Complex TCR repertoire dynamics underlie the CD8+ T-cell response to HIV-1

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Number of figures/tables: 8 (main), 6 (supplementary)
Abstract

Although CD8+ T-cells are important for the control of HIV-1 in vivo, the precise correlates of immune efficacy remain unclear. In this study, we conducted a comprehensive analysis of viral sequence variation and T-cell receptor (TCR) repertoire composition across multiple epitope specificities in a group of antiretroviral treatment-naive individuals chronically infected with HIV-1. A negative correlation was detected between changes in antigen-specific TCR repertoire diversity and CD8+ T-cell response magnitude, reflecting clonotypic expansions and contractions related to alterations in cognate viral epitope sequences. These patterns were independent of the individual, evidenced by discordant clonotype-specific evolution against different epitopes in single subjects. Moreover, long-term asymptomatic HIV-1 infection was characterized by evolution of the TCR repertoire in parallel with viral replication. Collectively, these data suggest a continuous bidirectional process of adaptation between HIV-1 and virus-specific CD8+ T-cell clonotypes orchestrated at the TCR/antigen interface.

Importance

We describe a relation between viral epitope mutation, antigen-specific T-cell expansion and the repertoire of responding clonotypes in chronic HIV-1 infection. This work provides insights into the process of co-adaptation between the human immune system and a rapidly evolving lentivirus.
Introduction

CD8+ T-cells are key determinants of immune efficacy in HIV-1 infection (reviewed in (1)). Although simple quantitative correlates of protection are generally lacking, previous studies have identified parameters that typically associate with effective HIV-specific CD8+ T-cell responses, including targeting specificity and breadth, antigen sensitivity, recall proliferation and polyfunctionality (reviewed in (2-4)). Nonetheless, these and other potentially important properties cannot fully explain the different disease outcomes associated with infection.

The inherent quality of a CD8+ T-cell response depends on the arsenal of T-cell receptor (TCR) clonotypes deployed to engage the targeted peptide-human leukocyte antigen class I (pHLA-I) complex. Current paradigms hold that diverse and/or cross-reactive TCR repertoires are beneficial in the face of rapidly evolving RNA viruses because they enable early recognition of emerging epitope variants (5-7). Indeed, restricted TCR diversity predisposes to immune escape in HCV infection (8). Moreover, diverse but highly biased repertoires can facilitate escape due to a lack of variant recognition (9). On the other hand, CD8+ T-cell repertoires that incorporate highly cross-reactive clonotypes are associated with delayed disease progression in SIV and HIV-1 infection (10-12). Nonetheless, a broad TCR repertoire per se is not necessarily protective, implicating additional attributes as clonotypic determinants of CD8+ T-cell efficacy (13). In this light, it has been shown previously that superior viral control can associate with enhanced antigen-specific clonal turnover, reflecting continual replenishment of the response with effective T-cell clonotypes (14-16). However, repertoire evolution is a variable phenomenon, even within CD8+ T-cell
responses directed against the same viral epitope (16). In addition, clonotype persistence has similarly been linked with long-term asymptomatic HIV-1 infection (17). These contrasting observations underscore the fact that the HIV-specific CD8+ T-cell response is highly heterogeneous.

Most antigen-specific repertoire studies to date in the HIV-1 field have focused on a single epitope with limited information on the circulating viral quasispecies. In contrast, we conducted a comprehensive analysis of cognate TCR sequences and viral epitope variation across four targeted specificities in a group of antiretroviral treatment-naïve individuals with chronic HIV-1 infection. All subjects carried the highly prevalent HLA-I alleles A*02 and B*08, enabling simultaneous analysis of more than one epitope-specific CD8+ T-cell response over time. A delicate balance was observed between HIV-1 variation and the virus-specific TCR repertoire, whereby only a few clonotypes reacted to changes in the viral milieu. These so-called ‘clonotypic shifts’ markedly affected CD8+ T-cell response magnitude in an antigen-driven manner. Moreover, long-term asymptomatic HIV-1 infection was achieved when the TCR repertoire adapted in response to viral replication.
Materials and methods

Study population

Eight initial participants with known seroconversion dates were selected from the Amsterdam Cohort Studies on HIV-1 infection and AIDS based on the presence of both HLA-A*02 and HLA-B*08; three individuals also carried the protective HLA-B*27 allele. All subjects were antiretroviral therapy-naïve prior to, and during, the time of sample collection. Peripheral blood mononuclear cell (PBMC) and serum samples were drawn from two time points per person: (i) early (t1), median 218 days post-seroconversion (range 169-568 days); and, (ii) late (t2), median 1133 days post-seroconversion (range 986-1226 days) (Table I). Further PBMC samples were collected from three participants: #5, #6 and #9, an additional seroprevalent subject exclusively selected for these extra analyses (Supplementary Table I). All individuals were in the asymptomatic, chronic phase of infection.

