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The Route of HIV Escape from Immune Response Targeting Multiple Sites is Determined by the Cost-Benefit Tradeoff of Escape Mutations

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Abstract

Cytotoxic T lymphocytes (CTL) are a major factor in the control of HIV replication. CTL arise in acute infection causing escape mutations to spread rapidly through the population of infected cells. As a result, the virus develops partial resistance to the immune response. The factors controlling the order of mutating epitope sites are currently unknown and would provide a valuable tool for predicting conserved epitopes. In this work, we adapt a well-established mathematical model of HIV evolution under dynamical selection pressure from multiple CTL clones to include partial impairment of CTL recognition, Δr, as well as cost to viral replication, Δf. The process of escape is described in terms of the cost-benefit tradeoff of escape mutations and predicts a trajectory in the cost-benefit plane connecting sequentially escaped sites, which moves from high recognition loss/low fitness cost to low recognition loss/high fitness cost and has a larger slope for early escapes than for late escapes. The slope of the trajectory offers an interpretation of positive correlation between fitness costs and HLA binding impairment to HLA-A molecules and a protective subset of HLA-B molecules that was observed for clinically relevant escape mutations in the

Pol gene. We estimate the value of Δr from published experimental studies to be in the range (0.01-0.86) and show that the assumption of complete recognition loss ($\Delta r = 1$) leads to an overestimate of mutation cost. Our analysis offers a consistent interpretation of the commonly observed pattern of escape, in which several escape mutations are observed transiently in an epitope. This non-nested pattern is a combined effect of temporal changes in selection pressure and partial recognition loss. We conclude that partial recognition loss is as important as fitness loss for predicting the order of escapes and, ultimately, for predicting conserved epitopes that can be targeted by vaccines.

Author Summary

Like many viruses, HIV has evolved mechanisms to evade the host immune response. As early as a few weeks after infection is initiated, mutations appear in the viral genome that reduce the ability of cytotoxic T lymphocytes (CTL) to control virus replication. However, of the many mutations in the viral genome that could potentially mediate viral escape from the CTL response, a specific subset are typically observed. This suggests that some mutations either entail too high a fitness cost for the virus, or are relatively inefficient escape mutations. A successful vaccine would target the CTL response to these regions in such a way that escape would not be possible. We use a computational model of HIV infection in order to study the factors that determine whether a given escape mutation will occur, how long it will be maintained in the population, and how these changes in the viral genome will affect the CTL response. Our analysis highlights the important role of partial recognition loss conferred by a mutation in producing the complex dynamics of escape that are observed during the course of infection.

Introduction

HIV replication continues for years despite a highly active immune response. Depletion of cytotoxic CD8+ T cells (CTL) in SIV infected animals causes rapid increase in viremia [1, 2] showing that CTL control HIV/SIV replication; that this response is antigen-specific is evident from rapid genetic evolution of HIV in antigenically important regions. Antigenic escape is one of the major mechanisms of HIV resilience in face of an active immune response, impeding effective vaccine design [3] and implicated in the progression to AIDS [4]. Shortly after infection is initiated, many CTL clones arise to target the transmitted virus

strain [5–7], each clone recognizing a distinct 8-10 amino acid viral peptide (epitope) presented on the surface of an infected cell by MHC molecules. Escape mutations in CTL epitopes begin to be selected within a month of infection and continue to be selected throughout chronic infection, sometimes causing a decrease in the intrinsic replication rate of the virus (fitness cost) [8–10]. However, despite a sustained CTL response, not all targeted epitopes escape. Moreover, among epitopes that do escape, the rate of escape slows dramatically over the first 100 days post infection. It remains unclear which parameters decide the timing and rate of escape in a given epitope as well as which epitopes escape and which are preserved throughout chronic infection [11–13].

Mathematical models of HIV evolution in the presence of multiple CTL clones have been applied to study the emergence of late escape mutations [14,15] and the effect of distributed CTL pressure on the rate of escape [12,16]. Previous work has emphasized two parameters, the mutation cost (Δf) and the number of active epitopes (n). It can be inferred that escape mutations come at a cost from the observation of occasional reversion of escape mutations upon transmission between MHC mismatched individuals [8, 9, 17, 18], as well as the frequent acquisition of compensatory mutations outside the epitope [19, 20]. Common escape mutations have been shown experimentally to have wide ranging fitness costs [10, 21, 22]. However, fitness costs and the number of CTL clones acting on the virus are not the sole determinants of the dynamics of escape. It has been observed in HIV infected individuals [23] and SIV infected animals [24] that CTL are capable of recognizing different variants of an epitope with different efficiencies. Thus, in general, an escape mutant does not fully abrogate recognition of the corresponding CTL clone.

In the present work, we address the process of antigenic escape in terms of a cost-benefit diagram. The benefit of an escape mutation is a partial CTL recognition loss and the mutation cost is a partial reduction in viral replication rate. We extend the basic model introduced by Althaus and De Boer [14] to include partially effective escape mutations and investigate how the two opposing evolutionary forces together determine the observed rate of escape from the CTL response [12,25]. The model predicts that a positive correlation between recognition and fitness losses emerges during sequential escape mutations, the strength of which changes over time as pressure from the immune system wanes. We compare our model predictions with existing data showing a correlation between fitness and recognition losses in clinically relevant escape mutations from the Pol gene [26] and estimate the range of recognition losses that occurs in commonly observed escape mutations from three published studies [27–29].

