HLA-Associated Clinical Progression Correlates with Epitope Reversion Rates in Early Human Immunodeficiency Virus Infection


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Human immunodeficiency virus type 1 (HIV-1) can evade immunity shortly after transmission to a new host but the clinical significance of this early viral adaptation in HIV infection is not clear. We present an analysis of sequence variation from a longitudinal cohort study of HIV adaptation in 189 acute seroconverters followed for up to 3 years. We measured the rates of variation within well-defined epitopes to determine associations with the HLA-linked hazard of disease progression. We found early reversion across both the gag and pol genes, with a 10-fold faster rate of escape in gag (2.2 versus 0.27 forward mutations/1,000 amino acid sites). For most epitopes (23/34), variation in the HLA-matched and HLA-unmatched controls was similar. For a minority of epitopes (8/34, and generally associated with HLA class I alleles that confer clinical benefit), new variants appeared early and consistently over the first 3 years of infection. Reversion occurred early at a rate which was HLA-dependent and correlated with the HLA class 1-associated relative hazard of disease progression and death ($P = 0.0008$), reinforcing the association between strong cytotoxic T-lymphocyte responses, viral fitness, and disease status. These data provide a comprehensive overview of viral adaptation in the first 3 years of infection. Our findings of HLA-dependent reversion suggest that costs are borne by some escape variants which may benefit the host, a finding contrary to a simple immune evasion paradigm. These epitopes, which are both strongly and frequently recognized, and for which escape involves a high cost to the virus, have the potential to optimize vaccine design.

The dynamics of viral replication in acute and early human immunodeficiency virus (HIV) infection are not well understood as longitudinal data from large cohorts of seroconverters are hard to assemble. Recent studies have shown that new HIV infections may be the result of a single transmitted variant, that new env gene mutations can be detected within a few weeks (25), and that early immune escape can be detected at sites across the HIV genome (9). These data add to a body of work showing that cytotoxic T cells act early, contributing to the early reduction in viremia (8, 30).

Whether early cytotoxic T-lymphocyte (CTL) immune responses influence longer-term clinical outcome is not clear. Antigen-specific CTLs capable of producing gamma interferon and other cytokines are detectable at all stages of HIV infection (1, 3, 24, 41). Much weight is placed on the macaque/simian immunodeficiency virus model in which nearly total peripheral blood CD8(+)-T-cell elimination using monoclonal antibodies results in rising viremia (42). The role of other forms of host immunity (e.g., neutralizing antibodies, natural killer cells, and macrophages) has, to some extent, been pursued with less intensity in light of persuasive evidence that CTLs can control retrovirus infection (46). The extent to which the simian model mirrors HIV infection has been questioned (5) and, despite exhaustive cellular assays of T-cell function—from gamma interferon enzyme-linked immunospot assays (1, 27, 38) to polyfunctional cytokine matrices (2, 6)—no CTL function correlates robustly with HIV plasma viral load or viral dynamics. Moreover, analyses of evolutionary data suggest that CTLs are inefficient at killing HIV-infected cells (4).

However, statistical analysis of data from large cross-sectional studies link HLA class I alleles with specific genomewide HIV polymorphisms, suggestive of a pervasive selection pressure exerted by CTLs (7, 10, 18, 36, 40). It is clear that associations between some HLA class I alleles and particular amino acid polymorphisms are robust although it is disputed whether immune escape influences disease progression. The viral fitness costs resulting from immune escape may even contribute to better clinical outcomes associated with the possession of HLA class I alleles such as B*27, B*57, and B*58 (18).

Evolutionary studies of HIV require longitudinal data from large cohorts of patients sampled since seroconversion to detect adaptation in new hosts as it accrues. HIV is one of the few pathogens where it is possible to do this within individuals because of the high viral turnover and rapid intrahost evolution. Here, we investigate a cohort of 189 acute seroconvert-
For each amino acid in every patient, the timing of the emergence of polymorphisms was identified over 3 years for all amino acid positions (amino acids 1 to 417 for pol and amino acids 1 to 379 for gag). We defined a forward mutation (FM) as a mutation away from the wild-type baseline (according to HXB2 sequence), whereas a backward mutation (BM), or reversion, was identified as a mutation present in the baseline sequence which subsequently changed to the HXB2 wild type. Although FM and BM are likely to represent immune escape and reversion, respectively, in the absence of documented cellular immune responses for each patient, other causes of variation cannot be excluded, and these generic terms are used. For FMs, the number of events at each time point was compared with the number of baseline wild-type sites—i.e., the number with potential to escape—and adjusted for 1,000 amino acid sites. For BMs, the number of events for each time point was compared with the number of baseline wild-type sites—i.e., the number with potential to escape—and adjusted for 1,000 amino acid sites.

