Induction of Gag-specific CD4 T cell responses during acute HIV infection is associated with improved viral control

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JVI Accepts, published online ahead of print on 16 April 2014
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Abstract
Effector CD4 T cell responses have been shown to be critically involved in the containment and clearance of viral pathogens. However, their involvement in the pathogenesis of HIV infection is less clear given their additional role as preferred viral targets. We previously demonstrated that the presence of HIV-specific CD4 T cell responses is rather associated with HIV control and that specific CD4 T cell function such as direct cytolytic activity can contribute to control of HIV viremia. However, little is known about how the induction of HIV-specific CD4 T cell responses during acute HIV infection influences disease progression and whether responses induced during the early phase of infection are preferentially depleted. We therefore longitudinally assessed in a cohort of fifty-five acutely HIV infected individuals HIV-specific CD4 T cell responses from acute to chronic infection. Interestingly, we found that the breadth, magnitude and protein dominance of HIV-specific CD4 T cell responses remained remarkably stable over time. Moreover, we found that the epitopes targeted in a high frequency in acute HIV infection were recognized at a same frequency by HIV-specific CD4 T cells in chronic HIV infection. Interestingly the induction of Gag-specific CD4 T cell responses in acute HIV infection was significantly inversely correlated with a lower viral set point in chronic HIV infection (R=-0.5, p=0.03), while the cumulative contribution of Env-specific CD4 T cell responses showed the reverse effect. Moreover, individuals with HIV-specific CD4 T cell responses dominantly targeting Gag over Env in acute HIV infection remained off antiretroviral therapy significantly longer (p=0.03, log-rank). Thus, our data suggest that the induction of HIV-specific CD4 T cell responses during acute HIV infection is beneficial for and does not fuel disease progression.
Importance

CD4 T cells are critical for the clearance and control of viral infections. However, HIV preferentially infects HIV-specific CD4 T cells. Thus, their contribution to the control of HIV viremia is uncertain. Here, we study HIV-specific CD4 T cell responses from acute to chronic HIV infection and show that the generation of certain CD4 responses is associated with control rather than disease progression.
Introduction

CD4 T cells are critical players in the clearance and control of viral infections. The presence of effective CD4 T cell help has not only been shown to enhance the ability of CD8 T cells to kill virally infected cells (1-4) but also aids in the development of a secondary recall response upon viral re-exposure (5, 6). Likewise, the generation of a high-affinity, long-lived antibody response is a CD4 T cell–or T follicular helper cell–dependent process (7). Moreover, CD4 T cells can also directly contribute to the elimination of virally infected cells through cytotoxic mechanisms in several viral infections such as EBV (8), Influenza (9, 10) and HIV (11); a function not normally attributed to CD4 T cells.

Indeed, many licensed anti-viral vaccines have been shown to induce a CD4 T cell component, stressing their importance in the prevention and containment of viral infection. However, despite the importance of anti-viral CD4 T cells in both the context of vaccination and natural infection, the role of HIV-specific CD4 T cells during HIV infection is less clear. HIV preferentially infects HIV-specific CD4 T cells (12) and thus the induction and presence of HIV-specific CD4 T cell responses may rather increase the pool of target cells and fuel HIV dissemination than contribute to the control of viral replication.

We have previously demonstrated that both the breadth and specificity of HIV-specific CD4 T cell responses are significantly associated with maintenance of low viremia during chronic infection (13). In particular, Gag-specific CD4 T cell responses detected during chronic HIV infection show a strong association with viral control, while Env-specific CD4 T cell responses is associated with rapid progression. Furthermore, we have found an HLA class II genetic association linked to CD4 T cell function that is correlated to durable control of HIV viremia (14). It is becoming increasingly evident, however, that events occurring during acute HIV infection set the stage for immunological outcome later on. We have previously demonstrated that expansion of virus-specific CD4 T cell responses—and in particular those with cytolytic activity—
during acute HIV infection is strongly associated with improved long-term viral suppression, suggesting a direct anti-viral role of these cells in individuals who spontaneously control HIV viremia (11).

Yet, the broader role of HIV-specific CD4 T cells during acute infection remains unclear. In particular, the degree to which the emergence of HIV-specific CD4 T cell targeting influences disease outcome remains elusive; it is unclear whether HIV-specific CD4 T cell responses during acute HIV infection have solely a positive impact on disease outcome or may otherwise additionally fuel disease progression by increasing the pool of viral target cells. Similarly, it is also unknown whether the massive depletion of CD4 T cells that occurs during acute HIV infection equally affects all HIV-specific CD4 T cells—resulting in an overall loss of CD4 T cell help—or rather preferentially depletes particular epitope-specific CD4 T cells.