Flow-assisted sorting of antigen-specific CD8+ T-cells

As no pre-screening information was available on the presence/absence of measurable epitope-specific CD8+ T-cell responses, we selected well-defined, dominant epitopes for the three HLA-I alleles of interest: B*08-FLKEKGGL (B8-FL8), B*08-EIYKRWII (B8-EI8), A*02-SLYNTVATL (A2-SL9) and B*27-KRWIILGLNK (B27-KK10). Antigen-specific CD8+ T-cells were labelled with pre-titrated concentrations of the respective fluorochrome-conjugated pHLA-I tetrameric complexes: (i) B*0801-FL8 and B*0801-EI8 (monomers produced in-house as described previously with...
minor modifications (18)), conjugated with QD705 and QD605 (Life Technologies), respectively; (ii) A*0201-SL9-APC (Sanquin); and, as applicable, (iii) B*2705-KK10-PE (Sanquin). Non-viable cells were eliminated from the analysis using Live/Dead Aqua (Life Technologies). Cells were then washed and surface-stained with the following monoclonal antibodies (mAbs): αCD3-APC-H7, αCD4-PE-Cy5.5, αCD8-PE-Cy7, αCD14-AlexaFluor700 and αCD19-AmCyan (Caltag/Invitrogen). After exclusion of non-viable/CD14+/CD19+ cells, up to four CD3+CD8+tetramer+ populations were sorted in parallel at >98% purity directly into RNAlater (Life Technologies) using a customized FACSria II flow cytometer (BD Biosciences) and stored at -80°C for subsequent TCRβ clonotype analysis.

TCRβ clonotype analysis

Clonotype analysis was performed as described previously with minor modifications (19). Briefly, mRNA from sorted CD8+ T-cell populations was extracted using the μMACS mRNA Isolation Kit (Miltenyi Biotec). An anchored template-switch RT-PCR was then used to amplify all expressed TCRβ chains linearly. Amplified products were ligated into the pGEM-T Easy vector (Promega) and transformed into chemically competent E. coli bacteria. Subcloned products were amplified using M13 primers and sequenced via capillary electrophoresis with the Big Dye Terminator v3.1 Cycle Kit (Life Technologies). Analysis of each TCRβ sequence and assignment of gene usage was performed using the international reference, web-based software from ImMunoGeneTics (20). At least 50 TCRβ
Sequences were successfully analyzed for each sample, a cut-off widely considered appropriate for antigen-specific memory T-cell responses (21).

Sequence analysis of HIV-1 epitopes

For Gag, viral RNA was isolated from serum using a Viral RNA Mini Kit (Qiagen) or silica particles as described previously (22). A combined cDNA synthesis and first-round PCR was then performed in 30 µl reactions using a Titan One Tube RT-PCR Kit (Roche). The following parameters were used: (i) 50°C for 30 min to synthesize cDNA; (ii) 95°C for 2 min to melt; (iii) 40 cycles of 95°C for 15 sec, 57°C for 30 sec and 68°C for 2.5 min (increased by 5 sec per cycle for the last 30 cycles) to amplify; and, (iv) 72°C for 10 min to complete extension. The second, nested, PCR was performed using 5 µl of the first-round product in 30 µl reactions with the Expand High Fidelity PCR System (Roche). The following parameters were used: (i) 95°C for 2 min to melt; (ii) 30 cycles of 95°C for 15 sec, 58°C for 30 sec and 68°C for 2.5 min to amplify; and (iii) 72°C for 10 min to complete extension. Primers KVL064 (forward, 5’-GTTGTGTGACTCTGGTAACTAGAGATCCCTCAGA-3’) and NCrev-2 (reverse, 5’-CCTTCCTTTCCACATTTCCAACAG-3’) were used for the combined cDNA synthesis/first-round PCR, and primers KVL066 (forward, 5’-TCTCTAGCAGTGCCGCCGAACAG-3’) and NCre-3 (reverse, 5’-CTTTTCTAGGGCCCTGCAATT-3’) were used for the second, nested, PCR.