Furthermore, the inclusion of partially effective escape mutations in the model can reproduce the

diverse patterns of intra-epitope escape that are routinely observed in HIV infected patients. During the majority of escape mutations that have been studied with time-resolved viral sequencing, 2-10 distinct epitope sequences grow in number to replace the transmitted sequence and eventually one mutant epitope spreads to the entire population. Furthermore, the dominant mutated epitope sequence changes over time [11, 30, 31]. Although, in some epitopes, mutations are added at new sites in a nested fashion, in a larger number of epitopes, mutations at new sites replace mutations at previous sites leading to a non-nested pattern. This pattern is atypical for models assuming constant selection pressure. The analysis below illustrates how time-dependent selection due to the changing CTL pressure and partial CTL recognition loss can produce the non-nested pattern of escape (see [32] for review).

Results

Phases of HIV infection

In order to study escape from a the immune response we consider a model that includes target cells, infected cells and multiple CTL clones which recognize various regions (epitopes) in the viral genome with equal avidities (Figure 1A and *Model*). The model predicts three distinct phases of HIV infection (Figure 1B), as follows. Phase 1: The transmitted HIV strain expands in the population of target cells. Phase 2: All CTL clones that recognize cells infected by the transmitted strain are activated, expand, and reduce the number of infected cells. A steady state is obtained with constant levels of infected cells and CTL, which represents chronic HIV infection (see Equations S1-S3 in *Text* S1). Phase 3: Escape mutations in viral epitopes emerge, changing the genetic composition of the population of infected cells and the clonal composition of the CTL population but only weakly affecting their overall sizes.

The dynamics of infected cells and CTL during the escape phase (Phase 3) depends on the number of epitopes that are targeted, n, and on the degree of recognition loss per mutation, Δr (Table 1). In the simplest case, if a single CTL clone is present in steady state and an escape mutation arises which completely abrogates CTL recognition ($\Delta r = 1$), the CTL clone contracts and the population of infected cells containing the escape mutation grows uncontrolled until target cells are depleted. In contrast, if the recognition loss conferred by the escape mutation is partial ($\Delta r < 1$), the population of infected cells grows only transiently, and the CTL clone expands until a new steady state is reached. When a group of CTL clones with similar avidity target the infected cell population (as shown in Figure 1B), the spread

of either partly or fully effective escape mutation causes the corresponding CTL clone to contract, since it recognizes infected cells less efficiently than other CTL clones (see Equation S5 in *Text* S2). Because infected cells continue to be recognized by other CTL clones, the population of infected cells does not grow out of control following the escape mutation. Thus, the model reproduces the three-phase dynamics that can be inferred from kinetic data in HIV infected individuals including the waning of CTL responses to escaped epitopes [11, 13, 30, 31].

The escape rate of a mutant strain is determined by the loss of CTL recognition and the loss of viral fitness

Escape mutations begin to be selected once CTL reach sufficiently high levels (third phase in Figure 1B). Mutations in all targeted epitopes begin to grow simultaneously, albeit with different rates due to variation in the amounts of fitness and recognition losses caused by a mutation. The exponential growth rates (escape rates) of mutant strains determine which mutant strain will have most selective advantage and grow to dominate the population. Here we describe the growth of a mutated strain (I_i) with a single mutation in epitope 1. An escape mutation results in both a fractional fitness cost, Δf_i, and a fractional loss of CTL recognition, Δr_i. The mutant strain begins to grow as $I_i(t) = I_i(0)e^{\epsilon_i t}$, with initial growth rate ϵ_i:

$$\epsilon_i = \frac{1}{I_i}\frac{dI_i}{dt} = kE_{\text{tot}}\left(\frac{\Delta r_i}{n_1} - \Delta f_i\right) - d_I \Delta f_i \tag{1}$$

where $1/n_1$ is the fraction of CTL population recognizing epitope 1 (see *Text* S1). Thus, the escape rate reflects the balance between the partial recognition loss and the partial fitness loss, which determines whether a mutant strain has a selective advantage ($\epsilon > 0$). In order to infer, for example, the underlying fitness cost of an escape mutation, it is necessary to measure not only the escape rate, but also the fraction of the total CTL recognition that is abrogated by the mutation.

When multiple epitopes are targeted and recognition and fitness losses vary across epitopes, the growth rates of escape mutants vary as well (Figure 1B). In data from HIV infected individuals, it is observed that the rate of escape slows dramatically over the first 100 days post infection [11–13]. The rise of escape mutants predicted by the model is consistent with these findings (Figure S2). Furthermore, assuming only a minor fitness cost, the entire observed variation of escape rates over time and across sites can be

simulated from the variation of the recognition loss, Δr.

The trajectory of escape mutations in the cost-benefit plane (Δr, Δf)

The process of gradual viral escape from the immune response of an infected host continues for years. At the same time, the virus has only a limited set of viable escape mutations ($10^2 - 10^3$). We investigate the trajectory connecting these escape mutations in the cost-benefit plane and predict how the average fitness costs and recognition losses incurred by an escape mutation will change as escape progresses. For this aim, we use a simplified version of the main model (see *Model*), focused only on the order of escape mutations (i.e., model dynamics are not considered explicitly). Parameters Δr and Δf are randomly generated for a genome with multiple epitopes and multiple sites per epitope (10 epitopes, with 10 sites per epitope in Figure 2) and epitope sites are ranked in the descending order of escape rate (Equation 1). When many random runs are compared, a correlation between Δr and Δf for escape mutations of a given rank is observed (Figure 2A). The average trajectory of escape in the cost-benefit plane moves from high recognition loss, low fitness cost to low recognition loss, high fitness cost. The maximum escape rate per epitope decreases over many rounds of escape (Figure 2B) and each epitope escapes at more than one site. This prediction is consistent with experimental observation, where the majority of escaping epitopes undergo more than one mutation [13, 30].

