Minireview

Effect of multiple infection of cells on the evolutionary dynamics of HIV *in vivo*: implications for host adaptation mechanisms

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Abstract

The dynamics between human immunodeficiency virus type 1 and the immune system have been studied both experimentally and mathematically, exploring aspects of host adaptation and viral mechanisms to escape host control. The majority of this work, however, has been performed assuming that any cell can only be infected by one copy of the virus. In recent years, it has become clear that multiple copies of the virus can infect the same cell, a process we refer to as co-infection. Here, we review this topic and discuss how immune control of the infection and the ability of the virus to escape immune control is affected by co-infection.

Keywords: mathematical model, co-infection, multiple infection, cytotoxic T lymphocytes, escape, immune control

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Introduction

The dynamics of human immunodeficiency virus and the immune system have been studied extensively, both experimentally and with mathematical and computational models. In this context, many strategies have been examined by which human immunodeficiency virus type 1 (HIV-1) and the less pathogenic HIV-1 viruses adapt to the host in order to escape immune mechanisms, to establish a persistent infection and to maximize its reproductive success. To counter this, the host fights the virus in a variety of ways, and various mechanisms have been discussed which can confer reduced susceptibility to infection (such as mutations in co-receptors) or which can minimize pathogenic effects and lengthen the time from infection to overt disease.[1] In animal hosts which are naturally infected by related members of the lentivirus genus of retroviruses, such as monkeys infected with simian immunodeficiency virus (SIV) or cats infected with feline immunodeficiency virus (FIV), co-evolution between the pathogen and the host has occurred over a much longer time, presumably resulting in adaptations that have reduced or slowed the incidence of morbidity and mortality.[2–5] Adaptation to reduced pathogenicity over time is a controversial proposition for many infectious agents;[6] however, it is generally thought that recent zoonotic transmission (within the past 90 or so years) of the chimpanzee virus (SIVcpz) into humans, resulting in modification of the virus to become HIV-1, is directly related to the more rapid disease course observed in humans than in chimps.[7] Whether HIV-1 eventually evolves to a form that has a more felicitous relationship with its human host, only time will tell.

An interesting aspect of the dynamics between HIV and the immune system is the ability of multiple HIV particles to enter and infect the same host cell. This leads to multiple simultaneous infection of cells, also referred to as co-infection. This has been well documented experimentally, which is reviewed below. Experimental and computational analysis has shown that co-infection can significantly alter the evolutionary dynamics between the viruses and its target cells as well as the interactions between viruses and the immune system.[8–15] Co-infection can lead to recombination between genetically different viruses residing in the same cells. In addition, different viruses within a cell can complement each other or antiagonize each other, correlating with altered outcomes of competition between virus strains, and hence altered evolutionary dynamics within hosts. This in turn is likely to have important implications for understanding disease progression and mechanisms by which the host can adapt to the virus to reduce pathology.

The aim of this paper is to review how co-infection can alter the dynamics between viruses and immune responses, and to discuss how observed characteristics of antiviral

immune responses relate to host adaptation to the infection. The paper is structured as follows. It starts with a brief review of the biology of HIV co-infection. This is followed by a theoretical discussion about how co-infection alters the viral dynamics and evolution in the context of immune responses and co-infection. The paper concludes with a discussion about how the theoretical findings relate to existing data and to host adaptation to the virus.

While evolutionary dynamics is a main focus of this paper, we do not discuss aspects of viral recombination here. Although this is likely to be a crucial aspect of the *in vivo* evolution of the virus, it is a very complex topic by itself that has been explored extensively in the literature[13,16–20] and that would go beyond the scope of this paper. Instead, we focus on the effect of co-infection on the basic competition dynamics between virus strains, which has been explored to a lesser degree thus far, but potentially has equally far reaching consequence for the dynamics of the infection.

The biology of HIV co-infection

Until fairly recently, the concept of co-infection has not played a prominent role in HIV research. This likely stems at least in part from the early observation that infection leads to the down-modulation of the CD4 receptor (reviewed in refs.[21,22]), and the more recent observation that HIV also down-modulates the CCR5 and CXCR4 viral co-receptors[23] from the cell surface, reducing the susceptibility of cells to re-infection over time. In fact, three separate HIV proteins, Nef, Vpu and Env, mediate CD4 down-modulation,[24,25] emphasizing its biological significance. Furthermore, it was also observed quite early in the epidemic that infection frequency of cells in blood is low, on the order of one in one thousand to one in one hundred thousand, leading to the incorrect assumption that the probability of two infection events in the same cell must be exponentially lower.

Over time it has become clear that this picture is not correct and that co-infection with two or more viruses, i.e. multiple infection of cells, is a frequent phenomenon that plays an important role in the natural history of HIV. First and perhaps foremost, HIV-1 replication occurs predominantly in the lymphoid tissues, where the concentration of target cells is higher than in the blood, and cell-to-cell contact facilitates transmission of multiple virions between cells. Even when few infected cells can be identified in mucosal tissues, they are observed to be infected with multiple viruses;[26] *in situ* staining in splenocytes of HIV-1 patients observed an average of 3–4 integrated proviruses per cell and sequencing of HIV-1 nucleic acids in these cells confirms multiple infection with divergent viruses and recombination between them.[27] During acute infection of macaques with a pathogenic strain of SIV, an average of 1.5 viruses per cell was observed, indicating co-infection of a large fraction of cells.[28] The recent description of cell-to-cell transmission of HIV via virological synapse formation[29–31] dramatically illustrates how multiple infection of cells can be locally generated.

Although CD4 loss from the cell surface is a consequence of HIV-1 infection, it is not clear that its primary function is to prevent re-infection (superinfection) of cells prior to virion production. Instead, removal of CD4 from the cell surface has been shown by several groups to increase the infectivity of the newly produced virions,[32,33] allowing more Env protein to associate with virions and increasing viral pathogenesis.[34] Further, there is an 18–24 h delay between infection of a cell and production of viral proteins which modulate CD4 expression, during which the cell remains susceptible to re-infection, so inhibition of superinfection is only operative during the productive phase of infection, when superinfection might be more toxic to the already stressed cell, causing premature cell death through apoptosis, lowering virus production (reviewed in refs.[21,35]). Since the lifespan of a productively infected T-cell *in vivo* is only about half a day to one day, once virus production is underway in the cell, superinfection at this late stage would most likely be unhelpful to the virus.

