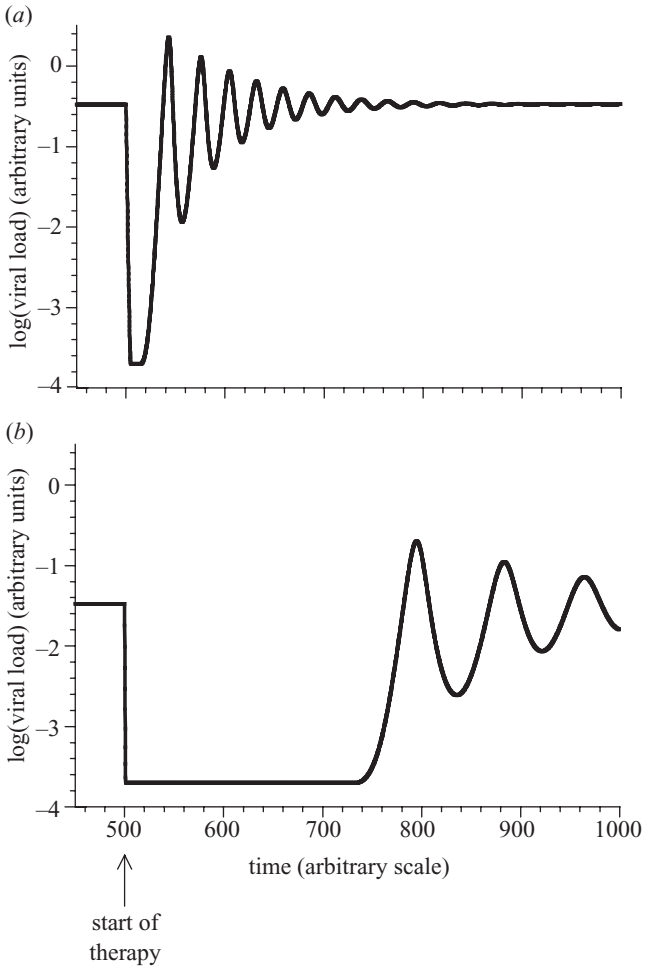

Figure 3. Viral dynamics during treatment of chronic infection. In (*a*) it is assumed that the immune response is relatively short-lived in the absence of antigen. As discussed in § 4, resistant virus emerges relatively quickly. In (*b*) it is assumed that the immune response has a longer lifespan in the absence of antigen. Resistant virus remains suppressed until the response has fallen below a threshold, which occurs after a longer period of time. If the response is sufficiently long-lived in the absence of antigen, resistant virus is expected to remain suppressed for the duration of treatment. Parameters were chosen as follows: $\lambda = 1$; $d = 0.1$; $a = 0.2$; $p = 1$; $c = 0.3$; $\beta_w = 0.8$; $\beta_r = 0.08$; $\beta'_w = 0.0201$; $\beta'_r = 0.08$. For (*a*) $b = 0.1$ and for (*b*) $b = 0.01$.