The dynamic interplay between CTL and partially effective escape mutations shape the overall course of HIV escape. As mentioned above, an escape mutation results in the decay of the cognate CTL population. In turn, the CTL decay causes the benefit of other escape mutations in the escaping epitope (in terms of recognition loss) to decrease, because the overall CTL pressure on the epitope is lessened. The presence or absence of CTL decay has observable consequences for the trajectory of escape mutations in the cost-benefit plane. Without the CTL decay, the average slope of the trajectory stays constant over time until no more escapes are possible (Figure 2A). In contrast, when CTL clones decay in response to the recognition loss, the trajectory bends towards X-axis (Figure 2C) as early escape in an epitope causes escape mutations on the other sites in that epitope to become progressively less advantageous. Therefore, a decreased number of escape mutations per epitope is observed when CTL decay is included in the model (fewer steps in Figure 2D as compared to Figure 2B).

Weak correlation between Δr and Δf has been observed in the *Pol* gene

The simulation example in Figure 2 offers an interpretation of the work by Mostowy et al [26], where a weak but statistically significant correlation was observed between fitness costs and HLA binding losses in clinically derived Pol sequences. The existence of the correlation follows from Equation 1, which states that more costly mutations will not appear unless they confer a large benefit to the virus. The model relates the weak strength of the correlation (slope = -0.12 between the fitness decrease and the impairment of HLA binding) to the large number of active epitopes. The existence of the CTL decay caused by escape can further reduce the slope: mutations sampled during the acute phase of HIV infection are predicted to have a much larger slope than those sampled during the chronic phase (Figure 3B). Since the majority of database sequences used in the cited work fall into the latter category, the small slope of the correlation observed can (at least partially) be caused by CTL decay.

There is an important caveat which must be considered when comparing the model with the cited work [26]. Loss of CTL recognition in the model is given by Δr, which is a composite parameter comprising changes in antigen processing, presentation and recognition, whereas Mostowy et al use the computationally predicted loss of HLA-epitope binding, ΔB. To convert the units (at least approximately), we analyzed data from several publications in which both overall loss of CTL recognition as well as HLA binding impairment was measured [27–29]. We found strong correlation between Δr and ΔB expressed as a linear relationship $\Delta B = 0.78\Delta r - 0.004$ (Figure S4, Equations S10 - S14), which justifies the comparison between the model and the data (see *Text* S5 for details).

Three patterns of antigenic escape in an epitope with two sites

Epitopes with many sites can produce different combinations of escape mutations (haplotypes) in response to CTL pressure. In [30] it was observed that the mutated sequence changed over time in the majority of epitopes that were studied longitudinally and, interestingly, the order in which escape haplotypes appeared varied from epitope to epitope. We divide the epitopes into three characteristic patterns, based on the order of dominant haplotypes: "simple", "nested" and "leapfrog". We can illustrate these patterns in an epitope with two sites, in which four haplotypes are possible. The presence or absence of an escape mutation at each site is denoted by a 1 or a 0, respectively. The infrequently observed "simple" pattern is characterized by a single escape haplotype. For example, in an epitope with two sites, the sequence of haplotypes observed is 00 → 10. The "nested" escape adds a new mutation sequentially to a previously

mutated sequence: $00 \rightarrow 10 \rightarrow 11$. This pattern is predicted if mutations at both sites are under a constant, positive selection pressure ($\epsilon > 0$ throughout the course of infection). The "leapfrog" pattern is characterized by a switch in the dominant single-site mutation: $00 \rightarrow 10 \rightarrow 01$. In the present model, the leapfrog pattern of escape arises due to time-dependent CTL selection pressure. Below we use the model to determine the distribution of fitness and recognition losses within an epitope that produces the leapfrog pattern of escape.

In order to study intra-epitope dynamics, we use the main model (Equations 6 to 8) and consider a genome composed of a large number of two-site epitopes. This simple case can be studied in detail, and the results can illustrate the general case, where epitopes are comprised of many sites. We use Equation 1 to determine the escape rates for each of the three mutant haplotypes has for a given level of CTL clone E_j recognizing epitope j:

$$\epsilon_{10} = kE_{\text{tot}} \left[\frac{\Delta r_1}{n_j(t)} - \Delta f_1 \right] \tag{2}$$

$$\epsilon_{01} = kE_{\text{tot}} \left[\frac{\Delta r_2}{n_j(t)} - \Delta f_2 \right] \tag{3}$$

$$\epsilon_{11} = kE_{\text{tot}} \left[\frac{\Delta r_1 + \Delta r_2 - \Delta r_1 \Delta r_2}{n_j(t)} - (\Delta f_1 + \Delta f_2 - \Delta f_1 \Delta f_2) \right] \tag{4}$$

where $1/n_j(t)$ is the time dependent fraction of the total CTL population comprised by CTL clone E_j and we have neglected the small parameter $d_I \Delta f$ (Table 1). We consider the case where the initial escape rate of haplotype 10 in epitope j (ϵ_{10}) is higher than the initial escape rate of haplotype 01 (ϵ_{01}).

