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Defective Escape Mutants of HIV

RICHARD M. BERRY† AND MARTIN A. NOWAK

Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

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The virological literature presents two broad types of defective virus mutants that can alter the outcome of viral infection. In some infections, defective interfering particles reduce the replication of wild-type virus and lead to an attenuated or persistent infection. In other cases, very specific and highly pathogenic defective mutants lead to virulent disease in the presence of a much less pathogenic but replication-competent helper virus.

Here, we outline the theoretical possibility that defective mutants of HIV, which escape from some of the immune responses directed at the wild-type virus, can have a positive effect on total virus growth in HIV infections. The high error rate of HIV may generate many mutants that have some altered epitope (escape mutants), but at the cost of greatly reduced or completely impaired reproductive abilities. If these mutants retain some ability to impair immune cell function, then the production of such "defective escape" mutants may enhance overall virus reproduction. This will be illustrated by a mathematical model.

1. Introduction

One of the striking features of the human immunodeficiency virus (HIV) is its high genetic variability. This follows from the inaccuracy of the process of viral replication. Reverse transcription of viral RNA into DNA introduces errors at a rate of the order of $>10^{-4}$–10^{-5} per base per cycle (Preston *et al.*, 1988; Roberts *et al.*, 1988), which implies a mutation rate of around one base per generation for the HIV genome of length $\simeq 10^4$ bases. This means that the probability that there are no errors during reverse transcription of the whole genome is less than about 0·4. Errors are also likely to occur during the production of new viral RNA from proviral DNA owing to the lack of a proofreading function in the host-cell RNA polymerase, and further mutation may occur as a result of recombination during the process of reverse transcription. These high mutation rates lead to the generation of many different variants during individual infections, which is likely to enable the virus population to evolve away from current immunological attack or to evolve resistance to drug treatment. Intra-host virus evolution may explain the long and variable asymptomatic period between HIV infection and development of AIDS (Nowak *et al.*, 1990, 1991; Nowak, 1991; Nowak & May, 1993).

The high mutation rate of HIV must also generate many mutants which are defective in the sense that they lack the ability to infect new host cells or to replicate in infected cells. The abundance of defective HIV mutants both *in vitro* and *in vivo* is confirmed by many different studies, a few of which are mentioned below. Goodenow *et al.* (1989) sequenced regions of the *gag* and *env* genes from infected peripheral blood mononuclear cells (PBMCs) and found high levels of base deletions, duplications and substitutions. In one isolate 15% (3/20) of sequences of a region covering only 0·6% of the HIV genome carried a stop codon, rendering them defective. Meyerhans *et al.* (1989) found that five out of 22 proviral *tat* genes in sequential isolates from a single patient contained stop codons or frameshift errors. They also demonstrated, by co-transfection of a plasmid containing a gene

†Current address: Department of Molecular and Cellular Biology Harvard University, 16 Divinity Avenue, Cambridge MA 02138, U.S.A.

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under the control of the *tat* protein, that nine out of 35 sequences were functionally defective, owing to stop codons, frameshift errors or amino acid substitutions. The *tat* gene represents only 2·3% of the total HIV genome. The above results, which suggest that the vast majority of HIV genomes are defective, may overestimate the incidence of lethal mutations as a result of *Taq* polymerase errors in the experimental procedure. Other reports (Balfe *et al.*, 1990; Brinchmann *et al.*, 1991) give more moderate estimates for levels of defective virus, but still suggest that something like half of all HIV genomes in PBMCs are defective. Li *et al.* (1991) found that only four out of 10 HIV-1 genomes cloned from unintegrated circular DNA in the brain tissue of an AIDS patient were complete, and that only one of these was able to replicate in donor lymphocytes or immortalized T-cell lines. Boulerice *et al.* (1990) cultured 18 single-cell-based clones of an infected monocyte cell-line and found that, while all 18 were positive for p24 antigen, five showed no reverse transcriptase (RT) activity and three of the four RT-positive clones tested were unable to infect other cell lines.

We might ask whether such high levels of defective virus are simply the unavoidable cost of viral mutation and evolution, or whether they may in fact play an important role in viral infections. At the most basic level, a virus producing defective mutants at too high a rate may be unable to sustain a self-replicating population, leading to the concept of an "error threshold" for the mutation rates (Eigen & Schuster, 1979). More specific roles for defective viruses have been postulated, a summary of which can be found in Holland (1990). Chief among these are so called "defective interfering" (DI) particles first postulated by Huang & Baltimore (1970). DI particles are defective viruses, which can only reproduce in the presence of helper virus, usually the wild type from which they are derived. They interfere specifically with the intracellular processes of the wild type, most commonly at the level of viral replication, with the typical result that the yield of non-defective virus is reduced. Once the DI strain appears it begins to replicate together with the standard virus and eventually becomes the predominant component in the viral progeny. It is believed that DI particles may play an important part in the persistence of viral infections and the evolution of viral pathogenesis, and their existence and properties are well documented *in vitro* (Bangham & Kirkwood, 1990). Despite this, it has proved difficult to demonstrate cases where virus disease and multiplication are being modulated by DI virus *in vivo* (Barratt & Dimmock, 1986). Defective viral genomes have also been shown to harbour highly pathogenic sequences that lead to virulent disease in the presence of a related replication-competent virus—for instance in the case of immunodeficiency-inducing feline (Overbaugh *et al.*, 1988) and murine (Aziz *et al.*, 1989; Chattopadhyay *et al.*, 1989) leukaemia viruses.

