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Intra-host competition between *nef*-defective escape mutants and wild-type human immunodeficiency virus type 1

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Various forms of *nef* genes with deletions at conserved positions along the sequence have been reported to persist in human immunodeficiency virus type 1 infected patients. We investigate the forces maintaining such variants in the proviral population. The main selection pressures are preservation of function and host immune response. The crippled Nef protein might have fewer epitopes, and as such be less visible to the specific immune response, but it will lose some function. Does a trade-off between avoidance of the immune response and loss of function explain the dynamics of the crippled virus found in the patients? To answer this question, we formulated a deterministic model of the virus–host interactions. We found that when the crippled protein presents few epitopes and suffers little loss of function, the two viral types can coexist. Otherwise, the wild-type comes to prevail. The mutant form might initially dominate, but as the selective pressure by the CD8+ T cells decreases over the course of infection, the advantage for the crippled form of losing epitopes disappears. Hence, we go from a situation of coexistence of wild-type and mutant, to a situation of only full-length *nef*. The results are discussed in the context of the suggested use of live attenuated vaccines having deletions in *nef*.

Keywords: HIV-1; competition; mathematical model; *nef*; defective escape; live attenuated vaccine

1. INTRODUCTION

As a result of the high mutation rate of human immunodeficiency virus type 1 (HIV-1) (Preston *et al*. 1988; Roberts *et al*. 1988), high replication rates (Ho *et al*. 1995; Wei *et al*. 1995), and host selection pressures, HIV-1 diversifies during the course of infection (Fisher *et al*. 1988; Hahn *et al*. 1986; Saag *et al*. 1988). In the viral quasispecies thus formed, HIV variants with functionally crippled *nef* genes due to in-frame stop codon mutations, deletions or frameshift mutations, have been documented in several patients (Deacon *et al*. 1995; Kirchhoff *et al*. 1995; Koenig *et al*. 1995; Mariani *et al*. 1996; Price *et al*. 1997; Salvi *et al*. 1998; Schwartz *et al*. 1996; Shugars *et al*. 1993). These forms have been found despite the substantial pressure to maintain an open reading frame (Kestler *et al*. 1991).

It has been argued that these seemingly defective proviral variants do not persist as a result of some selective advantage, but simply because of the longer life span of cells infected with defective provirus (Chun *et al*. 1997; Nowak *et al*. 1997). However, simian immunodeficiency virus (SIV) and the chimeric SIV–HIV (SHIV) with deletions in *nef* can successfully establish infection and persist (Daniel *et al*. 1992; Igarashi *et al*. 1998), suggesting viruses with these deletions do replicate, and are laid down in proviral form anew. What selective forces acting on a virus in a host would explain the persistence of a crippled gene?

First, preservation of function is of importance. Nef enhances viral replication and is necessary for full pathogenic potential (Kestler *et al*. 1991). Second, it is thought to activate quiescent cells (Spina *et al*. 1994); Baur *et al*. (1994) postulate that Nef activates quiescent cells depending on the subcellular pattern of expression of the gene. It downregulates CD4 expression on the surface of infected cells (Mariani & Skowronski 1993). Finally, there is evidence that Nef downregulates MHC I *in vitro* (Collins *et al*. 1998), and that it enhances apoptosis (Xu *et al*. 1997). For reviews, see Geleziunas *et al*. (1996), Harris (1996), Piguet & Trono (1999) and Ratner & Niederman (1995). The extent to which function is reduced in a truncated protein is unclear. Gibbs *et al*. (1994) observed no effect of deletion of *nef* on the replication of viruses in cell culture. However, infection with HIV-1 expressing crippled *nef* is associated with slow or non-progression in several patients, suggestive of reduced gene function (Deacon *et al*. 1995; Kirchhoff *et al*. 1995, 1997; Mariani *et al*. 1996; Miller & Greene 1996, p. 173; Salvi *et al*. 1998).

