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Quantifying factors determining the rate of CTL escape and reversion during acute and chronic phases of HIV infection

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Abstract

Human immunodeficiency virus (HIV) often evades cytotoxic T cell (CTL) responses by generating variants that are not recognized by CTLs. However, the importance and quantitative details of CTL escape in humans are poorly understood. In part, this is because most studies looking at escape of HIV from CTL responses are cross-sectional and are limited to early or chronic phases of the infection. We use a novel technique of single genome amplification (SGA) to identify longitudinal changes in the transmitted/founder virus from the establishment of infection to the viral set point at 1 year after the infection. We find that HIV escapes from virus-specific CTL responses as early as 30-50 days since the infection, and the rates of viral escapes during acute phase of the infection are much higher than was estimated in previous studies. However, even though with time virus acquires additional escape mutations, these late mutations accumulate at a slower rate. A poor correlation between the rate of CTL escape in a particular epitope and the magnitude of the epitope-specific CTL response suggests that the lower rate of late escapes is unlikely due to a low efficacy of the HIV-specific CTL responses in the chronic phase of the infection. Instead, our results suggest that late and slow escapes are likely to arise because of high fitness cost to the viral replication associated with such CTL escapes. Targeting epitopes in which virus escapes slowly or does not escape at all by CTL responses may, therefore, be a promising direction for the development of T cell based HIV vaccines.

Keywords: acute HIV infection, escape mutations, CTL response, cost of escape, mathematical model.

Abbreviations: CTL, cytotoxic T lymphocyte, SGA, single genome amplification.

Short running title: escape in acute HIV infection

1 Introduction

A hallmark of HIV infection of humans is the generation of viral variants that are not recognized by the virus-specific CTL responses (1, 2). Many of such mutants, although not all, result from point mutations in epitopes presented by the host MHC class I molecules and recognized by the CTL response (1, 3–5).

The importance of escape from the CTL response for the disease progression is not well established (for a critical overview, see (6)). Several studies have documented an increase in the viral load and disease progression following escape from the CTL response (7, 8). However, CTL escape in other cases has no effect on disease progression (9, 10). Escape from CTL responses has been considered as one reason for the failure of T cell based HIV vaccines (11–16). However, not always failure of a vaccine is due to viral escape (17). Escape from CTL response also occurs in a non-pathogenic SIV infection of non-human primates such as sooty mangabeys which do not progress to the disease (18). It has been suggested that some CTL escapes may be beneficial since such escapes could also lead to a reduction in the replicative fitness the escape variant (19, 20).

Mathematical models have been proposed to understand importance, timing and kinetics of CTL escape in HIV/SIV infection (21–31). Nowak et al. (21) proposed a model based on the escape of HIV from the immune response to explain disease progression of HIV infected individuals. More recently, Fernandez et al. (25) derived a simple mathematical model for simian-human immunodeficiency virus escape from the CTL response and for the first time estimated the rate at which such CTL escapes accumulate in the virus population. Following studies have shown the importance of taking into account changes in the virus replication rate and CTL response in determining the rate of viral escape over the course of HIV/SIV infection (27–29, 31). In another important study by analyzing a large number of CTL escapes in HIV infected patients, Asquith et al. (26) concluded that CTL response specific for a single epitope of HIV is not very efficient at killing virus-infected cells. The authors estimated that, on average, a single CTL response kills HIV-infected cells at the rate of 0.01 per day (26) which is about 1-2% of the death rate of cells, productively infected with HIV (32). Interestingly, this study also found that the rate at which virus escaped from the CTL response was significantly higher during the early phase than that in the chronic phase of HIV infection (26). A similar observation for several viral epitopes has also been made during SIV/SHIV infection of non-human primates (1, 31, 33, 34).

Since many of these previous studies employed cross-sectional data it is unclear if the same conclusions on the timing and kinetics of CTL escape hold in a given HIV infected patient. Some earlier studies did involve analysis of viral escape from the CTL response over the course of infection (35, 36) but the rates of viral escape have not been quantified. In this paper we use data from our recently published study in which we followed several individuals from the very early stages of acute HIV infection to the viral set-point (37).

Using a single genome amplification (SGA) technique (38, 39), were were able to predict the viral sequences that founded the current infection and map the CD8 T cell response to the founder virus. Analysis of the sequence data revealed that there is a rapid escape of HIV from several CTL responses occurring during the acute phase of the infection. In the chronic phase, however, the rate of viral escape was significantly reduced. Comparing the data on viral escape with the dynamics of CTL responses, we concluded that the slow rate of escape of the virus from the CTL response in the chronic phase of the infection is likely to arise because of the reduction in viral (intrinsic) fitness due to CTL escape.

2 Materials and Methods

2.1 Basic mathematical model of viral escape from a single CTL response

A model for the dynamics of escape of a virus from a single CTL response has been described in detail previously (25–27, see also Supplementary Information). In brief, we assume that the founder (wild-type) virus replicates at a rate r and cells infected with the virus are killed by the CTL response at the rate k. The CTL escape mutant, however, has a lower replication rate $(1-c)r$ (due to fitness cost c) and cells infected with the mutant virus are not killed by the epitope-specific CTL response. When both viral variants (wild type w and the escape mutant m) are present in an infected host, the dynamics is described by the following equations (see Supplementary Information):

$$\frac{\mathrm{d}w}{\mathrm{d}t} = rw - (\delta + k)w, \tag{1}$$

$$\frac{\mathrm{d}m}{\mathrm{d}t} = (1-c)rm - \delta m, \tag{2}$$

where w and m is the number of cells infected with the founder, wild-type and CTL mutant viruses, respectively, c is the cost of the escape mutation defined as a selection coefficient and δ is the death rate of productively infected cells due to viral pathogenecity. Since both SIV and HIV particles are known to be short-lived *in vivo* (32, 40, 41), densities of virus particles are likely to be proportional to the densities of cells productively infected with each virus variant given in eqns. (1)–(2).

Note that in our previous study we found that some mutations can simultaneously lead to escape from the CTL response and to restoration of viral fitness by reverting to a population consensus sequence (37), suggesting that cost of escape c can be negative. Note that the

model eqn. (1)–(2) is very general and may incorporate implicit or explicit changes in the rate of virus replication, changes in the death rate, and/or epitope-specific CTL response over time (see also Supplementary Information). As we have shown previously and as was confirmed in other studies, changes in the rate of virus replication in HIV/SIV infection (for instance, because of the depletion and recovery of target cells) can dramatically affect the kinetics of escape of the virus from CTL response and reversion of escape variants upon transmission to MHC-mismatched hosts (27, 29, 42, see also Results section).

It is useful to rewrite eqns. (1)–(2) to describe the dynamics of the ratio of the mutant to the wild-type density $z = m/w$:

$$\frac{\mathrm{d}z}{\mathrm{d}t} = \frac{\mathrm{d}m/w}{\mathrm{d}t} - z\frac{\mathrm{d}w/w}{\mathrm{d}t} = z\,(k - cr)\,, \tag{3}$$

where cr is the absolute difference in the replication rates of the wild-type and the mutant (43, 44). Note that this equation is also valid if all parameters in the model are dependent explicitly (or implicitly) on time. Integrating eqn. (3) we find

$$z(t) = z_0 \mathrm{e}^{\varepsilon t}, \tag{4}$$

where $z(t)$ and z_0 is the ratio of the frequency of the escape mutant to the frequency of the wild type virus in the population at some time t and at time $t = 0$, respectively, and $\varepsilon = \langle k - cr \rangle$ is the net average rate of accumulation of the mutant in the virus population which we call the escape rate. The average is taken because in general, the rates k and r could change during an infection (27, 29). Since the frequency of the mutant virus in the viral population is given by $f = z/(1 + z)$, changes in the frequency over time are given by

$$f(t) = \frac{z(t)}{1 + z(t)} = \frac{f_0}{f_0 + (1 - f_0)\mathrm{e}^{-\varepsilon t}}, \tag{5}$$

where f_0 is the frequency of the escape mutant in the population at time $t = 0$. This equation is similar to one proposed in previous studies (25–27). The time at which the escape variant reaches frequency of 50% in the population is calculated as $t_{50} = \ln(f_0^{-}1 - 1)/\varepsilon$.

2.2 Incorporating CTL and virus dynamics into the model

As defined above, the rate of viral escape ε is an average of the rate k at which cells that express the wild type epitope are killed by the CTL response and the cost of escape c multiplied by the virus replication rate r, $\varepsilon = \langle k - cr \rangle$ (27, see above). Both the killing rate

k and viral replication rate r clearly will change over time over the course of HIV infection. The killing rate should be proportional to the magnitude of the epitope-specific CD8 T cell response, and in the simplest case of mass-action killing, $k = k_E E(t)$ where k_E is the per capita killing efficacy of CTLs and $E(t)$ is the magnitude of the epitope-specific CTL response (see also Supplementary Information). In the case of a similar killing efficacy of T cells of different specificities, on average, the rate of escape should be proportional to the magnitude of the epitope-specific CD8 T cell response.

