Aus dem Institut für Mikrobiologie und Hygiene
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

HIV-spezifische CD4 T-Zell-Antworten in der akuten HIV-Infektion

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Inhaltsverzeichnis **Seite**

Abstract

Es ist bekannt, dass CD4 T-Helferzellen eine entscheidende Rolle in der Immunantwort gegen virale Pathogene spielen. Eine Ausnahme bildet dabei die HIV-Infektion, da CD4 T-Zellen gleichzeitig die bevorzugten Zielzellen des HI-Virus sind und damit die Grundlage für die Replikation des Virus darstellen. Es wurde bereits gezeigt, dass das Vorhandensein von HIV-spezifischen CD4 T-Zellen in der chronischen Infektion mit einer Kontrolle der HIV-Infektion einhergeht. Neben bisher ungeklärten Mechanismen trägt die direkte zytotoxische Funktion der CD4 T-Zellen in der chronischen Infektion zu einer geringen Viruslast bei. Bisher ungeklärt ist allerdings, welche Rolle eine Induktion der CD4 T-Zellen in der akuten HIV-Infektion unter Berücksichtigung des weiteren klinischen Verlaufs spielt und ob HIV-spezifische CD4 T-Zellen in der frühen Phase der HIV-Infektion bevorzugt depletiert werden. Um diesen Fragen zu begegnen, haben wir longitudinale Daten einer Kohorte von fünfundfünfzig akut HIV-infizierten Patienten über einen Zeitraum von zwölf Monaten erhoben. Interessanterweise beobachteten wir, dass die Intensität, das Spektrum und die Proteindominanz der HIV-spezifischen CD4 T-Zell-Antworten über den gesamten Zeitraum, von der akuten bis zur chronischen Infektion, stabil blieben. Epitope, die in der akuten Infektion in hoher Intensität CD4 T-Zell-Antworten provozierten, wurden in derselben Intensität auch in der chronischen Infektion von HIV-spezifischen CD4 T-Zellen erkannt. Außerdem sahen wir eine inverse Korrelation zwischen einer starken Gag-spezifischen CD4 T-Zell-Antwort in der akuten Infektion und der Viruslast in der chronischen Infektion (R=0.5; P=0.03), während die spezifischen CD4 T-Zell-Antworten gegen das Env-Protein den umgekehrten Effekt zeigten. Des Weiteren wurden die Patienten, die bereits in der akuten Infektion bevorzugt HIV-spezifische CD4 T-Zell-Antworten gegen das Gag-Protein und nicht gegen das Env-Protein hatten, signifikant später mit einer antiretroviralen Therapie behandelt, als die Patienten ohne dominante Gag-spezifische CD4 T-Zell-Antworten (P=0.03; log rank). Unsere Daten zeigen insofern, dass bestimmte HIV-spezifische CD4 T-Zell-Antworten in der frühen HIV-Infektion einen positiven Einfluss auf den weiteren Verlauf der Infektion haben und nicht wie ebenfalls diskutiert wurde, einen Progress der Infektion durch eine erhöhte Zahl an Zielzellen fördern.

Abstract

The important share of CD 4 T cells in the elimination of viral pathogens is well known. In the particular case of HIV infections the role of CD 4 T cells is less clear as CD 4 T cells are the preferred viral target. Previously we demonstrated a strong association between specific CD4 T cell responses and a low viremia during chronic HIV infection. Still the question remains, if the presence of CD4 T cell responses during acute HIV infection determines a beneficial clinical outcome or if the CD4 T cells, as a main target of HIV, present an extended contact surface for viral replication. To investigate this question we longitudinally assessed the HIV-specific CD4 T cell responses in a cohort of fifty-five individuals with primary HIV-1 infection. Whilst looking at breadth and magnitude of the CD4 T cell responses in a cross-sectional analysis and paired analysis in single individuals, we found a surprising stability in responses from acute to chronic infection. In addition we saw a remarkable conformity in the immunodominance pattern comparing the baseline to further stages of infection. The responses targeting Gag show a non-significant decrease, while the overall contribution of Gag-specific CD4 T cell responses to the overall HIV-specific responses remained stable. Interestingly we observed an inverse correlation of a lower viral set point and the overall contribution of Gag-specific CD4 T cell responses ($R=-0.5$, $p=0.03$). A contrary tendency showed the cumulative contribution of Env-specific CD4 T cell responses. Moreover we could evaluate already during acute HIV infection, that a dominant targeting of Gag over Env significantly determines a lower progression of infection as measured by the number of days until initiation of an antiretroviral therapy ($p=0.03$, log-rank). Our results indicate that an emerging CD4 T cell response in acute HIV-Infection is correlated with long-term disease control.

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Ausführliche Anteilserklärung an der erfolgten Publikation

Publikation :

Schieffer M, Jessen HK, Oster AF, Pissani F, Soghoian DZ, Lu R, Jessen AB, Zedlack C, Schultz BT, Davis I, Ranasinghe S, Rosenberg ES, Alter G, Schumann RR, Streeck H. Induction of Gag-specific CD4 T cell responses during acute HIV infection is associated with improved viral control. J Virol. 2014 Jul;88(13):7357-66.

Beitrag im Einzelnen (bitte **ausführlich** ausführen):

- Einschluss und Aufklärung von 34 Studienpatienten in Berlin, Praxis Jessen.
- Betreuung der Patienten über einen Studienzeitraum von 12 Monaten
- Organisation von Terminen zu den vorgesehenen Messpunkten bei 0, 2, 4, 6 und 12 Monaten
- Erhebung der demographischen Daten der Patienten und der Time-to-Treatment
- Blutentnahmen im Rahmen der regulären Labortermine der Patienten
- Arbeit in der Zellkultur
    - Isolation von PBMC
    - Depletion der CD 8 T Zellen
    - Präparation von Elispot-Platten zur Stimulation der CD 4 T Zellen
    - Entwicklung der Elispot-Platten nach 40 stündiger Inkubation
    - Einfrieren von Plasma- und Zellproben
    - Versand der Elispot-Platten und Kontrollproben in die USA
- Formulierung des Abstracts
- Formulierung der Methoden
- Beschriftung der Grafiken
- Korrekturlesen der gesamten Publikation

Dr. Heiko Jessen ist der betreuende Allgemeinmediziner der eingeschlossenen Patienten und Inhaber der Räumlichkeiten, in der die Zellkultur eingerichtet wurde. Aufgrund seiner erheblichen konzeptionellen Bedeutung für das Projekt wurde er als zweiter Erstautor eingetragen. Die praktische Arbeit zur Erhebung der Daten in Berlin wurde ausschließlich durch Miriam Schieffer verrichtet.