Several publications have reported on the possible role of functional HIV-specific CD4 T cells during chronic HIV infection (4, 11, 15-18), but HIV-specific CD4 T cell responses during acute HIV infection are less studied (11, 18-20). Furthermore, little is known about the progression of the epitope targeting over the course of infection, from the acute to chronic phases. We therefore address this issue by studying the longitudinal evolution of epitope-specific CD4 T cell targeting from acute to chronic HIV infection in a cohort of acutely HIV-infected individuals and determined whether and how the emergence of HIV-specific CD4 T cell responses are associated with long-term clinical outcome.

**Material and Methods**

**Patient characteristics**

Fifty-five subjects with acute HIV infection were recruited for this study (HIV clinic Jessen, Berlin, Germany; Fenway Health, Center, Boston, USA and Massachusetts General Hospital, ...
Boston, United States). Clinical and socio-demographic characteristics of study participants can be found in Table 1. The cohort was a male (100%), Caucasian (83.6%) study population and homogenous in terms of demographics. Acute HIV infection was classified using the Fiebig staging as previously described (21). The study subjects gave written informed consent and the study was approved by the respective institutional review boards.

**Assessment of HIV-specific CD4 T cell responses**

HIV-specific CD4 T cell responses were assessed as previously described (13). Briefly, freshly isolated PBMC were depleted of CD8 T cells prior to Ficoll-Hypaque density gradient centrifugation by incubation with anti-CD8 RosetteSep antibody (Stem Cell Technologies). HIV-specific CD4 responses were screened against a panel of overlapping-peptides (OLPs) spanning Gag, Nef and gp120 of the HIV-1 clade B consensus 2001 proteome using a modified IFN-γ enzyme-linked immunospot (ELISPOT) assay. Other HIV proteins were excluded based on previous findings that HIV-specific CD4 T cell responses are rarely detectable in these regions (13). Fresh CD8-depleted PBMC were plated in 96-well polyvinylidene plates (Millipore, MA, USA) pre-coated with 2 µg/ml anti-IFNγ monoclonal antibody 1-D1K (Mabtech, Stockholm, Sweden). A total of 65,000-100,000 CD8-depleted cells per well were added in 140 µl of RPMI 1640 containing 10% heat-inactivated fetal calf serum, 2 mM l-glutamine, 50 U of penicillin/ml, 50 µg of streptomycin/ml, and 10 mM HEPES. Each well contained a single OLP at a concentration of 14 µg/ml. As a negative control, CD8-depleted PBMC were incubated in medium alone for a minimum of 5 wells per plate. As a positive control, phytohemagglutinin (Sigma) was added at 1.8 µg/ml. The plates were incubated for 40 hrs at 37°C, 5%CO₂ to elicit the maximal cytokine secretion as previously described (22). The Elispot plates were then processed as previously described(23). The AID ELISpot Reader (Autoimmun Diagnostika GmbH, Strasbourg, Germany) was used to determine the spot-forming cells (SFC) per million CD8-depleted PBMC. The number of antigen-specific CD4 T cells was calculated by subtracting the mean negative control values. An antigen-specific CD4 T cell response was considered...
positive only if it was ≥55 SFCs/10^6 CD8-depleted PBMC, at least >3 times the mean background and also >3 times the standard deviation of the SFC/10^6 CD8-depleted PBMC within the negative controls.

Statistical Analysis

Statistical analysis and graphical presentation was done using Graph Pad Prism 5.0 and Microsoft Excel. Results are given as mean ± standard deviation (SD) or median with range unless otherwise indicated. Correlations were assessed by Spearman-rank analysis. Statistical analysis of significance (p-values) was based on two-tailed t-tests and linear regression analysis. Survival Kaplan-Meyer analysis was performed using log-rank test.

Results

The clinical characteristics of study participants are shown in Table 1. The majority (30/55) of study participants were diagnosed during Fiebig stages 2 and 3 of acute HIV infection, (7/55) in Fiebig 4, (12/55) in Fiebig 5, and (2/55) in Fiebig 6. Fiebig staging was not possible in 4 individuals. The average HIV viral load at baseline visit was 2.96*10^6±9.9*10^6 HIV RNA copies/ml and CD4 count averaged at 466±219 cells/μl. Nineteen individuals remained off therapy over one year after infection and of those twelve individuals had a matched assessment of HIV-specific CD4 T cell responses at both baseline and the one-year time point. Twenty-six enrolled participants elected to begin HAART during the first year of infection due to persistent high viremia. The average time to treatment initiation for all study participants was 230 days after diagnosis with acute HIV infection. Seventeen individuals were lost to follow-up.