All of the above effects depend upon mutant sequences that have specific abilities to alter viral or host cell activity not possessed by the standard virus. Here, we propose that immunopathogenic viruses, such as HIV, may produce large numbers of defective mutants that carry some unspecific mutation that destroys (or reduces) their replication ability, but which retain some of the immunopathogenic properties. It is not known exactly how HIV damages the immune system of its host, but many of the possible mechanisms (Weiss, 1993) do not require viral replication in infected cells. If the mutation also allows the mutants to escape recognition by the immune response to the wild type, they may accumulate to high levels and thus increase the chances of viral success by attacking the host's immune system. In this paper we present a simple mathematical model that demonstrates the possibility of this new role for defective viral mutants. We will show that the generation of such defective mutants can be beneficial to the wild-type strain and can enhance overall virus replication, under circumstances that are characterized in the paper.

Defective escape mutants may represent a successful viral strategy because their lack of replication means that any particular variant will exist in vanishingly small quantities, and thus will not generate a significant specific immune response against itself. This allows a cluster of defective offshoots of a replicating strain to build up to levels where their ability to impair the host's immune system is a significant advantage to the wild-type virus. Throughout the paper we shall use the term "escape mutant" for escape from recognition by some of the current immune responses. A "defective escape mutant" is thus a mutant which escapes immune recognition, but has lost the ability to complete a full reproductive cycle. Such mutants are expected to occur frequently because of the high mutation rate. In this paper we show that they may have an effect on HIV persistence and pathogenesis.

2. Mathematical Model

2.1. TOTAL ESCAPE, UNCHANGED IMMUNE SUPPRESSION

First, we consider the simplest case of defective mutants that escape from the current helper-dependent immune responses, but retain the capacity to

impair CD4$^+$ cell function. The model has three variables. The population of replicating virus is denoted by v_1, non-replicating escape mutants which retain the ability to impair immune function by v_2, and the helper-dependent immune response specific to the virus by X. We may then write

$$\dot{v}_1 = v_1(rQ - d - pX) \quad (1)$$

$$\dot{v}_2 = v_1 r(1 - Q) - v_2 d \quad (2)$$

$$\dot{X} = kv_1 - uvX. \quad (3)$$

Here r is the rate at which the wild-type v_1 produces offspring, which are either replication competent or not with probabilities of Q and $(1 - Q)$, respectively. We assume that both v_1 and v_2 are equally able to deplete helper-dependent immune responses, giving the term $-uvX$ in eqn (3), where v is the total viral population, $v_1 + v_2$. The parameters k and p are measures of the immune response's ability to respond to and neutralize virus via helper-dependent pathways (which are vulnerable to the virus), while d is the per capita death rate of virus by helper-independent means.

If we define the fraction of the total viral population which is replication-competent, $\rho = v_1/v$, we have

$$\frac{\dot{\rho}}{\rho} = R_1 - R, \quad (4)$$

where R_1 is the relative growth rate of replicating virus given by

$$R_1 = \frac{\dot{v}}{v_1} = rQ - d - pX \quad (5)$$

and R is the relative growth rate of the total virus population given by

$$R = \frac{\dot{v}}{v} = r\rho - d - p\rho X. \quad (6)$$

Assuming steady state for the helper-dependent immune response we have

$$\hat{X} = \frac{k}{u}\rho. \quad (7)$$

and the total viral growth rate is then given by

$$R(\rho) = r\rho - d - \frac{pk}{u}\rho^2. \quad (8)$$

The quadratic form of this expression indicates that the total growth rate of the virus might be maximized at values of ρ less than 1 and thus that the production of non-replicating escape mutants v_2 could be advantageous to the virus. The equilibrium fraction of replicating virus, $\hat{\rho}$, is defined by ($\dot{\rho} = 0$), which, from eqn (4) gives $R_1(\hat{\rho}) = R(\hat{\rho})$. From eqns (5), (6) and (7) we find that an equilibrium fraction $\hat{\rho}$ requires

$$Q = \hat{\rho} + a\hat{\rho}(1 - \hat{\rho}), \quad (9)$$

where the dimensionless parameter $a = pk/ru$ is a measure of the strength of the helper-dependent immune response compared to viral growth and immune suppression.