For Nef, viral RNA was isolated from serum using a Viral RNA Mini Kit (Qiagen). cDNA was synthesized with SuperScript III Reverse Transcriptase...
(Invitrogen) using a Nef-specific primer (Nef rv1, 5'-
GCTTATATGCAGGATCTGAGG-3') and purified on silica-based columns
(Macherey-Nagel). Template-specific amplification was performed as described
previously (23).

Amplified Gag and Nef products were gel-purified (Macherey-Nagel), A-
tailed and ligated using the pGEM-T Easy Vector System (Promega). Ligated
products were then transformed into chemically competent E.coli bacteria and
sequenced as described above (4-48 clones per sample).

**TCRβ diversity analysis**

A T-cell clonotype was defined as a TCRβ chain encoded by a unique
nucleotide sequence. Sample clonality was estimated by counting the relative
number of distinct clonotypes and by using Simpson’s diversity index (Ds) (21). This
index is defined as:

\[
D_s = 1 - \sum_{i=1}^{c} \frac{n_i (n_i - 1)}{n(n-1)}
\]

where \( n_i \) is the clonal size of the \( i \)th clonotype (i.e. the amount of copies of a specific
clonotype), \( c \) is the number of different clonotypes and \( n \) is the total number of
analyzed TCRβ sequences. This index uses the relative frequency of each clone to
calculate a diversity index ranging between 0 and 1, indicating minimal and maximal
diversity, respectively. To account for differences in sample size (i.e. the number of
successfully analyzed TCRβ sequences), all samples were normalized by random
sampling (without replacement) to an equal number of sequences (n=50) prior to the
calculation of TCRβ diversity (i.e. the relative number of unique clonotypes and
Simpson's diversity index). This process was repeated 1000 times, after which
median values of TCRβ diversity were determined and used for subsequent
analyses.

Statistical analysis

Sample normalization and statistical analyses were performed using SPSS 20.0.0
(SPSS Inc.). A p-value ≤0.05 was considered statistically significant. Graphics were
generated using GraphPrism 5.04 (GraphPad Software Inc.). Note that in some
analyses (Fig. 2A, Fig. 3), data from multiple T-cell populations per individual and
different epitope specificities were pooled; in these instances, the data points cannot
be considered fully independent of each other.
Results

Isolation and analysis of antigen-specific CD8+ T-cells

Eight treatment-naïve individuals with chronic HIV-1 infection were selected for co-expression of the HLA-A*02 and HLA-A*B08 alleles. Each subject was studied at two time points, approximately one (0.5-1.5) and three (2.7-3.5) years post-seroconversion (Table I). Initially, we used pHLA-I tetramers to characterize CD8+ T-cell responses directed against the frequently targeted epitopes A*02-SLYNTVATL (A2-SL9, p17-Gag), B*08-EIYKRWII (B8-EI8, p24-Gag), B*08-FLKEKGGL (B8-FL8, Nef) and B*27-KRWIILGLNK (B27-KK10, p24-Gag). Response magnitude varied as a function of specificity, with B27-KK10 and A2-SL9 eliciting the biggest and smallest CD8+ T-cell responses, respectively (Fig. 1A).

To compare the clonotypic composition of distinct antigen-specific CD8+ T-cell populations, we used a template-switch anchored RT-PCR to amplify all expressed TCRβ chains from pHLA-I tetramer+ cells sorted by flow cytometry to high levels of purity. Two measures of diversity were calculated: (i) the relative number of clonotypes (i.e. each unique TCRβ nucleotide sequence after normalization); and, (ii) Simpson’s diversity index (Ds), which accounts for clonotype frequency (21). CD8+ T-cell populations directed against B8-EI8 and A2-SL9 selected repertoires with the highest and lowest degrees of diversity, respectively (Fig. 1B and 1C, Supplementary Fig. S1, Supplementary Fig. S2). Of note, highly focused and polyclonal responses were observed within each specificity; these patterns were not associated with either
the time of sampling (closed vs open symbols) or the magnitude of the CD8+ T-cell population (data not shown).