HIV typing. Patients’ HLA types were determined by the oligo-allelic level using DYNal RELITM reverse sequence-specific oligonucleotide kits for the HLA-A, HLA-B, and HLA-C loci (DYNal Biotech). To obtain four-digit typing, DYNal Biotech sequence-specific priming kits were used, in conjunction with the Sequence-Specific Oligonucleotide type.

Relative hazards for disease progression. Hazards for disease progression were obtained from previous data using Cox model analyses based on the Caucasian samples from four cohorts for which patients had known dates of seroconversion: the Multicenter AIDS Cohort Study, the Multicenter Hemophilia Cohort Study, the San Francisco City Clinic Cohort, and AIDS Linked to Intra-venous Experience (11, 21, 22, 37).

### Table 1. Optimal epitopes used in the sequence analysis of the seroconverter cohort

<table>
<thead>
<tr>
<th>Epitope no.</th>
<th>Reference</th>
<th>Optimal epitope</th>
<th>HLA restriction</th>
<th>HXB2 position (aa)*</th>
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<tr>
<td>Pol epitopes</td>
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<tr>
<td>1</td>
<td>GPVKQVWPL</td>
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<td>RT 18–26</td>
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<tr>
<td>2</td>
<td>ALVEITEMEK</td>
<td>A*0301</td>
<td>RT 33–43</td>
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<tr>
<td>3</td>
<td>TVLVDGDAY</td>
<td>B*3501</td>
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<tr>
<td>4</td>
<td>VLDVFENFSF</td>
<td>B*2705</td>
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<tr>
<td>5</td>
<td>VPLDIDFRRK</td>
<td>B*3501</td>
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<td>TAFITPSI</td>
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<td>7</td>
<td>SPAIQSNSMT</td>
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<td>Gag epitopes</td>
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<td>GIKKXYKLK</td>
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<td>p17 24–31</td>
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<td>KYKLLKHIWV</td>
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<td>GEIYKRWH</td>
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<td>DCKTLIKL</td>
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<td>GPGHKARVL</td>
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<td>p24 223–231</td>
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*All epitopes are derived from the Los Alamos National Laboratory A list of optimal epitopes (http://www.hiv.lanl.gov/content/immunology/pdf/2008/optimal_cel_article.pdf), except for those indicated and referenced separately.

*aa, amino acids.
Statistics. Fisher’s exact tests, unpaired t tests, and linear regression were used in statistical analyses with Prism, version 4.0 (GraphPad Software).

RESULTS

Gene-specific rates of viral evolution following acute infection. The rates of FMs and BMs were compared in the gag and pol genes. For FMs, which represent possible immune escape, there was a difference between the two genes (P < 0.001) up to 12 months (Fig. 1A). For pol, over the first 6 months the prevalence was 0.27 FM/1,000 amino acid sites, and there was a 10-fold greater prevalence of 2.2 FM/1,000 amino acid sites in gag. Only after the first 12 months did the rate of escape in pol approximate that in gag.

For both genes, mutations present at baseline reverted early. For pol and gag, in the first 6 months, there were 26.2 reversions/1,000 amino acid sites and 29.9 reversions/1,000 amino acid sites, respectively. These rates were maintained for the first 2 years of infection, illustrating that transmitted mutations continued to revert (Fig. 1B). For a cohort of this size, it is not feasible to obtain donor sequences, and so it is possible that not all of these mutations were transmitted. Nevertheless, the changes observed are consistently toward wild type, with little evidence of recurrence of the mutations, suggesting that they were not originally selected, even if only temporarily, very early in infection.

As pol is also under selection pressure from antiretroviral therapy, we identified amino acid sites (Stanford University Drug Resistance Database [http://hivdb.stanford.edu/]) susceptible to drug resistance mutations. The proportion of both reversion and putative escape polymorphisms that arose at drug resistance residues was less than 5% of the new pol
mutations observed although baseline variants were identified at M41 (four patients), T69 (five), A98 (three), K103 (five), V108 (four), V118 (six), V179 (two), Y181 (one), M184 (one), Y188 (one), L210 (three), and T215 (nine), the majority of which persisted over time. These were randomly distributed among HLA class I alleles, showing that the rates of observed mutation within CTL epitopes were not biased by drug resistance mutations. Although the median time between sampling and the estimated date of seroconversion was 60 days (interquartile range, 39 to 86), it is possible that the \( p \) gene rate has been underestimated due to very early escape missed by our sampling. Although one might expect this bias to affect both genes equally, in order to answer this question robustly, one would need to analyze a cohort of acute and hyperacute patients identified within 4 weeks of infection.