Once an escape haplotype has reached a sufficiently high frequency, CTL clone E_j will decay monotonically in time, but the virus load will stay stable, since the population of infected cells will be controlled by the other CTL clones. As $1/n_j(t)$ decreases and the CTL-induced pressure on the epitope declines, the fitness losses at the two sites, Δf_i, begin to dominate the escape rates (Equations 2 - 4), and the favored haplotype will change. Eventually, the decay of the CTL clone will cause the transmitted haplotype (00) to regain fitness advantage and become a dominant strain again (the existence of compensatory mutations may cause stabilization of the last escaped clone, see *Discussion*). The sequence of dominant haplotypes can follow one of the patterns, as follows:

Simple Pattern. The simple pattern of escape (Figure 4A) can occur if the second site cannot escape

($\epsilon_{01} < 0$, Equation 3). It can also occur if the second site is weakly advantageous, yet the double mutant (11) does not have time to grow to appreciable frequency before the transmitted haplotype (00) regains the advantage in growth rate. Observation of this pattern implies a large difference in the fitness costs and/or recognition loss magnitudes between the two sites in an epitope.

Leapfrog Pattern. The leapfrog pattern can occur if haplotype 01 gains the advantage over 10 before the 10 loses the advantage over 00 (Figure 4C and D). In order for this to occur, site 1 must have both a greater fitness and a greater recognition loss than site 2:

$$\Delta r_1 > \Delta r_2, \Delta f_1 > \Delta f_2 \tag{5}$$

CTL to the escaping epitope decays at a faster rate during the time period when variant 10 dominates the population than than during the period when 01 dominates the population. The difference is due to the relatively higher recognition loss of the mutation at the first site (inset Figure 4D). The exact conditions required for this pattern are given in *Text* S3.

Nested Pattern. When both single-mutant haplotypes continue to have a positive growth rate throughout the course of infection, as in the case of constant selection pressure, the double-mutant haplotype has the highest escape rate (Figure 4E, F). The nested pattern may occur if the escape rates of both single-mutant haplotypes are similar ($\epsilon_{10} \sim \epsilon_{01}$).

We use numeric computation to determine which pattern of escape is observed over a range of recognition and fitness losses at each of the two epitope sites (*Text* S4). The number of epitopes is fixed, n, the escape rate for the first haplotype, ϵ_{10}, and the ratio of the fitness costs in the two epitope sites, $\Delta f_1/\Delta f_2$, at values that are representative of acute infection (Figure 5) or chronic infection (Figure S3) (see Figure S2 for the range of escape rates observed in HIV infected patients). For large escape rates, the leapfrog pattern can be observed for large values of Δr_1 and a broad range of Δr_2 (Figure 5). Smaller values of Δr_1 produce smaller escape rates, and the leapfrog is observed in a narrow range of small Δr_2 (Figure S3). In some cases, haplotype 11 is observed as a short intermediate between haplotypes 10 and 01 (labeled "nested leapfrog" in Figure 5). The time interval during which a given haplotype dominates the population depends on parameters of loss and recognition at epitope site, Δf_k and Δr_k. The less costly haplotype can dominate the population from months to years (shown as t_{01} in the inset of Figures S3 and 5). CTL to the escaping epitope will decay at the fastest rate when variant 11 dominates

the population (inset of Figure 4F).

Model

Model of HIV Dynamics

We model SIV/HIV evolution in the presence of multiple CTL clones (Figure 1A). Each CTL clone recognizes a distinct viral epitope that is presented by an infected cell. Mutations occur in the proviral genomes of infected cell that allow an infected cell to partially evade CTL recognition, but come at a cost in terms of viral replication. The model is given by the following equations:

$$\frac{dT}{dt} = \lambda - d_T T - \beta T \sum_i f_i I_i \tag{6}$$

$$\frac{dI_i}{dt} = \beta T f_i I_i - d_I I_i - \kappa I_i \sum_j r_{ij} E_j \tag{7}$$

$$\frac{dE_j}{dt} = \sigma + c E_j \sum_i \frac{r_{ij} I_i}{h_j + \sum_i r_{ij} I_i} - d_E E_j \tag{8}$$

where f_i and r_{ij} are the relative viral replication rate of viral genome i and the relative CTL recognition of sequence i by clone j, as compared to the transmitted sequence, respectively:

$$f_i \equiv \exp\left(-\sum_{j,k} s_{jk} a_{jk}^i\right) \tag{9}$$

$$r_{ij} \equiv \exp\left(-\sum_k \alpha_{jk} a_{jk}^i\right) \tag{10}$$

The processes described are, as follows. Target cells (T) are replenished at a rate λ cells per day, die with a rate d_T and are infected by virus at a rate that depends on the fitness on the infecting strain, with maximum rate per cell β, and the number of productively infected cells (I_i) in the system. We consider n epitopes, each consists of m amino acid positions (sites), giving a total of 2^{mn} possible strains. An cell infected is labeled by proviral genome i with n epitopes denoted $g^i = e_1^i, e_2^i, ..., e_n^i$, where each epitope, e_j^i, has m sites $a_{j1}^i, a_{j2}^i, ..., a_{jm}^i$, and $a \in 0, 1$ indicates the presence or absence of a mutation at an epitope site. The fitness of genome i is reduced by mutations. A mutation in epitope j at site k contributes cost

s_{jk} to the logarithm of the reduction in replication rate. The strain with all 0 is the transmitted strain, which has fitness 1.