Experimental systems to study the dynamics of multiple infection have frequently utilized recombinant viruses bearing different reporter genes, allowing quantification of cells infected with one or both viruses.[8,9,36] These studies, carried out in tissue culture or *in vivo* within human thymic tissue in SCID mice (SCID-hu Thy/Liv mice) have made it abundantly clear that multiple infection is a natural consequence of HIV-1 replication. Over many rounds of replication in tissue culture or in the SCID-hu Thy/Liv system, multiple infection proceeds without apparent inhibition, despite the ability of HIV-1 to inhibit re-infection, resulting in frequent recombination.[8] The inference is that the pace of HIV-1 replication exceeds the inhibition effect, and that fostering multiple infection, rather than inhibiting it, may be to the benefit of the virus.

Recombination is the best studied outcome of multiple infection. It can have important implications for the evolution of HIV *in vivo*. Recombination can potentially speed up the rate of evolution by bringing together different advantageous alleles into a single genome. On the negative side (from the virus' standpoint), recombination can also break up existing advantageous allelic combinations or it can lead to the inactivation of viable viruses if they are co-infected and recombine with defective viruses. The effect of recombination on the evolutionary dynamics *in vivo* is complex, and can depend on several population genetic phenomena, such as the degree of epistasis. This has been studied in a variety of theoretical papers.[13,16–20]

There are other important consequences of co-infection for virus dynamics. Viruses defective in vital functions can be phenotypically complemented during co-infection, resulting in chimeric virions bearing mixtures of genes and proteins from more than one parental strain,[9,10] and recombination can repair the defect at the genetic level.[9,10] Viruses with essentially zero fitness can replicate as a result of complementation during co-infection.[9]

Escape from cytotoxic T lymphocytes

Cytotoxic T lymphocytes (CTLs) are a branch of the adaptive immune system that play a crucial role in controlling

many viral infections.[37] CTLs kill virus-infected cells, recognizing viral peptides (epitopes) that are displayed on the surface of infected cells in association with Class I major histocompatibility complex (MHC) molecules. However, if the virus mutates its epitope, it may become invisible to this specific CTL response, and the cells infected with this mutant are not killed by CTLs anymore, a process called CTL escape.[38–56] In this environment, the escape mutants enjoy a higher fitness and replicate to higher levels than the wild-type virus. Such immune escape or antigenic escape need not be limited to CTLs but can occur in the context of all branches of the adaptive immune system (e.g. Class II MHC-restricted T responses and antibody responses) and is thought to be an important strategy for several viruses to avoid clearance. One of the best known viruses where immune escape has been extensively documented is HIV. It is thought that immune escape contributes both to the ability of the virus to establish a productive and persistent infection, and to the ability of the virus to drive the disease process from an asymptomatic state towards AIDS.[42,48,53] Prevention of escape or minimizing the impact of escape mutants on disease progression would be an important adaptation on the part of the host.

Co-infection is likely to have a significant impact on the effect of CTL escape mutants. If the same cell is infected with an escape mutant and a wild-type virus, the cell should still be vulnerable to CTL recognition and killing because the wild-type virus expresses the wild-type epitopes that are recognized. Consequently, the escape mutant loses some of its advantage. In the rest of this paper, these dynamics are discussed in the context of computational models.

Please note that we concentrate on lytic CTL responses. In addition, however, CTLs can secrete soluble factors that inhibit viral replication, and these have been shown to play a role in HIV infection.[57] For example, interferons can be produced that inhibit viral replication. Our results and arguments about immune escape do not apply to these types of responses. In fact, it is possible that such soluble mediators, which can be produced by other immune system components as well, can modulate the kinetics of co-infection itself.

A model for co-infection and CTL escape

Here, a mathematical model is discussed that describes virus replication in the context of co-infection and a CTL response,[14] which is based previously published mathematical work on co-infection dynamics.[58,59] A model is considered that takes into account two virus strains: the wild-type virus and the CTL-escape mutant. The population of uninfected, susceptible cells is denoted by x; the population of cells infected by the wild-type virus is denoted by y_1; the population of cells infected by the escape mutant is denoted by y_2; the population of cells infected by both virus strains is denoted by y_{12}; and the population of CTLs is denoted by z. The model is given by the following set of ordinary differential equations that describe the development of these populations over time.

$$\begin{aligned}
\frac{dx}{dt} &= \lambda - dx - \beta_1 x(y_1 + y_{12}) - \beta_2 x(y_2 + y_{12}) \\
\frac{dy_1}{dt} &= \beta_1 x(y_1 + y_{12}) - (a_1 + d)y_1 - \beta_2 y_1(y_2 + y_{12}) - py_1 z \\
\frac{dy_2}{dt} &= \beta_2 x(y_2 + y_{12}) - (a_2 + d)y_2 - \beta_1 y_2(y_1 + y_{12}) \\
\frac{dy_{12}}{dt} &= \beta_2 y_1(y_2 + y_{12}) + \beta_1 y_2(y_1 + y_{12}) - (a_i + d)y_{12} - py_{12} z \\
\frac{dz}{dt} &= c(y_1 + y_{12}) - bz
\end{aligned} \tag{1}$$

This modeling approach is based on extensive literature on virus dynamics modeling, summarized in refs.[1,60–63] The basic principles of the model are illustrated in Figure 1. Uninfected cells that are permissive to infection are assumed to be produced with a rate λ and die or lose the ability to become infected (e.g. by leaving the activated state) with a rate d. They become infected by either virus strain. Infection with both strains is assumed to be equal to the product of the probability of being infected with either strain, that is, co-infection is assumed to proceed without inhibition. The wild-type strain is derived from infected cell populations y_1 and y_{12}, while the escape mutant is derived from infected cell populations y_2 and y_{12}. Infected cells in turn produce new virus particles. The