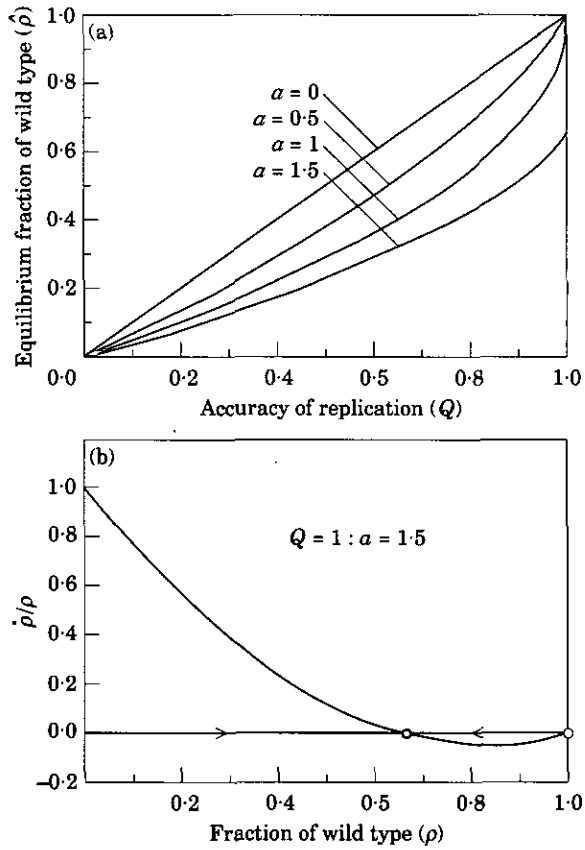

FIG. 1. (a) The equilibrium fraction of wild-type virus $\hat{\rho}$ as a function of Q, the fraction of replication-competent viral offspring, for different values of the parameter $a = pk/ru$. Non-replicating mutants both totally escape and retain the ability to impair the specific immune responses of the host to the wild type. If $a < 1$ there is a 1:1 correspondence between Q and $\hat{\rho}$ in the range from zero to one, while if $a > 1$ the maximum stable fraction of wild-type virus is $1/a$. (b) The relative rate of change of the fraction of wild-type virus as a function of that fraction for $Q = 1$ and $a = 1{\cdot}5$. The equilibrium at $\rho = 1$ is unstable against the introduction of mutants v_2, while the equilibrium at $\rho = 1/a = 2/3$ is stable.

Figure 1(a) shows $\hat{\rho}$ vs. Q for various values of a. If $a > 1$ then the equilibrium fraction of wild-type virus, $\hat{\rho}$, cannot ever exceed $1/a$, even as $Q \to 1$. This may be understood by considering the function $\dot{\rho}/\rho = R_1 - R$, which is plotted against ρ for $Q = 1$ and $a = 1{\cdot}5$ in Fig. 1(b).

We see that any $\rho < 1$ leads to the equilibrium at $\rho = 1/a$ and thus that the equilibrium at $\rho = 1$ is unstable against any small mutation. If $a < 1$ or

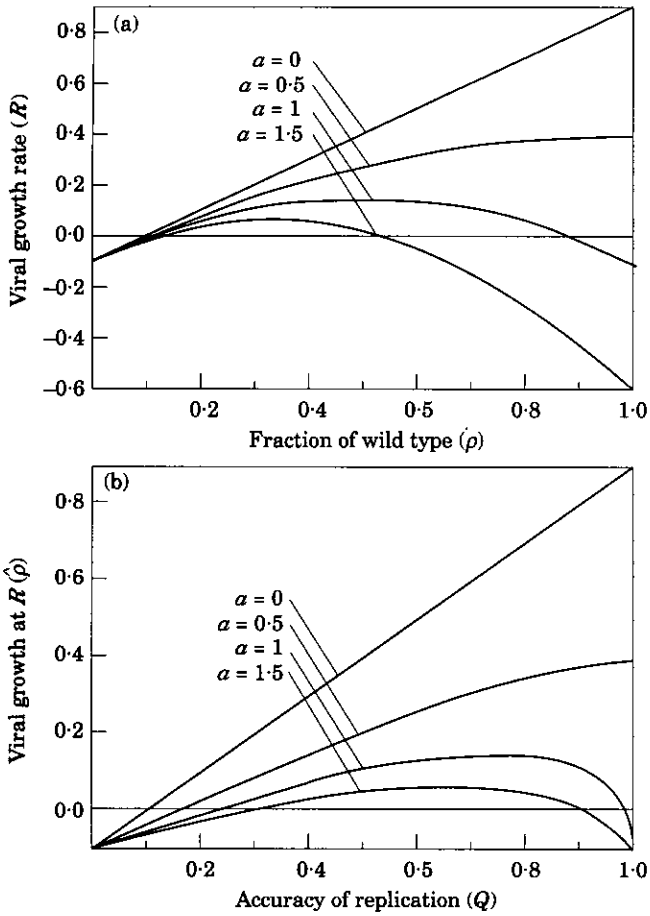

FIG. 2. (a) The overall growth rate of virus $R = \dot{v}/v$ at a fraction ρ of wild-type for $a = 0$, 0·5, 1 and 1·5. The maximum viral growth rate is achieved at $\rho = 1/2a$ which lies in the range $0 < \rho < 1$ so long as $a > 0{\cdot}5$. If $a < 0{\cdot}5$, the viral growth rate is maximized at $\rho = 1$ and is lowered by the presence of any non-replicating mutants. (b) Viral growth rates $R = \dot{v}/v$ at the equilibrium fraction of wild type as a function of the replication accuracy Q. $R(Q) = R(\hat{\rho}(Q))$, where $\hat{\rho}(Q)$ and $R(\rho)$ are as shown in Figs 1(a) and 2(a), respectively.