Alternatively, variability in escape rates could arise because of different fitness costs associated with escape and/or because of changes in the viral replication rate over time. Indeed, eqn. (3) suggests that at high rates of virus replication, the rate of escape is expected to be smaller ($\varepsilon \approx k - cr$) than that when there is no virus replication ($\varepsilon \approx k$). This is simply because at a high replication rate, the wild type virus has the opportunity to offset its killing by many rounds of replication while escape variant suffering replicative fitness cost, will not replicate as fast (27). Therefore, we expect that during the decline of viral load when virus replication is limited, escape should occur at a faster rate than during the chronic phase where the replication rate is higher (see Figure S3 in Supplementary Information). Changes in the viral replication rate over the course of HIV infection can be calculated using the following algorithm. In general, the dynamics of the viral load $V(t)$ is given by the following equation

$$\frac{\mathrm{d}V(t)}{\mathrm{d}t} = (r - d)V(t) \tag{6}$$

where r and d are the per capita time-dependent viral replication and death rate, respectively. From eqn. (6), the rate of virus replication at time t is given by

$$r = d + \frac{\mathrm{d}\ln V(t)}{\mathrm{d}t} \approx \delta + \frac{\mathrm{d}\ln V(t)}{\mathrm{d}t}, \tag{7}$$

where $d \approx \delta \approx 0.5\ day^{-1}$ is the death rate of cells, that are productively infected with the virus; the death rate δ does not change significantly during the acute or chronic phases of the infection (45–47). Thus, given changes in the viral load in experimental data, eqn. (7) allows one to calculate changes in the rate of virus replication over time.

2.3 Mathematical model for multiple escapes

A simple model given in eqns. (1)–(2) tracks changes in the density of the wild type (transmitted) virus and a single variant that has escaped recognition by one CTL response. In acute HIV infection, the virus escapes from multiple CTL responses (37, 48), and therefore

to track the dynamics of viral escape from multiple responses, we extend eqns. (1)–(2) in the following way. We assume that the there are in total n CTL responses that control viral growth and, potentially, the virus can escape from all n responses. A CTL response that recognizes the i^{th} epitope of the virus kills the virus-infected cells at the rate k_i, and escaping from the i^{th} CTL response leads to a viral replicative fitness cost c_i. Assuming that all viral variants are present in the population initially, the dynamics of the wild type and the escape from CTL responses is given by (see also Supplementary Information)

$$\frac{dw}{dt} = \left[r - \sum_{i=1}^{n} k_i - \delta \right] w, \tag{8}$$

$$\frac{dm_{\mathbf{i}}}{dt} = \left[(1 - c_{\mathbf{i}})r - \sum_{i \in \mathbf{i}} k_i - \delta \right] m_{\mathbf{i}}, \tag{9}$$

where $w = m_{\mathbf{0}}$ is the density of the virus-infected cells infected with the founder, wild type virus (that has not accumulated any escape mutations), $m_{\mathbf{i}}$ is the density of cells, infected with an escape variant denoted by a vector $\mathbf{i} = (i_1, i_2, \ldots, i_n)$ with $i_j = 0$ if there is no mutation in the j^{th} CTL epitope and $i_j = 1$ if there is a mutation leading to escape from the j^{th} CTL response. The death rate of an escape variant due to other CTL responses is then simply $\mathbf{k}^T \times \mathbf{i} = \sum_{j=1}^{n} k_j i_j$ where $\mathbf{k} = (k_1, k_2, \ldots, k_n)^T$ is the vector denoting the death rate of infected cells due to killing by the CTL responses. It should be noted that we assume that killing of infected cells by different CTL responses is additive. Some experimental data on killing of targets in vitro appear to support this assumption (49, 50) although in vivo evidence is lacking. Of note, most of current models of virus and CTL dynamics assume additive killing (e.g., (26, 30)).

Escape from a given CTL response incurs a fitness cost to the virus. Assuming multiplicative fitness, the fitness cost of a variant $\mathbf{i}$ is $1 - (\mathbf{1} - \mathbf{c})^T \times \mathbf{i} = 1 - \sum_{j=1}^{n} (1 - c_j) i_j$ where $\mathbf{c} = (c_1, c_2, \ldots, c_n)^T$ and $\mathbf{1} = (1, 1, \ldots, 1)^T$. Although there is evidence for positive epistasis for drug resistance mutations in HIV, the relative magnitude of epistasis is rather small (51), and therefore the assumption of multiplicative fitness is not expected be strongly violated. The initial density of a variant that has escaped from j different CTL responses is μ^j, where $\mu = 5 \times 10^{-5}$ is the mutation rate of HIV (52). Thus the initial density of the founder virus is set to $m_{\mathbf{0}}(0) = \mu^0 = 1$. The model that includes the generation of escape mutants by mutation has produced similar dynamics and will be presented elsewhere. The model eqn. (8)–(9) could also be modified to include limitation of viral growth by depletion of target cells or by explicitly modeling the dynamics of CTL responses (e.g., see eqn. (A.1) in Supplementary Information).

2.4 Statistics

The data on the escape were fit using eqn. (5) using the least squares algorithm (53) to estimate the average rate of escape ε and the initial frequency of the escape variant in the population f_0. In several cases of viral escape, frequency of the escape variant in the population changed from 0 to 1 in a given time period, precluding precise estimation of the average escape rate. To estimate the minimal escape rate, we substituted the observed null frequency of the escape variant with $1/(n+1)$ and when frequency was 1 with $n/(n+1)$ where n is the number of sequenced viral genomes at these time points. This methodology underestimated the rate of viral escape (26, 27). To calculate the 95% confidence intervals (CIs) for the estimated rate of escape we used bootstrap approach to resample data on escape (54). More specifically, for a given time point where m mutant out of total N sequences were detected, we generated a sample frequency of the mutant as $B_N(m/N)/N$ where $B_N(p)$ is a binomial distribution for N trials with the success rate per trial $p = m/N$. Resampling were repeated for each escape variant 1000 times.

3 Results

3.1 Estimating rates of viral escape during early HIV infection

In our earlier study, three patients (CH40, CH77, and CH58) were diagnosed with acute HIV infection and the dynamics of the HIV-specific CTL response and the virus was followed longitudinally (37, see Figure S2 in Supplementary Information). After the peak of viral load, virus accumulated mutations that became fixed in the population. Many of these mutations were selected by CTL responses that have arisen around peak of viremia (Figure S2). Using previously proposed model (see Materials and Methods) we estimated the rate of escape ε of HIV from CTL responses for every variant observed in these three patients, assuming that these escapes occur independently (see Figure 1 and Figures S4–S6 in Supplementary Information).

The estimated rate of escape ε of HIV in a particular epitope is equal to the difference between the killing efficacy of the epitope-specific CD8 T cell response and the difference in the replication rate of the wild-type virus and the escape variant (the latter is proportional to the cost of escape). Higher rates of escapes imply stronger immune pressure and low fitness cost, and slow escape implies low immune pressure or high fitness cost. For several escape variants, especially in cases when escape was very rapid, we can only estimate the minimal escape rate (i.e., minimal selective advantage) since there were no two data points in which both the wild type and the escape mutant are present (see Figure S4-S6 and Table S1-S3 in Supplementary Information). This implies that at these early time points, the actual rate

at which escape occurred could be even higher.

As we have discussed previously (37), in the acute phase of the infection HIV escapes from CD8 T cell responses at significantly higher rates than was suggested previously (26). On average, within first 50-70 days since infection, HIV escapes from the a single CTL response at the rate of 0.24 day^{-1} (median is 0.22 day^{-1}). Interestingly, the rate of viral escape declined with the time since infection such as one year after the infection, the escape rate was 10 fold lower than that in the acute phase (Figure 2). A similar observation was made earlier using cross-sectional data for humans and macaques (26, 31, 34).

Most of the detected escape variants arose after the peak of viremia. This could be explained by two processes. First, HIV-specific CTL response arises only around the peak of viremia (see Supplementary Information), and therefore, it is unlikely that an escape mutant becomes fixed in the population before the epitope-specific CD8 T cell response has been generated. Second, after the peak of viremia, the virus has used most of target cells available for infection (55, 56), and therefore has a reduced rate of replication. As the simple model shows, reduction in the rate of virus replication r leads to a faster accumulation of the escape mutant (see eqn. (3)). This in turn is because an escape mutant has a growth disadvantage as compared to the wild type, and less virus replication will speed up the accumulation of the escape variant (see also next section).