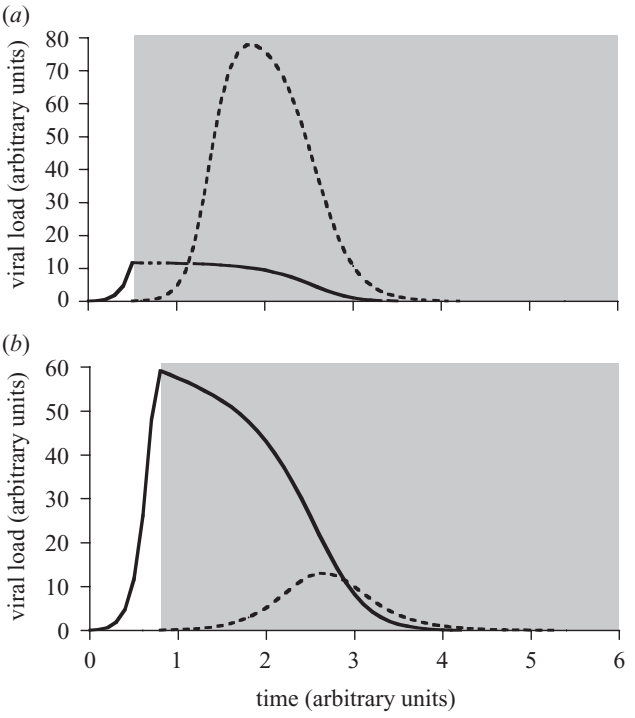

Figure 4. Dynamics of wild-type and resistant viral strains during the treatment of an acute infection, assuming that resistant virus pre-exists. (*a*) If treatment is started early, the resistant virus variant can grow. This is because immunity has not yet expanded above a threshold level (immune responses not shown). (*b*) If treatment is started when the wild-type virus has already reached higher levels and has thus already induced some immunity, the peak load of the resistant virus is low. Parameters were chosen as follows: $\lambda = 10$; $d = 0.1$; $a = 0.2$; $p = 1$; $c = 0.05$; $b = 0.01$; $\beta_w = 0.1$; $\beta_r = 0.09$; $\beta'_w = 0.0021$; $\beta'_r = 0.09$. Solid line, wild-type; dashed line, drug-resistant mutant. Shaded areas represent treatment.

5. ACUTE-INFECTION DYNAMICS

We now apply the analytical insights gained from the examination of drug therapy in the equilibrium situation to the acute-infection setting. In self-limiting acute infections, the virus is normally cleared by the immune system after the onset of symptoms. As our model is deterministic, we do not observe absolute clearance in the simulations, but rather a reduction of viral load to very low levels. Hence, we assume that the infection is cleared once viral load has been reduced below a threshold value. As before, we first assume that the resistant virus variant pre-exists in the viral population at the start of infection; we defer the question of the probability of resistant mutants being generated for now.

Upon introduction of infection, the immune response in the model first rises to a peak before declining and then oscillating towards an equilibrium level. The immune response can prevent the growth of drug-resistant strains if the level of immunity lies above a threshold level, as discussed in § 4. The exact level of this threshold is different from the expression presented for the chronic-phase scenario and is difficult to obtain because the level of uninfected cells is constantly changing during this period of time. The general properties of this threshold, however, remain the same. As before, the higher the cost of resistance, the lower this threshold level.

Whether resistant virus can grow during treatment, therefore, depends on the level of the immune response that has developed by the time therapy is started (figure 4). If treatment is started after the time at which the immune response reaches the threshold level, immunity can block the growth of the resistant mutant.[1] Alternatively, if treatment is started too early then the drug-resistant virus can grow as the immune response is not yet strong enough to suppress the strain. In this case, the resistant virus replicates to a peak and subsequently declines, as immunity builds up in response to the growth of the resistant virus. The peak abundance of the resistant strain is largely determined by the level of immunity that the wild-type virus has generated before the onset of therapy. The earlier therapy is

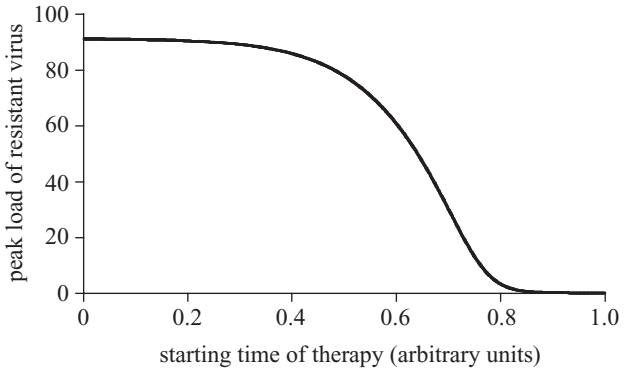

Figure 5. The peak load of resistant virus (arbitrary units) as a function of the time at which treatment is started. If treatment is started very early, before the immune response has developed sufficiently, the growth of the resistant strain is not significantly inhibited by the immune response, and the peak load is high. The later therapy is started, the more the wild-type virus will have grown, and the higher the level of immune expansion that has occurred. Hence, the peak load of resistant virus becomes lower, until the resistant virus entirely fails to grow. Parameters were chosen as follows: $\lambda = 10$; $d = 0.1$; $a = 0.2$; $p = 1$; $c = 0.05$; $b = 0.01$; $\beta_w = 0.1$; $\beta_r = 0.09$; $\beta'_w = 0.0021$; $\beta'_r = 0.09$.

started, the lower the level of immunity that the wild-type virus will have generated, and the higher the peak of the resistant virus variant (figures 4 and 5).