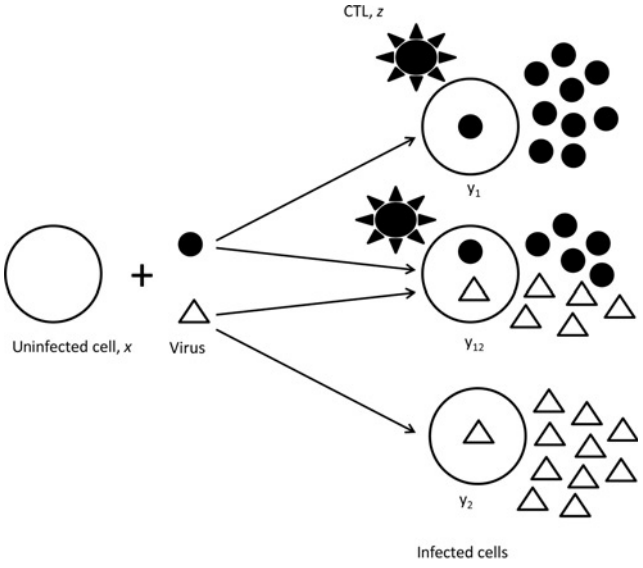

Figure 1 Schematic diagram explaining the principles that underlie the mathematical model. We assume the existence of two virus strains: wild-type (i.e. susceptible to cytotoxic T lymphocyte [CTLs]) and escape mutants (i.e. resistant to CTLs). Virus infection of cells can result in three types of infected cells: cells infected with the wild-type alone, cells infected with the escape mutant alone and cells infected with both viruses simultaneously. Only infected cells that contain a wild-type virus are recognized and killed by CTLs. This includes cells infected with the wild-type alone and cells infected with both viruses. Cells infected only with the escape mutant are not killed by CTLs. Infected cells of all types produce progeny virus, and the different virus strains can do this at different rates. Note that this schematic does not make a statement about the exact timing of events. For example, the infection arrows do not imply that two viruses infect the same cell simultaneously. The model assumes that co-infection occurs sequentially, as this is realistic

model does not explicitly take into account the free virus population. Rather, the population of free viruses is assumed to be in a quasi-steady state since their turnover is significantly faster than that of infected cells.[64,65] In addition, this formulation can describe cell-to-cell transmission which is also thought to occur in HIV infection.[30,66] As a consequence, the parameter β_1 and β_2 describe the overall replication kinetics of the wild-type and the escape strain, respectively. This includes the rate of infection, the rate of virus production and the death rate of viruses. The quasi-steady-state assumption has been made for simplicity. Explicit inclusion of the free virus population does not change the results described here. Note that the model assumes that doubly infected cells produce twice the amount of virus as singly infected cells. In the opposite extreme, the total amount of virus produced by a cell is independent of the number of resident viruses. We have studied this scenario with numerical simulations and found that it does not change the results on a qualitative level. It is currently not known how the number of virus particles in a cell influences the replication kinetics of the viruses, and is a current focus of our research. Cells infected with the wild-type strain only die as a result of virus replication with a rate a_1, and are killed by CTLs with a rate p. Cells infected with the escape mutant are killed by the virus with a rate a_2 and are not killed by the CTLs. Cells infected with both strains are killed by CTLs with a rate p, and are assumed to be killed by the virus with the rate determined by the faster killing strain, denoted by a_i in the model. It is also possible that the rate at which co-infected cells are killed, a_i, is higher than the rate at which any of the singly infected cells are killed. For example, this could be an additive effect, $a_i = a_1 + a_2$, although this relationship is not known at the moment. Our work indicates that the type of analysis performed here does not depend on the details of this relationship on a qualitative level. It will be assumed that fitness differences between the two strains are determined by the viral replication rates independent of the rate of virus-induced cell killing.

The CTL population is stimulated to expand by the wild-type virus with a rate c, and declines with a rate b in the absence of antigenic stimulation. It is important to comment on the term that describes CTL expansion. Note that it is a 'total production' term rather than a proliferation term. The mechanisms by which CTLs react to stimulation are not completely understood.[67–73] There is considerable information about the processes that occur during acute infections, where exponential expansion of antigen-specific CTLs is observed, but less information is available for persistent infections, where the dynamics of CTL expansion and contraction are less well known. What is clear is that CTL expansion is driven by the continued presence of the virus, and that virus load is determined in part by the characteristics of the CTL response. This is what the simple model tries to capture. Apart from the expression used in the model above, there are alternative mathematical ways in which these simple assumptions can be expressed. Analysis, combined with numerical simulations, however, indicate that results remain robust on a qualitative level. The current term has been chosen because it leads to relatively stable dynamics and does not include the possibility that virus-specific CTLs go extinct completely at low levels of antigenic stimulation (as some other models do).

The effect of the strength of the CTL response, or the CTL responsiveness (parameter c in the model) on the competition between wild-type and escape virus strains, will be discussed. First, it will be assumed that the viruses do not differ in their replication parameters, but only differ in their ability to be recognized by CTLs. Then, this analysis will be extended to include differences in the viral replication rates between the two strains as well. In the model written down above, it is assumed that the escape mutant is not recognized by any CTLs at all. This assumption corresponds to complete escape from all CTL responses, and provides a good basis for exploring the properties of the model. However, it is also possible that once a mutant has escaped from a CTL response, an alternative CTL clone, directed against the escape mutant, rises and suppresses the escape mutant to a certain degree.[74,75] This will be explored at the end of this discussion.

The case of virus strains which differ only in immunogenicity

Here, we examine how virus competition is affected by the presence of a CTL response, assuming that the viruses do not differ in basic replication parameters. That is $\beta_1 = \beta_2$ and $a_1 = a_2$. It is assumed that the virus strains can establish a persistent infection in isolation from each other. In this case, the two virus strains will always coexist in the context of co-infection and the presence of a CTL response. This coexistence is described by a stable equilibrium, the expressions for which are too complex to write down here.

We will investigate how the CTL responsiveness (parameter c) influences the outcome of the infection (Figure 2). A higher value of c correlates with a lower level at which the wild-type virus population persists. We will examine how variation in this parameter influences the relative abundance of the two virus strains at equilibrium, and how it influences overall set-point virus load.