A second force shaping the viral quasispecies is the immune response, particularly the specific immune response. Nef is relatively immunogenic (Culmann-Penciolelli *et al*. 1994, 1995; Goulder *et al*. 1997*a*,*b*). HIV-1 epitopes have as yet been mapped in *nef* in about half the HLA types described (Brander & Walker 1998; Brander *et al*. 1998). In those individuals having immunogenic Nef, an advantage of deletions in a viral gene is that epitopes might be removed, allowing the virus to escape cytotoxic T lymphocyte (CTL) responses (Koenig *et al*. 1995; Price *et al*. 1997). There are less functionally detrimental mechanisms to evade immune response, such as changes in the epitopes rather than dropping them altogether, which would give defective mutants even lower chances to persist (Haydon & Woolhouse 1998; Holmes & de Zanotto 1998). Here, we confine ourselves to the case in which the loss of epitopes is always favourable.

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Overall, the crippled form of the gene is faced with a trade-off: it might lose some of its function, but can present fewer epitopes, evading specific immune response. In this paper, we investigate whether this trade-off can explain the persistence of a crippled virus in a host.

Competition models have been formulated earlier in the context of the dynamics of virus–host interactions (Frost & McLean 1994; McLean & Nowak 1992), and it was established that defective virus could enhance population growth under certain conditions (Berry & Nowak 1994). Here we use a model of virus–host interactions incorporating the number of epitopes presented by wild-type and mutant virus in the expressions of the immune responses against the two viral types. We focus on the *nef* gene, and allow for varying degrees of loss of replicative capacity.

2. METHODS

We formulated a deterministic model of the interaction between the immune system and HIV-1 during infection. The virus has two forms: one is full length and fully functional, the other is truncated or has deletions. The latter has the disadvantage of losing some function, but has the possible advantage of dropping epitopes, evading immune recognition. We assumed that only the function of enhancement of replication was impaired in the crippled *nef* variant. The function of activation of latently infected cells is considered in an electronic appendix to this paper which can be found on The Royal Society Web site; other functions of Nef are still debated.

Uninfected CD4+ T cells (T) are produced at rate λ and have a per capita death rate δ_T. Uninfected cells are infected at rate $\beta(V_1 + V_2)$, where V_1 and V_2 are the two types of virions present. Subscript 1 refers to full-length *nef* viral products, and subscript 2 to *nef* with deletions. Cells productively infected with the wild-type (I_1) arise at rate βV_1. They die at rate δ_I and are removed by CTLs at rate kE_n, where k is the killing rate and E_n is the number of CD8+ T lymphocytes against full-length *nef*. CD4+ T cells infected with crippled *nef* viral particles (V_2) become productively infected with the mutant genotype (I_2). These cells die at rate δ_I and are killed by CTL at rate kE_m, E_m being the number of CD8+ T lymphocytes against *nef* with deletions. Virions V_1 and V_2 disappear at the same rate c, but are generated by the two types of infected cells at rates p_1 and p_2, respectively, with $p_1 \geqslant p_2$: the full-length form of the virus has the highest replication rate.

Three main assumptions were made. There is no superinfection, and cross-reactivity of CD8+ T cells only occurs between epitopes of the same viral genotype. The humoral immune response is negligible compared with the cellular immune response.

Cells infected with full-length *nef* virus present n epitopes, whereas the crippled form presents m epitopes, with $m \leqslant n$. The variables E_m and E_n refer to the CD8+ T cells directed at any epitope between 1 and m, and 1 and n, respectively. These variables are derived by summation of the responses to the individual epitopes. Let ξ_i represent the number of CD8+ T cells against epitope i:

$$E_m = \sum_{i=1}^{i=m} \xi_i \quad \text{and} \quad E_n = \sum_{i=1}^{i=n} \xi_i.$$