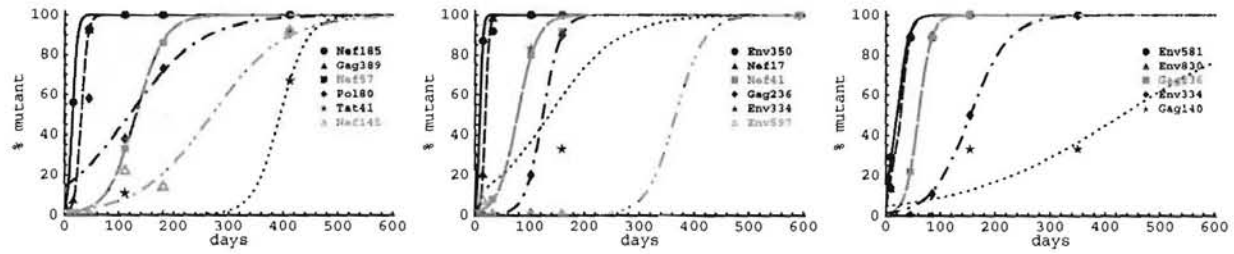

Figure 1: The dynamics of viral escape from the CD8 T cell responses in acute and chronic phases of HIV infection. Points represent the percent of a given variant at different times after infection and lines represent the best fit of the model given in eqn. (5) to these data. We show only a selected set of data and the model fits for clarity; complete plots are given in Figures S4–S6 in Supplementary Information. The estimates of escape rates with calculated confidence intervals are given in Tables S1–S3 in Supplementary Information. Mutations are labeled in accord with the peptide from the peptide pool in which the mutation occurred (37). Data are for patients CH40 (panel A), CH77 (panel B), and CH58 (panel C).

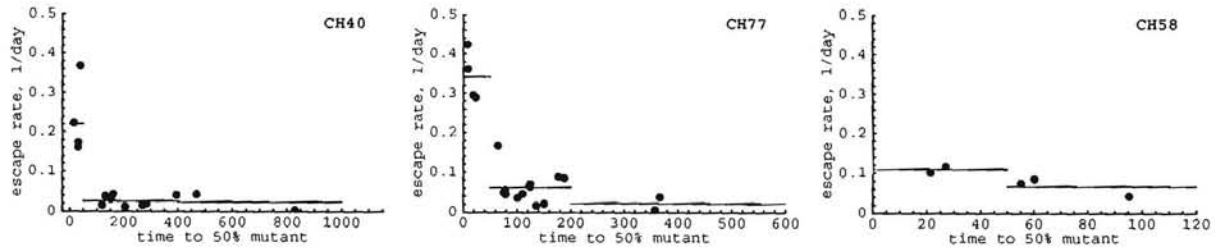

Figure 2: Distribution of the escape rates as the function of time since infection. For each escape variant we calculate the time t_{50} in which the escape mutant is predicted to reach the frequency of 50% in the virus population. Horizontal bars show the average escape rate in the several time intervals. The decline of the escape rate with time since infection was highly significant (linear regression of $\log \varepsilon \sim t_{50}$, $p < 0.002$, t-test).

3.2 Contribution of CTL killing efficacy, fitness cost and viral replication rate in determining rate of viral escape

It is not clear, however, why early escapes occur at faster rates than late escapes. In general, th rate of viral escape from a single CTL response is determined by 3 parameters: the killing efficacy of CTL response k against the wild type epitope, the fitness cost of the escape c and the rate of virus replication r (27, see Materials and Methods). Therefore, slower escape rate of HIV during late stages of the infection could be because in the chronic phase: 1) killing efficacy of the CTL response is lower; 2) cost of viral escape is higher; 3) the rate of virus replication is higher than that during the acute phase of the infection.

Decline in the magnitude of the HIV-specific CTL responses (and as the result CTL killing efficacy) over the course of acute HIV infection has indeed been observed at least for some epitopes (37, 48) and is expected from general properties of T cell responses during chronic viral infections (57). However, other CTL responses are often increased in the chronic phase of the infection and therefore at least for some epitopes we might expect a higher rate of escape in the chronic phases of the infection (37, 48). To investigate this further, we analyzed a simple mathematical model that tracks the dynamics of different viral variants that escape from multiple CTL responses (see Material and Methods and Supplementary Information for detail). Results of the analysis suggest that even if there is a no change in the magnitude of the CTL response over time, we do expect to see that escapes that occur early during the infection will escape at higher rates than those escapes that occur late in the infection (Figure 3A). The simple reason is that at everything else being equal, the virus will first escape from the strongest CTL response (at the fastest rate), and the last escape will be from the weakest CTL response (at the slowest rate). Interestingly, we found no significant correlation between the magnitude of the epitope-specific CD8 T cell response as measured by the ELISPOT assay and the rate of viral escape (Figure 4). This

suggests that the change in the magnitude of CTL response with the time since infection cannot account for the decreased rate of viral escape in the chronic phase of HIV infection (see also Discussion).

Several studies have established that escape from some CTL responses incurs a replication cost to HIV when measured in vitro (5, 58, 59), although recent analysis of the in vivo data suggest minimal cost of escape during HIV infection (26). A simple model that describes the dynamics of multiple escape variants (see eqns. (8)–(9) in Materials and Methods) indeed suggests that even when CTL pressure is similar on several viral epitopes, the virus will first escape at positions that confer lowest fitness cost and will escape the last at positions incurring highest possible fitness cost ($c_{\max} = k/r$, Figure 3B). At everything else being equal, early escape will be associated with a more rapid escape, because of a lower fitness cost on the escape variant than late escapes (see eqn. (3))

Finally, if there a fitness cost of viral escape, higher rates of viral replication will lead to a slower rate of escape (27). This is because higher rates of replication will offset the selective advantage of the escape mutant due to a faster replication rate of the wild type virus. Interestingly, we found a very strong correlation between the rate of virus replication during the infection and the rate of viral escape (Figure 5) suggesting that indeed changes in the rate of virus replication and/or cost of escape may be responsible for slow escape rate of late viral escapes. However, one needs to treat this result carefully since there is a strong correlation between the rate of virus replication and the time of infection (Figure S3).

`Increase in the breadth of the immune response`

3.3 Escape in B57 epitopes

3.4 Reversion of the founder virus to the consensus sequence

In these patients not all mutations that have accumulated in the virus population over time are due to escape from the CTL response (37). Some mutations such as in peptides Vpr74 in CH40, Rev9 and Tat55 in CH77, and Gag73 in CH58 suggest reversion to the HIV clade B consensus form, and thus indicating fitness cost to the virus due to mutations in these positions (37). These potential reversions follow a similar pattern as do true CTL escape mutations: if a reversion occurs early in the infection (Vpr74 and Tat55), it occurs at a high rate while late reversions (Gag73) are generally slower (see Tables S1–S3 in Supplementary Information). Our previous analysis suggested that the rate of virus replication may have a dramatic influence on the rate of reversion of the escape variant to the consensus (wild-type) sequence following infection of MHC-mismatched hosts (27). Indeed, a simple mathematical model for multiple CTL escapes that also tracks changes in the rate of virus replication due to depletion of target cells, suggests that if cost of escape is sufficiently high, reversion to