Assume that the CTL response is relatively strong and maintains the wild-type virus at relatively low levels. This means that most cells remain uninfected by the wild-type virus and are available for infection with the escape strain. Since these cells are only infected with the escape virus, it enjoys a significant selective advantage and grows to high levels. Thus, setpoint virus load is high and dominated by the escape strain. If the strength of the CTL response, c, is lowered, the following is observed. The wild-type virus population persists at higher levels. Therefore, more cells are available for co-infection, and less cells are available for the escape mutant to infect all by itself. Hence, the advantage enjoyed by the escape mutant is reduced, as a greater number of escape viruses are killed in the co-infected cells. Because a significant number of both wild-type and escape viruses are killed, overall setpoint virus load is lower, and the relative abundance of the two virus strains is more balanced (Figure 2). If the strength of the CTL response is reduced further, overall setpoint virus

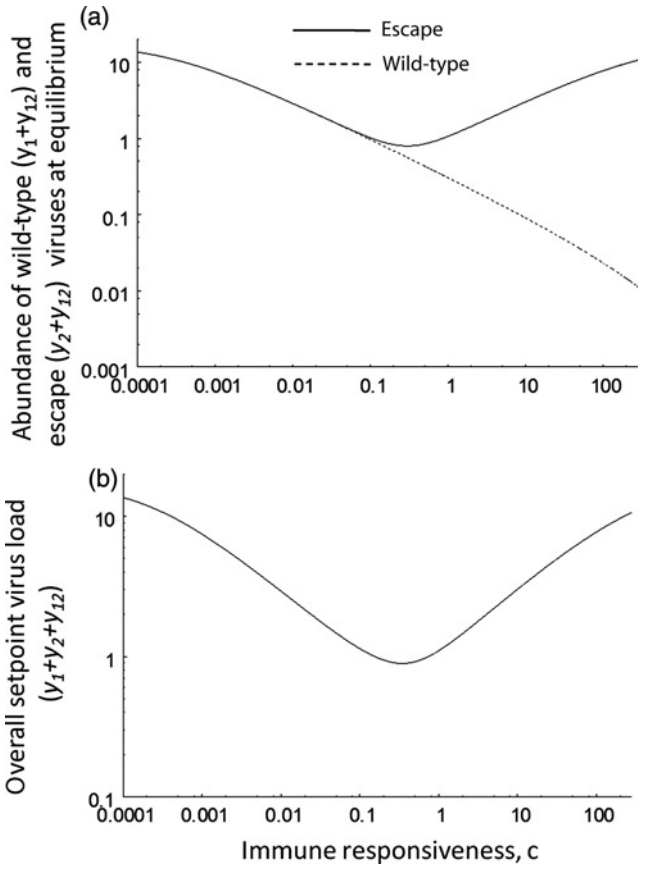

Figure 2 Dependence of the virus competition outcome on the cytotoxic T lymphocyte (CTL) responsiveness, *c*, in the co-infection model that assumes no replicative cost of the escape virus. The figure is based on numerical simulations and shows the equilibrium abundances of the viruses as the CTL responsivenss, *c*, is increased. (a) Dependence of the wild-type and escape virus levels on the CTL responsiveness. The stronger the CTL responsiveness, the lower the number of wild-type viruses. The number of escape viruses first declines towards a minimum, and then rises again as the CTL responsiveness is increased. (b) Dependence of overall setpoint virus load on the CTL responsiveness. Overall setpoint virus load first declines to a minimum, and then rises again as the CTL responsiveness is increased. That is, lowest setpoint virus load is observed at an intermediate CTL responsiveness. While in this parameter region, the number of wild-type infected cells is sufficiently high such that a sizable amount of co-infection can occur, the rate of CTL-mediated killing is still strong enough to remove both wild-type and escape viruses in the co-infected cells with a significant rate. Parameter values were chosen as follows for the purpose of illustration: $\lambda = 1$, $d = 0.01$, $a_1 = 0.05$, $a_2 = 0.05$, $\beta_1 = 3$, $\beta_2 = 3$, $b = 0.1$, $p = 1$. Note that the CTL responsiveness, *c*, is varied over a very wide range in this diagram. This does not imply that there is such large variation in this parameter among patients, i.e. the realistic variation might be through one or two logs. A wide parameter space has been plotted to show the complete parameter space ranging from no immune control of the wild-type virus to suppression of the wild-type virus to insignificant levels. The exact width of this parameter space will vary according to the parameter combinations selected. See model description for discussion of parameter selection. Further, note that if the CTL responsiveness values are weak, the wild-type and escape mutants are almost equally abundant, meaning that most cells are co-infected. The reason is that with almost no immunity, virus load is around maximum levels and almost all susceptible target cells have been infected. Consequently, it is much more likely that the virus enters an already infected cell rather than an uninfected cell, thus increasing the fraction of co-infected cells.[8,15,59] Such a high fraction of co-infected cells could occur at peak virus load during acute infection or towards the end stage of HIV disease. However, if the virus does not grow to maximum levels and does not use up all susceptible target cells in culture experiments or *in vivo* (which is likely to be the case in many circumstances), then this extreme end of the spectrum is not observed and the fraction of co-infected cells is lower

load rises again (Figure 2). This is because the wild-type virus population itself is now maintained at higher levels. The escape mutant is also maintained at similar levels because more cells will be co-infected.

In summary, an intermediate strength of the CTL response minimizes the detrimental effect of an existing escape mutant in the context of co-infection (Figure 2). The wild-type virus is maintained at relatively low levels, but still high enough to ensure that a sufficient amount of co-infection occurs. In this case, a significant number of escape genomes are destroyed by CTLs. Too strong a CTL response leaves too many uninfected cells that are solely available for infection by the escape virus. At the moment, it is not possible to determine the exact degree of co-infection that is necessary to observe these results. This depends on the particular parameter values of the model, many of which are not known.

Note that for low viral replication rates, the existence of an intermediate CTL strength that minimizes the effect of the escape mutant on virus load can break down. In this case, increasing the strength of the CTL response leads to a reduction in total setpoint virus load towards an asymptote. This occurs if the basic reproductive ratio of the virus is close to one, and virus load is low even in the absence of immunity. This is likely to be a biologically unrealistic parameter region. For all other parameter combinations, the results described here hold true and can thus be considered applicable to HIV infection.