Effector CTL (E_j) are replenished with a constant rate σ cells per day, divide with a rate dependent on the number of infected cells that they recognize and their avidity, $1/h_j$, with maximum rate c, and die with rate d_E. CTL clones each respond to a distinct viral epitope that is presented by an infected cell and kill infected cells at a maximum rate κ. The recognition ability of the CTL clone to epitope j in strain i is reduced by mutations, as given by Equation 10. A mutation in epitope j at site k contributes α_{jk} to the logarithm reduction in CTL recognition. In the present work, we assume CTL of equal avidities, $h_j \equiv h$. Throughout the text, we describe the relative loss in fitness and recognition due to escape mutations in terms of notation $\Delta r_{ij} \equiv 1 - r_{ij}$ and $\Delta f_i \equiv 1 - f_i$.

Mutations are generated randomly with rate $\mu = 3 \cdot 10^{-5}$ per site per generation of infected cells (i.e., the average lifespan of an infected cell, $1/d_I$) between strains that differ by one site. Strains with average copy number above one infected cell are simulated deterministically according to the above equations; below this threshold a strain is considered extinct. The basic model with one escape-conferring site per epitope ($m = 1$) has been introduced by Althaus and De Boer [14]. In this work, we adapt the model to focus on the effect of partial recognition and fitness losses during a narrow time interval after acute infection (see *Discussion* for a detailed comparison of the two models). Further information on model parameters and the estimated range of parameter values are listed in Table 1.

Following the original work [14], the model assumes that the majority of infected cells are killed by CTL rather than by viral cytopathicity. It has also been proposed that CTL decrease the viral production in infected cells in a non-lytic fashion, and that cells die within a fixed period of time ($\sim$ 24h) due to viral cytopathicity [33]. A specific choice of the mechanism of infected cell death (CTL or viral cytopathicity) is not likely to affect the main conclusions about the dynamics of CTL escape. Furthermore, due to the existence of the eclipse phase of viral production, it is difficult to distinguish between these mechanisms using data from *in vivo* studies [34, 35]. Thus, we prefer to use the CTL-induced death model, which is simpler to implement and easier to support immunologically.

Simplified model to study the order of escape mutations

We introduce a simplified model, which does not explicitly consider dynamics, in order to study the sequence of escape mutations for a realistic size genome. Fractional fitness costs (Δf) and recognition

losses (Δr) are randomly generated from a uniform distribution $[0, 1]$ for 100 sites (10 epitopes with 10 sites per epitope) in order to study the sequence of escaped sites. All sites are ranked in order of $\Delta r - \Delta f \cdot n$ and escape sites in similar ranking over many runs are considered together. CTL decay is introduced for all additional sites in an epitope once a site in the epitope escapes: after each round of escape, Δr is reduced for all sites in the epitope by: $\Delta r(n + 1) = \Delta r(n) \exp[-d'_E \sum_i \Delta r_i(0)]$ for all i in the epitope that have escaped. Here parameter d'_E is defined as the decay rate per escape, in contrast to d_E in the main model which is defined per day.

Discussion

The recent availability of time-resolved, deep-sequencing data from HIV infected patients have illuminated the complexity of interaction between CTL clones and the genetically diverse HIV population. Using a model including multiple CTL clones, we demonstrate that the rate of viral escape depends on both the partial recognition losses and fitness costs associated with an escape mutation as well as the number of active CTL clones. Furthermore, the model predicts that CTL populations change their relative sizes in response to viral escape, which has observable consequences for the sequence and number of escape mutations that are possible over time. Changing selection pressure on an epitope due to declining CTL levels can also cause the dominant escape variant in an epitope to change in a non-nested fashion. The model contains several assumptions and simplifications, as follows.

Pre-existing mutations. We assume that the population of infected cells in acute infection and steady state is large, so that all single escape mutants exist in the population before the rise of CTL (as in [14,15]). The assumption is supported by estimates of the large effective HIV population size [4,36,37]. We can also infer the fact of preexistence of CTL escape mutations from the observed preexistence of drug-resistant mutations evident in the early emergence of resistance to mono-therapy (e.g. [38]). Furthermore, since the number of escape mutations per epitope is typically larger than the number of drug resistance sites per drug, the mutation cost of the least costly escape mutation can be assumed to be lower – and the frequency of preexisting mutations higher – for escape mutations. The preexistence of single mutants casts doubts on the interpretation of very late escape mutations as the result of the late appearance of an escape mutation not initially present in the population [39].

Eventual reversion to the wild type is prevented by compensatory mutations. Mutations compensating for fitness losses are widely spread in HIV genome [43], including drug-resistance mutations [40–42] and immune-escape mutations [10, 19, 20]. They have been hypothesized to be a major driving force of HIV evolution and high diversity [44]. However, the dynamics of compensation remains poorly understood. Therefore, our model does not explicitly include compensatory mutations and, as a result, predicts the eventual reversion of any escape mutation with a fitness cost. Instead of including compensation, we calculate the period of time that a given mutation would be maintained in the population before reversion occurs, which is the same time interval where compensation would be necessary in order to prevent reversion (inset of Figures 5 and S3). When the non-nested pattern of escape is observed in infected patients, the first escape variant is typically short-lived compared to the second escape variant, and the transmitted variant is not observed after the initial escape [11, 30, 31]. Our interpretation is that the fitness cost of the first variant is large relative to the second variant and does not have time to be compensated before the second variant gains the advantage. The second variant either has a very small fitness cost or is compensated gradually during its lifetime (compensation is not explicitly simulated). Our calculation of the lifetimes of different escape variants in an epitope is a first step towards understanding the timescales associated with compensation.