To determine the contribution of HIV-specific CD4 T cell responses to long-term control, we performed a cross-sectional analysis of epitope-specific CD4 T cell responses at different stages of infection (baseline, two months, six months, and twelve months post diagnosis with acute HIV infection). Strikingly, we found that 81% of all individuals had a detectable HIV-
specific CD4 T cell response at baseline. Similarly to our previous report in chronic HIV infection, we observed a tight clustering of CD4 T cell responses within the N-terminus of p17 and a 20-amino acid region within the p24 protein that are essential for the formation of the matrix protein and capsid dimerization (13) that was detectable even at the time of initial presentation of acute HIV infection. This suggests an early establishment of effector responses that are subsequently maintained throughout infection. Interestingly, we found that the frequency and epitope-recognition hierarchy of HIV-specific CD4 T cell responses at baseline was remarkably similar to that detected at two, six or twelve months post HIV infection. Moreover, we found the same overall recognition pattern of HIV-specific CD4 T cell responses in acute HIV infection as compared to our previous published findings in chronic HIV infection (shown for comparison) (13). Indeed, at no time point were significant differences observed in the recognition frequency of the most dominant HIV-specific CD4 T cell responses at baseline compared to any other time point.

In particular, the most frequently targeted epitopes within Gag were OLP41 (50%), followed by OLP37 (28%) and OLP6 (27%)—similar to what we reported in chronic HIV infection (OLP41: 38%, OLP37: 16% and OLP6: 21%, respectively) (13) (Table 2). Likewise, responses to OLP91 (within Nef) were detected in comparable frequencies during acute (20%) and chronic (13%) HIV infection. A different pattern was discernable for gp120-specific CD4 T cell responses. We observed a minor shift in the epitope hierarchy in gp120. While OLP301 was similarly targeted throughout infection (11% in acute versus 13% in chronic), the OLP316 epitope became the most frequently targeted epitope in chronic infection (7% versus 16%, respectively). Overall, however, we unexpectedly found that HIV-specific CD4 T cell responses targeted the same epitopes during acute HIV infection at a comparable frequency as in chronic HIV infection. Moreover, we found no shift in the epitope hierarchy or significant changes in the frequency of CD4 T cell epitope recognition at any time point of infection.
Previous studies have shown that the breadth of HIV-specific CD8 T cell responses—defined as the total number of epitopes recognized by T cells at a given time in an individual—increases from acute to chronic HIV infection (24). We therefore assessed whether similar changes occur for HIV-specific CD4 T cell responses over time, or rather whether we observe a contraction and depletion of the HIV-specific CD4 T cell response pool. In contrast to HIV-specific CD8 T cell responses, a cross-sectional analysis from baseline to one year post infection indicates that the overall breadth of the HIV-specific CD4 T cell responses decreases slightly, albeit non-significantly, from acute (average breadth: 5.5) to chronic (average breadth: 3.3) HIV infection (Figure 2A).

To investigate whether the slight changes in HIV-specific CD4 T cell breadth were affected by averaging effects of the cross-sectional assessment of multiple participants, we compared the breadth within certain individuals for whom matched samples were available at baseline and then at twelve months post infection (Figure 2B). Nonetheless, while the breadth of HIV-specific CD4 T cell responses decreased in half (6/12) of these participants, the breadth increased in five and remained stable in one. Thus, we found no evidence that the breadth of HIV-specific CD4 T cell responses significantly changes from acute to chronic HIV infection even using subject-matched specimens. Moreover, we found the same pattern when we restricted our analysis to incorporate only individuals identified during Fiebig II/III, indicating that neither a time-dependent loss of CD4 responses in this subgroup nor the time point during acute HIV infection that individuals were identified is contributing to these results (supplemental figure 1). Thus, our data suggest that at least during the first year of infection, HIV-specific CD4 T cell responses are not measurably depleted from the overall immune response. We next assessed whether the magnitude of HIV-specific CD4 T cell responses changes over time, as an increase in magnitude for HIV-specific CD8 T cell responses has been previously described (24). Similar to changes in the breadth of HIV-specific CD4 T cells, however, we found that the overall magnitude of HIV-specific CD4 T cell responses did not differ between the different stages in
cross-sectional analyses (Figure 2C). While we found an overall decrease in the magnitude
from baseline (1333 ± 1721 SFC/M) to twelve months (703.9 ± 877.5 SFC/M) post infection, this
difference did not reach statistical significance and was also not significant in a similar matched
paired analysis as described above (Figure 2D). Moreover, the change in breadth or magnitude
(increase or decrease) of HIV-specific CD4 T cell responses did not result in faster or slower
disease progression (data not shown). Thus, while we observed a trend of slightly decreasing
breadth and magnitude of HIV-specific CD4 T cell responses over time, changes within one
year of infection are not significant and appear to remain stable during this time.