When comparing the model properties described here to a model without co-infection, it is found that in the

absence of co-infecton, only the escape virus persists and the wild-type virus goes extinct. Overall setpoint virus load does not depend on the CTL responsiveness, c.

The case of virus strains which also differ in replication parameters

Here, we assume that there can be a difference between the two strains in the basic replication parameters. In particular, it has been found that HIV-1 CTL escape mutants often show impaired reproductive fitness, i.e. that they replicate less efficiently than wild-type strains, and that in the absence of a CTL response, a virus population dominated by escape mutants reverts back to wild-type.[76–80] A similar finding has been made in the context of hepatitis C virus infection.[81] In the mathematical model, this corresponds to $\beta_2 < \beta_1$. In the following, we examine the dynamics under these assumptions, in particular how the CTL responsiveness influences the outcome. We distinguish between two parameter regions. The model outcomes in both parameter regions are summarized schematically in Figure 3 and are explained as follows. These results were obtained by a combination of extensive numerical simulations and some invasion analysis where possible.

(a) First assume that $\beta_2 > (a+d)^2/\lambda$ (Figure 3a). This means that the escape virus can in principle be maintained by replication in wild-type infected cells only, and can thus persist even if the number of uninfected cells converges to zero. If the difference between the replication rates β_1 and β_2 is relatively low, then the effect of the CTL responsiveness on the outcome is the same as observed in the simplified scenario where there is no difference between the two viral replication rates. That is, the wild-type and escape strains always coexist and minimum setpoint virus load is observed for an intermediate CTL responsiveness. If the difference in the viral replication rates (β_1 and β_2) is larger, however, a different behavior is observed (Figures 3 and 4a). For a low CTL responsiveness, both virus strains coexist and setpoint virus load is relatively high because immunity is weak. If the CTL responsiveness is increased beyond a threshold, only the wild-type virus persists and the escape mutant goes extinct, correlating with a drop in overall setpoint virus load. The reason is that the wild-type virus now persists at higher levels. Therefore, more cells are co-infected, leading to the removal of more escape strains by the CTL. As a result, the escape strain cannot spread fast enough and goes extinct. A further increase in the CTL responsiveness leads again to the coexistence of the two virus strains and to a rise in overall setpoint virus load. The stronger CTL response suppresses the wild-type virus to lower levels. This correlates with the existence of a higher number of uninfected target cells that are solely available for infection by the escape strain. Thus, the escape mutant gains in fitness and can replicate without being significantly affected by the CTL response. A computer simulation that shows a time series of wild-type and escape virus populations is given in Figure 5, showing both extinction of the escape strain and convergence of both virus strains to a stable equilibrium.

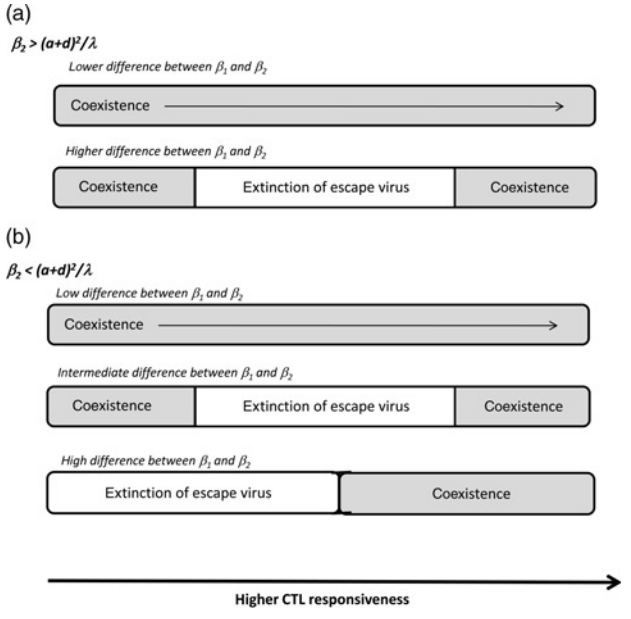

Figure 3 Schematic, summarizing the dependence of the competition outcome between wild-type and escape virus, assuming that the escape virus carries a replicative cost. Two parameter regions are distinguished corresponding to (a) faster and (b) slower replication of the escape virus. Note that in (b), outcome (ii) is not observed if the replication rate of the escape mutant, β_2, is low such that its basic reproductive ratio in isolation is close to one

(b) Now, assume that $\beta_2 < (a+d)^2/\lambda$ (Figure 3b). This means that the escape mutant cannot be maintained by replication in wild-type infected cells alone, but requires a certain number of uninfected cells to persist as well. In this case, the following outcomes of competition are observed (Figure 3b). (i) If the replication rate of the wild-type, β_1, is only slightly larger than that of the escape mutant, β_2, then the two virus strains always coexist, regardless of the CTL responsiveness. (ii) Next, assume that the difference in the replication rates β_1 and β_2 is larger. Now coexistence of the two virus strains occurs if the CTL responsiveness is low or high, but the escape mutant goes extinct if the CTL responsiveness is intermediate. (iii) If the difference between the viral replication rate is sill larger, then the escape mutant goes extinct if the CTL responsiveness lies below a threshold, and coexistence is observed if the CTL responsiveness lies above that threshold. This outcome was not observed in the previous parameter region explored above. The reason is that in the current regime, the escape virus does not replicate fast enough to be maintained in the population of already infected cells alone. In other words, it needs a sizable pool of uninfected cells to persist. If immunity is weak, most cells will be infected with the fast replicating wild-type, leaving very few uninfected cells available for infection by the escape strain. The escape virus can only invade if immunity suppresses the wild-type virus sufficiently such that the availability of uninfected target cells is relatively high. In all cases, the dependence of overall setpoint virus load on the CTL responsiveness is similar as before. That is, setpoint virus load first decreases towards a minimum and then rises again as the CTL responsiveness is increased.