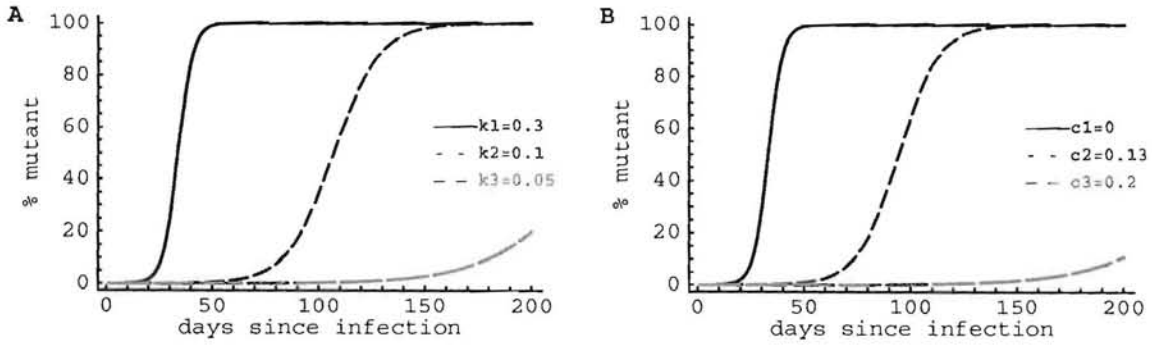

Figure 3: Killing efficacy of HIV-specific CTL response and the cost of escape determine the sequence of CTL escapes during infection. We simulate the dynamics of HIV escape from multiple CTL responses using a simple mathematical model (see Materials and Methods). The model demonstrates that if there are 3 CTL response from which the virus can escape, with everything else being equal the first escape occurs from the strongest immune response ($k_1 = 0.3\ day^{-1}$), and the latest escape is from the weakest CTL response ($k_3 = 0.05\ day^{-1}$, panel A). Similarly, if the CTL killing efficacy is similar for all epitopes, the first escape to occur is in epitope that induces the lowest fitness cost ($c_1 = 0$), and late escapes are those that incur the highest fitness cost ($c_3 = 0.20$, panel B). In both cases, early escapes occur at a faster rate than late escapes (panels A and B). In panel A, parameters are $c_1 = c_2 = c_3 = 0.005$, $k_1 = 0.3\ day^{-1}$, $k_2 = 0.1\ day^{-1}$, $k_3 = 0.05\ day^{-1}$. In panel B, parameters are $k_1 = k_2 = k_3 = 0.3\ day^{-1}$, $c_1 = 0$, $c_2 = 0.13$, $c_3 = 0.20$. Other parameters for both panels are $r = 1.5\ day^{-1}$, and $\delta = 0.5\ day^{-1}$. The initial density of viral variants is given by μ^j where j is the number of mutated epitopes in a given viral variant and $\mu = 5 \times 10^{-5}$. (i.e., $m_{(0,0,0)} = 1$, $m_{(1,0,0)} = m_{(0,1,0)} = m_{(0,0,1)}\mu$, etc). As predicted by a simple model, the rate of escape from the i^{th} CTL response is given by the difference $k_i - c_i r$ (see eqn. (4)). Results were quantitative similar if we explicitly included the dynamics of target cells in the model (results not shown).

the consensus sequence will take place in the acute phase of the infection and will occur at a high rate (Figure 6C, $c = 0.5$). This is simply because as soon as the consensus (wild type) sequence has arisen, it will quickly substitute the founder/escape variant provided that the rate of virus replication is high. On the other hand, if cost of escape is low, then the consensus, wild type virus does not have sufficient selective advantage to substitute the escape variant early in the infection. Following depletion of target cells and subsequently, decrease in the rate of virus replication (see Figure 6A&B), substitution of the escape variant by the wild type will occur at a slower rate that is proportional to the rate of virus replication in this time period (Figure 6C). In general, therefore, we do expect early reversions to arise at a higher rate than late reversions. This pattern, however, could be affected in situation when escape variant and the consensus, wild type form are both transmitted/founder viruses (e.g., see (39)). In this case, the wild type virus will rapidly accumulate during the viral expansion phase and even moderate fitness costs in the CTL escape variant will lead to

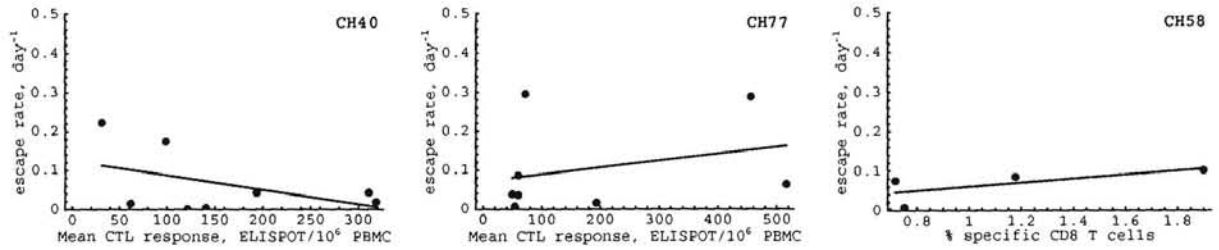

Figure 4: The absence of significant correlation between the rate of escape of HIV from the CTL response and the average magnitude of the response in three patients (CH40, CH77, and CH58; $p > 0.20$ t-test). From a simple analysis it is expected that CTL responses of a larger magnitude should select for more rapid escapes. This is however not observed for several measures of the CTL response such as the mean response (shown in the figure), maximal response or the total response (results not shown). This result suggests that the slow rate of accumulation of late escapes is not due to weaker CTL responses in the chronic phase.

a rapid substitution of the escape variant by the consensus, wild type sequence (results not shown). The importance of the initial inoculum on the speed of reversion was clearly demonstrated in a recent study (42).

Another potential indication of the fitness cost incurred by the CTL escape variant is the reversion of the viral sequence to consensus following disappearance of the epitope-specific CTL response. Slow escapes could arise if the escape mutations incurs a high fitness cost to the virus. Such reversions to the founder/transmitted virus sequence has indeed been observed in several cases (e.g., Vif113 in patient CH40 or Pol657 in CH77). The rate of loss of the escape variant after the peak can potentially be used to estimate the fitness cost of the escape mutation (28). For one escape variant that appears to revert upon loss of the CTL response (Vif113 in patient CH40), the estimated cost $c \approx 8\%$ that implies a significant reduction in viral fitness following CTL escape.

4 Discussion

There is only limited quantitative data suggesting an important role of CTL response in control of HIV replication in the acute phase of infection. We have recently shown that CTLs, specific to one epitope of HIV, could be responsible for 15 to 35% of death of infected cells during the decline of the viral load in the acute phase of the infection (37). Here we extend this finding by showing that the rate of viral escape decreases dramatically as infection progresses reaching 10 fold lower levels. Similar differences in escape rates between early and chronic phases of infection have also been reported for HIV and SIV infections (26, 34).

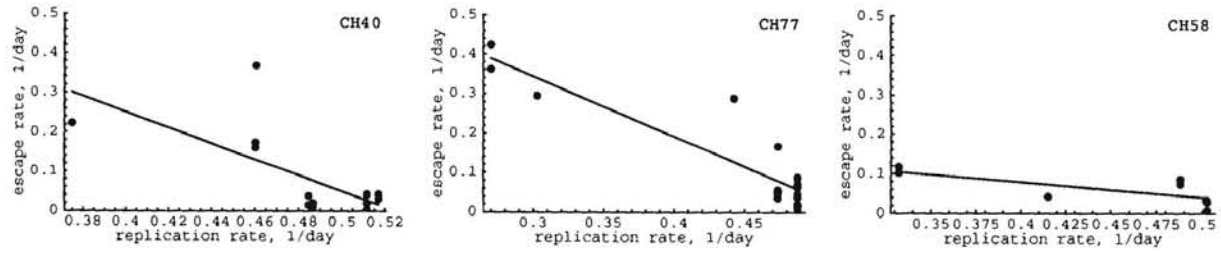

Figure 5: The rate of escape is inversely correlated with the rate of virus replication during HIV infection. A simple model predicts that if reduction in viral fitness due to escape is the main determinant of the rate of virus escape from the CTL response, then we expect to see a positive correlation between the rate of escape and viral replication rate. This is simply because at high rates of viral replication, killing of the wild-type virus by the CTL response is offset by its higher replicative capacity as compared to the CTL escape variant, hence, lower rate of viral escape from the CTL response (see Materials and Methods and the main text). All correlations are significant with $p < 0.05$ (in panel A and B, $p < 0.002$). The rate of virus replication was calculated using eqn. (7) with $\delta = 0.5\ day^{-1}$ and results were not highly sensitive to choosing lower or higher values of the death rate of virus-infected cells δ (results not shown).

Based a simple model of viral escape from the CTL response, changes in the rate of escape with the time since infection could occur due to 1) differences in the killing efficacy of early and late CTL responses, 2) higher fitness cost associated with late escapes, 3) increase in the rate of viral replication after the peak of infection to the chronic phase. All these factors combined will lead to a synergistic situation where early escapes occur rapidly and late escapes occur slowly.

Indeed, modeling suggests that with everything else being equal escapes that occur early in infection are aimed at avoiding strongest CTL responses. Previous studies have also suggested that changes in the magnitude of individual CTL response in the chronic HIV infection could be responsible for driving sequential viral escapes (22, 30, 60). However, despite this logic, we found no correlation between the rate of escape and the magnitude of CTL response (Figure 4). This contrasts with a conclusion reached in a recent study of SHIV infection of macaques where a strong (although nonlinear) correlation between the magnitude of the CTL response as measured by tetramer staining and the rate of viral escape has been observed (31). Also, our result is inconsistent with another analysis of SIV infection of monkeys that predicted faster escape with a larger CTL response (28). The difference in conclusions could arise because of difference in infection type (HIV vs SHIV/SIV) or because of different methods of measuring CTL response (ELISPOT vs tetramers). Indeed, several previous studies have argued that ELISPOT may not be highly predictable of the efficiency of CTL responses in vitro (61–63). It is also possible that the lack of correlation between CTL response and rate of viral escape is due to only a few responses analyzed or because the per capita killing efficacy of HIV-specific CTLs depends strongly on specificity. Indeed, in