These variables change over time as follows, a being the activation rate and δ_E the death rate of CD8 + T cells,

$$\left.\begin{aligned} \frac{\mathrm{d}\xi_i}{\mathrm{d}t} &= a(I_1 + I_2) - \delta_E \xi_i \quad \text{for } i < m \\ \frac{\mathrm{d}\xi_i}{\mathrm{d}t} &= aI_1 - \delta_E \xi_i \quad \text{for } m < i < n \end{aligned}\right\},$$

so

$$\left.\begin{aligned} \frac{\mathrm{d}E_m}{\mathrm{d}t} &= \sum_{i=1}^{i=m} \frac{\mathrm{d}\xi_i}{\mathrm{d}t} = ma(I_1 + I_2) - \delta_E E_m \\ \frac{\mathrm{d}E_n}{\mathrm{d}t} &= \sum_{i=1}^{i=n} \frac{\mathrm{d}\xi_i}{\mathrm{d}t} = naI_1 + maI_2 - \delta_E E_n \end{aligned}\right\}.$$

Our model can be summarized as follows.

$$\left.\begin{aligned} \frac{\mathrm{d}T}{\mathrm{d}t} &= \lambda - \delta_T T - \beta(V_1 + V_2)T \\ \frac{\mathrm{d}I_1}{\mathrm{d}t} &= \beta V_1 T - \delta_I I_1 - kE_n I_1 \\ \frac{\mathrm{d}I_2}{\mathrm{d}t} &= \beta V_2 T - \delta_I I_2 - kE_m I_2 \\ \frac{\mathrm{d}V_1}{\mathrm{d}t} &= p_1 I_1 - cV_1 \\ \frac{\mathrm{d}V_2}{\mathrm{d}t} &= p_2 I_2 - cV_2 \\ \frac{\mathrm{d}E_m}{\mathrm{d}t} &= ma(I_1 + I_2) - \delta_E E_m \\ \frac{\mathrm{d}E_n}{\mathrm{d}t} &= maI_2 + naI_1 - \delta_E E_n \end{aligned}\right\}. \tag{1}$$

The parameter values were drawn from literature (Ho *et al.* 1995; Krakauer & Nowak 1999; Perelson *et al.* 1996; Wei *et al.* 1995): $\lambda = 1$, $\delta_T = 0.01$, $\delta_I = 0.5$, $\beta = 0.05$, $k = 0.1$, $p_1 = 100$, $c = 3$, $a = 0.01$, $\delta_E = 0.02$, $p_2 = 75$ when it does not vary between 0 and p_1, $n = 8$, m varies but $m \leqslant n$.

Some parameter values lacked experimental backing, and thus were chosen conveniently. However, the qualitative statements on the outcome of intra-host competition between viral genotypes are not dependent on the particular choice of parameter values.

3. RESULTS

Let us look at a host infected with wild-type virus. How successful is a crippled genotype when infecting this patient? Is it capable of invading in a population of full-length viruses? To answer this question, we analyse the viral dynamics in a host infected only with the full-length variant.

In the absence of crippled virus, system (1) has a non-trivial equilibrium at

$$\bar{T} = \theta c, \quad \bar{I}_1 = \frac{\delta_E(\beta p_1 \theta - \delta_I)}{kan}, \quad \bar{V}_1 = \frac{p_1 \delta_E(\beta p_1 \theta - \delta_I)}{ckan},$$
$$\bar{E}_m = \frac{m(\beta p_1 \theta - \delta_I)}{kn}, \quad \bar{E}_n = \frac{\beta p_1 \theta - \delta_I}{k}, \quad \bar{I}_2 = 0, \quad \bar{V}_2 = 0, \tag{2}$$

which exists if $\theta > \delta_I/\beta p_1$, where θ is the real positive solution of

$$\beta^2 p_1^2 \delta_E \theta^2 + (\delta_T kanc - \delta_E \delta_I p_1 \beta)\theta - \lambda kan = 0. \tag{3}$$

Numerical simulations using LOCBIF and CONTENT software (Khibnik *et al.* 1992; Kuznetsov 1998) show that this equilibrium is stable.