Patterns of HLA-associated variation in HIV epitopes. To determine how these patterns of forward mutation and reversion related to immune pressure, we studied 34 well-defined optimal epitopes restricted by HLA class I alleles (Table 1). For these epitopes we had previously documented HLA-restricted immune responses using gamma interferon enzyme-linked immunospot assays in a cohort of chronically infected patients (41). Epi
tope sequences were grouped according to whether patients carried the requisite restricting HLA class I allele. Any measure of variation needs to be controlled for sequence variation when the site is not acting as an antigen, i.e., in the absence of the restricting HLA class I allele. (Even then, confounding effects such as overlapping epitopes may occur.) For each time point the proportion of wild-type epitopes (those with the same sequence as the index peptide occur). For each time point the proportion of wild-type epitopes (those with the same sequence as the index peptide) was plotted for both the HLA-matched and unmatched patients (mostly epitopes that become more variable in the presence of HLA pressure or structural constraint. Of interest are the epitopes that persist over time and no significant difference between the two (Fig. 2b). The examples shown are SLYNTVATL (A*0201), EERLQHLLRW (B44), and GGKK YKLK (B*0801). It is possible that there is so much background variation at these sites that we are not able to identify small HLA-attributable changes. However, since HLA-matched and HLA-unmatched patients are so similar, HLA class I is unlikely to be the major source of variation within these epitopes.

The third group (\( n = 8/34 \)) contained epitopes in which there was a divergence between the proportion of wild-type sequences in the HLA-matched and -unmatched patients over time (Fig. 2c). The HLA-matched epitopes became increasingly variant while baseline variation in unmatched epitopes reverted toward wild-type sequences. This third group of discordant epitopes included ISPRTLNAW (B*5701), KR WILGLNK (B*2705), TAFTIPI (B*5101), TLYCVHQRI (A*1101), TSITLSEQIGW (B*5701), PIVLPEKDSW (B*5701), and QASKEVKNW (B*5701). This third group is dominated by epitopes restricted by HLA class I alleles which have previously been associated with lower viral loads (28), better clinical outcomes (37), and a high prevalence of variation in chronic infection (18). For some epitopes there were more mutant sequences in the HLA-matched patients than in the HLA-unmatched controls at baseline, which is suggestive of early escape prior to first sampling. For example, the HLA B*51-restricted epitope TAFTIPI was mutant at the position 8 isoleucine in 10/13 patients at baseline compared with 68/151 B51-negative controls (\( P = 0.021 \)), and the B*57/58-restricted TSITLSEQIGW was mutant at position 3 in 4/12 patients compared with 12/135 controls (\( P = 0.003 \)). So, although the median interval between estimated seroconversion and first sampling is 60 days, at certain epitopes escape had clearly already taken place.

Epitopes restricted by beneficial HLA class I alleles show greater variability over time than less favorable epitopes. We plotted the initial rates of change for each epitope in the presence and absence of HLA class I restriction (Fig. 3). By intersecting the axes of the figure through (0, 0), four quadrants are defined. The inset figure shows the inferred behavior of epitopes located in each section of the plot. Most epitopes (20/34; 59%) are located on the left side of the y axis, suggesting that these epitopes become increasingly variant over time in the absence of HLA-associated pressure; i.e., there is ongoing nonsynonymous change over time not necessarily attributable to HLA class I-dictated immunity. We cannot rule out the possibility that overlapping or undefined epitopes might contribute to this. Twenty-three epitopes lie below the x axis, showing that variants accumulate over time in the HLA-matched patients. The less-variant epitopes lie close to the intersection of the x and y axes, suggestive of weaker selection pressure or structural constraint. Of interest are the epitopes located in the lower right quadrant. In this quadrant are epitopes that become more variable in the presence of HLA but that revert once this pressure is removed. They include KR WILGLNK (restricted by HLA B*2705), ISPRTLNAW (HLA B*5701), TSITLSEQIGW (HLA B*5701), QASKEVKNW (HLA B*5701), and TLYCVHQRI (HLA A*1101). The epitopes in the other three quadrants show little evidence of HLA-specific selection.