$Q < 1$ there is only one equilibrium in the range $0 < \rho < 1$, and this equilibrium is always stable.

Figure 2 (a) shows the overall viral growth rate $R(\rho)$. The peak value of R occurs at $\rho_p = 1/2a$. This optimum fraction, ρ_p, will be the equilibrium fraction if the mutation rate satisfies

$$Q_p = \frac{1}{4a} + \frac{1}{2}. \tag{10}$$

The total viral growth rate at the equilibrium fraction of wild-type, $R(\hat{\rho})$, can be expressed as a function of Q by combining eqns (8) and (9) and is displayed in Fig. 2(b). The virus can benefit from mutation only if $\rho_p < 1$, or if

$$a > \frac{1}{2}. \tag{11}$$

This condition also ensures that $Q_p < 1$. If it is satisfied then the virus will benefit from mutation, i.e. $R(\hat{\rho}) > R(1)$, so long as $\hat{\rho} > (1 - a)/a$ or equivalently if $Q > 2(1 - a)$.

For the virus population to grow we require $R > 0$. Without mutation this implies

$$R(1) = r\left(1 - a - \frac{d}{r}\right) > 0, \tag{12}$$

or

$$\frac{d}{r} < 1 - a, \tag{13}$$

while the growth rate at the peak is

$$R_p = R(\rho_p) = r\left(\frac{1}{4a} - \frac{d}{r}\right), \tag{14}$$

which will be positive if

$$\frac{d}{r} < \frac{1}{4a}. \tag{15}$$

Figure 3(a) shows regions where the virus population can grow ($R > 0$) with and without mutation. The horizontal axis is $a = pk/ru$; the vertical axis is d/r. Without mutation the virus can only grow below the straight line, $d/r = 1 - a$, while with the optimum mutation rate the virus may grow below the curved line, $d/r = 1/4a$. Note that the curved line is only drawn for $a > 1/2$ as below this value mutation cannot increase the viral growth rate.

We see that there are four distinct possibilities. In region 1, generating defective escape mutants is a disadvantage to the virus, which is able to grow without mutation; in region 2 mutation may be an advantage if $Q > 2(1 - a)$, but is not necessary for the virus to grow; and in region 3 mutation is not only advantageous to the virus, but is necessary for positive viral growth. In region 4, the viral population cannot grow with or without mutation, but the rate at which it declines can be reduced if $a > 1/2$. This reduction could be important for pathogenesis because it could significantly increase the length of time for which certain HIV strains are maintained during infection and thus increase, for example, the likelihood of producing new replicating escape mutants. Figure 3(a) illustrates that non-replicating escape mutants of the type in our model are most beneficial to the virus when a is high and d/r is low, so that the helper-dependent component of the immune response, which is vulnerable to viral attack by these mutants, is large compared to other causes of viral death.

Figure 3(b) shows the optimum value of Q as a function of a, which is equal to 1 (no mutation) for $a < 1/2$ and falls towards 1/2 as $a \to \infty$.