We previously reported that the hierarchy of HIV-specific CD8 T cell responses based on the
HLA class I expression is very predictable during acute HIV infection (25). However, viral
escape (26), superinfection (27) and viral recombination (28) have been shown to significantly
alter the frequency of recognized HIV-specific CD8 T cell responses from acute to chronic
infection, leading to a shift in the overall epitope immunodominance pattern of these cells.
Indeed, the epitopes recognized by HIV-specific CD8 T cells are often not recognized in the
chronic phase of infection, where other epitopes are more frequently targeted (25). We were
therefore interested in assessing whether shifts in the protein immunodominance hierarchy exist
from acute to chronic HIV infection in CD4 T cells. Interestingly, in both acute and chronic HIV
infection, HIV Gag was consistently the most dominantly targeted; responses to gp120 and Nef
subsequently followed. We observed a non-significant decline in the breadth and magnitude of
CD4 T cell responses against Gag and gp120 from baseline to twelve months post infection
(Figure 3A and B), while the Nef-specific CD4 T cell responses appeared to remain stable over
time. Interestingly, the overall contribution of Gag-specific CD4 T cell responses to the total HIV-
specific responses remained stable over time, while gp120-specific responses slightly
diminished (Figure 3C). To further dissect potential changes in the intensity patterns of epitope
recognition of HIV-specific CD4 T cells, we followed individual responses longitudinally where
sufficient data was available. Similar to HIV-specific CD8 T cell responses (24) we found that
individual HIV-specific CD4 T cell responses alternated between expansion and contraction over time (Figure 3D). While some HIV-specific CD4 T cell responses were longitudinally maintained, others fully contracted or only emerged later in HIV infection. However, despite the changes in intensity of existing and newly emerging CD4 T cell responses, no overall change could be observed. Changes in the intensity and fluctuation of the responses were not due to changes in CD4 count (data not shown).  

The induction of HIV-specific CD4 T cell responses can potentially have two diametrically opposed outcomes: increased control of HIV infection or, alternatively, increased susceptibility to infection. Given the remarkable high frequency of HIV-specific CD4 T cell responses during acute HIV infection, we investigated whether the induction of HIV-specific CD4 T cell responses during acute HIV infection has an impact on clinical prognosis—as defined by early viral set point—as this has been previously shown to be highly predictive for long-term disease outcome (29-31). We were able to measure the early viral set point for nineteen HIV infected individuals. Interestingly, we observed that the contribution of Gag-specific CD4 T cell responses to the total HIV-specific CD4 T cell response at baseline showed a significant inverse correlation with viral set point ($R=-0.5$, $p=0.03$) (Figure 4A). In contrast, we found the opposite effect for Env-specific CD4 T cell responses in that the contribution of Env-specific responses to the total HIV-specific response at baseline was associated with a higher viral set point, albeit without reaching statistical significance ($R=0.4$, $p=0.06$) (Figure 4B). We previously demonstrated that the Env/Gag ratio of HIV-specific CD4 T cell responses is a strong indicator of HIV control in chronic HIV infection (13) and, interestingly, we found that a low Env/Gag ratio already at baseline (during which no differences in HIV viremia exists between groups) similarly predicts a low viral set point ($R=0.49$, $p=0.03$) (Figure 4C). Moreover, using a Kaplan-Meyer Analysis, we found that individuals whose HIV-specific CD4 T cell responses targeted more Gag, compared to Env, remained significantly longer of antiretroviral therapy than individuals with more Env-specific CD4 T cell responses (median time to ART of 596 days versus a median time to ART of 275  

11
days; \( p=0.03 \) (Figure 4D). Taken together, the induction and presence of HIV-specific CD4 T cell responses –in particular targeting the Gag protein– shows an overall association with better disease outcome despite potential preferential depletion by HIV.