These arguments assume that the immune responses decline at only a very slow rate once wild-type viral load has been reduced by drugs (low value of *b*). If immunity declines at a relatively fast rate when the wild-type virus is suppressed by therapy (higher value of *b*), the resistant virus variant can grow once immunity has fallen below the threshold level. As explained in § 4, this might occur in the presence of impaired immune responses.

A note of caution: the exact mechanisms by which acute immune responses are generated are not known. In the context of cytotoxic T lymphocyte (CTL) responses, the idea of programmed proliferation has received some attention recently (Kaech & Ahmed 2001; Van Stipdonk *et al.* 2001). We have used a relatively simple model, which includes the same term for immune expansion as does the chronic-infection model. As we consider immune-response dynamics in general (which can include CTLs, antibodies or CD4 cells), we have chosen to describe immune expansion is this simplistic way. The results considered here should not, however, depend on such details. They depend only on the well-documented assumption that immunity rises in response to wild-type infection; furthermore, immunity has to attain significant levels to suppress the resistant mutants.

6. THE PROBABILITY OF GENERATING RESISTANT VIRUS VARIANTS

In §§ 3–5, we assumed that the drug-resistant virus variants pre-existed in viral populations, and investigated the conditions under which they could grow to appreciable levels. Here, we explore the probability of generating a resistant virus variant both before and during therapy, assuming the presence of a normal immune response and assuming that only drug-sensitive viral strains are present

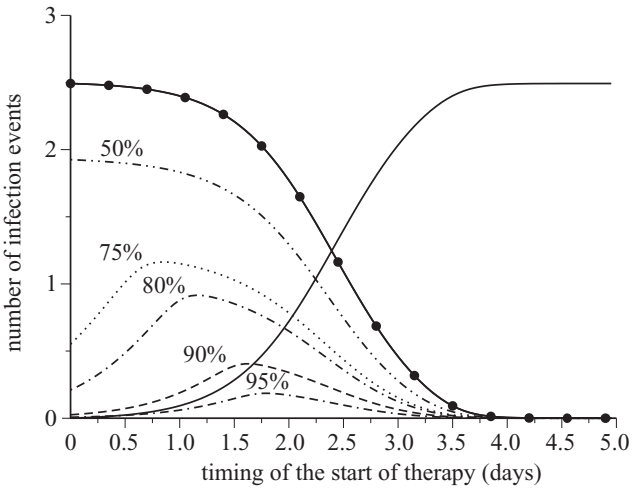

Figure 6. The chances of generating a resistant strain as a function of the time when therapy is started. As discussed in § 6, the chance of generating a resistant strain is proportional to the number of infection events occurring. At each time point, the solid curve shows the total number of infection events that have occurred up to the given time during the course of an untreated infection. The solid curve with circles denotes the complementary quantity, namely the number of infection events that occur after the given time in an untreated infection. The remaining broken curves show the number of infection events that are seen during the course of therapy as a function of the time at which therapy is started. These curves are shown for a range of treatment efficacies (from the bottom to the top of the figure the dashed curves represent 95%, 90%, 80%, 75% and 50% effective therapies, respectively). Other parameters were taken as follows: $\lambda = 0.25$; $d = 0.1$; $\beta = 1.0$; $a = 0.25$; $c = 4.0$; $p = 4.0$; $b = 0.1$.

initially. The chance of generating a resistant strain is proportional to the number of replication cycles for the sensitive strain, because each replication cycle provides an opportunity for a genetic change to occur, which might lead to resistance. The relative chance of generating a resistant mutant before and during therapy can, therefore, be estimated by calculating the number of infection events that occur before and during therapy.

