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HIV-Specific CD4 T Cell Responses to Different Viral Proteins Have Discordant Associations with Viral Load and Clinical Outcome

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A successful prophylactic vaccine is characterized by long-lived immunity, which is critically dependent on CD4 T cell-mediated helper signals. Indeed, most licensed vaccines induce antigen-specific CD4 T cell responses, in addition to high-affinity antibodies. However, despite the important role of CD4 T cells in vaccine design and natural infection, few studies have characterized HIV-specific CD4 T cells due to their preferential susceptibility to HIV infection. To establish at the population level the impact of HIV-specific CD4 T cells on viral control and define the specificity of HIV-specific CD4 T cell peptide targeting, we conducted a comprehensive analysis of these responses to the entire HIV proteome in 93 subjects at different stages of HIV infection. We show that HIV-specific CD4 T cell responses were detectable in 92% of individuals and that the breadth of these responses showed a significant inverse correlation with the viral load ($P = 0.009$, $R = -0.31$). In particular, CD4 T cell responses targeting Gag were robustly associated with lower levels of viremia ($P = 0.0002$, $R = -0.45$). Importantly, differences in the immunodominance profile of HIV-specific CD4 T cell responses distinguished HIV controllers from progressors. Furthermore, Gag/Env ratios were a potent marker of viral control, with a high frequency and magnitude of Gag responses and low proportion of Env responses associated with effective immune control. At the epitope level, targeting of three distinct Gag peptides was linked to spontaneous HIV control ($P = 0.60$ to 0.85). Inclusion of these immunogenic proteins and peptides in future HIV vaccines may act as a critical cornerstone for enhancing protective T cell responses.

The role of CD4 T cell responses in the control of several chronic viral infections has been well characterized (28), yet surprisingly little is known about the presence of these responses in the setting of HIV infection. In particular, the contribution of HIV-specific CD4 T cell responses to viral control is unclear, and the qualitative differences between efficacious responses and inadequate responses have not been comprehensively investigated at the level of individual proteins and peptides. The identification of the specificities and efficacies of these responses is likely to be vital for HIV vaccine design, since any protein-based vaccines will most likely induce some degree of HIV-specific CD4 T cells. Indeed, a modest protective effect was recently observed in the RV144 "Thai trial" HIV vaccine (11, 19), which not only elicited nonneutralizing antibody responses but also induced HIV-specific CD4 T cell responses in vaccinees. Moreover, it will be important to identify which HIV-specific CD4 T cells are induced in natural HIV infection in order to successfully augment the efficacy of these responses in future vaccines.

There is a growing body of evidence that HIV-specific CD4 T cells may enhance immunological control of HIV viremia either by providing help for CD8 T and B cells (5) or by direct antiviral effects (14, 22). In particular, studies have demonstrated that the presence of HIV-specific CD4 T cells is enriched in individuals spontaneously able to control viral replication in the absence of antiretroviral therapy (8, 16, 21). Furthermore, the presence of HIV-specific CD4 T cells in highly exposed but HIV-seronegative individuals also suggests a protective role for this cellular subset (20). In addition, it is important to note that although a small percentage of HIV-specific CD4 T cells are preferentially targeted by HIV, the vast majority of these cells remain uninfected at all times *in vivo* (6), where they are likely to mediate an important antiviral role.

Currently, only a small fraction of HIV-specific CD4 T cell responses have been identified. The specificities of the individual peptide responses have not been analyzed, since previous studies predominantly utilized peptide pools (2, 9, 18) or assessed the polyfunctionality of these responses to a single HIV protein (8). Thus, our study represents the first comprehensive analysis conducted at the population level to identify HIV-specific CD4 T cell responses to individual HIV protein subunits and peptides and to elucidate the immunodominance profile of these responses in a large cohort of HIV controllers and progressors. Our results demonstrate that the breadth of total HIV-specific CD4 T cell responses and, also, the breadth and magnitude of Gag-specific responses are inversely correlated with viral load. Furthermore, differences exist in the patterns of immunodominant peptide targeting of subjects with differing clinical outcomes. Dominant targeting of Gag was exhibited by HIV controllers, while HIV progressors frequently targeted Env epitopes that have previously been underestimated in chronic infection. Indeed, the ratio of Gag/Env responses was a strong marker of viral control. In addition, three distinct Gag peptides were identified that linked to spontaneous HIV control. Together, these data are consistent with our hypothesis that HIV-specific CD4 T cells are likely to make an important contribution to the durable control of HIV replication.