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Induction of Gag-Specific CD4 T Cell Responses during Acute HIV Infection Is Associated with Improved Viral Control

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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 somewhat associated with HIV control and that specific CD4 T cell functions, 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 55 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 at a high frequency in acute HIV infection were recognized at the 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 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 overall 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.

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 virus-infected cells (1–4), but also aids in the development of a secondary recall response upon reexposure to virus (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 virus-infected cells through cytotoxic mechanisms in several viral infections, such as Epstein-Barr virus (EBV) (8), influenza virus (9, 10), and HIV (11), a function not normally attributed to CD4 T cells.

Indeed, many licensed antiviral 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 antiviral CD4 T cells in the context of both 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 increase the pool of target cells and fuel HIV dissemination rather 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 are associated with rapid progression. Furthermore, we have found an HLA class II genetic association linked to CD4 T cell function that is correlated with durable control of HIV viremia (14). It is becoming increasingly evident, however, that events occurring dur-

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ing acute HIV infection set the stage for the 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 antiviral role of these cells in individuals who spontaneously control HIV viremia (11).

However, 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 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 epitope targeting over the course of infection from the acute to chronic phases. We therefore addressed 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 is associated with the long-term clinical outcome.

MATERIALS 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, MA, USA; and Massachusetts General Hospital, Boston, MA, USA). The clinical and sociodemographic characteristics of the study participants can be found in Table 1. The cohort was a male (100%), Caucasian (83.6%) study population and homogeneous in terms of demographics. Acute HIV infection was classified using Fiebig staging, as previously described (21). The study subjects gave written informed consent, and the study was approved by the institutional review boards of the HIV Clinic Jessen, Berlin, Germany; Fenway Health Center, Boston, MA, USA; and Massachusetts General Hospital, Boston, MA, USA.

Assessment of HIV-specific CD4 T cell responses. HIV-specific CD4 T cell responses were assessed as previously described (13). Briefly, freshly isolated peripheral blood mononuclear cells (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 gamma interferon (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) precoated with 2 μg/ml anti-IFN-γ monoclonal antibody 1-D1K (Mabtech, Stockholm, Sweden). A total of 65,000 to 100,000 CD8-depleted cells per well were added to 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 h at 37°C and 5% CO_2 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 number of spot-forming cells (SFC) per million CD8-depleted PBMC (SFC/M). 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 SFC/10^6 CD8-depleted PBMC, at least >3 times the mean background, and also >3 times the standard deviation (SD) of the number of SFC/10^6 CD8-depleted PBMC within the negative controls.

Statistical analysis. Statistical analysis and graphical presentation were done using Graph Pad Prism 5.0 and Microsoft Excel. Results are given as means ± 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-Meier analysis was performed using a log rank test.

TABLE 1 Summary of clinical characteristics of study participants[a]

Characteristic	Value for Fiebig stage:				
	2/3	4	5	6	ND
Viral load (HIV RNA copies/ml)					
Minimum	7,240	3,030	291	50,600	25,100
25th percentile	550,250	27,700	6,785	75,950	56,300
Median	1,000,001	93,200	27,300	101,300	71,750
75th percentile	4,295,000	125,500	132,750	126,650	120,100
Maximum	69,649,052	706,000	2,010,000	152,000	250,000
Mean	5,186,200	158,376	240,329	101,300	104,650
SD	12,980,985	246,689	593,242	71,701	99,450
CD4 count (cells/μl)					
Minimum	127	206	285	443	369
25th percentile	290	291	368	571	434
Median	388	395	469	699	478
75th percentile	515	755	721	827	550
Maximum	743	989	1119	955	550
Mean	398	526	556	699	507
SD	160	305	263	362	141

[a] The races of the participants (100% male; $n = 55$) were as follows: Caucasian, 83.6% (46/55); African-American, 1.8% (1/55); Asian, 10.9% (6/55); other/mixed, 3.6% (2/55). The Fiebig stages were represented as follows: Fiebig 2/3, 54.6% (30/55); Fiebig 4, 12.7%, (7/55); Fiebig 5, 21.8% (12/55); Fiebig 6, 3.6% (2/55); not determined (ND), 7.2% (4/55). Time to treatment was 138 (range, 3 to 1,155) days.

RESULTS

The clinical characteristics of the study participants are shown in Table 1. The majority (30/55) of the 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 load at the baseline visit was $2.96 \times 10^6 \pm 9.9 \times 10^6$ HIV RNA copies/ml, and the CD4 count averaged 466 ± 219 cells/μl. Nineteen individuals remained off therapy over 1 year after infection, and of those, 12 individuals had a matched assessment of HIV-specific CD4 T cell responses at both baseline and the 1-year time point. Twenty-six enrolled participants elected to begin highly active antiretroviral therapy (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 and 2 months, 6 months, and 12 months postdiagnosis 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 on chronic HIV infection, we observed tight clustering of CD4 T cell responses within the N terminus of p17 and a 20-amino-acid region within the p24 protein, which 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 early establishment of effector responses that are subsequently maintained throughout the infection. Interestingly, we found that the frequency and epitope recognition hierarchy of HIV-specific CD4 T cell responses at baseline were remarkably similar to those detected at 2, 6, or 12 months post-HIV infection (Fig. 1). Moreover, we found the same overall recognition pattern of HIV-specific CD4 T cell responses in acute HIV infection as in 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 (56%), followed by OLP37 (25%) and OLP6 (33%), similar to what we reported in chronic HIV infection (OLP41, 38%; OLP37, 16%; and OLP6, 21%) (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 discernible 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 infection), 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 frequency comparable to that 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 observed 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 1 year postinfection indicates that the overall breadth of the HIV-specific CD4 T cell responses decreases slightly, albeit nonsignificantly, from acute (average breadth, 5.5) to chronic (average breadth, 3.3) HIV infection (Fig. 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 breadths within certain individuals for whom matched samples were available at baseline and then at 12 months postinfection (Fig. 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 when using subject-matched specimens. Moreover, we found the same pattern when we restricted our analysis to incorporate only individuals identified during Fiebig 2/3, indicating that neither a time-dependent loss of CD4 responses in this subgroup nor the time point during acute HIV infection at which individuals were identified contributed to these results (see Fig. S1 in the supplemental material). 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 (Fig. 2C). While we found an overall decrease in the magnitude from baseline (1,333 $\pm$ 1,721 SFC/M) to 12 months (703.9 $\pm$ 877.5 SFC/M) postinfection, this difference did not reach statistical significance and was also not significant in a similar matched-pair analysis, as described above (Fig. 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 (measured as the amount of time without requiring ART intervention) (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 most dominantly targeted; responses to gp120 and Nef followed. We observed a nonsignificant decline in the breadth and magnitude of CD4 T cell responses against Gag and gp120 from baseline to 12 months postinfection (Fig. 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 (Fig. 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 were 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 (Fig. 3D). While some HIV-