To demonstrate how epitope variation unfolds over time, we present key epitopes from Fig. 2 in a format that highlights the differences between the rates of mutation. On these plots the axes refer to the prevalence of variation within the cohort for each epitope at each time point. The figures reveal the patterns of epitope behavior through the movement of each epitope around the plot over 3 years. Figure 4a shows examples of neutral epitopes that remain close to the line \( x = y \) over time, with no strong evidence of HLA-driven adaptation, and that might represent the less effective “passenger” epitopes (29). Figure 4b shows the protective or dynamic “driver” epitopes.
(a.)
- high proportion of wild-type virus in HLA-matched and un-matched hosts
- slow rates of escape and reversion

(b.)
- high proportion of mutant virus in HLA-matched and un-matched hosts
  - slow rates of escape and reversion

(c.)
- reversion: fitness cost associated with escape
  - rapid escape: strong CTL selection pressure
(29) evolving over time, with specific adaptation to the HLA class I immune response. The figure includes the HLA B*5701-restricted ISPRTLNAW, the B*2705-restricted KRWIILGLNK, the B*5101-restricted TAFTIPSI, TSTLQEQIGW (B*5701), and QASKEVKNW (B*5701). As well as moving down the plot, these epitopes also move farther right over time. This reveals new mutations in the HLA-matched patients combined with reversion in the HLA-unmatched patients. This pattern is not seen in Fig. 4a. Of interest in Fig. 4b is the plot for the HLA B51-restricted TAFTIPSI. This is an epitope that is restricted by a favorable HLA class I allele (37) and mutates early (19) but is not associated with a fitness cost according to this in vivo analysis. One might expect, therefore, mutations in TAFTIPSI to accumulate in the population over time and to be prevalent in populations with a high frequency of HLA B*51-positive individuals.

HLA-associated rates of reversion, but not escape, correlate with relative hazard of disease progression. Recent data suggest that the frequencies with which epitopes are targeted by CTLs correlates with both the rate of escape in early infection (9) and the HLA-specific relative hazard of disease progression (41). The rate at which epitopes acquire new variation in early infection has not been directly compared with the risk of disease progression. In the absence of virological or clinical outcome data from the SPARTAC cohort, which remains blinded, the well-described relative hazards obtained from large Western seroconverter cohorts were used (11, 21, 22, 37). We correlated the rate of variation for each epitope in the absence and presence of the restricting HLA class I allele with the documented relative hazards for disease progression for specific HLA class I alleles for the time to reach a CD4 cell count of less than 200 cells/µl, time to AIDS using the CDC 1987...
FIG. 4. The evolution of epitopes over time. The figure shows the transit of neutral (a) and dynamic (b) epitopes across the plot over time. Each epitope is plotted at each time point according to the relative prevalence of wild-type (WT) sequences in HLA-matched (y axis) and unmatched (x axis) patients. The epitopes shown in panel a are KYKLKHIIVW, restricted by A*2402 (A24); SLYNTVATL, A*0201 (A2); ALVEICTEMEK A*0301 (A3); and GPKVKQWPL, B*0801 (B8). The epitopes shown in panel b are TAFTIPSI, restricted by B*5101 (B51); ISPRTLNAW, B*5701 (B57); KRWIILGLNK, B*2705 (B27); QASKEVKNW, B*5701 (B57); and TSTLQEIQGW, B*5701 (B57).
definition (time to AIDS 1987) (12), time to AIDS using the CDC 1993 definition (time to AIDS 1993) (13), and time to death. As previously described, the population HLA frequencies in these cohorts and our United Kingdom patients are strongly correlated (41).

There was no correlation between the rates of epitope mutation in the presence of their restricting HLA class I alleles and the associated relative hazards of disease progression (CD4 of \( \frac{200}{H11021} \), \( P = 0.08 \) and \( R^2 = 0.09 \); time to AIDS 1987, \( P = 0.16 \) and \( R^2 = 0.06 \); time to AIDS 1993, \( P = 0.09 \) and \( R^2 = 0.09 \); death, \( P = 0.18 \) and \( R^2 = 0.06 \)) (data not shown). However, when a variant epitope is transmitted to an HLA-mismatched patient, the rate at which the epitope reverts (percent wild type/year) is strongly associated with the relative hazard for the restricting HLA class I allele (Fig. 5a to d). Measuring the epitope variation rate in an HLA-unmatched patient is effectively an in vivo fitness assay. The statistical correlation of reversion rates with the parameters for HLA-associated relative hazard is strong evidence that viral escape from beneficial HLA class I alleles results in strong fitness costs in the epitopes that they restrict.