We use the concept of the basic reproductive number, R_0, to answer the question of whether the mutant genotype can establish itself. At the population level, R_0 is defined as the number of secondary infections generated by one infected individual in an otherwise susceptible population (Anderson & May 1991; Diekmann *et al.* 1990). Here, R_0 is the number of cells infected with the crippled virus, generated by one cell infected with crippled virus, in a population exclusively infected with full-length virus.

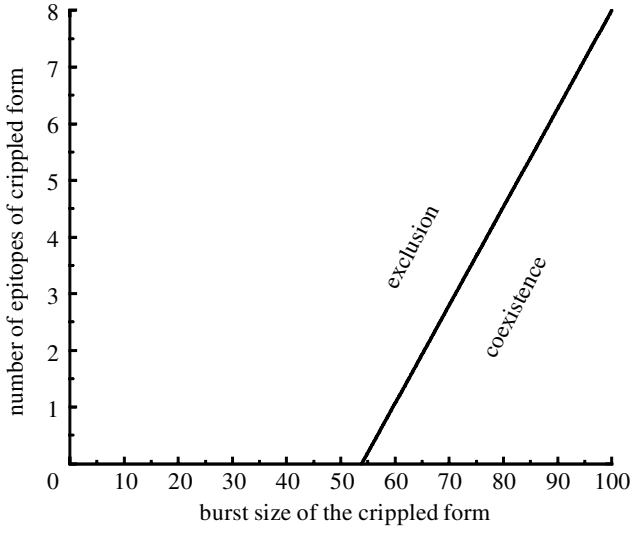

Figure 1. Outcome of the competition between the two genotypes as a function of the number of epitopes and burst size of cells infected with the defective variant. Either the two viral forms coexist ('coexistence') or the full-length form outcompetes the crippled variant ('exclusion'). The boundary is given by the condition $R_0 = 1$ (equation (5)).

R_0 can be calculated as follows: it is the average life span of a cell infected with the crippled variant multiplied by the number of infected cells generated by one crippled virus per unit of time. The latter is the product of the number of virions produced per infected cell (p_2), the life span of the virion ($1/c$), its infectivity (β) and the number of susceptible cells when the crippled form enters the population ($\overline{T}$).

When the immune response variables are at equilibrium, the average life span of a cell infected with the truncated variant is $1/(\delta_I + k\overline{E}_m)$, as an infected cell either dies 'naturally' or is killed by the immune response.

Hence,

$$R_0 = \left(\frac{1}{\delta_I + k\overline{E}_m}\right)\left(\frac{p_2}{c}\right)\beta\,\overline{T} = \left(\frac{p_2}{p_1}\right)\left(\frac{\delta_E\delta_I + kan\,\overline{I}_1}{\delta_E\delta_I + kam\,\overline{I}_1}\right). \tag{4}$$

When $R_0 > 1$, the crippled form can invade a host infected with only full-length virus; when $R_0 < 1$, it cannot.

We are interested to see for what burst size and number of epitopes of the defective virus this transition takes place.

At this invasion boundary, $R_0 = 1$, and hence

$$p_2 = p_1\frac{\delta_E\delta_I + kma\,\overline{I}_1}{\delta_E\delta_I + kna\,\overline{I}_1}. \tag{5}$$

Because $\overline{I}_1$ does not depend on p_2 or m, there is a linear relationship between p_2 and m when $R_0 = 1$.

To find out whether the two forms can coexist, we analyse the reciprocal situation, in which we let a full-length variant invade a population of defective virus at equilibrium. If each type can invade in a viral population consisting of the other type, they can coexist. We compute R_0 for the situation in which a single full-length virus infects a population infected with the crippled variant (R_0').