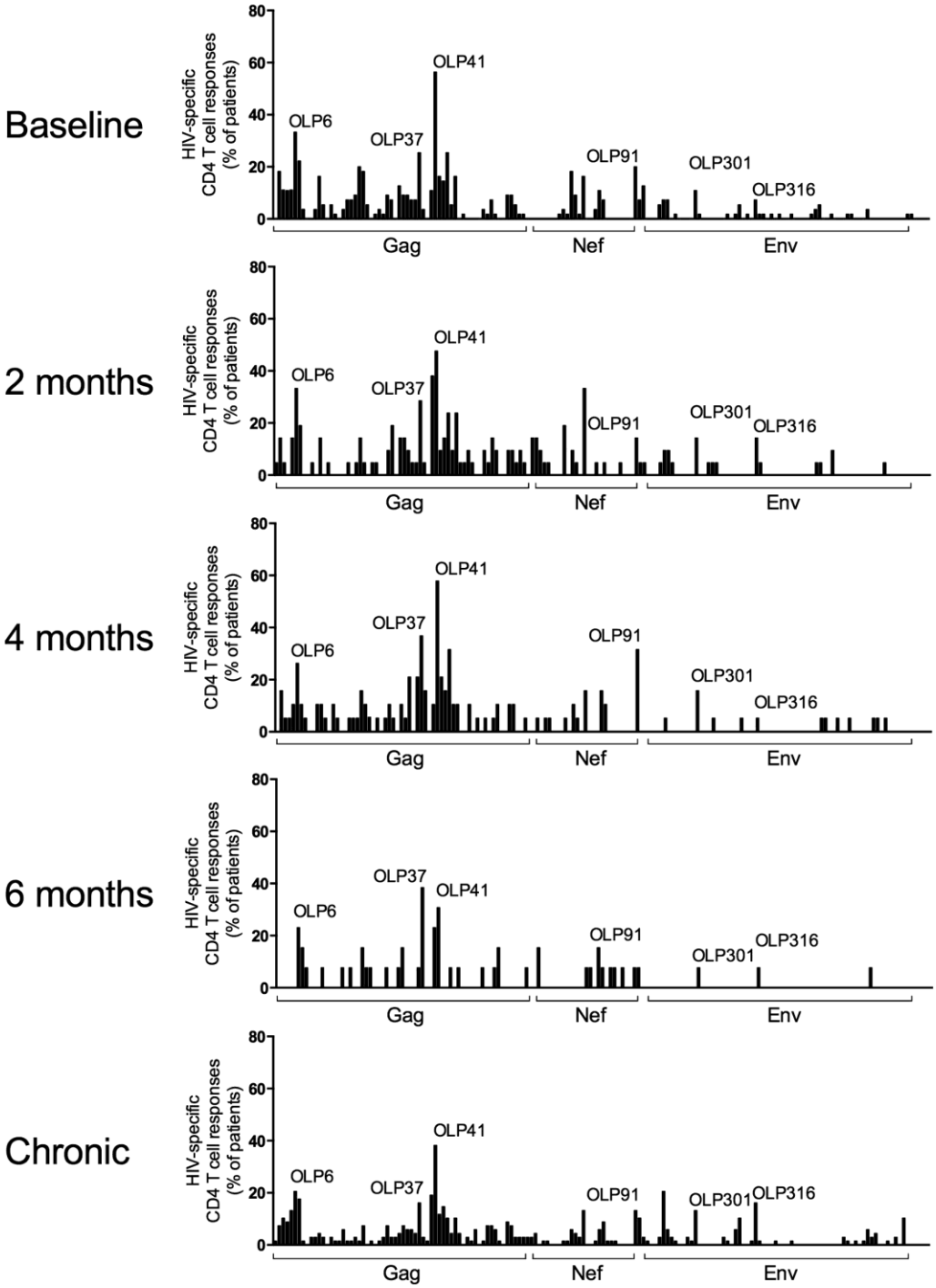


FIG 1 HIV-specific CD4 T cell responses showed similar frequencies and immunodominance profiles in acute and chronic infection. HIV-specific CD4 responses from different time points postpresentation were screened against a panel of OLPs spanning HIV's proteome of Gag, Nef, and gp120. HIV-specific CD4 T cell responses obtained from a chronically infected cohort, as published previously (13), are shown in comparison (bottom). The *y* axes show the frequencies of epitope-specific CD4 T cell responses to the respective OLPs. The HIV proteins corresponding to the OLP positions are shown on the *x* axes. Particular OLPs of interest are shown above the corresponding values.