**DISCUSSION**

We present a study of a large cohort of serially sampled HIV-infected seroconverters, with data analyzed for up to 3 years after infection. There are three main findings. First, the rates of FM in the two study genes vary, with escape mutations being more common in the more immunogenic gag gene although in both genes there is rapid and early reversion of transmitted mutants. Second, the patterns of variation are driven by changes within epitopes, with the most variant being restricted by beneficial HLA class I alleles. Third, we show a correlation between the HLA class I-associated hazard and the rate of reversion in HLA-unmatched epitopes, providing strong evidence for a role of viral fitness in the benefit conferred by certain HLA class I alleles.

In both the gag and pol genes, reversion occurred early—generally within the first year of infection—whereas FMs arose faster in the gag gene. There may be a number of explanations for this. In new hosts, the controlling effect of the previous donor’s immune response on HIV variability has been relaxed before the new recipient has mounted his own T-cell response. Accordingly, an initial limiting factor to viral replication will be the fitness cost of the transmitted mutations. If one assumes that HIV can produce extensive de novo variants early in infection, then one might expect the most costly escape mutations to revert quickly and early. Our data agree with the findings of Li et al., who reported early reversion on clonal analysis of seven acute seroconverters, also predominantly within 6 months (32). These data are in contrast to the transmission of drug resistance mutations to drug-naïve hosts. Here,
it would appear that transmitted variants—mostly in pol—are
maintained, often for a number of years (20). Why should
there be a different dynamic for the transmission of immune
escape and drug resistance mutations? Possibilities include the
different potencies of the selection pressures driving variation,
the role of compensatory mutations, and the pathways for
reversion. For certain antiretroviral agents including nucleo-
side reverse transcriptase inhibitors and protease inhibitors, a
number of mutations cluster together to confer the reduction
in drug susceptibility, some of which require two nucleotide
mutations within the same codon. It is feasible, therefore, that
the reversion pathways for these drug resistance mutations
require much greater kinetics on behalf of the virus and are
more likely to persist once transmitted.

We found that escape occurs more quickly in the gag gene.
This is not surprising as Gag has consistently been reported as
one of the main targets of the immune response in acute
infection, and the early detection of escape reflects this. In
addition, the enzymatic function of Pol may impose greater
sequence conservation than the structural Gag protein. It is
possible that we have underestimated the rate of escape in pol
as in some patients escape mutations may have occurred prior
to sampling; however, data from Brumme et al. (9) also reveals
a similar discrepancy in gag and pol.

We find that, in early infection, the variation appears most
rapidly within epitopes restricted by HLA class I alleles that
have been associated with lower viral loads and delayed pro-
gression. In particular HLA B*2705, B*5101, and B*5701 were
associated with faster rates of mutation, but this was not the
case for all the epitopes restricted by these alleles. A key
finding from this study is the correlation between the rates of
reversion of transmitted mutations and the relative hazard of
disease progression reported for the alleles that select them.
The rate of reversion for a transmitted mutation, when assayed
across a large number of patients, is an in vivo measure of its
associated fitness cost. Accordingly, our data reflect the corre-
lation between HLA class I alleles that are linked with a de-
layed progression to AIDS and the fitness costs incurred by
the virus due to the strong selection pressures these alleles impose.
We cannot comment on whether transmission of an escape
variant to an HLA-mismatched recipient is of clinical advan-
tage or disadvantage. This is a separate analysis that will be
undertaken once the SPARTAC trial has been unblinded.

Recently, the clinical significance of reverting sites and their
association with lower viral loads have been inferred from
cross-sectional data from chronically infected patients (34).
Although we agree with the findings of that study, to comment
on reversion requires longitudinal analysis of acutely infected
patients, ideally, although not necessarily, with viral sequence
data from the donor. Cross-sectional analyses from chronic
infection make assumptions on the nature and frequency of
mutation transmission. Although our study is a longitudinal
analysis of acutely infected patients, we do not know the se-
cence of the donor virus for the members of our cohort. How
might this bias our results? In the analysis, we identified any
variant epitope present in the baseline sample, i.e., the sample
taken at first clinical presentation within a median of 60 days of
the estimated date of seroconversion. Variation was defined
against the sequence of the wild-type optimal epitope in the
Los Alamos National Laboratory HIV database (www.lanl.gov). If the variant remained unchanged over time, this was
classified as a nonrevertant. There are two potential sources of
error that arise in the absence of the donor sequence. First,
rather than being transmitted the variant may have arisen anew
in the recipient, reflecting adaptation to the new selective
environment. If such a variant changed to a wild-type genotype
at a later time point, then this would suggest a transient
escape, a process which we encountered rarely in our cohort
(0.15% of all amino acids) (data not shown). Accordingly, a
change from a variant to wild-type sequence is likely to
reflect true reversion. Alternatively, if the variant persisted,
this could reflect a nonreverting transmitted mutation or a
stable new mutation in the new host. Without the donor
sequence it is impossible to distinguish between these two
outcomes. However, when the reverting mutation is a well-
documented escape mutation in a patient who does not carry
the restricting HLA class I allele, this additional information
supports the argument that this is true reversion. We also
cannot exclude the influence of overlapping epitopes with
escape mutations at the same amino acid sites.