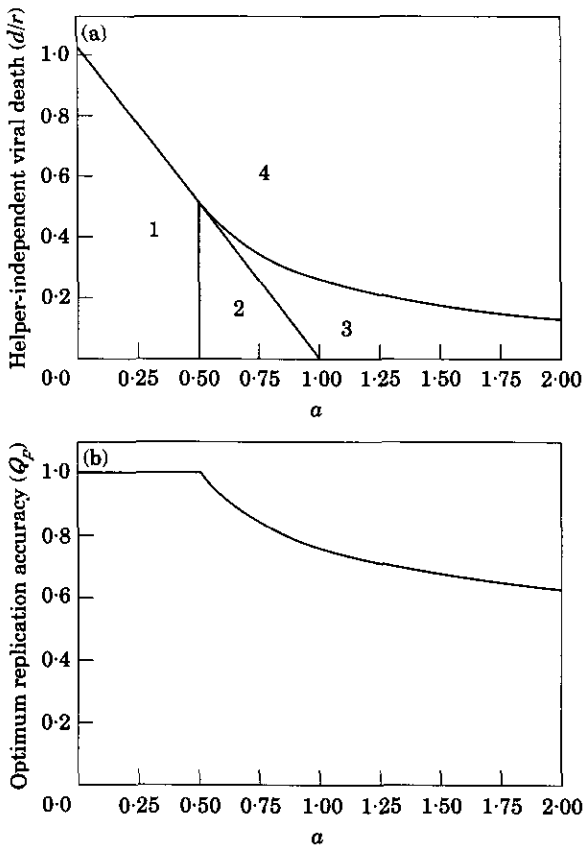

FIG. 3. (a) The straight line $d/r = 1 - a$ marks values of a, r and d, where the growth rate of a purely wild-type population, $R(1)$, is zero. Above this line $R(1)$ is negative and the virus will die out, below the line $R(1)$ is positive and the virus grows. The curve $d/r = 1/4a$; $a > 0{\cdot}5$ also marks the boundary between positive and negative viral growth, but now for a virus producing the optimum fraction of non-replicating escape mutants among its offspring. In region 1 the viral growth rate of a pure wild-type population is positive and is reduced by all levels of mutation ($a < 0{\cdot}5$). In region 2 mutation may be an advantage ($a > 0{\cdot}5$) but is not neccessary for positive viral growth. In region 3 mutation not only increases the viral growth rate but is actually neccessary if viral growth is to be positive. In region 4 the virus cannot grow with or without mutation but the viral growth rate can be made less negative if $a > 1/2$. (b) The optimum replication accuracy given by $Q_p = 1/4a + 1/2$ for $a > 1/2$ and by $Q_p = 1$ for $a < 1/2$.

2.2 PARTIAL ESCAPE OR ALTERED IMMUNE SUPPRESSION

In the previous section we assumed that the non-replicating mutants totally escape the helper-dependent immune response, which is specific to the wild type, and that they are able to suppress immune responses at the same rate as the wild type. Here we allow for the possibility that the escape is only partial and that the degree of immune suppresion by the mutants may be different to that of the wild type. Equations (1–3) then become

$$\dot{v}_1 = v_1(rQ - d - p_1 X) \tag{16}$$

$$\dot{v}_2 = v_1 r(1 - Q) - v_2(d + p_2 X) \tag{17}$$

$$\dot{X} = k_1 v_1 + k_2 v_2 - (u_1 v_1 + u_2 v_2)X, \tag{18}$$

where k_1, k_2 and u_1, u_2 represent the different rates of inducing and impairing immune responses of the replicating and non-replicating mutants which are cleared according to the parameters p_1, p_2. Both p and k will depend upon the ability of the immune system to recognize particular viral epitopes, and are therefore likely to vary in the same way for different viral strains. We shall use the notation $u_1 = u$, $u_2 = (1 + \alpha)u$, $p_1 = p$, $p_2 = (1 - \beta)p$ and $k_1 = k$, $k_2 = (1 - \beta)k$. Equations (4) and (5) for $\dot{\rho}/\rho$ and $R_1 = \dot{v}_1/v_1$ are unchanged, while eqn (8) becomes

$$R = \frac{\dot{v}}{v} = r\left(\rho - a\frac{[1 - \beta(1 - \rho)]^2}{1 + \alpha(1 - \rho)} - \frac{d}{r}\right). \tag{19}$$

Setting $R_1 = R$, as before, we obtain

$$Q = \hat{\rho} + a\frac{\beta(1 - \hat{\rho})[1 - \beta(1 - \hat{\rho})]}{1 + \alpha(1 - \hat{\rho})} \tag{20}$$

for the mutation rate required to obtain a stable equilibrium fraction, $\hat{\rho}$, of wild-type virus. Notice that, with $\beta = 1$ and $\alpha = 0$, eqns (19) and (20) reduce to eqns (8) and (9) for total escape mutants with unchanged immune suppression.

The parameter β describes the degree at which the defective mutant escapes from immune recognition by responses directed at the wild type. In general many defective mutants will have a β close to zero; i.e. they are more or less completely recognized by responses directed at the wild type. Such mutants cannot cause any enhancement of overall growth. This can only be caused by defective *escape* mutants, which, by definition, have a larger β (i.e. closer to 1).

2.2.1. *Partial escape*

First, we assume that the defective mutants can impair immune responses as well as the wild type ($\alpha = 0$), but can only partially escape from the immune responses directed at the wild type ($0 < \beta < 1$). Equation (19) then reduces to

$$R = r\left(\rho - a[1 - \beta(1 - \rho)]^2 - \frac{d}{r}\right). \tag{21}$$

This is again quadratic in ρ, with a peak at

$$\rho_p = \frac{1 - 2a\beta(1 - \beta)}{2a\beta^2}. \tag{22}$$

Equation (20) becomes

$$Q = \hat{\rho} + a\beta(1 - \hat{\rho})[(1 - \beta)(1 - \hat{\rho})], \tag{23}$$