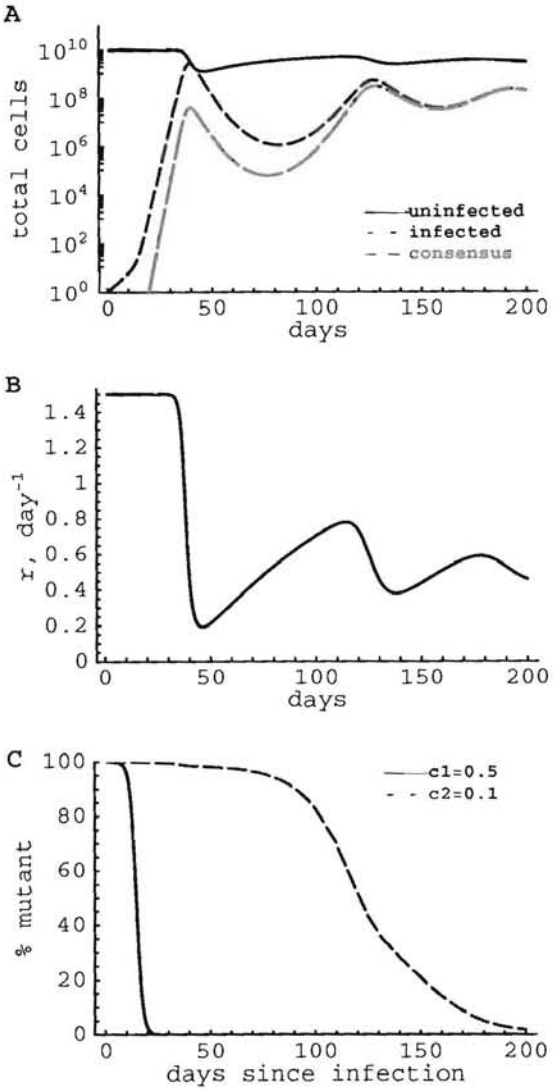

Figure 6: Rapid reversions to the consensus sequence occur early in acute HIV infection. We simulate the dynamics of uninfected target and virus-infected cells and changes in the composition of the virus population following infection of CTL escape mutant in HLA-mismatched hosts. Specifically, in the model given in eqns. (8)–(9) we let the initial virus population to have 2 mutations having costs $c_1 = 0.5$ and $c_2 = 0.1$, and allow the virus to revert to the consensus (wild type) sequence assuming that viral dynamics in acute infection is limited only by target cells (see eqn. (A.1) in Supplementary Information). Including changes in the death rate of targets due to CTL responses did not affect significantly the kinetics of viral reversion (results not shown). In panel A we show the dynamics of target cells, infected cells, and cells infected by the consensus sequence virus (i.e., virus reverted to the consensus sequence in both epitopes). In panel B we plot changes in the rate of virus replication given by $r = \beta T(t)$. In panel C we plot changes in the frequency of the virus that has reverted in first ($c_1 = 0.5$) and second ($c_2 = 0.2$) epitopes. Early reversion occurs at a much higher rate than the late reversion because of the higher cost and higher rate of virus replication before the peak of viremia (panel B). The rate of reversion in an i^{th} epitope is given by $c_i r$ where r is the rate of virus replication at the time of escape and $i = 1, 2$. Other parameters of the model are $T_0 = 10^{10}$ *cells*, $s = 0.01$ day^{-1}, $\beta = 1.5 \times 10^{-10}$ day^{-1} $cell^{-1}$, $\delta = 0.5$ day^{-1}. The initial density of viral variants is given by μ^j where j is the number of mutated epitopes in a given viral variant and $\mu = 5 \times 10^{-5}$. (i.e., $m_{(0,0)} = 1$, $m_{(1,0)} = m_{(0,1)} = \mu$, and $m_{(1,1)} = \mu^2$). Modifying the model to include generation of reversions by mutation produced quantitatively similar results (results not shown).

several recent studies it was indeed found that the per capita killing efficacy of mouse CTLs does depend on TCR specificity (64, 65, results not shown).

In contrast, we found that there is a significant inverse correlation between the rate of viral escape and the rate of virus replication (Figure 5) suggesting that cost of escape and/or changes in the rate of virus replication may be responsible for the observed changes in the rate of virus escape over the course of infection. Indeed, several studies suggest that late escapes do often arise in conserved regions of the viral genome such as Gag (1, 66, 67)

In our analysis we assumed that all mutations accumulated by the virus during the first year of infection are due to escape from the CTL response. We have previously discussed that it is not likely to be case as some mutations may represent reversions to a consensus sequence (HIV clade B) and other may simply be associated with true escape mutations (37). Restricting analysis to only true CTL escape mutations did not affect the main conclusions of this paper (results not shown).

Overall, our analysis suggests that most likely explanation for the slow rate of accumulation of late escapes is the high fitness cost associated with the escape mutations. A recent study also suggests that slow escape of the virus does not necessarily implies little pressure from the CTL response (30). Therefore, it is possible that targeting regions mutations in which lead to a high fitness cost may represent a valuable strategy to control viral growth early and may potentially lead to a lower viral set-point.

We also found that the rate of reversion of the transmitted/founder virus to the consensus sequence (e.g., CTL escape variant to its wild type form) occurs more rapidly during the acute phase of the infection than during the chronic phase. This is likely to arise because 1) mutations with a higher cost are likely to revert more rapidly, and 2) early reversions are assisted by high rates of viral replication prior to peak viremia (27, 29). Recent study has also shown that the composition of the infecting virus population may also have a dramatic influence on the rate of reversion of the escape variant to the consensus form (42).

Several assumptions have been made in our analysis of the viral escape from the CTL response. First, we assumed that CTL escapes occurred independently. Analysis of the general model, that was proposed in this paper and is aimed at tracking densities of all viral variants in the population, suggests that escapes for the analyzed range of parameters may occur independently with the rates that are predicted by the simple model given in eqns. (1)–(2). We have also assumed that all infected cells harbor only one viral variant. One study has found that lymphocytes in the spleen are often infected by multiple viruses (68), although another study found that only a small percent of peripheral blood lymphocytes are infected with two or more viral variants (Palmer & Coffin, HIV dynamics and Evolution Conference, 2008). These differences could arise due to different lymphocyte populations sampled or could be due to different methods used. Importantly, recent work suggests that including a possibility of cells to be infected with multiple viral variants can dramatically

affect the kinetics of viral escape from the CTL response (69). Whether a change in the rate of co-infection of cells with multiple viral variants over time could explain the decrease in the rate of viral escape from CTL response will be investigated elsewhere.

Another important assumption of our model is that the death rate of cells, infected with the founder, wild-type virus is the sum of the death rates due to killing by CTL responses specific to different viral epitopes. In this model escape from several CTL response is expected to lead to a decrease in the death rate of cells infected with the escape variant. However, the current view is that the death rate of infected T cells does not depend on the time since infection (47). Several potential explanations could be offered to explain this difference between the model prediction and the data. First, it is possible that as virus escapes from a given CTL response and as this CTL response decreases in magnitude (due to loss of recognition of cognate antigen, (37)), other CTL responses compensate for this loss by increasing in magnitude (30). Alternatively, CTLs may be able to remove a large fraction of infected cells before these cells start producing the virus (69, 70). In this case, escape from the CTL response may have little influence on the death rate of cells, producing the virus (results not shown). Finally, CTLs may reduce virus production by infected cells by releasing anti-viral cytokines and chemokines (27), and therefore, escape from the CTL response will not affect the rate at which infected cells die. How these mechanisms influence the kinetics viral escape and changes in the escape rate over time will be investigated elsewhere.

It has been suggested that slow rate of accumulation of CTL escape variants in the chronic phase of HIV/SIV infection could simple arise from sparse sampling in the data (30, 34). More frequent sampling and using advances sequencing techniques such as 454 sequencing (71) may allow to investigate this hypothesis further. It should be noted, however, that slow CTL escape during the chronic phase SHIV infection of macaques has been observed even with extremely frequent sampling (31).

Our study raises several important questions that have not been addressed previously and require future investigation. While we document rapid escape of HIV from several CTL responses in the acute phase of the infection, the relative contribution of simple point mutations and recombination for the generation of such escape variants is unknown. Many previous models addressed the question of importance of target cell limitation and CTL response in control of HIV replication during the acute phase (72–74) but these studies did not include the ability of the virus rapidly escape from the CTL response during this time period. Therefore, the importance of these CTL escapes as well as CTL response in determining viral decline during acute HIV infection and viral set point is also unclear. Our understanding of HIV infection will clearly benefit from addressing these questions in future research.