Discussion

The induction of HIV-specific CD4 T cells during acute HIV infection is a double-edged sword. Although it is well established that an effective CD8 and antibody response relies on the presence of CD4 T cell mediated helper signals (7), HIV also preferentially infects and depletes HIV-specific CD4 T cells (12). Thus, the generation of CD4 T cell responses during acute HIV infection may increase the availability of target cells for HIV propagation and may accelerate disease progression. We have previously demonstrated that, in chronic HIV infection, the breadth and specificity of HIV-specific CD4 T cell responses is significantly associated with maintenance of low viremia (13). Moreover, we demonstrated that CD4 T cells targeting Env versus Gag is an indicator for HIV progression while Gag-specific CD4 T cell responses are associated with control viral replication (13). However, it is unknown whether this is the cause or consequence of low viremia, and whether the expansion of Gag-specific CD4 T cell responses directly contributes to HIV control or rather if these cells persist by virtue of being spared in the environment of controlled HIV infection is unclear. Here we assessed the longitudinal development of early epitope-specific CD4 T cell responses during acute HIV infection until one year after initial presentation. We unexpectedly found that the overall HIV-specific CD4 T cell response remained relatively stable over this time in terms of frequency, breadth and magnitude. While the stability of the frequency and dominance of HIV-specific CD4 T cells over time is surprising, we cannot exclude the possibility that these cells have a shorter life-span, and thus stable levels are maintained by a higher cell turnover rate, as has been previously described (32). Interestingly, we found that in some individuals, the breadth and magnitude of HIV-specific CD4 T cell responses declined, while an increase was observed in others.
Previous studies designed to understand the earliest kinetics of HIV-specific CD4 T cell responses during acute HIV infection are overall consistent with our findings. Riou et al. defined in a cohort of twelve acute HIV infected individuals, three patterns of HIV-specific CD4 T cell response kinetics using multicolor flow cytometry after stimulation with HIV peptide pools: decreasing, undetectable/stable and increasing (33). Similarly, Gloster et al. found that in patients that gained relative control over viral replication, HIV-specific CD4 T cell responses increased from acute to chronic HIV infection, while a decreasing or lack of HIV-specific CD4 T cell responses were observed in patients that did not control viral replication in the chronic phase of HIV infection (34). Indeed, in a matched-controlled cohort study, we previously described that in individuals that control HIV infection, a significant increase of HIV-specific CD4 T cell responses early after acute HIV infection could be observed (11). This expansion was most pronounced in the population of cytolytic CD4 T cell responses, suggesting a direct antiviral, cytotoxic activity of HIV-specific CD4 T cells in the control of HIV infection. However, consistently all studies observe that overall HIV-specific CD4 T cell responses are decreasing over time in the majority of patients. Riou et al. described declining HIV-specific CD4 T cell responses in 7/12 individuals and the opposite in 2/12 individuals, while we found 6/12 individuals had diminishing CD4 T cell responses and increasing CD4 T cell responses in 5/12. In contrast, while Lubong-Sabado et al. found decreasing HIV-specific CD4 T cell responses in most (8/9) individuals from baseline to 1 months post infection, he found in the three patients that remained off antiretroviral therapy six months of infection all patterns as described by Riou et al. (HIV-specific CD4 T cell responses remained stable in 1/3 of individuals, decreased in 1/3 of individuals and increased in 1/3 of individuals). Thus, we observed a slight decrease in the breadth and magnitude of HIV-specific CD4 T cells over time, in line with other previous studies of primary infection (19, 35), although these decreases were not statistically significant and mirrored the overall (and similarly not significant) decrease of the CD4 T cell count.
Comparable to HIV-specific CD8 T cell responses, we found the intensity of HIV-specific CD4 T cell responses fluctuating at different time points within individual participants, which has been suggested to be associated with different levels of antigenemia (24). Moreover, we cannot exclude that this may be due to functional impairment or higher depletion rate of HIV-specific CD4 T cells at different time points. In addition, this study was not set up to identify changes within a short period of time in the same individual. Here observed that the breadth and magnitude of both Env- and Gag-specific responses decline over the first year of infection, but in agreement to Malhorta et al. (36), the contribution of Gag-specific responses to the overall CD4 response seems to slightly increase, while that of Env declines.