Viral epitope variation does not correlate directly with CD8+ T-cell repertoire diversity

Previous studies from our group and others have suggested that the T-cell repertoire is shaped primarily by the presented epitope (24,25). To assess the relationship between TCRβ diversity and viral epitope mutation, we conducted an extensive analysis of autologous HIV-1 sequences in targeted regions of the viral genome. Modest variations were detected across viral populations. A single epitope sequence was usually dominant, deviating from wildtype (WT) most prominently in the B8-FL8 and B27-KK10 regions (Table II). Of note, the majority of these variants were predicted to bind their respective HLA-I molecules (73% via NetMHC3.4, 82% via NetMHCpan; Supplementary Table II) (26,27). No significant correlations were detected between the frequency of the WT epitope and the diversity of the corresponding TCRβ repertoire, either in terms of Simpson’s diversity index (Fig. 2A) or the relative number of clonotypes (Supplementary Fig. S3). Similarly, there were no associations between TCRβ diversity and either the presence of epitope variants or the number of different epitope sequences (Fig. 2B, Supplementary Fig. S3). A direct analysis of viral epitope variation quantified using Simpson’s diversity index also failed to reveal significant correlations with TCRβ diversity (data not shown). These findings indicate that the composition of the viral epitope population does not necessarily associate with TCRβ repertoire diversity at any given time point,
although a relationship between these parameters cannot be excluded from the current data.

Parallel evolution of the TCR repertoire, viral quasispecies and CD8+ T-cell responses

It is well established that CD8+ T-cell response magnitude, TCR diversity and viral epitope sequences can evolve significantly during the course of HIV-1 infection (16). Accordingly, we examined a subset of HIV-specific CD8+ T-cell responses (n=10) over time. Repertoire diversity varied between the early and late time points without a common tendency to increase or decrease (Fig. 3A and 3B). Next, we studied how changes in TCRβ diversity (measured as the ratio of the number of normalized clonotypes or the Simpson index at timepoint 2 divided by the corresponding values at timepoint 1) related with longitudinal changes in CD8+ T-cell response magnitude (measured as the ratio of the response magnitude at timepoint 2 divided by the corresponding value at timepoint 1) (Fig. 3C and 3D). A significant negative correlation was observed between these two parameters, indicating that the repertoire became less diverse with CD8+ T-cell expansion and more diverse with CD8+ T-cell contraction. Similar correlations were observed between changes in response magnitude and changes in TCRβ diversity (data not shown). These observations suggest that shifts in response magnitude over time were associated with inflation and deflation of particular clonotypes.

On this basis, we examined the relationship between clonotypic stability and viral epitope diversity. All longitudinal CD8+ T-cell responses were first stratified
according to changes in magnitude over time (ratio t2 to t1): (i) large decreases in
magnitude (ratio ≤0.5); (ii) conservation of magnitude (ratio >0.5 and <2.0); and, (iii)
large increases in magnitude (ratio ≥2.0) (Fig. 4, upper panel). The corresponding
TCRβ repertoires (Fig. 4, middle panel) and circulating viral epitopes (Fig. 4, lower
panel) were then compared across categories. Interestingly, the observed shifts in
CD8⁺ T-cell response magnitude were often linked with changes in the TCRβ
repertoire and viral epitope over time. For example, the two decreasing responses
(subject #1 and subject #7, B8-FL8) were accompanied by deflation of one (subject #1)
or two (subject #7) dominant clonotypes (Fig 4, panel 1). Similarly, two increasing
responses (subject #2 and subject #6, B27-KK10) were paralleled by inflation of
previously subdominant clonotypes (Fig 4, panel 3). In both scenarios, viral epitope
sequences changed over time. For CD8⁺ T-cell responses that remained relatively
stable, however, fewer mutations were detected in the targeted viral epitopes and
TCRβ repertoire composition remained largely unchanged (Fig 4, panel 2).

To validate these observations, we calculated changes in the absolute
numbers of tetramer-binding CD8⁺ T-cells (Supplementary Table III). Based on these
values, the majority of subjects adhered to the categories determined above with
respect to changes in response magnitude. The only exception was the B8-EI8-
specific response in subject #7, where the ratio in absolute numbers fell below 2.0 into
the 0.5-2.0 range. Notably, more pronounced shifts were apparent in the
 corresponding TCRβ repertoire compared to other HIV-specific CD8⁺ T-cell
responses in this category.
Together, these results suggest that preferential inflation or deflation of specific clonotypes within the available repertoire may relate to viral epitope mutations and drive changes in the magnitude of the antigen-specific CD8+ T-cell response.