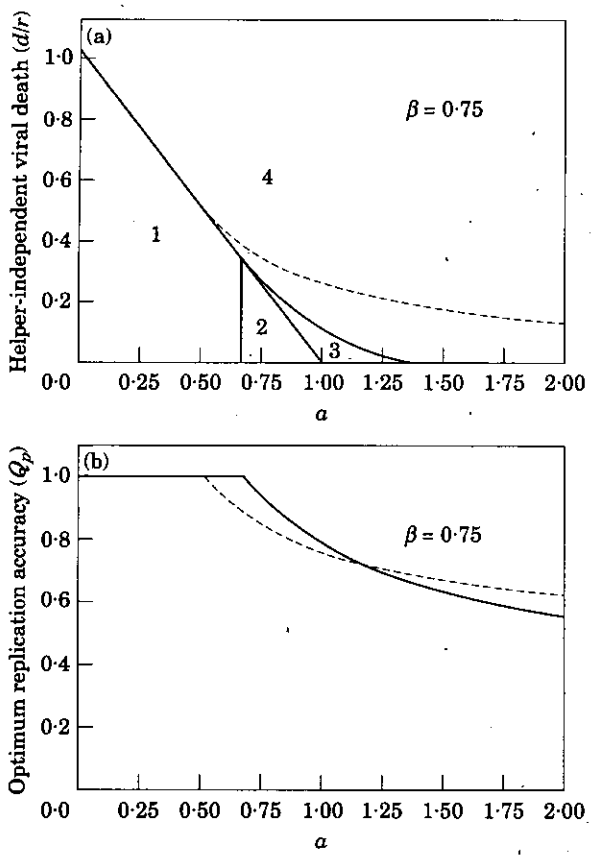

FIG. 4. (a) Conditions for positive viral growth as in Fig. 3(a), but now for non-replicating mutants, which only partially escape the immune response to the wild-type ($k_2 = (1-\beta)k$; $\beta = 0{\cdot}75$). The broken line is $d/r = 1/4a$; $a > 0{\cdot}5$ as in Fig. 3(a) for comparison with a total escape mutant ($\beta = 1$). The minimum value of a where mutation can increase the growth rate is now $1/(2\beta)$ compared to 1/2 for total escape mutants and there is a maximum value of $a = 1/4\beta(1-\beta)$ above which positive viral growth is impossible. (b) The optimum replication accuracy for partial escape mutants with $\beta = 0{\cdot}75$ (solid line) compared to that for total escape mutants (broken line).

which gives

$$Q_p = \frac{1-2a\beta}{4a\beta^2} + 1 \tag{24}$$

for the optimum fraction of wild type vs. mutant offspring, Q_p. The virus can benefit from mutation only if $\rho_p < 1$ or if

$$a > \frac{1}{2\beta}, \tag{25}$$

which also ensures that $Q_p < 1$. The optimum total growth rate of virus is

$$R_p = R(\rho_p) = r\left(\frac{1-4a\beta(1-\beta)}{4a\beta^2} - \frac{d}{r}\right), \tag{26}$$

which will be positive if

$$\frac{d}{r} < \frac{1-4a\beta(1-\beta)}{4a\beta^2}. \tag{27}$$

The expressions of eqns (25) and (27) reduce to those of eqns (11) and (15) for total escape mutants where $\beta = 1$.

Figure 4(a) shows regions where, as in Fig. 2(a), the viral growth rate is positive. The growth rate without mutation $R(1)$ is unchanged and the straight line, given by $d/r = 1 - a$, indicates, as before, the boundary between parameter regions where a pure wild-type population will have positive or negative growth. The solid curve is given by

$$\frac{d}{r} = \frac{1-4a\beta(1-\beta)}{4a\beta^2}, \tag{28}$$

and marks the same boundary for a virus population producing the optimum fraction of mutant offspring. Regions 1–4 are defined as in Fig. 3(a), and Fig. 4(b) shows the optimum replication accuracy, Q_p. The broken curves show the same functions for total escape mutants for comparison with Fig. 2. Partial escape mutants, unlike the total escape mutants of the previous section, are unable to ensure positive viral growth rates as $a \to \infty$. This is because the helper-dependent immune responses represented by a are now able to control the escape mutants which before could accumulate to infinity as $d \to 0$.

If region 3, where the production of mutants is necessary for viral growth, is to exist, we require not only that mutation can increase R [eqn (25)], but also that

$$a < \frac{1}{4\beta(1-\beta)}, \tag{29}$$

which ensures that $R_p > 0$ as $d/r \to 0$.

The shaded region in Fig. 5 represents values of a and β where mutation can hold the balance between growing and shrinking viral populations [that is, region 3 in Fig 4(a) exists]. At points outside this region and above the solid curve $a = 1/2\beta$, mutation increases the total viral growth rate, but never sufficiently to make it positive, even as $d/r \to 0$. Figure 5 illustrates the importance of immunological escape if the production of non-replicating mutants is to be of any advantage to the virus. In particular, it shows that the possibility that production of such mutants with unchanged immune suppression can be essential for viral growth requires that $\beta > 1/2$.