TABLE 2 Longitudinal assessment of select epitopes[a]

Epitope sequence	Protein	Peptide no.	Targeting frequency (%)				
			Baseline	2 mo	6 mo	12 mo	Chronic
YVDRFYKTLRAEQASQEV	p24	41	56	48	58	31	38
WIILGLNKIVRMYSPTSI	p24	37	25	29	37	38	16
ASRELERFAVNPGLL	p17	6	33	33	26	23	21
MTETLLVQNANPDCKTIL	p24	44	25	24	32	8	10
PEKEVLVWKFDSRLAFHH	Nef	91	20	14	32	8	13
TILKALGPAATLEEMMTA	p24	46	16	24	11	8	10
YKAAVDLSHFLKEKGGL	Nef	78	16	33	16	8	13
WVKVVEEKAFSPEVIPMF	p24	22	20	14	16	15	1
NVTENFNMWKNNMVEQMH	gp120	301	11	14	16	8	13
KVSFEPIPIHYCAPAGFA	gp120	316	7	14	5	8	16

[a] Frequently targeted HIV-specific CD4 T cell responses from acute to chronic HIV infection.

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 the 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 remarkably 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 the clinical prognosis—as defined by the early viral set point—as this has been previously shown to be highly predictive of the long-term disease outcome (29–31). We were able to measure the early viral set point for 19 HIV-infected individuals. Interestingly, we observed that the contribution of Gag-specific CD4 T cell responses to the total

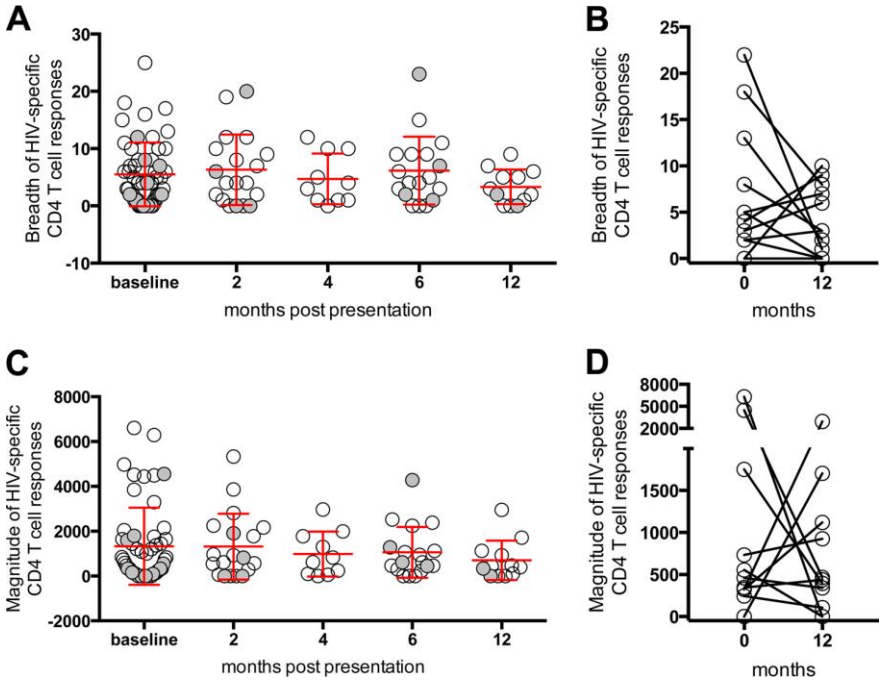
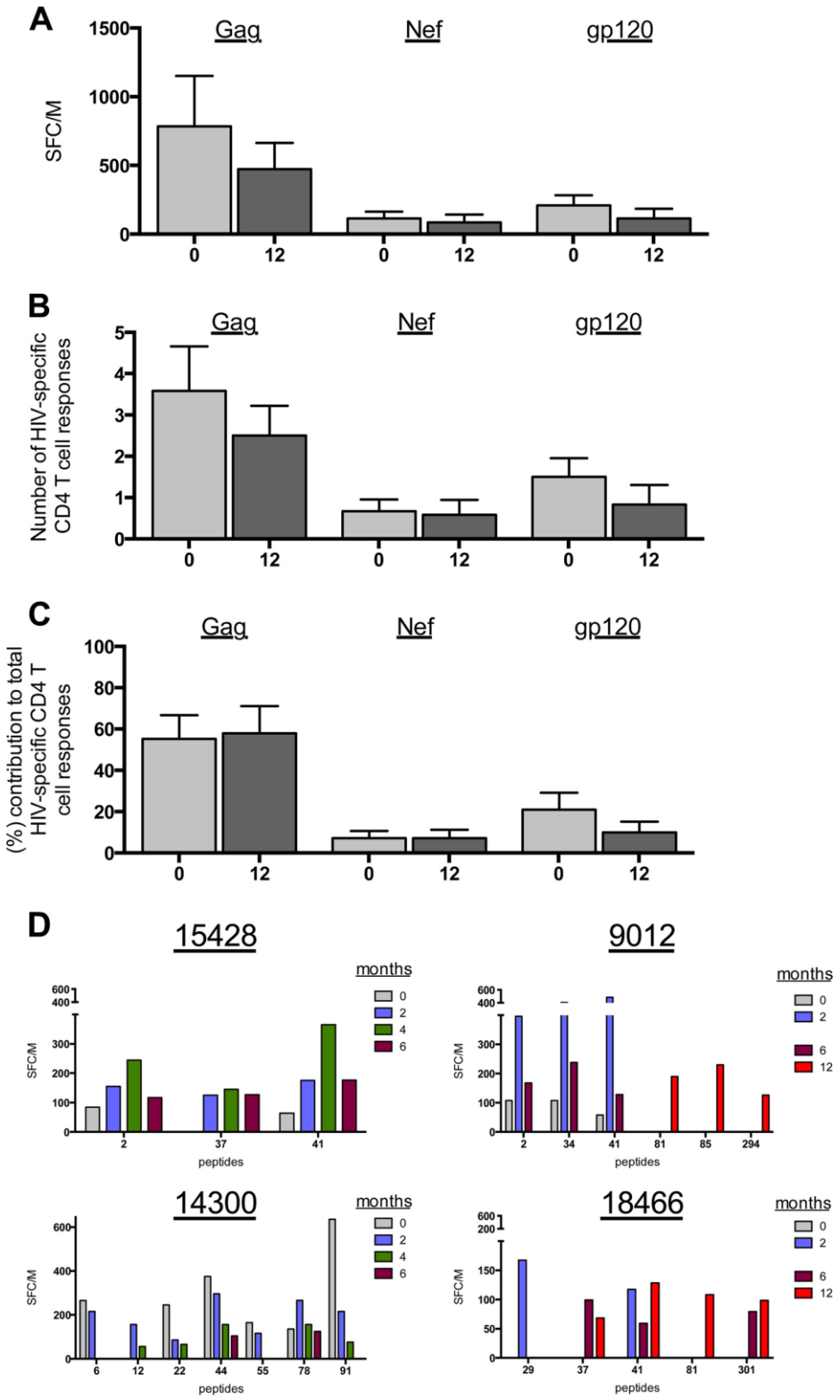