Discordant evolution of CD8+ T-cell responses within individuals

Next, we conducted a longitudinal analysis of HIV-specific CD8+ T-cell responses in three individuals (Fig. 5). Differences in response magnitude over time (upper panel) were again linked with TCRβ repertoire stability (lower panel), now stratified per person. Discordant patterns of evolution across epitope specificities were also apparent in each donor. For example, the B8-EI8-specific response in subject #1 showed minimal changes in magnitude and clonotypic representation over time, whereas the corresponding B8-FLK response varied substantially across both parameters. Similarly, some responses were stable in subject #6 (B8-EI8) and subject #7 (A2-SL9, B8-EI8), while others changed dramatically in terms of magnitude and TCRβ repertoire composition (subject #6: B27-KRW; subject #7: B8-FL8, B27-KK10).

Together, these data indicate that HIV-specific CD8+ T-cell responses evolve independently of the host, most likely driven by TCR/antigen interactions.

TCR repertoire evolution and viral load dynamics
A minority of individuals infected with HIV-1 maintain control of viral load at low or undetectable levels. To determine the long-term impact of such low-level viral replication and antigen presentation, we analyzed TCRβ repertoire composition in CD8+ T-cell populations specific for B8-FL8 and B8-EI8 using additional samples from subjects #5, #6 and #9 (Fig. 6), all of whom showed signs of delayed disease progression (asymptomatic with stable viral loads and CD4+ T-cell counts >300 cells/μl at least 7 years after seroconversion).

Subject #9 maintained undetectable viral loads 14 years after entry into the cohort (Fig. 6A). Subjects #5 and #6 similarly controlled viral loads to low or undetectable levels after acute infection, although progressive increases approximately seven years after seroconversion warranted subsequent antiretroviral therapy (Fig. 6B and 6C). Different patterns of TCRβ repertoire evolution were observed in these individuals. Clonotypic representation remained stable in some epitope-specific CD8+ T-cell populations (B8-EI8 in subject #9; B8-FL8 and B8-EI8 in subject #6 during early infection), whereas considerable changes in the constituent TCRβ clonotypes were observed in others (B8-FL8 and B8-EI8 in subject #5; B8-FL8 and B8-EI8 in subject #6 during late infection). Moreover, these clonotypic characteristics often paralleled viral load trajectories. Subject #6 displayed stable viral loads during early infection in conjunction with largely constant TCRβ repertoires specific for B8-FL8 and B8-EI8. As viral loads increased during late infection, however, dramatic changes in clonotypic composition were apparent for both specificities. Similar patterns were observed in subject #5. In this case, epitope-specific TCRβ repertoire instability mirrored viral load fluctuations during both early
and late infection. Conversely, the B8-EI8-specific TCRβ repertoire in subject #9 remained stable in the presence of undetectable viral loads, although clear clonotypic shifts were observed in the B8-FL8-specific CD8+ T-cell population. Collectively, these data suggest that the HIV-specific TCR repertoire evolves more rapidly with changes in viral load. Thus, viremic control can be associated with relatively conserved repertoires, whereas higher levels of viral replication tend to drive clonotypic turnover.
Discussion

Although it is widely accepted that HIV-1 evades CD8+ T-cell immunity via epitope mutation, the clonotypic correlates of this phenomenon remain poorly understood. Accordingly, we investigated antigen-specific CD8+ T-cell repertoire dynamics in relation to viral epitope variation in antiretroviral therapy-naïve seroconverters with asymptomatic HIV-1 infection. In line with previous work, we found no time-matched correlations between viral epitope composition and clonotypic diversity within the cognate HIV-specific CD8+ T-cell response (28). However, longitudinal analyses revealed a more nuanced picture. A negative correlation across multiple specificities was initially detected between changes in TCR repertoire diversity and CD8+ T-cell response magnitude. More detailed investigations showed that this association reflected clonotype-specific expansions and contractions related to alterations in cognate viral epitope sequences. These patterns were discordant within individuals, suggesting an antigen-driven process. Moreover, clonotype turnover was related to viral load, as noted previously (16). Although tempered by sampling limitations, these data suggest a continuous bidirectional process of adaptation between HIV-1 and virus-specific CD8+ T-cell clonotypes that could ultimately govern immune efficacy and the outcome of infection.