The starting equilibrium situation is

$$\overline{T} = \phi c, \quad \overline{I}_2 = \frac{\delta_E(\beta p_2\phi - \delta_I)}{kam}, \quad \overline{V}_2 = \frac{p_2\delta_E(\beta p_2\phi - \delta_I)}{ckam},$$
$$\overline{E}_m = \overline{E}_n = \frac{\beta p_2\phi - \delta_I}{k}, \quad \overline{I} = 0, \quad \overline{V}_1 = 0, \tag{6}$$

which exists if $\phi > \delta_I/\beta p_2$, where ϕ is the real positive solution of

$$\beta^2 p_2^2\delta_E\phi^2 + (\delta_T kamc - \delta_E\delta_I p_2\beta)\phi - \lambda kam = 0.$$

Following the reasoning set out to obtain equation (4), we find: $R_0' = p_1/p_2$.

We are primarily interested in the case in which the crippled form has reduced function compared with the full-length genotype ($p_2 \leqslant p_1$). It follows that in the parameter region of interest, $R_0' > 1$. Hence, the wild-type can always invade a mutant population. As a result, $R_0 = 1$ corresponds to the transition from a situation in which there is an equilibrium of only full-length virus to a situation in which the crippled form can successfully co-infect a host.

Modified models show that the invasion criterion of wild-type virus in a mutant population is always fulfilled, as it is only dependent on the ratio of the function of the two genotypes. Invasion of the mutant in a wild-type population depends on both function and immunogenicity of the variants (see the electronic appendix). Hence, there are always two possible outcomes: wild-type only or both variants coexisting. Thus, results are rather robust against changes in model structure.

In figure 1, the invasion boundary given by the relationship of equation (5) is plotted. The area above the curve refers to the situation in which the full-length variant outcompetes the crippled variant. The area below the curve corresponds to a situation of coexistence of the two genotypes. In this region the system is permanent (Hofbauer & Sigmund 1989); it follows that an equilibrium must exist here, in which the two types can coexist. Simulations have shown that this equilibrium is stable. Intuitively, we might expect that a crippled form presenting very few or no epitopes, and with little loss of function, would win in the competition against the wild-type. However, within the region of interest, there is no parameter set for which the crippled form outcompetes the full-length variant. Essentially, as the loss of function is more severe, more epitopes are to be lost by the crippled form in order for it to coexist with full-length Nef. If the crippled genotype does not lose enough epitopes, it is outcompeted by the wild-type.

In figure 2*a*, the ratio at equilibrium of cells infected with the full-length virus over those infected with the crippled virus is indicated as a function of the number of epitopes in the crippled form. The advantage lies with the crippled form when it has 75% of function and fewer than about two epitopes, as the ratio is smaller than one. Figure 2*b* shows that a crippled variant with two epitopes is only in the majority when the burst size of the crippled form is over 75% of the wild-type burst size. In the electronic appendix we show that after a perturbation in the equilibrium, the average time needed to return

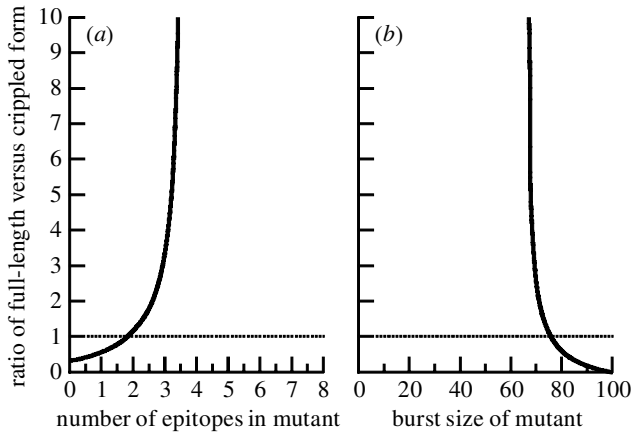

Figure 2. (*a*) Ratio of cells productively infected with full-length virus over cells productively infected with crippled virus in the coexistence equilibrium, against the number of epitopes in the crippled form; $p_2 = 75$. (*b*) Ratio of cells productively infected with full-length virus over cells productively infected with crippled virus in the coexistence equilibrium, against the burst size of the crippled form; $m = 2$.