FIG 2 HIV-specific CD4 T cell responses remain stable within the first year of infection. (A) Breadth of HIV-specific CD4 T cell responses for 55 subjects shown at baseline and 2, 4, 6, and 12 months postpresentation. We found a nonsignificant decrease in breadth from acute infection (average, 5.5) to 12 months postinfection (average, 3.3). The open circles represent individuals identified during Fiebig stages 2, 3, and 4; the gray circles represent individuals identified during Fiebig stages 5 and 6. (B) Matched-pair analysis of the breadths of HIV-specific CD4 T cell responses in 12 individuals at baseline and 12 months postinfection showed divergent results: decrease of breadth in 6/12, increase of breadth 5/12, and no change in breadth in 1/12. (C) Cross-sectional analysis of the total magnitudes of HIV-specific CD4 T cell responses for 55 subjects at baseline and 2, 4, 6, and 12 months postpresentation. We found an overall tendency of decrease in magnitude from acute (baseline, 1,333 ± 1,721 [SD] SFC/M) to chronic (12 months postpresentation, 703.9 ± 877.5 SFC/M) infection that was not significant. (D) Matched-pair analysis of the magnitude of HIV-specific CD4 T cell responses in 12 individuals at baseline and 12 months postpresentation showed an overall tendency of a decrease of magnitude that was not statistically significant.


A
Gag
Nef
gp120
SFC/M
1500
1000
500
0
0
12
0
12
0
12
B
Gag
Nef
gp120
Number of HIV-specific CD4 T cell responses
5
4
3
2
1
0
0
12
0
12
0
12
C
Gag
Nef
gp120
(%) contribution to total HIV-specific CD4 T cell responses
100
80
60
40
20
0
0
12
0
12
0
12
D
15428
months
0
2
4
6
SFC/M
600
400
300
200
100
0
2
37
41
peptides
9012
months
0
2
6
12
SFC/M
600
400
300
200
100
0
2
34
41
81
85
294
peptides
14300
months
0
2
4
6
SFC/M
600
400
200
0
6
12
22
44
55
78
91
peptides
18466
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SFC/M
600
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50
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29
37
41
81
301
peptides

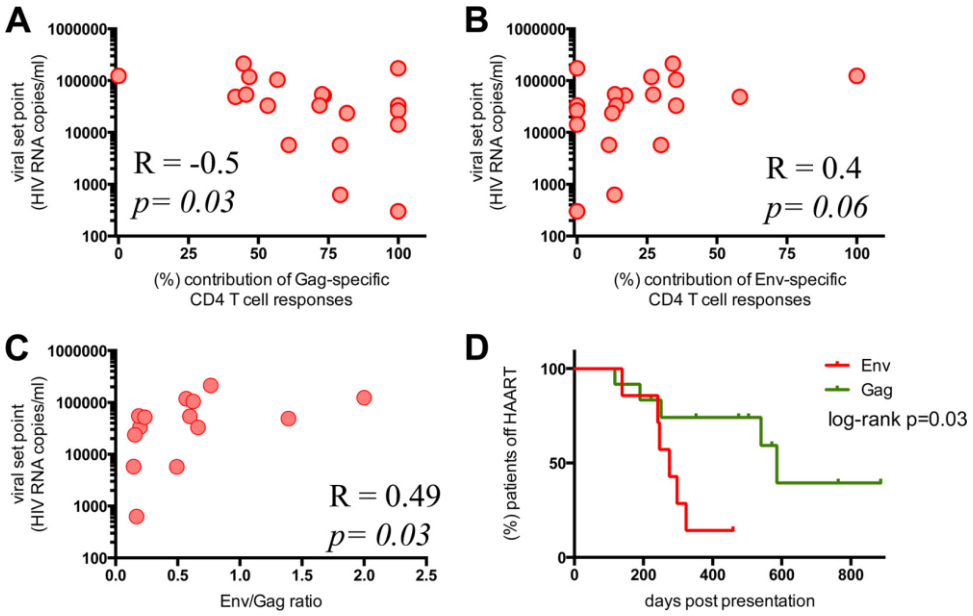

FIG 4 Association of Gag-specific CD4 T cell responses and clinical outcome. (A) The contribution of Gag-specific CD4 T cell responses as a percentage of the total HIV-specific CD4 T cell response was significantly inversely correlated with a low viral set point ($R = -0.5$; $P = 0.03$; Spearman rank test). (B) The contribution of Env-specific CD4 T cell responses tended 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 rank test). (D) Survival analysis of the time patients remained off antiretroviral therapy. Individuals with higher Env-specific ($n = 7$) than Gag-specific ($n = 12$) CD4 T cell responses at baseline initiated HAART significantly earlier than individuals with higher Gag-specific than Env-specific CD4 T cell responses (median time to HAART, 275 days, versus median time to HAART, 596 days, respectively; $P = 0.03$; log rank test).