Previous studies have highlighted such dynamic interplay between clonotypic adaptation and lentiviral pathogens. This is perhaps best exemplified in the B27-KK10 system, where the early mobilization of public TRBV4-3/TRBJ1-3 clonotypes drives the emergence of TCR escape mutations, which can subsequently
be controlled by cross-reactive TRBV6-5/TRBJ1-1 clonotypes in some individuals (11,29). Adaptive plasticity in the B27-KK10-specific repertoire may even underlie the protective phenotype conferred by this HLA-I allele (30). Similarly cross-reactive clonotypes are also thought to confer preferential outcomes in the context of non-protective HLA-I alleles. Indeed, a B8-FL8-specific TCR previously associated with long-term non-progressive disease was detected in this study (12). Nonetheless, it is possible that clonotypic adaptation represents a double-edged sword, in some cases exhausting immune resources without demonstrable benefit. Further detailed studies will be required to clarify these issues in relation to specific epitopes and restriction elements.

*In silico* analysis predicted sufficient affinities for the majority of epitope variants detected in this study to bind the relevant HLA-I molecules. It therefore seems likely that many of these mutations arose to circumvent TCR recognition. However, the efficacy of this evasion strategy in the presence of a potentially vast cognate TCR repertoire is almost certainly limited to specific scenarios, in contrast to viral mutations that abrogate epitope presentation via effects on antigen processing and/or HLA-I binding. Nonetheless, our data suggest that such “shifting” epitope structures shape the virus-specific TCR repertoire over time. It is notable in this context that clonotypic overlap between CD8+ T-cell populations directed against WT and variant epitopes has been reported previously and may even be commonplace (17,28).

Despite the primary roles of antigen quantity and quality as determinants of TCR repertoire dynamics, it is important to note that other factors are implicated by
the heterogeneous patterns observed in our study. For example, the A2-SL9-specific TCR repertoire in subject #4 remained stable despite substantial sequence variation in the cognate viral epitope and changes in response magnitude. In this case, it seems likely that both dominant clonotypes were equally responsive to the emerging variant, suggesting the operation of other selection pressures during viral evolution. Conversely, the B8-FL8-specific TCR repertoire in subject #9 shifted substantially over time despite a consistently undetectable viral load. In contrast, the corresponding B8-EI8-specific response remained clonotypically stable over the same prolonged time period. Thus, antigen drive alone does not fully explain the evolutionary patterns observed across distinct epitope-specific TCR repertoires in this study.

In summary, our data show that the antigen-specific CD8+ T-cell repertoire is intimately linked with viral load and epitope variation during chronic HIV-1 infection. This complex dynamic interplay confounds simplistic interpretation and hinders the search for clonotypic determinants of CD8+ T-cell efficacy.
Acknowledgements

This work was supported by a grant from the AIDS Fonds Netherlands (number 2011033) and a Fellowship from the Fundação para a Ciência e Tecnologia. D.A.P is a Wellcome Trust Senior Investigator. The authors are grateful to Gerrit Spierenburg and Koos Gaiser for technical assistance. A.I.C. and D.K. performed experiments, analyzed data and wrote the manuscript. K.L. performed experiments and analyzed data. J.E.M. performed experiments. B.P.X.G. assisted with data normalization. I.M.M.S. and C.K. critically revised the manuscript. P.H. assisted with experiments. M.N. assisted with experiments and revised the manuscript. J.A.M.B. and D.A.P. wrote the manuscript. D.B. designed the study and experiments, analyzed data and wrote the manuscript. The authors declare no conflict of interest.
References


Figure 1: Analysis of CD8+ T-cell populations directed against A2-SL9, B8-EI8, B8-FL8 and B27-KK10. (A) Antigen-specific CD8+ T-cells were labelled with pHLA-I tetramers and quantified by flow cytometry. Response magnitude is shown as the frequency of tetramer+ events in the total CD8+ T-cell population. (B, C) TCRβ diversity was quantified using the relative number of clonotypes (B) and Simpson’s diversity index (C). Data are shown for each individual at both the early (closed circles) and late (open circles) time points.