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6 Supplementary Information

6.1 Deriving a model for viral escape from a single CTL response

To derive the simple model for the dynamics of the wild-type and the escape variant viruses we follow previous publications (27, 29) and start with the standard model for virus dynamics:

$$\frac{dT}{dt} = s(T_0 - T) - \beta(TV_w + V_m), \quad \text{(A.1)}$$

$$\frac{dI_w}{dt} = \beta TV_w - \delta I_w - k_E I_w E, \quad \text{(A.2)}$$

$$\frac{dI_m}{dt} = \beta TV_m - \delta I_m, \quad \text{(A.3)}$$

$$\frac{dV_w}{dt} = p_w I_w - cV_w, \quad \text{(A.4)}$$

$$\frac{dV_m}{dt} = p_m I_m - cV_m, \quad \text{(A.5)}$$

where T is the number of uninfected target CD4 T cells, T_0 is the preinfection level of uninfected targets, s is the rate of turnover of uninfected target cells, β is virus infectivity, I_w and I_m is the number of cells infected with the wild-type and the escape variant viruses, respectively, δ is the death rate of infected cells due to viral pathogenecity, $k_E E$ is the death rate of virus infected cells due to killing by CTLs that are specific for the wild-type epitope (E), V_w and V_m is the density of the wild-type and escape viruses, respectively, p_w and p_m is the rate of virus production by cells that are infected with the wild-type and escape viruses, respectively, and c is the death rate of free viral particles. In this model we made several simplifying assumptions. We assumed that the wild-type and escape viruses differ only in the rate of virus production; generally $p_w \geq p_m$ (but see (37)). It is also possible that mutations that lead to escape from the CTL response also affect viral infectivity β especially if they occur in the envelope region of the genome. We assumed that the rate of virus replication is proportional to the density of target cells, $r = \beta T$. Some recent experimental data supports this conclusion for the acute phase of SHIV/SIV infection (75, 76), but it is still unclear if this functional form is true if virus dynamics in the chronic phase is also considered (results not shown). Given that in vivo, viral particles are short-lived (32, 40, 41), due to a quasi steady state, the density of viruses is simply proportional to the density of infected cells, $V_w = p_w/cI_w$ and $V_m = p_m/cI_m$. Now, by replacing variables $r = p_w\beta/cT$, $c = 1 - p_m/p_w$, $w = I_w$, $m = I_m$, $k = k_w E_w$, $d = \delta + kE$ we arrive to the model given in eqns. (1)-(2) for the dynamics of cells, infected with the wild type (w) or mutant (m) viruses. This model can easily be extended to track viral escape from several CTL responses (see below).

6.2 Model with multiple CTL escapes

In the main text we formulate a mathematical model that describes the dynamics of viral escape from n CTL responses. In a particular case when $n = 3$, there are 6 different viral variants present in the population. We denote a given viral variant by a vector $\mathbf{i}$ of the length $n = 3$ with values equal to 0 (no escape) or 1 (escape). The density of a given variant is then given by $m_{\mathbf{i}}$. For example, the density of a viral variant that has escaped only from the second CTL response is $m_{\mathbf{i}} = m_{(0,1,0)}$. The dynamics of all viral variants are given by the following model (see eqns. (8)–(9) in the Main text):

$$\frac{dm_{(0,0,0)}}{dt} = [r(t) - (k_1 + k_2 + k_3) - \delta]\, m_{(0,0,0)}, \tag{A.6}$$

$$\frac{dm_{(1,0,0)}}{dt} = [(1 - c_1)r(t) - (k_2 + k_3) - \delta]\, m_{(1,0,0)}, \tag{A.7}$$

$$\frac{dm_{(0,1,0)}}{dt} = [(1 - c_2)r(t) - (k_1 + k_3) - \delta]\, m_{(1,0,0)}, \tag{A.8}$$

$$\frac{dm_{(0,0,1)}}{dt} = [(1 - c_3)r(t) - (k_1 + k_2) - \delta]\, m_{(1,0,0)}, \tag{A.9}$$

$$\frac{dm_{(1,1,0)}}{dt} = [(1 - (1 - c_1)(1 - c_2))r(t) - k_3 - \delta]\, m_{(1,1,0)}, \tag{A.10}$$

$$\frac{dm_{(1,0,1)}}{dt} = [(1 - (1 - c_1)(1 - c_3))r(t) - k_2 - \delta]\, m_{(1,0,1)}, \tag{A.11}$$

$$\frac{dm_{(1,1,1)}}{dt} = [(1 - (1 - c_1)(1 - c_2)(1 - c_3))r(t) - \delta]\, m_{(1,1,1)} \tag{A.12}$$

where $m_{(0,0,0)}$ is the density of the wild-type virus, c_i is the cost of escape of the virus from the i^{th} CTL response, and k_i is the rate at which cells expressing the i^{th} epitope are killed by the i^{th} CTL response, and δ is the death rate of virus-infected cells due to virus cytopathogenecity (see also Main text for details). The fraction of viral variants that have escape recognition from an i^{th} CTL response is simply the sum of viral densities of variants that have escaped from the i^{th} CTL response over the total density of all variants in the population, $M = \sum_{\mathbf{i}} m_{\mathbf{i}}$. For example, the fraction of the variant that has escaped from the 1st CTL response is simply

$$f_1 = \frac{m_{(1,0,0)} + m_{(1,1,0)} + m_{(1,1,1)}}{M}. \tag{A.13}$$

In this model we made a very important assumption that the death rate of cells, infected with the wild-type virus, is the sum of the killing by the CTL responses specific to the different viral epitopes. It is unclear if the pressure by CTL responses of different specificities are additive although some in vitro data are consistent with this assumption (49, 50).

6.3 Late and slow escape in B57 epitopes

Escape variant	ε (95% CIs), day^{-1}	t_{50}
Gag113	0.17 (0.101–0.839)	31
Gag389	0.17 (0.103–0.855)	31
Gag481	0. (0.–0.143)	3688
Pol80	0.02 (0.009–0.301)	119
Vif57	0.03 (0.01–0.144)	152
Vif113	0.04 (0.007–0.326)	160
Vif161	0.37 (0.102–0.839)	38
Vpr74	0.16 (0.101–0.836)	30
Tat41	0.04 (0.005–0.143)	395
Rev49	0.02 (0.007–0.367)	253
Env401	0.02 (0.01–0.143)	284
Env435	0.02 (0.01–0.143)	282
Env765	0.04 (0.–0.143)	469
Env830	0. (0.–0.143)	829
Nef57	0.04 (0.015–0.146)	131
Nef145	0.02 (0.008–0.143)	270
Nef185	0.22 (0.079–0.574)	15

Table S1: Estimates of the relative fitness advantage of different escape variants in the patient CH40 and the predicted time at which the mutant is present at 50% frequency in the virus population (t_{50}). Fits are shown in Figure S4. 95% confidence intervals were calculated by recreating the samples from the sequence data (see Materials and Methods).

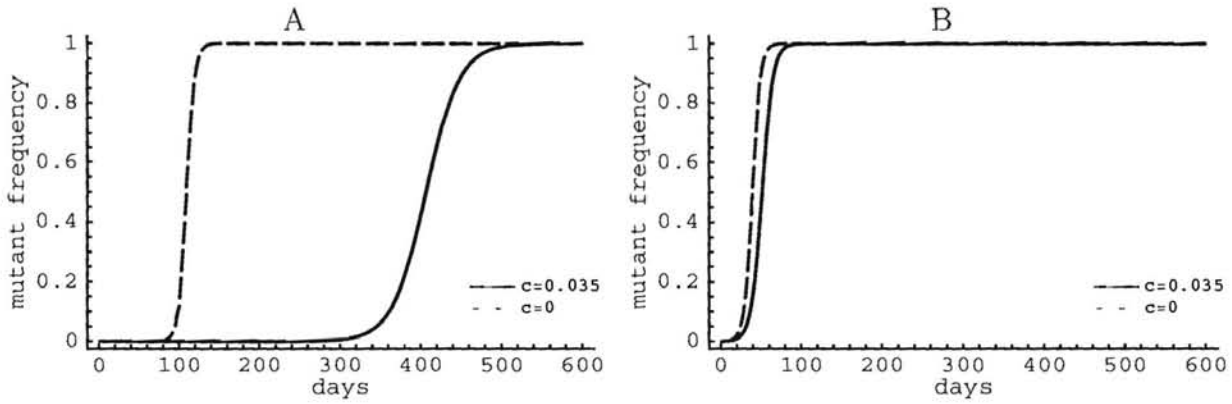

Figure S1: Escape requiring multiple mutations for evasion of the CTL response could lead to slow and late escape.