2.2.2. *Altered immune suppression*

In this section we consider the effect of altering the degree to which an escape mutant can impair the host immune response. Equations (19) and (20) are

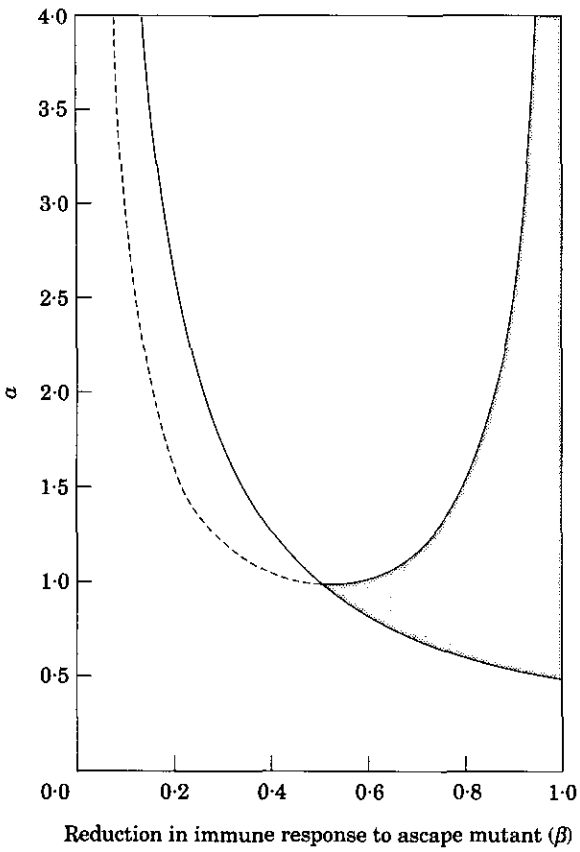

FIG. 5. The falling solid curve is $a = 1/(2\beta)$ and represents the lower limit on a if non-replicating partial escape mutants are to increase the viral growth rate. The broken curve is $a = 1/4\beta(1-\beta)$, above which the viral growth rate, even at the optimum mutation rate, cannot be positive. Points in the shaded region represent values of a and β where mutation can hold the balance between growing and shrinking viral populations [that is, region 3 in Fig. 4(a) exists]. At points outside this region and above the solid curve mutation increases the total viral growth rate, but never sufficiently to make it positive, even as $d/r \to 0$.

singular at $\rho = 1 + 1/\alpha$, which falls outside the range $0 < \rho < 1$ so long as $1 + \alpha > 0$ or $u_2 = (1+\alpha)u$ is positive. Setting $dR/d\rho = 0$ in eqn (19) we find that the viral growth rate has a peak at $\rho_p < 1$ so long as

$$a < \frac{1}{2\beta + \alpha}. \tag{30}$$

This also ensures that $Q_p < 1$. Figure 6(a) shows thresholds for overall viral growth at the optimum mutation rate, $R(\rho_p)$, and Fig. 6(b) shows the optimum replication accuracy, Q_p, and Fig. 6(b) shows the optimum replication accuracy, Q_p, both as functions of a for $\beta = 0{\cdot}9$ and various values of α. As mutants become more pathogenic, their production becomes more and more advantageous to the virus, owing to reduced levels of immune response. It should be noted, however, that we have not considered the fact that reducing CD4 cell counts may also reduce viral replication, because CD4 cells are the major host cells for HIV. Nonetheless, the possibility exists that highly pathogenic non-replicating mutants could play an important role in the immune suppression observed in AIDS.

3. Many Different Strains

The model of Section 2 is highly simplified. The total viral population is divided into two categories: replicating virus (v_1) and non-replicating mutants (v_2). In reality, we would expect many strains in each category ($v_{1,i}$ and $v_{2,j}$), each with its own replication rate and interaction with the immune system. Another objection which might be raised is the lack, in the model, of a specific helper-dependent immune response to the mutants If we assume n antigenically distinct classes of escape mutants, $v_{2j}; j = 1 \ldots n$, each with its own specific helper-dependent immune response we can replace eqn (2) by