Interestingly, we observed that a high contribution of Gag-specific CD4 T cell responses to the total HIV-specific CD4 T cell response is detectable even early during acute HIV infection. Likewise, a low Env/Gag ratio was significantly associated with a lower viral set point and thus may be predictive for disease outcome. A previous study of six primary HIV infected individuals described an association between greater breadth of HIV-specific CD4 T cell responses in acute HIV infection and better disease outcome (34). However, while we observed a similar trend for an association of breadth with one-year viral set point (R=-0.25, p=ns, data not shown), a significant association was found only with the relative recognition of epitopes within Gag. Given our previous findings of Gag-specific CD4 T cell responses and HIV control in chronic infection, it raises the question of whether Gag-specific CD4 T cell responses directly contribute to the control of HIV replication, potentially through cytotoxic mechanisms (11). Moreover, these Gag epitopes overlap with defined immunodominant HIV-specific CD8 T cell responses, also raising the possibility that CD4 help is provided to HIV-specific CD8 T cells and therefore enhancing HIV control. Interestingly, previous studies demonstrate that the efficacy of pathogen-specific CD8 T cell responses in chronic infection is dependent on specific CD4 T cell signals. Moreover, these signals appear to be delivered repeatedly to ensure the ability of CD8 T cell responses to
contain persistent infection. We previously described a short region within p24 that is targeted
by HIV-specific CD8 T cell responses associated with better disease outcome (25).
Interestingly, this region as well as proximal regions is also preferentially targeted by HIV-
specific CD4 T cell responses, supporting the hypothesis that HIV-specific CD4 T cell responses
may support these efficient HIV-specific CD8 T cell responses.

Indeed, it is possible that HIV-specific T cells responding to different antigens may have
divergent functional roles. For example, Env-specific T cells may be more critical to support
neutralizing antibody production, while Gag-specific CD4 T cell responses may mainly be
important to provide help for HIV-specific CD8 T cell responses. Thus, our data cautiously
suggest that the specific induction of Gag-specific responses in acute HIV infection may have
an active involvement in control of viremia. Accordingly, only Env-specific antibodies can protect
from initial viral acquisition are ineffective at controlling viremia and disease progression.
Indeed, we observed that a fine balance between beneficial Gag-specific and detrimental Env-
specific responses is significantly associated with better disease outcome measured as time the
patients stayed off HAART after diagnosis with acute HIV infection. However, we cannot
exclude the possibility that Env-specific responses may be beneficial in a vaccination setting. In
conclusion, our data support the notion that the induction and emergence of HIV-specific CD4 T
cell responses during acute HIV infection is associated with long-term disease control rather
than detrimentally increasing the potential target pool for HIV propagation.

Acknowledgements
This study was funded by the US National Institutes of Health (NIH) (R01 AI091450-01 and R01
AI094602-01) and supported by the Bill and Melinda Gates Foundation.

Note: the views expressed herein are those of the authors and should not be construed to
represent the positions of the US Army or the Department of Defense.
References


**FIGURE 1.** HIV-specific CD4 T cell responses showed similar frequency and immunodominance profiles in acute and chronic infection. HIV-specific CD4 responses from different time points post presentation (from top to bottom: baseline, 2 months, 6 months, and 12 months) were screened against a panel of overlapping-peptides (OLPs) spanning HIV’s proteome of Gag, Nef and gp120. HIV-specific CD4 T cell responses obtained from a chronically infected cohort are shown in comparison as published previously (Ranasinghe et al., 2012) (bottom). Y-axis shows the frequency of epitope-specific CD4 T cell responses to the respective OLP. The HIV protein corresponding to the OLP position is illustrated in the X-axis. Particular OLPs of interest are inscribed above corresponding value.

**FIGURE 2.** HIV-specific CD4 T cell responses remain stable within the first year of infection. (A) The breadth of HIV-specific CD4 T cell responses for 55 subjects is shown at baseline and 2, 4, 6 and 12 months post presentation. We found a non-significant decrease of breadth from acute infection (avg.: 5.5) to 12 months post infection (avg.: 3.3). Open circles represent individuals identified during Fiebig stages 2, 3 and 4; grey circles represent individuals identified during Fiebig stages 5 and 6. (B) Matched-pair analysis of breadth of HIV-specific CD4 T cell responses in twelve individuals at baseline and twelve months post infection showed divergent results. Decrease of breadth in 6/12, increase of breadth 5/12, no change in breadth 1/12. (C) The cross-sectional analysis of the total magnitude of HIV-specific CD4 T cell responses is shown for 55 subjects at baseline and 2, 4, 6 and 12 months post presentation. We found an overall tendency of decrease in magnitude from acute (baseline 1333 (SD) ±1721 SFC/M) to chronic infection (12 months post presentation 703.9 (SD) ±877.5 SFC/M), which was not significant. (D) Matched-pair analysis of the magnitude of HIV-specific CD4 T cell responses in twelve individuals at baseline and twelve months post presentation showed an overall tendency of a decrease of magnitude, not statistically significant.
FIGURE 3. Stable dominance pattern of Gag-specific CD4 T cell responses over time.