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TABLE 1 Clinical characteristics of study participants

Cohort	No. of subjects	Subcohort definition (viral load & T cell count)	Median (range) viral load (copies/ml)	Median (range) $CD4^+$ cell count (cells/μl)	Gender (% male)	Race (% white)	Median (range) age (yr)	Risk factor[a] (%)
Controllers	42							
EC	17	<50 copies/ml & >500 $CD4^+$ T cells	<48	832 (528-1,583)	82	82	53 (20-65)	47
VC	25	51–2,000 copies/ml & >450 $CD4^+$ T cells	282 (65–1,680)	795 (450–1,101)	76	60	49.5 (31–64)	48
Chronic progressors	41							
LVP	12	2,001–10,000 copies/ml	4,076 (2,550–9,240)	511 (301–1,023)	75	25	37.5 (25–49)	75
IVP	16	10,001–50,000 copies/ml	19,673 (10,200–43,431)	550 (386–989)	100	69	39.5 (22–66)	88
HVP	13	>50,001 copies/ml	65,804 (50,904–255,000)	367 (191–594)	93	64	47.5 (33–63)	71
ART-treated subjects	10	Fully suppressed on ART for >6 months	<48	473 (110–1,097)	100	60	44.5 (26–50)	30

[a] Men who have sex with men.

MATERIALS AND METHODS

Subjects. A total of 93 HIV-infected individuals were recruited from the Massachusetts General Hospital after providing informed consent for participation in the study. The HIV-infected individuals were stratified into three major groups: 42 HIV controllers with spontaneously controlled infection (viral loads of <2,000 HIV RNA copies/ml for >1 year) in the absence of antiretroviral therapy (ART), 41 HIV chronic progressors (treatment naïve and with >2,000 HIV RNA copies/ml), and 10 ART-treated individuals (fully suppressed viral load during continuous ART for >6 months). The ART-naïve groups were further stratified by viral load into the defined subgroups elite controller (EC), viremic controller (VC), low-viremic progressor (LVP), intermediate-viremic progressor (IVP), and high-viremic progressor (HVP), as outlined in Table 1.

CD8 depletion and modified IFN-γ ELISPOT assay. 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). Flow cytometry confirmed at least 98% CD8-negative PBMC prior to use in the IFN-γ ELISPOT assay. HIV-specific CD4 responses were screened against a panel of 410 overlapping peptides (OLPs) spanning the entire HIV-1 clade B consensus 2001 proteome using a modified gamma interferon (IFN-γ) enzyme-linked immunospot (ELISPOT) assay. Fresh CD8-depleted PBMC were plated in 96-well polyvinylidene plates (Millipore, MA) precoated with 2 μg/ml anti-IFN-γ monoclonal antibody 1-D1K (Mabtech, Stockholm, Sweden). Total amounts of 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 h at 37°C, 5% CO_2 to elicit the maximal cytokine secretion (12, 17) and 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. 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 $\geq$55 SFC/10^6 CD8-depleted PBMC, at least 4 times the mean background, and also, $\geq$4 times the standard deviation of the number of SFC/10^6 CD8-depleted PBMC within the negative controls. The breadth of responses is defined as the sum of IFN-γ-positive peptide responses within a given individual across the entire HIV proteome or within a group for a specified protein. Positive responses to two adjacent overlapping peptides were treated as a single epitope to ensure that the breadth of CD4 T cell responses was not overestimated.