Multiple CTL clones of equal avidity. The present analysis is focused on the first year post-infection, the time interval in which most escape mutations occur. Therefore, we consider a group of CTL clones with similar avidities that are present initially in similar numbers. This is a reasonable assumption after escape mutations have occured in the first few immunodominant epitopes during the resolution of acute viremia. At this time, a large number of CTL clones are activated and are maintained for many months at simular levels [6, 45]. Once all CTL clones are activated, our model predicts that the steady state viral load is proportional to the inverse avidity of the most avid CTL clone (Equation S2). Kadolsky and Asquith [46] estimated that the average viral load increases by only 0.051 log copies/ml per CTL escape. This small increase, which we interpret to be the average difference avidity spacing between CTL clones, justifies our assumption that CTL avidities are, indeed, very closely spaced. Our analysis shows that, given closely spaced avidities, it is recognition and fitness losses that govern the order and timing of escape mutations, rather than variation in CTL avidity.

Other assumptions. The model includes additional simplifying assumptions, as follows. i) The proliferation rate of CTL clones saturates with the infected cell number, but not with the total CTL level.

In the original model [14], the authors postulate this saturation to predict that the total number of CTL limits the growth of individual CTL clones, which causes clones to interact and enables the co-existence of multiple CTL clones with different avidities. We believe that further study is needed to verify the existence and the possible origin of the interclonal interaction. (Note that the target availability may not be the cause of interclonal interaction, because most CTL are not bound to their targets even at the peak of infection, and the overall effector to target ratio is less than two [47].) ii) CTL are short-lived in the absence of antigen, with an average lifetime of 10 days, as is consistent with early studies of CTL dynamics in SIV system [48, 49] and mathematical modeling of these data [50]. iii) We ignore recombination, which may increase the rate of emergence of escape mutations among different epitopes [15] as well as the long term rate of evolution [51, 52]. For the intra-epitope dynamics of escape, recombination between neighboring sites will be a small correction. (iv) CTL clones against escape mutants are not included in the model. By including partial rather than full recognition loss, we allow CTL to escaped epitopes to continue to exert selection pressure without incorporating additional CTL clones. Though clones to escape mutants may exist, introducing extra CTL clones (5 clones for each two-site epitope, instead of 1) would complicate analysis of the model without changing the essential results. (v) Small fitness costs are assumed. Equation 1 states that costly escapes $\Delta f \sim 1$ cannot arise when recognition losses are partial and many CTL clones target the viral genome. We choose to focus on the time interval corresponding to the first year after the resolution of acute infection when these assumptions are fulfilled. Escape mutations entailing a high fitness cost can only escape when only one or two CTL clones dominate strongly, which we estimate from previous data to be during the 3-4 weeks post-infection. (vi) Fitness costs are positive. A fraction of mutations that are transmitted to an individual that fall outside the individuals HLA-restricted epitopes revert (i.e. may have a negative fitness cost). However, the rate of reversion is very small [17] and hardly interferes with a faster dynamics of the within-epitope mutations. (vii) We consider escape processes in different epitopes separately, because typically they do not overlap much in time. In general, however, linkage effects (clonal interference, background selection) between epitope mutations and compensatory mutations of same or different epitopes may complicate the picture (see [32] for review of recent research in this area).

Thus, our model has demonstrated that partial recognition losses, in addition to fitness costs and the breadth of the CTL response, dramatically affect the rate and the order of escape mutations during an HIV infection. These findings help to interpret the positive correlation between fitness costs and

recognition losses observed in the Pol gene [26] and make the testable prediction that the strength of the correlation should decrease with the time post-infection. Our results call for direct measurements of recognition losses for different escape mutations. Combined with the proposed trajectory approach (Figure 2), these data will serve as a basis for improved prediction of conserved epitopes for use in vaccines.

Acknowledgments

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Table 1. Model Parameters

Parameter	Realistic Value	Description	Reference
n	1- 8	Number of epitopes recognized during first 100 days	$[11, 13, 30, 31]$
m	2 - 10	Number of sites per epitope important for recognition	$[11, 13, 30, 31]$
d_T	1 d^{-1}	Target cell death rate	$[50, 53]$
λ/d_T	$5 \cdot 10^8$ cells	Activated target cell level	$[50, 54, 55]$
d_I	1 d^{-1}	Virus-induced infected cell death rate	$[56]$
$\sum_j \kappa E_j^{ss}$	4 d^{-1}	CTL-induced infected cell death rate	$[50, 54, 55]$
β	$1.1 \cdot 10^{-8} (\text{d} \cdot \text{cell})^{-1}$	Basic efficiency of target cell infection	$[50, 54, 55]$
s	$[0 - \infty]$	Intrinsic mutation cost	$[10, 21, 22, 27\text{--}29]$
α	$[0 - \infty]$	Reduction of CTL recognition	$[27\text{--}29]$
Δf_i	$[0 - 1]$	Fractional reduction in intrinsic replication rate	
Δr_{ij}	$[0 - 1]$	Fractional reduction of CTL recognition	
κ	$1 \cdot 10^{-9} (d \cdot cell)^{-1}$	CTL killing efficiency. Gives $10^8 - 10^9$ CTLs in chronic infection	$[48]$
σ/d_E	10^3 cells	Initial population of CTLs	$[54]$
c	1 d^{-1}	Maximum growth rate of effector cells	$[54]$
d_E	0.1 d^{-1}	Death rate of effector cells	$[50, 54, 55]$
h_j	$2.5 \cdot 10^8$ cells	Number of recognized infected cells for half maximal proliferation of CTL (inverse avidity). Gives an infected cell level of $10^7 - 10^8$ in chronic infection	$[47]$