(A) The overall magnitude (in SFC/M) of HIV-specific CD4 T cell responses is shown for Gag, Nef and gp120 each at 0 and 12 months post diagnosis of acute HIV infection. While there was a non-significant decrease of magnitude in responses against Gag and gp120, the responses against Nef remained stable. Error bars indicate SEM. (B) Breadth of HIV-specific CD4 T cell responses is shown for Gag, Nef and gp120 each at 0 and 12 months post diagnosis of acute HIV infection. Against Gag and gp120 the breadth of responses decreased non-significantly, while the responses against Nef remained stable. Error bars indicate SEM. (C) The contribution to total HIV-specific CD4 T cell responses is shown for Gag, Nef and gp120 each at 0 and 12 months post diagnosis of acute HIV infection. Gag has the highest percentage of HIV-specific CD4 T cell responses, followed by gp120 and Nef. While the responses against Gag and Nef remained stable, the responses against gp120 showed a non-significant decrease in the contribution to the total HIV-specific CD4 T cell responses from acute to chronic infection. Error bars indicate SEM. (D) The Magnitude (in SFC/M) of HIV-specific CD4 T cell responses against selected overlapping peptides is shown for four subjects at different stages of infection from 0 to 12 months post presentation where data was available. A prevalent pattern is the initial expansion of responses against specific peptides, followed by a contraction. While some of the responses were present at all stages of infection, several fully contracted or appeared only during chronic infection.


(A) The contribution of Gag-specific CD4 T cell responses as percentage of the total HIV-specific CD4 T cell response was significantly inversely correlated a low viral set point (R=0.5, p=0.02; Spearman rank test). (B) The contribution of Env-specific CD4 T cell responses trended to be associated with a higher viral set point (R=0.4, p=0.06; Spearman rank test). (C) A low Env/Gag ratio was significantly associated with a low viral set point (R=0.49, p=0.03; Spearman test).
rank test). (D) Survival analysis of time patients remained off antiretroviral therapy. Individuals with higher Env- (n=7) than Gag- specific (n=12) CD4 T cell response at baseline initiated HAART significantly earlier than individuals with higher Gag- then Env-specific CD4 T cell response (median time to HAART: 275 days versus median time to HAART: 596 days, respectively (p=0.03; log rank test).
rank test). (D) Survival analysis of time patients remained off antiretroviral therapy. Individuals with higher Env- (n=7) than Gag-specific (n=12) CD4 T cell response at baseline initiated HAART significantly earlier than individuals with higher Gag- then Env-specific CD4 T cell response (median time to HAART: 275 days versus median time to HAART: 596 days, respectively (p=0.03; log rank test).