Statistical analysis. L1-regularized logistic regression (26) was used to predict an association with viral control (HIV controllers versus chronic progressors) based on OLP-specific CD4 T cell responses. Specifically, this predictive model was applied to 1,000 bootstraps of the data to determine the frequency with which the feature corresponding to an OLP target appeared in the model. All peptide specificities were tested, and a P value threshold of >0.5 was used to infer the likelihood that a peptide response was associated with loss or gain of HIV control. The Lasso penalty for each prediction was determined by nested 10-fold cross-validation. Analysis of variance (ANOVA) was used to compare continuous outcomes among groups, and adjustment was made for multiple comparisons using the Bonferroni multiple testing correction procedure. Spearman's rank test was used for correlation analyses. Viral-load values below the limit of detection of 50 HIV RNA copies/ml for EC were set at 49 HIV RNA copies/ml. Five EC for whom additional single-copy analysis values were available were utilized for the correlation analysis.

RESULTS AND DISCUSSION

To evaluate the role of HIV-specific CD4 T cells in the control of viremia at the population level, we comprehensively assessed a cohort of 93 HIV-infected individuals for the presence and specificity of HIV-specific IFN-γ-positive CD4 T cell responses in the setting of progressive or spontaneously controlled HIV infection. We utilized 410 overlapping peptides (OLPs) spanning the entire HIV clade B proteome in a modified *ex vivo* IFN-γ ELISPOT assay to screen HIV-specific CD4 T cell responses in our large cohort and to accurately map these T cell responses at the level of single peptides (23).