In the absence of therapy, the chance that a resistant virus variant is generated, as expected, increases with time because the virus continuously replicates (figure 6, solid curve). A plateau is reached at *ca.* 4 days, corresponding to the time at which the immune system clears the virus in this simulation. (Notice that an alternative way of presenting the same information is to plot the number of infection events that occur after a given time point in the absence of treatment: figure 6, solid curve with circles.) Therefore, the later therapy is started the higher the chance that resistant strains have been generated before the start of treatment.

In the presence of therapy, the number of infection events that occur over the course of therapy will depend on the treatment efficacy (more effective treatment leads to greater suppression of the virus and hence fewer replication events) and the timing of therapy initiation (as both viral load and the strength of the immune response vary during acute infection). Figure 6 (broken curves) shows that the chance of generating a resistant virus from the wild-type virus during therapy generally decreases with

increasing treatment efficacy. When treatment is sufficiently effective, imposition of therapy causes the levels of the virus to fall immediately: the number of infection events during such therapy roughly reflects the viral load at the start of treatment. (The broken curves representing 90% and 95% effective therapies resemble the time-course of viral load during an untreated infection.) As a consequence, the probability of generating resistance during therapy is greatest if treatment is started near the peak of infection.

When therapy is less effective, levels of the virus can still increase despite treatment (although at a lower rate than in the absence of treatment), and so the course of infection follows the typical pattern seen in the absence of treatment, albeit at a somewhat blunted level. In these cases, the probability of generating resistance during treatment follows a pattern similar to that of the number of infection events after a given time point in the untreated case, and so is a decreasing function of time. (Notice that the broken curve depicting 50% effective therapy in figure 6 has a similar shape to the solid curve with circles showing the number of infection events after a given time-point in the untreated case.)

Notice that the discussion in this section merely considers the chance of a resistant mutant being generated; it does not address the issue of whether such a mutant would be able to grow to appreciable levels. A complete understanding of the evolution of resistance requires both considerations to be taken together, but this is beyond the scope of this study.

7. DISCUSSION AND CONCLUSION

Using mathematical models, we have shown that the ability of drug-resistant virus variants to rise can crucially depend on the quality of the immune response established. The model suggests that the rise of resistance is unlikely if immune responses are maintained above a threshold level during therapy. Alternatively, if immune responses fall rapidly below a threshold during treatment, a drug-resistant virus will grow. This result requires the following assumptions: there is immunological cross-reactivity between wild-type virus and resistant virus variant; the mutation conferring resistance carries a cost; and immunity expands in response to antigenic stimulation. If therapy is started very early, before sufficient immunity has been established, drug-resistant strains are more likely to grow if they have been generated. The probability that they have been generated is, however, very low in this case, as the early start of therapy limits the number of replication events and hence gives the virus little chance to evolve.

These results suggest that there is an important difference in the evolutionary dynamics of drug resistance between chronic infections (such as HIV) and acute self-limiting infections (such as influenza or rhinovirus). In HIV infection, specific immunity is impaired by the virus (Kalams & Walker 1998; Kalams *et al.* 1999*a*). HIV-specific CD4 T-cell responses have been shown to be absent or present at impaired levels even early in the infectious process (Rosenberg *et al.* 2000). This can have various consequences for the dynamics of the specific effector branches of the immune system. CD8 T-cell responses are thought to be particularly important in limiting HIV replication. It has been observed that, upon starting drug therapy, the levels of CTL precursors and effectors drop to low levels at a relatively fast rate (Kalams *et al.* 1999*b*). Thus, the CTLs do not seem to be maintained at reduced levels of antigenic stimulation during treatment. According to our model, this is the condition required for the rise of drug-resistant virus. A note of caution: it has not been directly established whether this decline of immunity during treatment is the effect of immune impairment; a recent discussion is given in Wodarz (2001). Alternatively, a defect in immune responses is not observed with acute viral infections caused by influenza virus or rhinovirus. Hence, we conclude that, in contrast to HIV infection, drug-resistant strains are unlikely to grow *in vivo* with influenza or rhinoviruses.