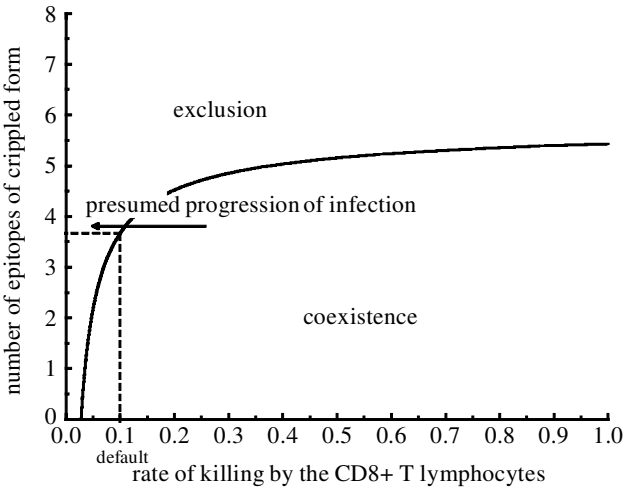

Figure 3. Outcome of the competition between the two genotypes as a function of the number of epitopes presented by cells infected with the defective variant and the killing rate of CD8 cells; $p_2 = 75$. Either the two viral forms coexist ('coexistence') or the full-length form outcompetes the crippled variant ('exclusion'). The boundary is given by the condition $R_0 = 1$.

halfway between the perturbation and the equilibrium (characteristic return time) is around three to seven days for the exclusion equilibrium, and 63 days for the coexistence equilibrium.

It is known that the number of CD4 cells is declining over the course of infection. Hence the number of CD4 T helper cells decreases too, leading to a possible decrease in the efficiency of virus killing by the CD8 cells (parameter k). The characteristic return time is a conservative estimate of the rate of approach of the equilibrium; for almost all initial conditions, the system initially moves more quickly to its equilibrium. k will change over periods of years, so the scale of change of k is bigger than that of the time to equilibrium of the system, especially if exclusion is to occur. We can therefore ask how a drop in the efficiency of killing by CD8 affects the outcome of the competition between the crippled and the full-length form. What is the maximum number of epitopes that the crippled form can carry, to be able to coexist with the full-length form, for varying degrees of CTL killing? To answer this question, we derived the critical number of epitopes from the boundary condition (equation (5)) in terms of the parameter k.

When CD8+ T cell killing rates drop, more epitopes are to be lost by the crippled form in order to coexist with the full-length variant (see the electronic appendix for calculations). Figure 3 shows the decreasing number of epitopes needed for the crippled form to survive for decreasing rate of CD8 killing. For killing rates above the default value, the critical number of epitopes is hardly affected by the killing rate, but below the default value, it drops very quickly. Hence, we predict the disappearance of the crippled form during infection.

We focused here on the function of Nef in enhancing replication. We generalized our model to describe a crippled form of Nef with reduced ability to activate quiescent cells (see the electronic appendix). The same qualitative results were obtained as in this model, although the ability of the virus to infect cells latently markedly reduces the average time to exclusion.

4. DISCUSSION

The outcome of the competition between the two genotypes is determined by the extent of loss of function and by the number of epitopes lost by the crippled variant. On one extreme, with high loss of function and little loss of epitopes, the crippled form will disappear from the population. On the other extreme, when the crippled variant maintains much of its functionality but loses many epitopes, the two forms are coexisting. Rather counter-intuitively, there never is a case where the crippled form outcompetes the full length. However, for low immunogenicity and little loss of function, the crippled form is in the majority. It is difficult to predict what coexistence between wild-type and crippled genotype with a majority of virus defective in *nef* means in terms of clinical outcome. At most, we can conjecture that overall pathogenicity of the viral population is reduced. A model including a quiescent phase predicts that the larger the fraction of cells going into quiescence, the higher the probability of the full-length outcompeting the crippled form (see the electronic appendix). The quiescent phase reduces exposure to killing by CTL, so the advantage of expressing fewer epitopes subsides, and the crippled form is generally worse off in a system with an extensive quiescent phase. If the crippled form of the virus were to have lower capacity to downregulate MHC I, it would gain immunogenicity, hence making it an even poorer competitor against the wild-type.