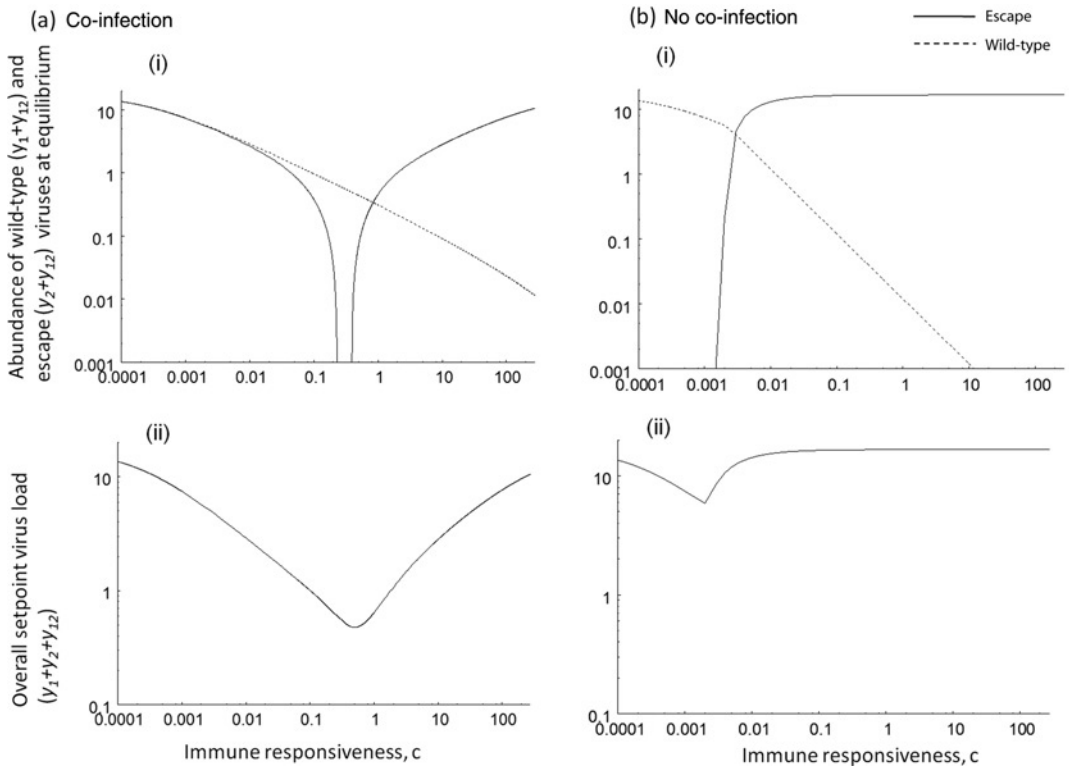

Figure 4 (a) Dependence of the competition outcome on the cytotoxic T lymphocyte (CTL) responsiveness, *c*, in the co-infection model that assumes the presence of a cost of the escape virus. The figure is based on numerical simulations and shows the equilibrium abundances of the viruses as the CTL responsiveness, *c*, is increased. Panel a,i shows the effect of the CTL responsiveness on the abundance of the wild-type and escape virus. At low CTL responsiveness values, the two virus strains coexist. At intermediate CTL responsiveness values, the escape virus goes extinct while the wild-type virus persists. If the CTL responsiveness is increased further, we again observe coexistence between the two virus strains, with a significant dominance of the escape strain. This is because at high values of *c*, strong CTL-mediated suppression of the wild-type virus occurs. Note that extinction of the escape virus at an intermediate CTL responsiveness does not occur in all parameter regions, see text and Figure 3. Panel a,ii shows that overall setpoint virus load first declines to a minimum, and then rises again as the CTL responsiveness is increased. (b) Same dependencies, using a model that assumes absence of co-infection. Panel b,i shows that the escape virus goes extinct at low CTL responsiveness values, while it coexists with the wild-type if the CTL responsiveness lies above a threshold. Overall setpoint virus load (panel b,ii) again first declines to a minimum and then rises again as the CTL responsiveness is increased. The minimum setpoint virus load is, however, comparatively high. Parameters were chosen as follows for illustrative purposes: $\lambda = 1$, $d = 0.01$, $a_1 = 0.05$, $a_2 = 0.05$, $\beta_1 = 3$, $\beta_2 = 1$, $b = 0.1$, $p = 1$. For notes regarding the relative abundance of wild-type and escape virus, as well as the parameter range explored, see legend to Figure 2

Finally, we compare the above findings to the dynamics between escape and wild-type strains in the absence of co-infection (Figure 4b). In this case the wild type excludes the escape strain in the absence of immunity or if the CTL responsiveness lies below a threshold. This occurs if $\beta_2 x^*/(a_2 + d) < 1$, where x^* is the equilibrium abundance of uninfected cells in the presence of the wild-type virus only (not shown here, because of expression). On the other hand, if the CTL responsiveness lies above this threshold and the condition is not fulfilled, then the escape strain can coexist with the wild-type strain. The reason is that suppression of the wild-type to low levels by CTL correlates with a high availability of susceptible target cells, allowing the escape strain to spread largely unopposed. At weak immunity, persistence of the escape strain is not possible because no co-infection occurs. Similar to the co-infection scenario, overall setpoint virus load first declines to a minimum and then rises again as the CTL responsiveness is increased. The magnitude of these changes in setpoint virus load is, however, relatively small in realistic parameter regions. Minimum virus load is given by $y_{\min} = (\beta_2\lambda/\beta_1 a_1) - (d/\beta_1)$. On an approximate level, $y_{\min} = (\beta_2/\beta_1)y_1^{(0)}$, where $y_1^{(0)}$ is the amount of wild-type virus in the absence of immunity (assuming that the viruses replicate efficiently and that the value of d/β_1 is relatively small). In other words, the fraction of minimum setpoint virus load in the presence of immunity relative to virus load in the absence of immunity is given by β_2/β_1. If the cost of the escape mutation is not excessive, no significant reduction of overall virus load is achieved by the CTLs. For example, if the escape mutant carries a 10% fitness cost, minimum setpoint virus load in the presence of CTLs is only 10% less than virus load in the absence of immunity.