HIV-specific CD4 T cell response at baseline showed a significant inverse correlation with the viral set point ($R = -0.5$; $P = 0.03$) (Fig. 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 ($R = 0.4$; $P = 0.06$) (Fig. 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 (at which time no differences in HIV viremia exist between groups) similarly predicts a low viral set point ($R = 0.49$; $P = 0.03$) (Fig. 4C). Moreover, using a Kaplan-Meier analysis, we found that individuals whose HIV-specific CD4 T cell responses targeted more Gag than Env remained significantly longer off antiretroviral therapy (ART) than individuals with more Env-specific CD4 T cell responses (median time to ART, 596 days versus 275 days, respectively; $P = 0.03$) (Fig. 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 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 main-

FIG 3 Stable dominance pattern of Gag-specific CD4 T cell responses over time. (A) Overall magnitudes (in SFC/M) of HIV-specific CD4 T cell responses for Gag, Nef, and gp120 at 0 and 12 months after diagnosis of acute HIV infection. While there was a nonsignificant decrease in magnitude in responses against Gag and gp120, the responses against Nef remained stable. The error bars indicate standard errors of the mean (SEM). (B) Breadth of HIV-specific CD4 T cell responses for Gag, Nef, and gp120 at 0 and 12 months after diagnosis of acute HIV infection. Against Gag and gp120, the breadth of responses decreased nonsignificantly, while the responses against Nef remained stable. The error bars indicate SEM. (C) Contributions to total HIV-specific CD4 T cell responses for Gag, Nef, and gp120 at 0 and 12 months after diagnosis of acute HIV infection. Gag had 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 nonsignificant decrease in the contribution to the total HIV-specific CD4 T cell responses from acute to chronic infection. The error bars indicate SEM. (D) Magnitudes (in SFC/M) of HIV-specific CD4 T cell responses against selected overlapping peptides for four subjects at different stages of infection from 0 to 12 months postpresentation where data were available. A prevalent pattern is the initial expansion of responses against specific peptides, followed by contraction. While some of the responses were present at all stages of infection, several fully contracted or appeared only during chronic infection.

tenance of low viremia (13). Moreover, we demonstrated that CD4 T cells targeting Env versus Gag is an indicator of HIV progression, while Gag-specific CD4 T cell responses are associated with control of 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. Here, we assessed the longitudinal development of early epitope-specific CD4 T cell responses during acute HIV infection until 1 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. Using multicolor flow cytometry after stimulation with HIV peptide pools, Riou et al. defined, in a cohort of 12 acute HIV-infected individuals, three patterns of HIV-specific CD4 T cell response kinetics: decreasing, undetectable/stable, and increasing (33). Similarly, Gloster et al. found that in patients who gained relative control over viral replication, HIV-specific CD4 T cell responses increased from acute to chronic HIV infection, while a decrease or lack of HIV-specific CD4 T cell responses was observed in patients who did not control viral replication in the chronic phase of HIV infection (34). Indeed, in a matched, controlled cohort study, we previously reported that in individuals who 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 the studies observed that HIV-specific CD4 T cell responses decreased overall 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, with 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 month postinfection, they found, in the three patients who remained off antiretroviral therapy at 6 months of infection, all patterns described by Riou et al. (HIV-specific CD4 T cell responses remained stable in 1/3 individuals, decreased in 1/3 individuals, and increased in 1/3 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 fluctuated at different time points within individual participants, which has been suggested to be associated with different levels of antigenemia (24). Moreover, we cannot exclude the possibility that this may be due to functional impairment or a 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 time in the same individual. Here, we observed that the breadth and magnitude of both Env- and Gag-specific responses decline over the first year of infection, but in agreement with Malhotra et al. (36), the contribution of Gag-specific responses to the overall CD4 response seems to increase slightly, while that of Env declines.

Interestingly, we observed that a large 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 of the disease outcome. Previous studies with 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, 37). However, while we observed a similar trend for an association of breadth with the 1-year viral set point ($R = -0.25$; P, not significant) (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, this 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 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 demonstrated 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 but 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 the time that 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 are associated with long-term disease control rather than

detrimentally increasing the potential target pool for HIV propagation.

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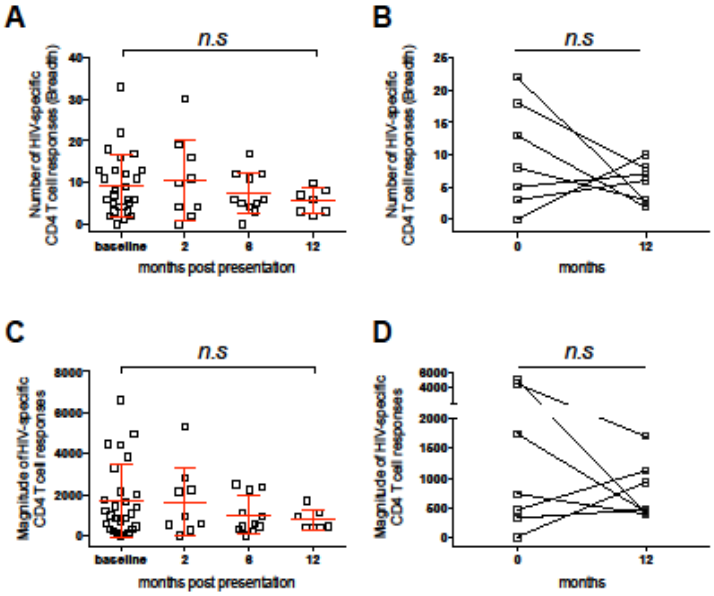
REFERENCES