Escape variant	ε (95% CIs), day^{-1}	t_{50}
Gag140	0.04 (0.018–0.367)	101
Gag236	0.06 (0.018–0.407)	124
Pol1	0.02 (0.005–0.327)	150
Pol472	0.04 (0.021–0.384)	79
Pol657	0. (0.–0.143)	∞
Vpr49	0.05 (0.026–0.391)	110
Tat9	0.42 (0.267–0.805)	8
Tat25	0.07 (0.028–0.411)	123
Rev9	0.01 (0.003–0.141)	357
Env1	0.05 (0.028–0.379)	75
Env334	0.02 (0.003–0.377)	136
Env451	0.07 (0.004–0.143)	124
Env311	0.09 (0.–0.143)	177
Env350	0.36 (0.203–0.805)	9
Env597	0.04 (0.004–0.143)	366
Env822	0.17 (0.029–0.376)	65
Env838	0.09 (0.004–0.143)	188
Nef17	0.3 (0.126–0.649)	19
Nef41	0.06 (0.027–0.38)	78
Nef73	0.29 (0.169–1.409)	24

Table S2: Estimates of the relative fitness advantage of different escape variants (and 95% confidence intervals) in the patient CH77 and the predicted time after symptoms at which the mutant is present at 50% frequency in the virus population. Fits are shown in Figure S5.

Escape variant	ε (95% CIs), day^{-1}	t_{50}
Gag140	0.01 (0.–0.336)	430
Gag236	0.08 (0.002–0.579)	60
Gag73	0.03 (0.–0.348)	154
Rev49	0.04 (0.–0.146)	95
Env581	0.1 (0.–0.881)	21
Env334	0.03 (0.–0.349)	154
Env830	0.12 (0.–0.777)	27
Nef113	0.07 (0.–0.212)	55

Table S3: Estimates of the relative fitness advantage of different escape variants (and 95% confidence intervals) in the patient CH58 and the predicted time t_{50} at which the mutant is present at 50% frequency in the virus population. Fits are shown in Figure S6.

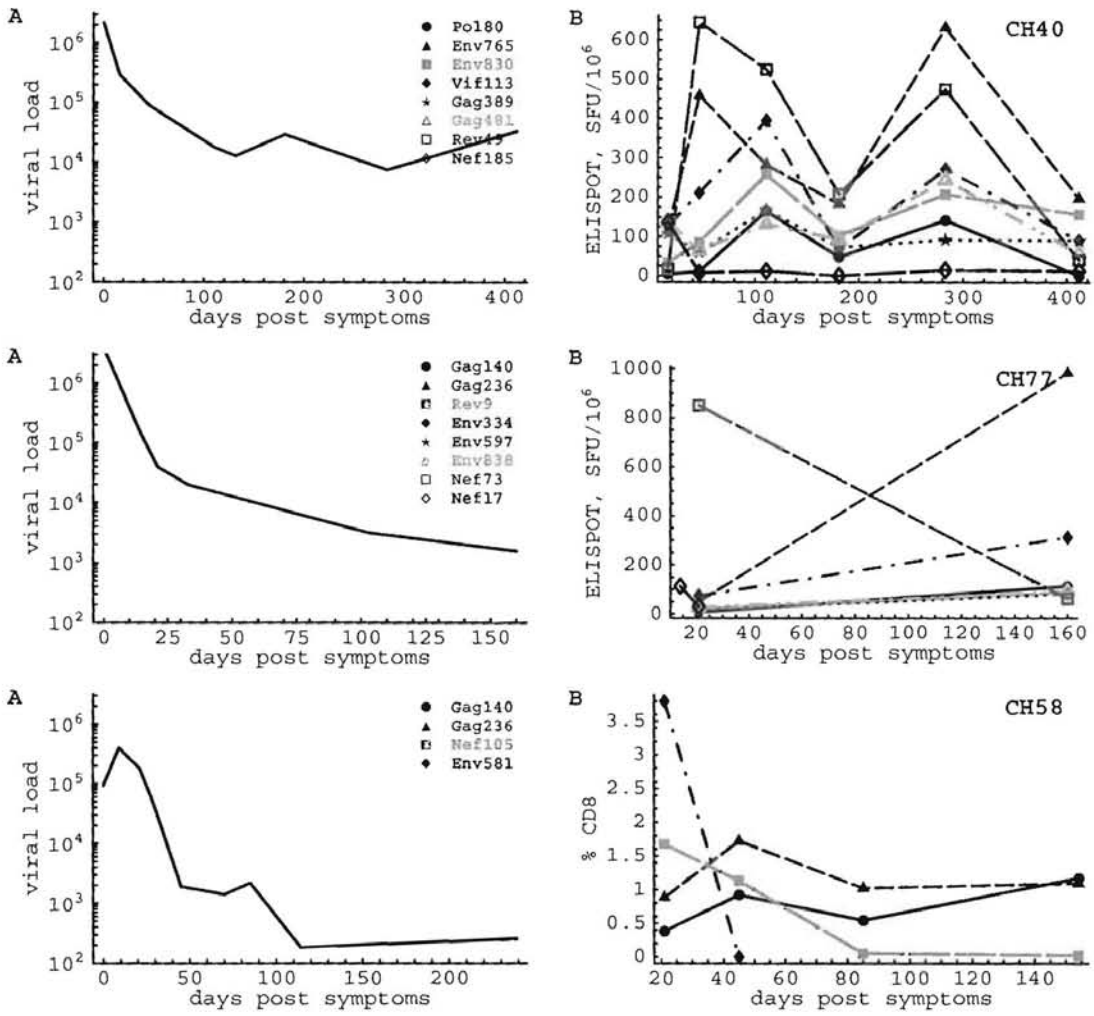

Figure S2: The dynamics of the virus and CTL response in which escapes occur for three patients following from the acute phase of HIV infection (CH40, CH77, and CH58). For simplicity, we only plot changes in CTL response from which we have observed viral escape. Full data have been published previously (37).

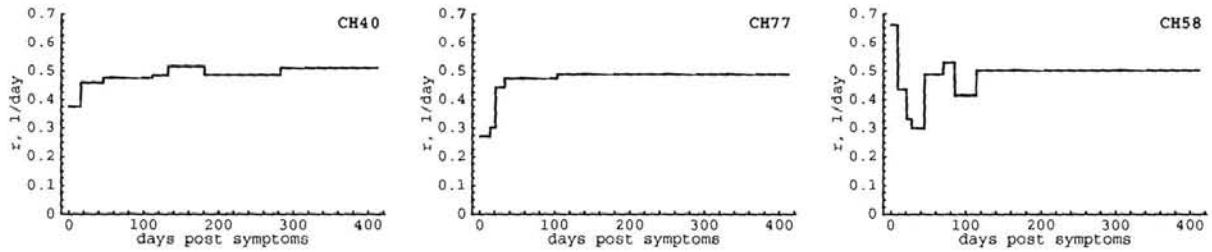

Figure S3: Changes in the rate of virus replication over the time of infection. We use a simple formula given in eqn. (7) in main text to calculate change in the rate of virus replication r over the time since infection. We assume that the rate of virus death is constant during the infection, $\delta = 0.5\ day^{-1}$ (47).