Recognition of escape strains by alternative CTLs

The above analysis assumed that a mutant which has escaped a given CTL response is not recognized by any other CTL response. This corresponds to total escape. It is possible, however, that new CTL clones rise to oppose the new escape mutant.[74,75] It is thought that such responses are usually weaker than the original response, but can nevertheless suppress the replication of the escape mutant to a certain degree.[82] Here, we investigate how this scenario

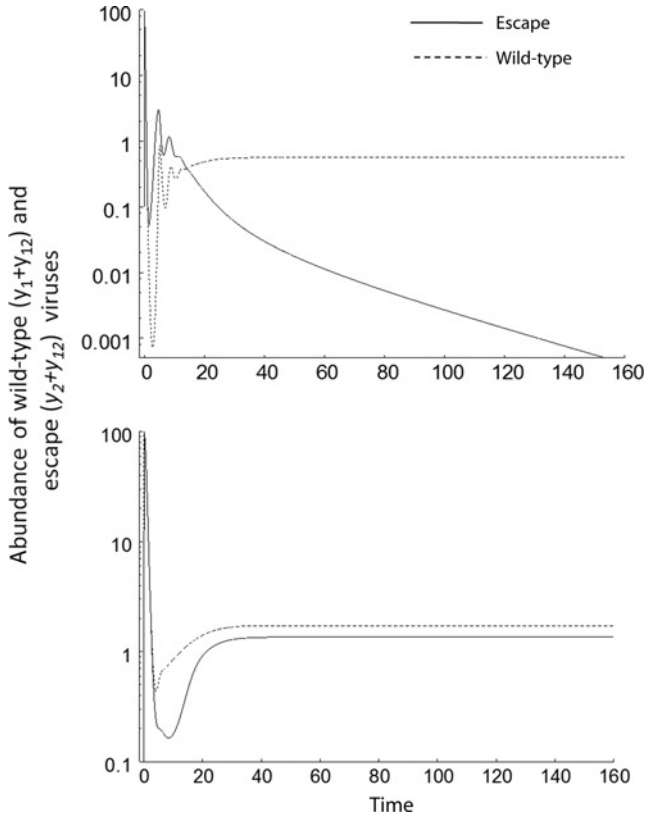

Figure 5 Time series for two possible outcomes of the interaction between a wild-type virus and a cytotoxic T lymphocyte (CTL) escape virus in the co-infection model that includes a CTL response, assuming that the escape mutant carries a replicative cost. (a) The escape virus goes extinct and only the wild-type virus persists, converging to a stable equilibrium. (b) The escape virus coexists with the wild-type virus. Both populations converge to a stable equilibrium. Note that the escape strain persists at lower levels than the wild-type. This is a consequence of the particular parameter combination chosen for this simulation. The escape strain can be less or more abundant than the wild-type strain at equilibrium, depending on the parameter values, see Figure 4. Parameters were chosen as follows for purposes of illustration: $\lambda = 1$, $d = 0.01$, $a_1 = 0.05$, $a_2 = 0.05$, $\beta_1 = 3$, $\beta_2 = 1$, $b = 0.1$, $p = 1$. (i) $c = 0.3$; (ii) $c = 0.03$

influences the model predictions. Thus, we include a second CTL population into the model, z_2, that is stimulated only by the escape mutant with a rate c_2. It is assumed that $c_2 = fc_1$, where $f < 1$. This CTL response declines with a rate b and kills cells infected with the escape mutant alone, y_2, and cells infected with both virus strains, y_{12}. The conditions under which the escape mutant goes extinct or coexists with the wild-type strain are the same as in the total escape scenario. The effect of the CTL responsiveness on the overall setpoint virus load, however, can be different. If the responsiveness of the alternative CTL clone is relatively low (value of f is small), then the behavior converges to the one observed in the total escape scenarios. On the other hand, if the responsiveness of the alternative CTL clone is higher (higher value of f), then the dependence of overall setpoint virus load on the CTL responsiveness is different. For relatively high values of f, there is no minimum setpoint virus load at an intermediate CTL responsiveness. Instead, setpoint virus load declines monotonically as the CTL responsiveness is increased. Although the escape strain can invade at higher CTL responsiveness values, its replication is limited by the alternative CTL response, keeping overall virus load low. In the total escape scenario, the escape mutant can replicate freely if the wild-type virus is significantly suppressed and can reach levels that are similar to those that the wild-type can reach in the absence of immunity, hence, the difference in behavior. Between these two extremes, it is possible that there is a minimum setpoint virus load for an intermediate CTL responsiveness. However, virus load at higher CTL responsiveness values is still relatively low because escape mutant replication is again suppressed to a certain degree by the alternative CTL response that emerges.

Strength of CTLs, occurrence of escape mutants and host adaptation to infection

The above discussion suggests that an intermediate strength of the CTL response against the wild-type is most detrimental to a CTL escape mutant, and can even lead to its extinction in some circumstances. The reason for this is that an intermediate CTL response still leads to a sufficient amount of killing to suppress viral load, while keeping the number of infected cells sufficiently high to make sure that a significant amount of escape virus is removed in co-infected cells. A stronger CTL response against the wild-type is beneficial for the escape mutant. This is because stronger CTLs suppress the wild-type virus more, leaving many uninfected cells available for infection by the escape mutant alone, which leads to protection from CTL-mediated activity. A weak CTL response allows the wild-type virus to replicate at high levels, which in turn allows the escape virus to persist as well. Because of these patterns, overall setpoint virus load is also lowest for an intermediate strength of the CTL response.

These patterns were observed in all models considered, including the simple case where the wild-type and the escape mutant are characterized by the same replication rate and differ only in immunogenicity, as well as the more complicated case where escape mutants were assumed to carry a replicative cost. In the latter scenario, these results are the strongest: in specific parameter regions, an intermediate CTL response not only leads to minimum virus load but can also lead to the extinction of the escape mutant. While the more complicated scenario, which assumes differences in viral replication, contains a variety of parameter regions with slightly different behaviors, the overall message remains the same across all parameter regions. The results break down in biologically unrealistic parameter regions in which the viral basic reproductive ratio is close to one (i.e. the virus infection can be barely maintained in the patient in the absence of immunity). In addition, if an escape virus elicits a CTL response that is comparable in strength to the response against the wild-type, then virus load is not minimized at an intermediate CTL responsiveness. However, it is clear that at least some escape mutants can potentially lead to substantially higher virus loads, indicating that they are not significantly opposed by CTLs with a new specificity. Therefore, within

the context of the model discussed here, our results apply to parameter regions that are representative of HIV.