Resistance studies of viral infections that are not immunosuppressive support this line of argument. An example is the use of acyclovir to treat herpes simplex viral infections. Epidemiological studies reported that the prevalence of resistance is significantly higher in populations of immunocompromised individuals than in immunocompetent individuals. Christophers *et al.* (1998) found resistant strains in 6% of immunocompromised patients, while among the immunocompetent patients, the prevalence of resistant strains was 0.1–0.6%. Englund *et al.* (1990) showed that 4.7% of immunocompromised patients carried resistant virus, while none of the immunocompetent patients tested positive for resistant strains. A variety of people can be immunocompromised, including those infected with immunosuppressive pathogens, bone-marrow-transplant patients and the elderly. While this group of patients is most susceptible to developing and transmitting resistance, they are the group that might benefit most from therapy. If this group of patients is only a small fraction of patients that are treated with an antiviral drug, the spread of resistance in the population might not be significant. An epidemiological-level model could prove useful in the assessment of this problem, addressing the trade-off between the individual-level benefit of treatment and the population-level problem of the emergence and spread of resistance.

We conclude by discussing a specific case study: the treatment of picornaviruses with pleconaril (Yasin *et al.* 1990; Groarke & Pevear 1999; Pevear *et al.* 1999; Hayden *et al.* 2003). Picornaviruses, which include enteroviruses and rhinoviruses, are the most ubiquitous pathogens of humans. Enteroviruses are associated with a number of human diseases including viral meningitis, encephalitis and respiratory infections, while rhinoviruses are the most frequent cause of upper respiratory infections. Pleconaril is a small-molecule drug that inhibits the capsid function of enteroviruses and rhinoviruses. The drug integrates into the viral capsid at a hydrophobic site in the VP1 protein. Consequently, viral attachment to cells and uncoating of the virus are blocked. This prevents the release of viral RNA and replication of the virus.

Under specific *in vitro* conditions, various drug-resistant variants have been identified. In all cases, resistance required amino acid substitutions in the drug-binding pocket of VP1. Experiments have shown that the resistant strains are significantly less stable than the wild-type drug-susceptible virus. They have also been observed to have a

reduced ability to replicate, resulting in lower viral loads, less virulence and less pathology (Yasin *et al.* 1990; Groarke & Pevear 1999). This indicates that the changes required for resistance are characterized by a significant fitness cost. The molecular analysis of the changes present in the resistant virus also suggests that these changes will not lead to differences in immunogenicity. Hence, the wild-type virus and resistant virus are likely to be recognized by the same immune responses (cross-reactivity).

Resistant virus has been recovered from a small fraction of patients treated with pleconaril in clinical trials (F. Hayden, personal communication, and see http://www.fda.gov/ohrms/dockets/ac/02/briefing/3847b1_01_viropharma.pdf). The mere presence of resistant virus, however, does not mean that it was ever present at a high enough level to enable transmission. It is also not clear whether resistance was confined to certain subgroups of the population (e.g. the elderly) who may have had less vigorous immune responses for some reason.

Given that unimpaired immune responses develop against this infection, resulting in sustained immunity and memory, our model suggests that treatment should have a public-health benefit, and that the rise of drug-resistant virus *in vivo* is unlikely to present a significant problem. As resistant virus is unlikely to grow to high levels *in vivo*, these strains are not expected to spread on an epidemiological level. The implications of drug resistance for rhinoviral therapy have been investigated on an epidemiological level in a separate study (Lloyd & Wodarz 2004). As mentioned earlier, however, the problem of treating the immunocompromised has to be considered in more detail.

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ENDNOTE

[1]Please note that the immune-response dynamics can show prolonged oscillations in the model. This means that once the response has risen above the threshold, it can subsequently fall below it temporarily. The occurrence of these oscillations is, however, highly model dependent, and experimental data on immune responses do not tend to show extensive oscillations. We do not place much significance, therefore, on the oscillations observed in the model.

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