Model parameters for the model of escape from multiple CTL shown in Figure 1.

Figure 1. A computational model of the interaction between HIV and multiple CTL clones. (A) The model given by Equations 6 to 8 comprises three interacting cell compartments: target cells (T), infected cells (I) and multiple CTL clones (E). Viral genomes contain multiple epitopes, which can mutate to partially abrogate CTL recognition. An escape mutation is denoted by an X. Each CTL clone recognizes a single viral epitope and is stimulated to divide at a rate proportional to the number of infected cells with recognizable epitopes. The model is designed to study the rate of escape in epitopes when CTL pressure is distributed across multiple epitopes, as well as study intra-epitope escape patterns when CTL respond dynamically to the infected cells that they recognize. Black arrows: flux of cells from one compartment to another. Blue arrows: dependence of the rate of flux from one compartment on another. Dotted lines represent attenuation of the interaction strength. (B) Simulation example showing three phases of HIV evolution. A single virus strain initiates the infection (transmitted strain, black). In response to the growing number of infected cells, multiple CTL clones are activated (colored lines), and the system reaches a steady state. Finally, virus strains with escape mutations (dashed, colored lines) replace the transmitted strain. In response to lowered activation signals, some CTL clones decline. The escape strains are colored to match the CTL against which an escape was most recently acquired. Model parameters: number of epitopes, $n = 6$; number of sites per epitope, $m = 1$. Epitopes 1-3 have parameters that allow escape $\Delta r_i = [0.1, 0.2, 0.3], \Delta f = 0.01$, epitopes 4-6 have parameters that prohibit escape, $\Delta f_i = \Delta r_i = 0.1$. Other parameters are listed in Table 1.

Figure 2. The escape trajectory in the cost-benefit plane bends over time due to CTL decay. Fitness costs and recognition losses are randomly generated for 100 sites (10 epitopes with 10 sites per epitope) in order to study the sequence of escaped sites (black line) in the whole genome without CTL decay (A,B) or with CTL decay (C,D) for 1000 simulation runs. (A) For each site that escapes, the fractional fitness cost, Δf, multiplied by the number of epitopes, $n = 10$, and fractional recognition loss, Δr) (Equation 1 and Table 1) is shown. Colors show the predicted rank of escape mutations, from early escape mutations (blue) to late escape mutations (red). The average trajectory over all runs (black) moves from high recognition loss, low fitness cost to low recognition loss, high fitness cost. Inset: The best-fit slope for each escape rank. A positive correlation is observed between the fitness and recognition losses for all epitopes that escape at a given rank. (B) The maximum escape rate of any epitope site for all 10 epitopes for a representative simulation run. (C-D) As in (A-B), except including CTL decay. CTL decay is simulated by reducing recognition losses for all epitope sites in epitopes that have partially escaped according to $\Delta r(n + 1) = \Delta r(n) \exp[-d'_E \sum_i \Delta r_i(0)]$, summing over all i sites in the epitope that have escaped with $d'_E = 0.1$ per escape . When CTLs decay in response to lost recognition caused by an escape in an epitope, the immune pressure and hence the recognition loss for all other sites in that epitope is decreased. The result is that the average trajectory in the cost-benefit plane bends towards the horizontal axis.

Figure 3. Fitness loss and recognition losses of escape mutations are predicted to correlate positively, with a slope that deceases in time. Sites are randomly sampled from the simulation described in the caption to Figure 2 in order to simulate the effect of acute samples (high ranking sites), late chronic samples (low ranking sites) or patient samples from random times (random ranks). (A) No CTL decay. The slope of the correlation from acute (blue) or late chronic (red) sampled escape mutations is the same, however, it is lower for escape mutations sampled at random times (green). (B) CTL decay causes the slope of the correlation to decrease in time, due to decrease of CTL selection pressure. CTL decay is introduced as described in the caption to Figure 2 and *Model*.

Figure 4. Dynamical selection pressure from CTLs causes three possible patterns of intra-epitope escape: example for an epitope with two sites. Intra-epitope escape in one epitope with two sites is studied for the model shown in Figure 1 and *Model*. The sequence in which haplotypes are selected depends on the distribution of fitness and recognition losses within an epitope. The fraction of the infected cell population containing each of the four haplotypes in the escaping epitope is shown in for "simple" (A), "leapfrog" (C) and "nested" pattern (E). For each pattern, the dependence of the escape rate (Equations 2 - 4) for each haplotype on the fraction of CTLs responding to the epitope (B, D, F) . The inset shows CTL dynamics: the size of the CTL clone to escaping epitope (red) and the total CTL number (black). Parameters: (A,B) $\Delta r_{1k} = [0.4, 0.1], \Delta f_{1k} = [0.01, 0.01]$, (C,D) $\Delta r_{1k} = [0.6, 0.2], \Delta f_{1k} = [0.1, 0.003]$, (E,F) $\Delta r_{1k} = [0.4, 0.25], \Delta f_{1k} = [0.003, 0.003]$ with $n = 2$, $m = 2$ for all panels; other parameters are given in Table 1.