Our data are intriguing since some escape is evident, but for
most epitopes (23/34) there is no significant difference in the
rates of variation in the HLA-matched and HLA-unmatched
patients. If one assumes that HIV can respond to selection
pressure by accepting mutations with fitness costs (26), the lack
of variation in so many epitopes suggests weak selection rather
than structural constraint and, by inference, a weak CTL im-
une response. Alternatively, these epitopes may simply be
critical sites of conservation within the HIV genome. If they
are conserved, then CTLs should have a “good” antigen to
target. If no escape is detected yet variation is tolerated, it
follows that the CTLs are weak, which is compatible with
recent mathematical models (4, 5). Cellular assays of the CTL
immune response are currently being undertaken on the sero-
converter cohort to resolve this issue. The results of these
studies in combination with the sequence variation data will
inform vaccine design by highlighting epitopes that are both
strongly and frequently recognized and for which escape in-
volves high cost to the virus. However, the observation that
variation and immune escape are limited to a few epitopes
associated with slower disease progression is compatible with
data from African and European cohorts of chronically in-
fected patients (18).

The evidence that immune escape is the precursor to a rise
in viral load and clinical progression is limited (14, 23). We
have previously argued that the fitness cost imposed by certain
immune escape mutations may be one of a number of factors
which benefit the host (18). These hypotheses have been borne
out by in vitro fitness assays (33). Since escape polymorphisms
occur in patients with protective HLA class I alleles, one has to
cconclude that the selection pressure imposed by CTLs dictated
by these alleles is so strong that it is still to the virus’s advan-
tage to escape, despite the fitness cost. Hence, there is rapid
reversion of these mutations once they are transmitted to an
environment where they are no longer beneficial. Some muta-
tions do not appear to carry a fitness cost (e.g., the HLA
B51-restricted TAFTIPS1), and mutants are therefore likely to
accrue over time, possibly rising to fixation and so becoming
the consensus sequence (31). Should our analysis include
epitopes that already contain an escape variant as part of the
consensus sequence ("negatopes"), this would present as apparent reversions in HLA-matched hosts and a slow rate of "escape" in HLA-mismatched hosts. Such events were extremely rare in this cohort (data not shown), and although negatopes have been described within the genome (31), we do not feel that they bias this analysis.

Our cohort is part of a clinical trial, so a majority of patients received a short course of antiretroviral therapy (ART) at seroconversion. Could our findings have been influenced by a short course of drug therapy? The reduction in viral load, replication, antigenic load, and the predictable decline in CTL immune responses that might be expected from a short course of ART would be expected to slow down viral evolution and immune escape and decrease genetic diversity. Our data show that mutations that could reflect both escape and reversion are present within 6 months, and in a number of epitopes (e.g., the pol epitope TAFTIPS1), the escape variants had already reached fixation on clonal analysis (data not shown). In addition, as therapy will be distributed randomly across the different HLA class I alleles, any difference in viral variation attributed to different HLA class I alleles should not be significantly biased. Evidence from unpublished data from a similar seroconverter cohort (Stephane Hue, personal communication) suggests that the impact on genetic diversity of a short course of HAART at seroconversion is limited although this needs to be formally assessed in a controlled trial. The epitopes that we have shown to mutate, do so quickly although it is possible that, if anything, we have underestimated this. Most other epitopes do very little; if there is a differential impact of a short course of ART on epitope variability, this warrants further analysis once the SPARTAC trial is unblinded.

Our results support the notion that HIV adaptation to new hosts begins quickly following transmission but also show that this process continues for at least 36 months, predominantly at the level of the secondary immune response. If fitness cost, rather than a weak selection pressure, is the key, then these will be epitopes worth targeting for vaccine design.

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