Figure 2: CD8+ T-cell repertoire diversity is not related to viral epitope variation. (A, B) Antigen-specific TCRβ repertoire diversity (Simpson’s diversity index) and viral epitope sequences were determined in parallel for a subset of samples (n=22). The percentage of wildtype (WT) epitope sequences (A) and the number of variant epitopes present at the time of analysis (B) were used as measures of epitope composition in the autologous viral quasispecies. All plots include data derived from the early (closed symbols) and late (open symbols) time points. Symbol shape denotes epitope specificity: A2-SL9 (circle), B8-EI8 (square), B8-FL8 (diamond) and B27-KK10 (inverted triangle). Correlation testing in (A) was performed using the Spearman Rank test. Note that Simpson’s diversity index was determined after data normalization for appropriate diversity comparisons (see Materials and methods for details).
Figure 3: Longitudinal variations in CD8+ T-cell response magnitude correlate negatively with TCRβ diversity. (A, B) TCRβ diversity was quantified at the early (t1) and late (t2) time points for a subset of antigen-specific CD8+ T-cell responses (n=10) using the relative number of clonotypes (A) and Simpson’s diversity index (B). Statistical analyses were performed using the Wilcoxon Signed-Rank test. (C, D) Changes in CD8+ T-cell response magnitude (ratio t2 to t1) were related to differences in TCRβ diversity (ratio t2 to t1) determined by the relative number of clonotypes (C) and Simpson’s diversity index (D). Each dot represents a CD8+ T-cell response analyzed at two time points. Symbol shape denotes epitope specificity: A2-SL9 (circle), B8-EI8 (square), B8-FL8 (diamond) and B27-KK10 (inverted triangle). Correlation testing was performed using the Spearman Rank test.

Figure 4: The relationship between CD8+ T-cell response magnitude, TCRβ diversity and viral epitope variation. Antigen-specific CD8+ T-cell responses were stratified according to changes in magnitude over time (ratio t2 to t1). Panel 1 (left, white bars) depicts CD8+ T-cell responses that subsided over time, panel 2 (middle, grey bars) depicts CD8+ T-cell responses that remained stable over time and panel 3 (right, black bars) depicts CD8+ T-cell responses that increased over time. The clonotypic composition of each CD8+ T-cell population is illustrated in the pie charts (middle panel) and the respective viral epitope sequences are shown (lower panel). Response magnitudes are indicated in the pie charts as the frequency of tetramer+
events in the total CD8+ T-cell population. Pie charts colors match clonotypes for each epitope pair, but do not correspond between pairs.

Figure 5: CD8+ T-cell response evolution is not dependent on the individual.
Antigen-specific CD8+ T-cell responses were stratified according to subject origin. Bar colors in the upper panel are adapted from Figure 4 and represent decreasing (white), stable (grey) and increasing (black) response magnitudes over time. The clonotypic composition of each CD8+ T-cell population is illustrated in the pie charts with response magnitudes indicated as the frequency of tetramer+ events in the total CD8+ T-cell population (lower panel). Pie chart colors match clonotypes for each epitope pair, but do not correspond between pairs.

Figure 6: TCR repertoire evolution and viral load dynamics during long-term asymptomatic infection. (A-C) Viral load trajectories and CD8+ T-cell repertoires specific for B8-FL8 and B8-EI8 are shown for subject #9 (A), subject #5 (B) and subject #6 (C). Pie chart colors match clonotypes for each epitope pair in each individual, but do not correspond between pairs or individuals. Single asterisks correspond to the time points described in Table I. Double asterisks correspond to additional time points (Supplementary Table I). T denotes the commencement of antiretroviral therapy.
Table I. Subject characteristics.

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<td>5</td>
<td>19342</td>
<td>A<em>01, A</em>02, B<em>0801, B</em>40</td>
<td>536</td>
<td>1166</td>
<td>490</td>
<td>800</td>
<td>670</td>
<td>860</td>
<td>~17000* (22000-7600)</td>
</tr>
<tr>
<td>6</td>
<td>18840</td>
<td>A<em>02, A</em>02, B<em>0801,B</em>27</td>
<td>568</td>
<td>1169</td>
<td>480</td>
<td>690</td>
<td>600</td>
<td>910</td>
<td>~1400* (&lt;1000-1640)</td>
</tr>
<tr>
<td>7</td>
<td>18839</td>
<td>A<em>02, A</em>02, B<em>0801,B</em>37</td>
<td>266</td>
<td>1100</td>
<td>360</td>
<td>420</td>
<td>440</td>
<td>850</td>
<td>~33000* (48000-19000)</td>
</tr>
<tr>
<td>8</td>
<td>18785</td>
<td>A<em>01, A</em>02, B<em>0801, B</em>07</td>
<td>189</td>
<td>1080</td>
<td>400</td>
<td>380</td>
<td>510</td>
<td>690</td>
<td>40000 210000</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>218</td>
<td>1133</td>
<td>485</td>
<td>415</td>
<td>625</td>
<td>820</td>
<td>625 (169-568)</td>
</tr>
</tbody>
</table>

* Trend viral load, estimated from the closest adjacent time points.
Table II-A. HIV-1 sequence analysis of epitopes restricted by HLA-A*02 and HLA-B*08.