Figure 5. The pattern of emergence of escape variants in a single epitope contains information about the fraction of recognition and fitness lost by single-site mutations in the epitope. The pattern of escape (Figure 4) is obtained from simulation of the model (Figure 1, Equations 6 to 8) with two sites per epitope, $m = 2$ for a range of recognition and fitness losses. The pattern that is obtained is plotted as a function of the parameters of recognition loss at the first and second site (Δr_1 and Δr_2, respectively). Equations S6 (red line) and S9 (blue line) determine the region where the leapfrog pattern can be observed. Regions that require $\Delta f_1 < 0$ are not allowed by definition (magenta line). The shaded regions between these three lines correspond to regions of parameter space where both sites escape. The corresponding patterns are: "leapfrog" ($10 \rightarrow 01$, Figure 4C), "nested" ($10 \rightarrow 11$, Figure 4E), "nested leapfrog" ($10 \rightarrow 11 \rightarrow 01$). Observation of the leapfrog pattern in an epitope tightly constrains the fraction of CTL recognition loss conferred by sites in an epitope. The inset shows the length of time during which haplotype 01 is dominant in the escaping epitope. Fixed parameters are: the escape rate of the first haplotype ($\epsilon_{10} = 0.5$) and the number of targeted epitopes ($n = 3$), values which correspond to escape mutations that occur in acute infection (see Figure S3 for parameters that correspond to later in infection). Fitness costs are chosen such that the second site is less costly than the first: $\Delta f_1/\Delta f_2$ equal to 3 (A) or much less costly than the first, $\Delta f_1/\Delta f_2 = 10$ (B). Other parameters given in Table 1.

Figure S1. Changing composition of CTL and infected cell populations. Simulation of model given by Equations 6 to 8 with the same parameters as in Figure 1B. (A) Escape mutations in viral epitopes spread through the population of infected cells to abrogate CTL recognition. Once the system has reached steady state, the number of infected cells is roughly constant, and the prevalence of an escape mutation can be measured as a fraction of the total infected cell population. (B) Once an escape mutation has spread to the majority of infected cells, the corresponding CTL clone begins to decay with a rate proportional to the fraction of recognition lost. Since the total CTL population remains roughly constant in steady state, the prevalence of a given CTL clone can be shown as a fraction of the total CTL population.

Figure S2. Escape rate and t_{50} are negatively correlated in two experimental studies. The frequency of a mutated epitope for epitopes over time is fit to the curve $f(t) = \{1 + \exp[-\epsilon(t - t_{50})]\}^{-1}$, which describes deterministic selection on a single site with selection coefficient ϵ, in order to determine parameters ϵ and t_{50}. Colored dots show data from a single patient studied in [11] (blue) and multiple patients studied in [30] CH40 (red), CH58 (green), CH77 (cyan). Inset: Simulation example showing the correlation between escape rate, ϵ, and the time that the mutation spreads to 50% of the population of infected cells, denoted t_{50}. Parameters ϵ and t_{50} are found for the three escape mutations shown in Figure 1B that occur in the first 200 days post infection. Thus, variation in recognition and fitness losses across many epitopes successfully reproduces this feature of escape dynamics.

Figure S3. CTL lifetime changes the frequency with which intra-epitope escape patterns are observed Here we show a modified version of Figure 5 for the case when CTL are long-lived, $d_E = 0.01$ (other parameters are the same as Figure 5). When CTLs are long-lived, the leapfrog pattern (Figure 4C)) is observed more often, since the haplotype 01 has time to grow to dominate the population. / Here, the death rate of CTL is $d_E = 0.01$, ten times smaller than the value used in Figure 5. The difference between the predicted leapfrog region as determined by Equations S6 (red line) and S9 (blue line) and the shaded region where leapfrog is actually observed is lessened for long-lived CTL. This is the case because each time that haplotype 01 gains the advantage over haplotype 10, it grows to dominate the population before CTL decay to the level where haplotype 01 also reverts.

Figure S4. Estimating the relationship between Δr and ΔB approximately from three published experiments. Our model contains one parameter for CTL recognition loss caused by a mutation, Δr. In order to compare model predictions with data from Mostowy et al [26], where HLA binding impairment caused by mutations was considered rather than overall CTL recognition loss, we sought to compare HLA binding impairment with overall CTL recognition loss. By combine data from three references, Schneidewind et al [27], Kawashima et al [28] and Matthews et al [29], we were able to demonstrate a strong correlation between the two parameters. This justifies our comparison of our model predictions with data from [26].

Text S1: Steady state and the escape rate

Text S2: Escape causes contraction of CTL clones

Text S3: General conditions for observing the leapfrog pattern of escape

Text S4: Simulation: Leapfrog pattern can occur in a broad range of fitness and recognition losses

Text S5: Finding the relationship between Δr and HLA binding loss from three different experiments