1. **Yi JS, Du M, Zajac AJ.** 2009. A vital role for interleukin-21 in the control of a chronic viral infection. Science **324:**1572–1576. http://dx.doi.org/10.1126/science.1175194.
2. **Elsaesser H, Sauer K, Brooks DG.** 2009. IL-21 is required to control chronic viral infection. Science **324:**1569–1572. http://dx.doi.org/10.1126/science.1174182.
3. **Frohlich A, Kisielow J, Schmitz I, Freigang S, Shamshiev AT, Weber J, Marsland BJ, Oxenius A, Kopf M.** 2009. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science **324:**1576–1580. http://dx.doi.org/10.1126/science.1172815.
4. **Chevalier MF, Julg B, Pyo A, Flanders M, Ranasinghe S, Soghoian DZ, Kwon DS, Rychert J, Lian J, Muller MI, Cutler S, McAndrew E, Jessen H, Pereyra F, Rosenberg ES, Altfeld M, Walker BD, Streeck H.** 2011. HIV-1-specific interleukin-21+ CD4+ T cell responses contribute to durable viral control through the modulation of HIV-specific CD8+ T cell function. J. Virol. **85:**733–741. http://dx.doi.org/10.1128/JVI.02030-10.
5. **Sun JC, Bevan MJ.** 2003. Defective CD8 T cell memory following acute infection without CD4 T cell help. Science **300:**339–342. http://dx.doi.org/10.1126/science.1083317.
6. **Sun JC, Williams MA, Bevan MJ.** 2004. CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. Nat. Immunol. **5:**927–933. http://dx.doi.org/10.1038/ni1105.
7. **Streeck H, D'Souza MP, Littman DR, Crotty S.** 2013. Harnessing CD4(+) T cell responses in HIV vaccine development. Nat. Med. **19:**143–149. http://dx.doi.org/10.1038/nm.3054.
8. **Omiya R, Buteau C, Kobayashi H, Paya CV, Celis E.** 2002. Inhibition of EBV-induced lymphoproliferation by CD4(+) T cells specific for an MHC class II promiscuous epitope. J. Immunol. **169:**2172–2179. http://dx.doi.org/10.4049/jimmunol.169.4.2172.
9. **Brown DM, Dilzer AM, Meents DL, Swain SL.** 2006. CD4 T cell-mediated protection from lethal influenza: perforin and antibody-mediated mechanisms give a one-two punch. J. Immunol. **177:**2888–2898. http://dx.doi.org/10.4049/jimmunol.177.5.2888.
10. **Wilkinson TM, Li CK, Chui CS, Huang AK, Perkins M, Liebner JC, Lambkin-Williams R, Gilbert A, Oxford J, Nicholas B, Staples KJ, Dong T, Douek DC, McMichael AJ, Xu XN.** 2012. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. Nat. Med. **18:**274–280. http://dx.doi.org/10.1038/nm.2612.
11. **Soghoian DZ, Jessen H, Flanders M, Sierra-Davidson K, Cutler S, Pertel T, Ranasinghe S, Lindqvist M, Davis I, Lane K, Rychert J, Rosenberg ES, Piechocka-Trocha A, Brass AL, Brenchley JM, Walker BD, Streeck H.** 2012. HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. Sci. Transl. Med. **4:**123ra25. http://dx.doi.org/10.1126/scitranslmed.3003165.
12. **Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, Casazza JP, Kuruppu J, Kunstman K, Wolinsky S, Grossman Z, Dybul M, Oxenius A, Price DA, Connors M, Koup RA.** 2002. HIV preferentially infects HIV-specific CD4+ T cells. Nature **417:**95–98. http://dx.doi.org/10.1038/417095a.
13. **Ranasinghe S, Flanders M, Cutler S, Soghoian DZ, Ghebremichael M, Davis I, Lindqvist M, Pereyra F, Walker BD, Heckerman D, Streeck H.** 2012. HIV-specific CD4 T cell responses to different viral proteins have discordant associations with viral load and clinical outcome. J. Virol. **86:**277–283. http://dx.doi.org/10.1128/JVI.05577-11.
14. **Ranasinghe S, Cutler S, Davis I, Lu R, Soghoian DZ, Qi Y, Sidney J, Kranias G, Flanders MD, Lindqvist M, Kuhl B, Alter G, Deeks SG, Walker BD, Gao X, Sette A, Carrington M, Streeck H.** 2013. Association of HLA-DRB1-restricted CD4(+) T cell responses with HIV immune control. Nat. Med. **19:**930–933. http://dx.doi.org/10.1038/nm.3229.
15. **Pitcher CJ, Quittner C, Peterson DM, Connors M, Koup RA, Maino VC, Picker LJ.** 1999. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat. Med. **5:**518–525. http://dx.doi.org/10.1038/8400.
16. **Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, Walker BD.** 1997. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science **278:**1447–1450. http://dx.doi.org/10.1126/science.278.5342.1447.
17. **Harari A, Petitpierre S, Vallelian F, Pantaleo G.** 2004. Skewed representation of functionally distinct populations of virus-specific CD4 T cells in HIV-1-infected subjects with progressive disease: changes after antiretroviral therapy. Blood **103:**966–972. http://dx.doi.org/10.1182/blood-2003-04-1203.
18. **Harari A, Rizzardi GP, Ellefsen K, Ciuffreda D, Champagne P, Bart PA, Kaufmann D, Telenti A, Sahli R, Tambussi G, Kaiser L, Lazzarin A, Perrin L, Pantaleo G.** 2002. Analysis of HIV-1- and CMV-specific memory CD4 T-cell responses during primary and chronic infection. Blood **100:**1381–1387. http://dx.doi.org/10.1182/blood-2001-11-0080.
19. **Oxenius A, Fidler S, Brady M, Dawson SJ, Ruth K, Easterbrook PJ, Weber JN, Phillips RE, Price DA.** 2001. Variable fate of virus-specific CD4(+) T cells during primary HIV-1 infection. Eur. J. Immunol. **31:**3782–3788. http://dx.doi.org/10.1002/1521-4141(200112)31:12<3782::AID-IMMU3782>3.0.CO;2-#.
20. **Zaunders J, Carr A, McNally L, Penny R, Cooper DA.** 1995. Effects of primary HIV-1 infection on subsets of CD4+ and CD8+ T lymphocytes. AIDS **9:**561–566. http://dx.doi.org/10.1097/00002030-199506000-00005.
21. **Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, Heldebrant C, Smith R, Conrad A, Kleinman SH, Busch MP.** 2003. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS **17:**1871–1879. http://dx.doi.org/10.1097/00002030-200309050-00005.
22. **Lagrelius M, Jones P, Franck K, Gaines H.** 2006. Cytokine detection by multiplex technology useful for assessing antigen specific cytokine profiles and kinetics in whole blood cultured up to seven days. Cytokine **33:**156–165. http://dx.doi.org/10.1016/j.cyto.2006.01.005.
23. **Streeck H, Frahm N, Walker BD.** 2009. The role of IFN-gamma Elispot assay in HIV vaccine research. Nat. Protoc. **4:**461–469. http://dx.doi.org/10.1038/nprot.2009.7.
24. **Alter G, Tsoukas CM, Rouleau D, Cote P, Routy JP, Sekaly RP, Bernard NF.** 2004. Assessment of longitudinal changes in HIV-specific effector activity in subjects undergoing untreated primary HIV infection. AIDS **18:**1979–1989. http://dx.doi.org/10.1097/00002030-200410210-00002.
25. **Streeck H, Jolin JS, Qi Y, Yassine-Diab B, Johnson RC, Kwon DS, Addo MM, Brumme C, Routy JP, Little S, Jessen HK, Kelleher AD, Hecht FM, Sekaly RP, Rosenberg ES, Walker BD, Carrington M, Altfeld M.** 2009. HIV-1-specific CD8+ T cell responses during primary infection are major determinants of the viral set point and loss of CD4+ T cells. J. Virol. **83:**7641–7648. http://dx.doi.org/10.1128/JVI.00182-09.
26. **Price DA, Goulder PJ, Klenerman P, Sewell AK, Easterbrook PJ, Troop M, Bangham CR, Phillips RE.** 1997. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. Proc. Natl. Acad. Sci. U. S. A. **94:**1890–1895. http://dx.doi.org/10.1073/pnas.94.5.1890.
27. **Altfeld M, Allen TM, Yu XG, Johnston MN, Agrawal D, Korber BT, Montefiori DC, O'Connor DH, Davis BT, Lee PK, Maier EL, Harlow J, Goulder PJ, Brander C, Rosenberg ES, Walker BD.** 2002. HIV-1 superinfection despite broad CD8+ T-cell responses containing replication of the primary virus. Nature **420:**434–439. http://dx.doi.org/10.1038/nature01200.
28. **Streeck H, Li B, Poon AF, Schneidewind A, Gladden AD, Power KA, Daskalakis D, Bazner S, Zuniga R, Brander C, Rosenberg ES, Frost SD, Altfeld M, Allen TM.** 2008. Immune-driven recombination and loss of control after HIV superinfection. J. Exp. Med. **205:**1789–1796. http://dx.doi.org/10.1084/jem.20080281.
29. **Lyles RH, Munoz A, Yamashita TE, Bazmi H, Detels R, Rinaldo CR, Margolick JB, Phair JP, Mellors JW.** 2000. Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. Multicenter AIDS Cohort Study. J. Infect. Dis. **181:**872–880. http://dx.doi.org/10.1086/315339.
30. **Mellors JW, Margolick JB, Phair JP, Rinaldo CR, Detels R, Jacobson LP, Munoz A.** 2007. Prognostic value of HIV-1 RNA, CD4 cell count, and CD4 cell count slope for progression to AIDS and death in untreated