<table>
<thead>
<tr>
<th>Subject</th>
<th>ACS #</th>
<th>HLA type</th>
<th>Time point</th>
<th>A2-SL9</th>
<th>B8-EI8</th>
<th>B8-FL8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SLNTVATL %Seqs</td>
<td>HIYKRWII %Seqs</td>
<td>FLKEKGGL %Seqs</td>
</tr>
<tr>
<td>1</td>
<td>19957</td>
<td>A<em>01, A</em>02, B<em>0801, B</em>15</td>
<td>T=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79.2%</td>
<td>19.2%</td>
<td>1.6%</td>
</tr>
<tr>
<td>3</td>
<td>19861</td>
<td>A<em>01, A</em>02, B<em>0801, B</em>51 or B*52</td>
<td>T=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79.2%</td>
<td>19.2%</td>
<td>1.6%</td>
</tr>
<tr>
<td>4</td>
<td>19453</td>
<td>A<em>01, A</em>02, B<em>0801, B</em>38</td>
<td>T=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>51.7%</td>
<td>48.3%</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86.7%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>19342</td>
<td>A<em>01, A</em>02, B<em>0801, B</em>40</td>
<td>T=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>18785</td>
<td>A<em>01, A</em>02, B<em>0801, B</em>07</td>
<td>T=1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86.1%</td>
<td>14.0%</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>93.9%</td>
<td>3.0%</td>
<td>3.0%</td>
</tr>
</tbody>
</table>
Table II-B. HIV-1 sequence analysis of epitopes restricted by HLA-A*02, HLA-B*08 and HLA-B*27.

<table>
<thead>
<tr>
<th>Subject</th>
<th>ACS #</th>
<th>HLA type</th>
<th>Time point</th>
<th>A2-SL9</th>
<th>B8-EI8</th>
<th>B8-FL8</th>
<th>B27-KK10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SLYNTVATL %Seqs</td>
<td>SIYKRWII %Seqs</td>
<td>FLKEKQGL %Seqs</td>
<td>KRWIKLGLNK %Seqs</td>
</tr>
<tr>
<td>2</td>
<td>19885</td>
<td>A<em>01, A</em>02, B<em>0801, B</em>27</td>
<td>T=1</td>
<td>--F-A--V-- 100%</td>
<td>--R----- 100%</td>
<td>--P----- 100%</td>
<td>--R----- 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T=2</td>
<td>--F----V-- 93.8%</td>
<td>--E----V-- 96.8%</td>
<td>--R----- 93.8%</td>
<td>--E----V-- 93.8%</td>
</tr>
<tr>
<td>6</td>
<td>18840</td>
<td>A<em>02, A</em>02, B<em>0801, B</em>27</td>
<td>T=1</td>
<td>-----I--- 90.3%</td>
<td>-----I--- 90.3%</td>
<td>-----I--- 90.3%</td>
<td>-----I--- 90.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T=2</td>
<td>-----I--- 90.3%</td>
<td>-----I--- 90.3%</td>
<td>-----I--- 90.3%</td>
<td>-----I--- 90.3%</td>
</tr>
<tr>
<td>7</td>
<td>18839</td>
<td>A<em>0207, A</em>0207, B<em>0801, B</em>27</td>
<td>T=1</td>
<td>--R----- 100%</td>
<td>--R----- 100%</td>
<td>--R----- 100%</td>
<td>--R----- 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T=2</td>
<td>--R----- 97.6%</td>
<td>--R----- 97.6%</td>
<td>--R----- 97.6%</td>
<td>--R----- 97.6%</td>
</tr>
</tbody>
</table>
A B
A2-SL9 early time point
A2-SL9 late time point
B8-FL8 early time point
B8-FL8 late time point
B27-KK10 early time point
B8-EI8 early time point
B8-EI8 late time point
B27-KK10 late time point

p = 0.271
r = 0.246

WT epitope sequence (%)

Simpson index TCR (D3)

WT epitope sequence
WT + 1 variant sequence
WT + 2 variant sequences

0.0 0.2 0.4 0.6 0.8 1.0

0 0.2 0.4 0.6 0.8 1.0

WT epitope sequence (%)

Simpson index TCR (D3)
A

\[ p = 0.012 \]
\[ r = -0.754 \]

B

\[ p = 0.029 \]
\[ r = -0.685 \]

C

\[ p = 0.445 \]

D

\[ p = 0.593 \]

\[ r = -0.685 \]