In models of competition between CXCR4 and CCR5 HIV variants, similar coexistence was found (Wodarz & Nowak 1998). This equilibrium is likely to be stable under therapy (Nijhuis *et al*. 1998), but unstable upon immune stimulation (Ostrowski *et al*. 1998). Just as in previous competition models (Wodarz & Nowak

1998), the outcome of the competition between these viral variants is dependent on the genetic background of the patient, and on time since infection, as shifts in immunodominance may alter the selective advantage of the crippled virus (Nowak *et al*. 1995).

Members of the Sydney Blood Bank cohort were infected through contaminated blood containing a *nef* gene with deletions. Despite early signs of slower disease progression (Deacon *et al*. 1995), declining CD4 counts were recently observed in cohort members (Greenough *et al*. 1999; Learmont *et al*. 1999). Sequencing of viral DNA revealed no full-length *nef* sequences. However, it is not clear how many clones were obtained, and regained virulence might have been due to the presence of a *nef* gene with an open reading frame. Most patients had high CTL levels (Dyer *et al*. 1999), but they were decreasing in one patient, concomitant with a declining clinical status. In this case, wild-type virus might have taken over, as would have been predicted by our model. Once wild-type virus appears it can grow in numbers very rapidly, although the equilibrium in which both variants coexist is reached slowly. As infection progresses, the gradual shift in CD8 killing efficiency may lead to a situation in which the system tends very rapidly to a steady state with only wild-type virus. Stochastic models describing competition between asexual species would predict a longer time to disappearance of defective virus than this deterministic model (Gerrish & Lenski 1998). Resurgence of the full-length *nef* could thus be delayed, possibly explaining the persistence of the defective virus.

Deletion of *nef* has been suggested for the synthesis of a potential live attenuated vaccine (Daniel *et al*. 1992; Farthing & Sullivan 1998), although SIV vaccine results are conflicting (Baba *et al*. 1995, 1999; Wyand *et al*. 1996). Our findings would in principle not support the proposed use of live attenuated *nef*-deleted vaccine. Administering such a vaccine amounts to infecting an individual with a virus having a defective *nef* gene. There are two scenarios of subsequent events: in the first case, the person gets infected with wild-type HIV-1. As we have shown, either the two variants coexist or the wild-type virus takes over. Our results suggest the latter is more likely to happen if the live attenuated virus is designed such as to generate a strong immune response to induce protective immunity. In the second case, the individual does not get infected. Full-length *nef* might still appear: *in vitro* studies have shown that the fitness of a crippled virus can increase after approximately two months, through insertion of gene sequence in the viral genome (Berkhout *et al*. 1999), and SIV repair is possible *in vivo* (Stahl-Hennig *et al*. 1996; Whatmore *et al*. 1995). If the defective viral variant were able to fully revert to the wild-type form, we would be thrown back in the first scenario. The possibilities of introducing a live attenuated vaccine in patients after previous exposure to HIV-1 have been considered (Bonhoeffer & Nowak 1995; Nowak & McLean 1991). Bonhoeffer & Nowak obtained a more favourable picture for the attenuated form than we did, using more general models than the ones applied here: cross-reactive immune response was included, and all parameters differed between the two variants. The worst case scenario they found was the dominance of the wild-type virus in a situation of coexistence, leading to slower disease progression.

In this light, the question arises what the properties of a good live attenuated vaccine are. Traditionally, they are loss of pathogenic potential combined with strong immunogenicity. A live attenuated retroviral vaccine can persist in the host for a long time. For such a vaccine, competitive exclusion of the wild-type genotype by the attenuated variant is another requisite.

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