### Table 1. Summary of clinical characteristics of study participants

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<th>75% Percentile</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>% (n)</td>
<td>7.240</td>
<td>25.0 (17)</td>
<td>93.200</td>
<td>126.500</td>
<td>4,295.000</td>
<td>5,196,200</td>
<td>12,980.985</td>
</tr>
<tr>
<td>25% Percentile</td>
<td>3.030</td>
<td>12.7 (7)</td>
<td>27.300</td>
<td>132.750</td>
<td>706.000</td>
<td>158,376</td>
<td>246.689</td>
</tr>
<tr>
<td>Median</td>
<td>291</td>
<td>21.8 (12)</td>
<td>101,300</td>
<td>126,650</td>
<td>2,010,000</td>
<td>240,320</td>
<td>593,242</td>
</tr>
<tr>
<td>75% Percentile</td>
<td>50,600</td>
<td>3.6 (2)</td>
<td>71,750</td>
<td>120,100</td>
<td>152,000</td>
<td>101,300</td>
<td>71,701</td>
</tr>
<tr>
<td>Maximum</td>
<td>75,950</td>
<td>7.2 (4)</td>
<td>120,100</td>
<td>250,000</td>
<td>250,000</td>
<td>104,650</td>
<td>99,450</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>CD4 count (cells/μl)</th>
<th>Minimum</th>
<th>25% Percentile</th>
<th>Median</th>
<th>75% Percentile</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>% (n)</td>
<td>127</td>
<td>250</td>
<td>388</td>
<td>515</td>
<td>743</td>
<td>388</td>
<td>160</td>
</tr>
<tr>
<td>25% Percentile</td>
<td>206</td>
<td>290</td>
<td>395</td>
<td>755</td>
<td>889</td>
<td>526</td>
<td>305</td>
</tr>
<tr>
<td>Median</td>
<td>285</td>
<td>291</td>
<td>469</td>
<td>721</td>
<td>1119</td>
<td>556</td>
<td>363</td>
</tr>
<tr>
<td>75% Percentile</td>
<td>443</td>
<td>368</td>
<td>699</td>
<td>827</td>
<td>955</td>
<td>699</td>
<td>362</td>
</tr>
<tr>
<td>Maximum</td>
<td>369</td>
<td>571</td>
<td>478</td>
<td>550</td>
<td>550</td>
<td>507</td>
<td>141</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Time to treatment</th>
<th>median (days; range: 3-1155)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>160 (range: 3-1155)</td>
</tr>
</tbody>
</table>
Table 2. Frequently targeted HIV-specific CD4 T cell responses from acute to chronic HIV infection

<table>
<thead>
<tr>
<th>epitope sequence</th>
<th>protein</th>
<th>peptide number</th>
<th>baseline (%)</th>
<th>2 months (%)</th>
<th>6 months (%)</th>
<th>12 months (%)</th>
<th>chronic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YVDRFYKTLRAEQASQEV</td>
<td>p24</td>
<td>41</td>
<td>50</td>
<td>48</td>
<td>58</td>
<td>31</td>
<td>38</td>
</tr>
<tr>
<td>WIILGLNKIVRMYSPTSI</td>
<td>p24</td>
<td>37</td>
<td>28</td>
<td>29</td>
<td>37</td>
<td>38</td>
<td>16</td>
</tr>
<tr>
<td>ASRELFHAVNPGLL</td>
<td>p17</td>
<td>6</td>
<td>27</td>
<td>33</td>
<td>26</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>MTETLLVQNAPDCKTL</td>
<td>p24</td>
<td>44</td>
<td>23</td>
<td>24</td>
<td>32</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>PEKELVWKSRLAFHH</td>
<td>Nef</td>
<td>91</td>
<td>20</td>
<td>14</td>
<td>32</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>TILKALGPAATLEEMMTA</td>
<td>p24</td>
<td>46</td>
<td>16</td>
<td>24</td>
<td>11</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>YKAADVLSHFLKEKGGL</td>
<td>Nef</td>
<td>78</td>
<td>16</td>
<td>33</td>
<td>16</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>WYKVVEEKAFSPEVIPMF</td>
<td>p24</td>
<td>22</td>
<td>15</td>
<td>14</td>
<td>16</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>NVTENFMWKNMVEQMNH</td>
<td>gp120</td>
<td>301</td>
<td>11</td>
<td>14</td>
<td>16</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>KVSFEPIPPIHCAPAGFA</td>
<td>gp120</td>
<td>316</td>
<td>7</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>
Figure 1

Baseline

2 months

6 months

12 months

Chronic
FIGURE 2

A

Breadth of HIV-specific CD4 T cell responses

Baseline 2 4 6 12 months post presentation

B

Breadth of HIV-specific CD4 T cell responses

0 10 20 30 months

C

Magnitude of HIV-specific CD4 T cell responses

Baseline 2 4 6 12 months post presentation

D

Magnitude of HIV-specific CD4 T cell responses

0 1000 2000 3000 months
**FIGURE 3**

A. Number of HIV-specific CD8+ T cell responses for Gag, Nef, and gp120.

B. Percentage contribution of HIV-specific CD8+ T cell responses for Gag, Nef, and gp120.

C. SFC/M for peptides A, B, C, D, and E over time for Gag, Nef, and gp120.

D. SFC/M for peptides F, G, H, I, and J over time for Gag, Nef, and gp120.
FIGURE 4

A

B

C

D

R = -0.5
p = 0.03

R = 0.4
p = 0.06

R = 0.49
p = 0.03

Env/Gag ratio
days post presentation

log-rank p=0.03