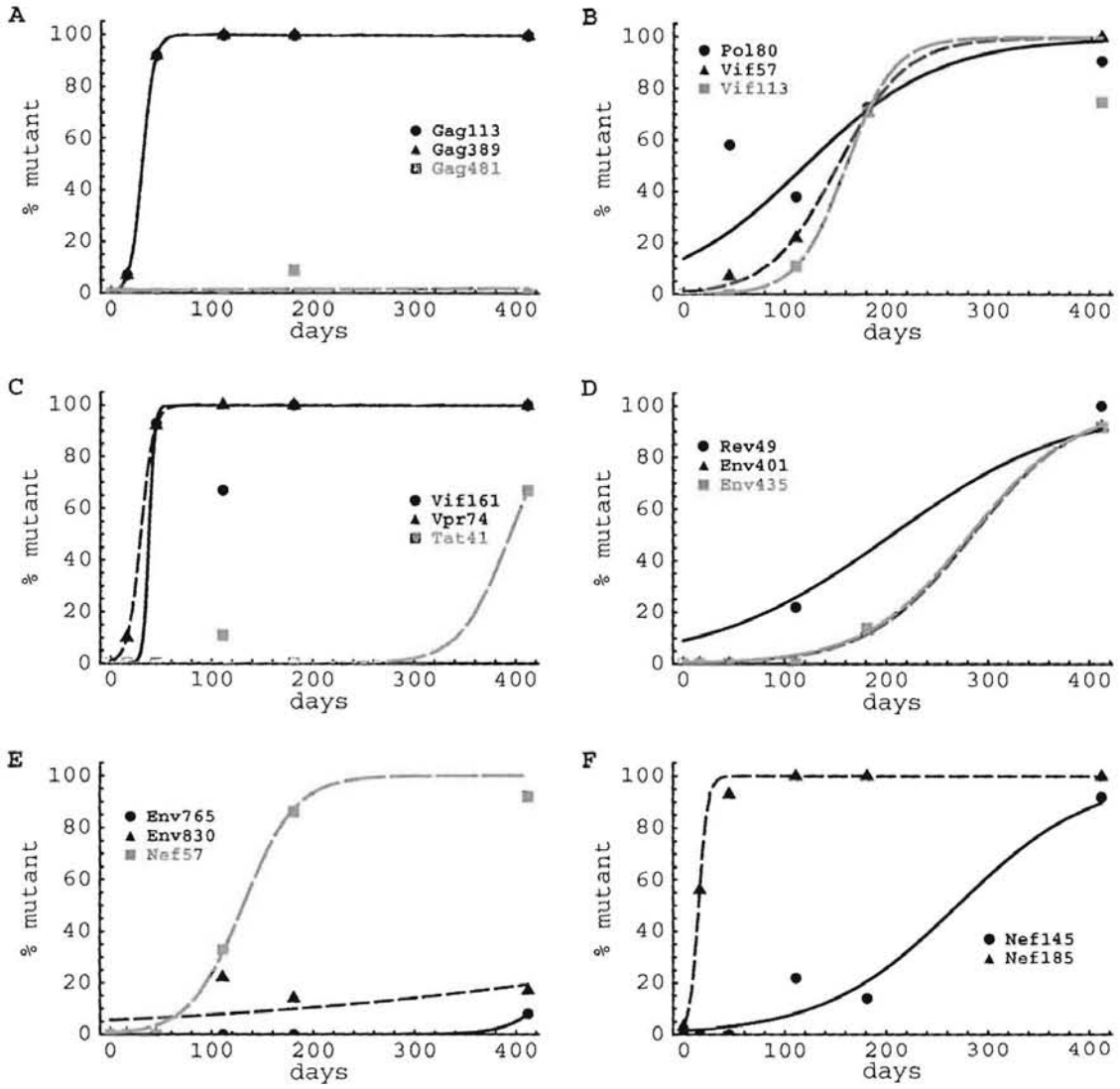

Figure S4: Fits of the data on escape of HIV from the CD8 T cell response in the patient CH40. Points represent the frequency of a particular escape variant detected at different times after symptoms and lines represent the best fit of the model given in eqn. (5) to the data.

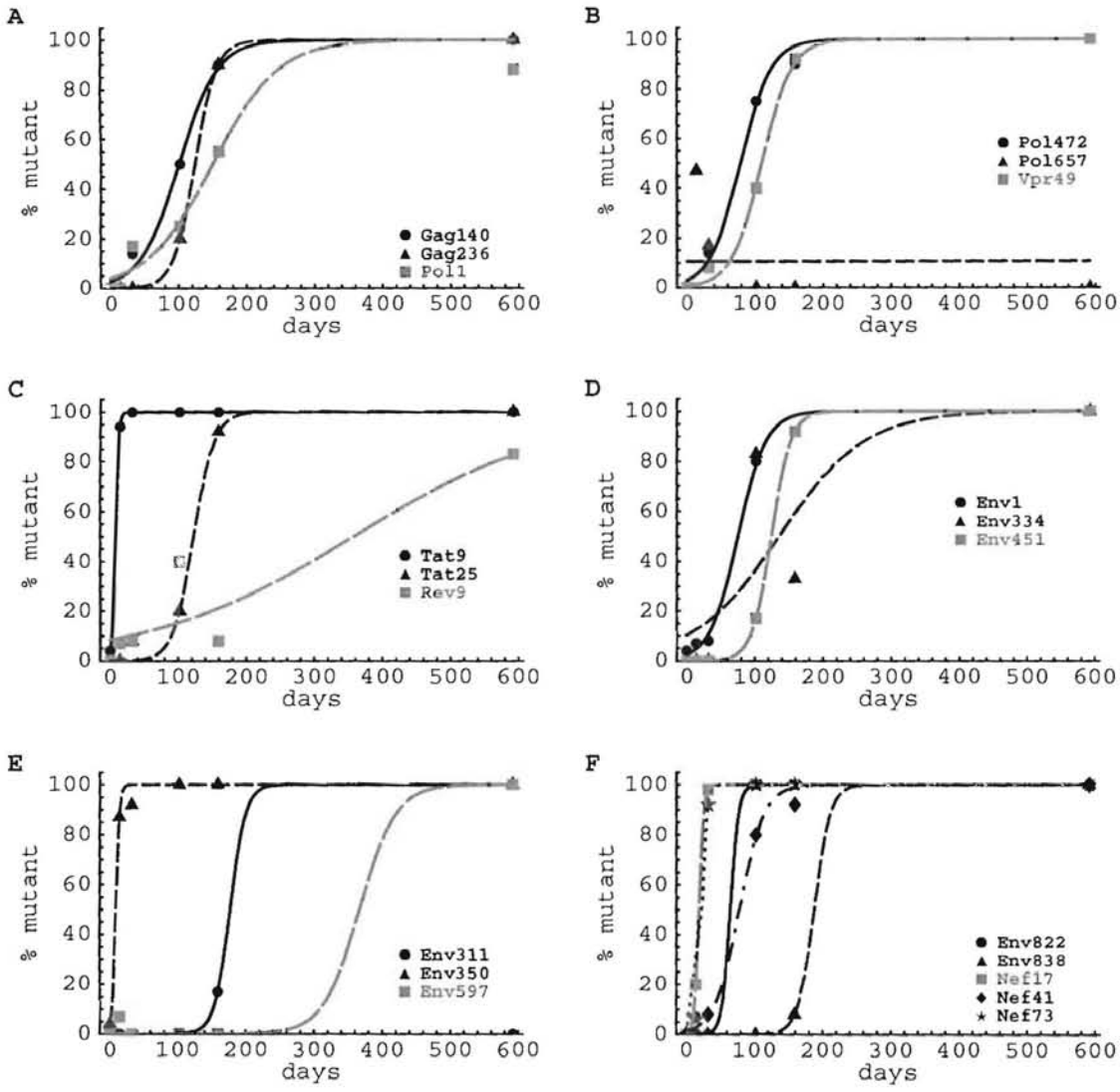

Figure S5: Fits of the data on escape of HIV from the CD8 T cell response in the patient CH77.

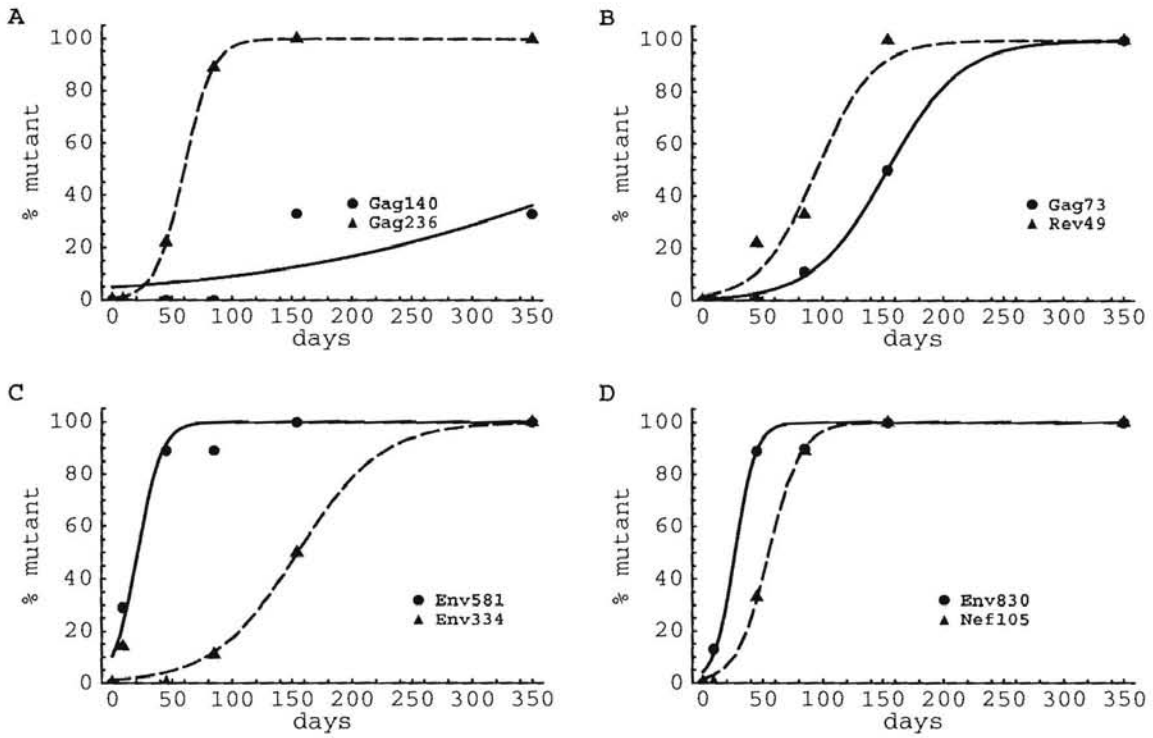

Figure S6: Fits of the data on escape of HIV from the CD8 T cell response in the patient CH58.