HIV-1 infection. JAMA **297:**2346–2350. http://dx.doi.org/10.1001/jama.297.21.2349.

31. **Mellors JW, Rinaldo CR, Jr, Gupta P, White RM, Todd JA, Kingsley LA.** 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science **272:**1167–1170. http://dx.doi.org/10.1126/science.272.5265.1167.
32. **Brenchley JM, Ruff LE, Casazza JP, Koup RA, Price DA, Douek DC.** 2006. Preferential infection shortens the life span of human immunodeficiency virus-specific CD4+ T cells in vivo. J. Virol. **80:**6801–6809. http://dx.doi.org/10.1128/JVI.00070-06.
33. **Riou C, Ganusov VV, Campion S, Mlotshwa M, Liu MK, Whale VE, Goonetilleke N, Borrow P, Ferrari G, Betts MR, Haynes BF, McMichael AJ, Gray CM.** 2012. Distinct kinetics of Gag-specific CD4+ and CD8+ T cell responses during acute HIV-1 infection. J. Immunol. **188:**2198–2206. http://dx.doi.org/10.4049/jimmunol.1102813.
34. **Gloster SE, Newton P, Cornforth D, Lifson JD, Williams I, Shaw GM, Borrow P.** 2004. Association of strong virus-specific CD4 T cell responses with efficient natural control of primary HIV-1 infection. AIDS **18:**749–755. http://dx.doi.org/10.1097/00002030-200403260-00005.
35. **Lubong Sabado R, Kavanagh DG, Kaufmann DE, Fru K, Babcock E, Rosenberg E, Walker B, Lifson J, Bhardwaj N, Larsson M.** 2009. In vitro priming recapitulates in vivo HIV-1 specific T cell responses, revealing rapid loss of virus reactive CD4 T cells in acute HIV-1 infection. PLoS One **4:**e4256. http://dx.doi.org/10.1371/journal.pone.0004256.
36. **Malhotra U, Holte S, Zhu T, Delpit E, Huntsberry C, Sette A, Shankarappa R, Maenza J, Corey L, McElrath MJ.** 2003. Early induction and maintenance of Env-specific T-helper cells following human immunodeficiency virus type 1 infection. J. Virol. **77:**2663–2674. http://dx.doi.org/10.1128/JVI.77.4.2663-2674.2003.
37. **Frater J, Ewings F, Hurst J, Brown H, Robinson N, Fidler S, Babiker A, Weber J, Porter K, Phillips RE.** 4 December 2013. HIV-1 specific CD4 responses in primary HIV-1 infection predict disease progression in the SPARTAC trial. AIDS http://dx.doi.org/10.1097/QAD.0000000000000130.



SUPPLEMENTARY FIGURE 1. *HIV-specific CD4 T cell responses remain stable within the first year of infection in donors in Fiebig 2/3.* **(A)** The number of HIV-specific CD4 T cell responses (breadth) for 27 subjects is shown at baseline and 2, 6 and 12 months post presentation. We found a non-significant decrease of breadth from baseline (avg.: 9.3) to 12 months post infection (avg.:5.6) when comparing individuals identified during Fiebig 2 and 3 only (Mann-Whitney *P*=0.27). Open circles represent individual donors. **(B)** Matched-pair analysis of breadth of HIV-specific CD 4 T cell responses in seven individuals at baseline and twelve months post infection showed divergent results. Decrease of breadth in 4/7 and increase of breadth 3/7. **(C)** The cross-sectional analysis of the total magnitude of HIV-specific CD4 T cell responses is shown for 27 subjects at baseline and 2, 6 and 12 months post presentation. We found an overall tendency of decrease in magnitude from acute (baseline 1704 SFC/M) to chronic infection (12 months post presentation 781 SFC/M), which was not significant (Mann-Whitney *P*=0.37). **(D)** Matched-pair analysis of the magnitude of HIV-specific CD4 T cell responses in seven individuals at baseline and twelve months post presentation showed an overall tendency of a decrease of magnitude, was not statistical significant (Mann-Whitney *P*=0.7).

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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