HIV-1 replication is characterized by a relatively high mutation rate, as well as an enormous amount of viral replication, especially during the acute phase of the infection. CTLs are thought to play a major role in down-regulating the very large number of infected cells that exist at peak virus load during acute infection.[83–91] Given the high mutation rate and the large amount of viral replication, it is possible that by the time virus load has reached its peak, escape mutants have been generated and pre-exist even before the CTL response kicks in. Even during the chronic phase of the infection, a substantial amount of viral replication and mutant generation occurs. Thus, it can be hypothesized that an intermediate strength of the CTL response, and thus a suboptimal suppression of HIV, could be favorable to the host because it minimizes the impact of these CTL escape mutants on setpoint virus load and thus on subsequent disease progression. While the suppression of the wild-type virus is suboptimal, this allows the occurrence of a sufficient level of co-infection such that a significant number of escape genomes are removed by CTLs in co-infected cells. This leads to limited replication of the escape mutant, and possibly extinction of the escape mutant if it carries a replicative cost. Hence, setpoint virus load is relatively low, potentially slowing disease progression.

If the CTL response is stronger and suppresses the wild-type virus to lower levels, then two outcomes are possible, depending on whether or not escape mutants have been generated. If escape mutants have not been generated, then such a strong CTL response will lead to low virus load and slow disease progression. On the other hand, if escape mutants have been generated, then strong wild-type suppression allows the escape mutant to replicate to high levels that are almost comparable to those observed for virus replication in the absence of immunity. This in turn would lead to fast disease progression.

If in the majority of HIV-1 infected patients, escape mutants are generated relatively early, then it would pay for the host to mount a suboptimal CTL response that limits the impact of the escape mutants on setpoint virus load. Therefore, the failure to suppress virus loads to very low levels in the majority of HIV-1 infected patients could be more of an adaptive state than we currently think and could prevent the immediate and fast progression to pathology. In this context, it is interesting to note that monkeys which are naturally infected with SIV (the monkey equivalent of HIV) and which never develop AIDS, show a range of virus loads during chronic infection.[4,92,93] This system has not evolved towards efficient control of viral replication, a state characteristic of most other persistent infections that do not typically cause pathology. In the light of the current analysis, one possibility is that lack of strong CTL-mediated virus suppression could limit the impact of immune escape mutations, thus contributing to lack of pathology. However, the reasons for lack of pathology in natural SIV infection and the role of CTL escape mutants in these dynamics are complex and currently not understood.

The model can also contribute to understanding the conditions when certain CTL escape mutants are expected to be present or absent in patients. While HIV-1 can readily accumulate point mutations that are necessary for escape, and while studies have clearly demonstrated the emergence of escape mutants in HIV-1 infection,[38,42,43,48,83,94–96] other studies have failed to find evidence for escape mutations in some HIV-1 infected individuals.[97–102] Such findings have lead to the suggestion that CTLs do not contribute significantly to virus control in these cases. This is in contrast to findings that link the decline of virus load in acute HIV-1 infection to the rise of CTL responses, and that correlate more vigorous CTL responses to lower setpoint virus loads and slower disease progression.[38,42,94,95,103] The evolutionary dynamics of CTL escape mutants appear to be more complex than those of drug resistant virus strains, which tend to appear readily under the appropriate selection pressures. While escape from CTL responses certainly confers a selective advantage to the mutant, it has recently been found that escape mutations can also have a detrimental effect on the virus because it induces less efficient viral replication.[76–80] Whether the escape mutant will rise or not therefore depends on the balance between the benefit and the cost of such a mutation in the presence of an ongoing CTL response. This is indeed indicated by experimental studies that have examined the evolutionary dynamics of various escape mutants *in vitro*.[104] The modeling presented here adds to this picture. It suggests that under conditions where the escape mutant invades in the absence of co-infection, the escape mutant can go extinct if co-infection occurs, even though a relatively high amount of CTL-mediated killing occurs. In fact, the CTL-mediated killing is the reason for the extinction of the escape mutant. If escape mutants are removed by CTLs in co-infected cells, then they cannot be maintained if they also bear a replicative cost, as suggested by experimental data. Therefore, the absence of escape mutants does not indicate that CTLs do not play a role in fighting HIV-1 replication. To the contrary, a weak CTL response that has little effect on the wild-type virus can lead to the coexistence of the escape and wild-type viruses, while a stronger and intermediate CTL response that suppresses the wild type to a larger extent can lead to extinction of the escape virus. Put another way, when the CTL response to the mutant is intermediate in strength, co-infection of cells with both wild-type and less fit escape mutants further reduces the fitness of the mutant to a point where it cannot compete against the wild-type virus. Of course, a very strong CTL response that suppresses the wild-type virus to very low levels can again lead to the emergence of the escape mutant because most cells will be infected with the escape mutant only, with co-infection events being negligible. Thus, according to the model, escape mutants are expected to be found in the virus population if the CTL response is relatively weak or relatively strong, while escape mutants can be driven to extinction when the CTL response is of intermediate strength and when there is a fitness cost to the escape mutation. In summary the model suggests that the presence or absence of escape mutants in the viral population in a patient does not correlate with the efficiency with which the CTL response controls the wild-type virus in a straightforward way. The mere presence or absence of escape

mutants cannot be used to judge the role of CTLs for controlling the virus *in vivo*.

Conclusion

Throughout this paper, we have emphasized that our understanding of the dynamics between viruses and immune responses, as well as the interpretation of experimental observations, can change significantly if the dynamics are investigated in the context of multiple infection of cells, or co-infection. While we concentrated on the evolution and emergence of immune escape mutants, such changes in our understanding are likely to apply to other aspects of the dynamics as well. With respect to host adaptation to HIV infection, it would be interesting to investigate the occurrence and kinetics of co-infection in hosts that are naturally infected with their respective immunodeficiency viruses but never develop pathology. Given that co-infection can substantially alter the outcome of competition between virus strains, and thus the outcome of the evolutionary dynamics of the virus in host, it is possible that differences in the occurrence and kinetics of co-infecion could provide clues to the exact mechanism by which pathology occurs in humans.

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