$$\dot{v}_{2j} = v_1 r \frac{(1-Q)}{n} - v_{2j}(d + pX_{2j}). \tag{31}$$

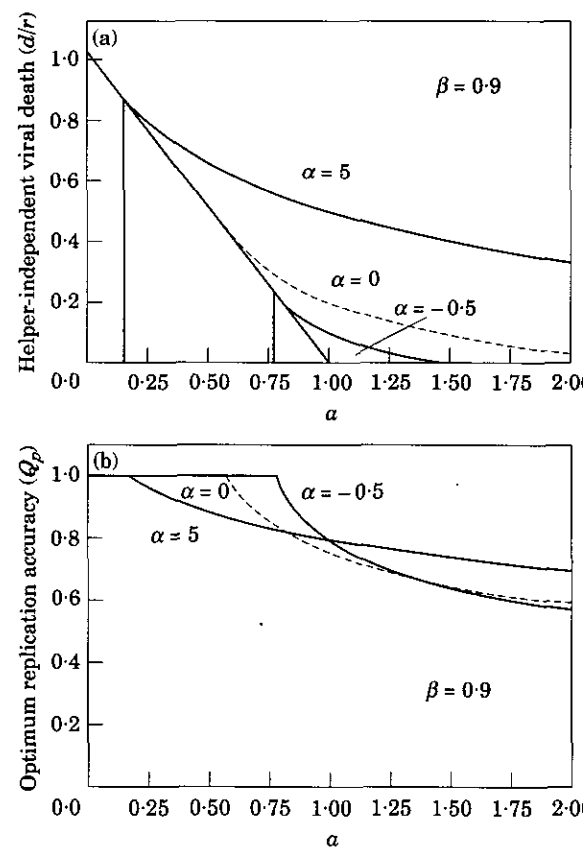

FIG. 6. (a) Conditions for positive viral growth at the optimum mutation rate as in Fig. 3(a), but for partial escape mutants ($\beta = 0{\cdot}9$) with altered ability to suppress the host immune response ($u_2 = (1+\alpha)u$, $\alpha = -0{\cdot}5$, 0, 5). The minimum value of a where mutation can increase the growth rate is now $1/(2\beta + \alpha)$. (b) The optimum replication accuracy Q_p for partial escape mutants ($\beta = 0{\cdot}9$) with different immune impairment to the wild type.

Here, all v_{2j} are recognized by a different strain-specific immune response, and they are all produced in equal numbers by mutations of the wild-type virus. The specific immune responses obey

$$\dot{X}_{2j} = kv_{2j} - uvX_{2j}, \tag{32}$$

where the total viral population is now

$$v = v_1 + \sum_1^n v_{2j}. \tag{33}$$

The equilibrium values of X_{2j} are then

$$\hat{X}_{2j} = \frac{k}{u}\frac{v_{2j}}{v} = \frac{k}{u}\frac{(1-\rho)}{n}. \tag{34}$$

As $n \to \infty$, $\hat{X}_{2j} \to 0$ and the model reverts to the form of eqns (1–3). Thus, while the total population of non-replicating mutants may be large, each individual variant will be produced in vanishingly small numbers and will pass away before any appreciable specific immune response can be raised against it, allowing us to neglect any immune response specific to the non-replicating mutants in eqns (1–3) and (17–18). If n is finite, which assumes that each of the immune responses x_{2j} cross-reactively covers a fraction $1/n$ of the non-replicating escape mutants, we have

$$R = r\left(\rho - \frac{d}{r} - a\rho^2 - \frac{a(1-\rho)^2}{n}\right) \tag{35}$$

for the total viral growth rate. This is again a quadratic expression, with a peak at

$$\rho_p = \frac{n+2a}{2a(n+1)}. \tag{36}$$

The mutation rate required for an equilibrium fraction of wild-type $\hat{\rho}$ satisfies

$$Q = (1+a)\hat{\rho} - a\hat{\rho}^2 - \frac{a(1-\hat{\rho})^2}{n} \tag{37}$$

which gives

$$Q_p = \frac{4a+n+2an}{4a+4an} \tag{38}$$

for the optimum mutation rate. The conditions for mutation to be able to increase viral growth, $\rho_p < 1$, and for ρ_p to be a possible equilibrium fraction of wild-type, $Q_p < 1$, are both satisfied if $a > 1/2$, just as in Section 2.1 and regardless of n. The expression above for R and Q, and thus also those for ρ_p and Q_p, all reduce to those of Section 2.1 as $n \to \infty$. It might at first appear surprising that the virus can benefit by producing non-replicating mutants that are no more or less pathogenic nor antigenic than the wild-type. The key lies in the increased antigenic diversity that these mutants cause by escaping the immune response to the wild-type. As in the diversity threshold model (Nowak *et al.*, 1990, 1991; Nowak, 1991; Nowak & May, 1993), all viral variants can impair all helper-dependent immune responses, while each immune response is only active against specific viral strains, shifting the balance of power towards the virus in a highly antigenically diverse population.

4. Discussion

The high mutation rate of HIV is likely to produce large numbers of escape mutants, which also harbour defective mutations. There are various possibilities for the nature of viral escape mutants, which are unable to replicate yet retain their ability to impair helper-dependent immune responses. Mutants may have defects in one or more genes, which prevent them from completing the viral life cycle but not from impairing the host immune response. There is some evidence for defective mutants of this kind. Ohki *et al.* (1991) and Li *et al.* (1991) report non-infectious HIV strains that are still able to kill $CD4^+$ T-cells by inducing the formation of giant multinucleated complexes of fused cells (syncytia). Other possibilities include defective mutants, which cannot produce infectious virions, but which can enter host cells and disrupt immune signalling or activation (Mann *et al.*, 1987; Linette *et al.*, 1988), or possibly disrupt the genetic regulation of the host cell leading to programmed cell death (apoptosis) (Terai *et al.*, 1991).

To summarize, the model presented here illustrates the theoretical possibility that, under certain conditions, the total growth rate of HIV in an infected individual may be increased by the production of defective mutants, so long as these mutants both escape and impair (to some degree) the host immune response to the replicating HIV population.

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