Strikingly, nearly all patients had at least one detectable HIV-

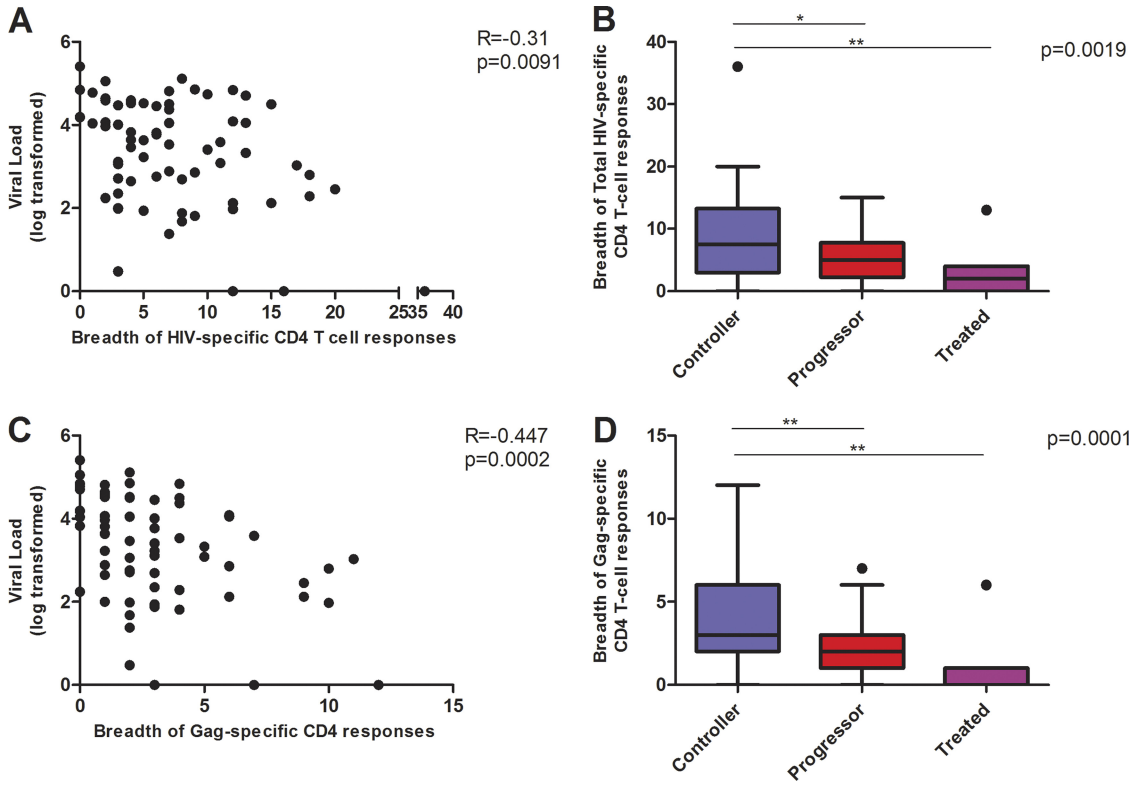

FIG 1 HIV- and Gag-specific CD4 T cell responses are associated with viral load and clinical outcome. (A) Correlation between the breadth of HIV-1-specific CD4 T cell responses and plasma viral load for treatment-naïve subjects. The breadth of HIV-specific CD4 T cell responses showed a significant inverse correlation with viral load ($R = -0.31$; $P = 0.009$). (B) Breadth of total HIV-specific CD4 T cell responses is shown for all 93 HIV-infected patients stratified into subgroups of HIV controllers, chronic progressors, and treated individuals. Significant differences were observed in the overall breadth of these responses based on ANOVA ($P = 0.0019$) with Bonferroni correction (*, $P < 0.05$; **, $P < 0.01$). The mean difference in breadth of HIV-specific CD4 T cell responses between controllers and progressors is 4.3 (95% confidence interval [CI], 1.3-7.4), and the mean difference between controllers and ART-treated individuals is 8.0 (95% CI, 4.3 to 11.2) Box and whisker plots (Tukey) are used to show the distribution of data, with filled circles representing individual values that are greater or less than 1.5 times the interquartile range. (C) Correlation between the breadth of Gag-specific CD4 T cell responses and plasma viral load for treatment-naïve subjects. The breadth of Gag-specific CD4 T cell responses showed a significant inverse correlation with viral load ($R = -0.45$; $P = 0.0002$). (D) Breadth of Gag-specific CD4 T cell responses is shown for all 93 HIV-infected patients stratified into subgroups. Significant differences were observed in the overall breadth of these responses based on ANOVA ($P = 0.0001$) with Bonferroni correction (**, $P < 0.01$). The mean difference between breadth of Gag-specific CD4 T cell responses between controllers and progressors is 2.9 (95% CI, 1.3-4.6), and the mean difference between controllers and ART-treated individuals is 4.5 (95% CI, 2.6-6.4).

specific CD4 T cell response (92%, 86/93), and most subjects demonstrated multiple responses (89%, 83/93). Moreover, 42 subjects were able to spontaneously control viral replication below a level of 2,000 HIV RNA copies/ml in the absence of any antiretroviral therapy. These individuals, referred to as "HIV controllers," showed on average significantly greater HIV-specific CD4 T cell responses (average number of peptides, 10.9; standard error of the mean [SEM], ±1.33) than the 41 subjects with progressive HIV infection (average number of peptides, 6.6; SEM, ±0.74) ($P = <0.05$), as defined in Table 1. Importantly, the total breadth of HIV-specific CD4 T cell responses was inversely correlated to viral load ($P = 0.009$, $R = -0.31$) (Fig. 1A). This association was found to be driven by CD4 T cell responses targeting the HIV Gag protein ($P = 0.0002$, $R = -0.45$) (Fig. 1C). Indeed, HIV controllers demonstrated a significantly higher breadth of Gag-specific responses than noncontrollers ($P < 0.01$) (Fig. 1D), and the magnitude of responses to Gag also showed a strong inverse correlation with viral load (see Fig. S1 in the supplemental material) ($P < 0.0001$, $R = -0.45$). While significant differences were observed between HIV controllers and chronic progressors, no statistical differences were detected between elite and viremic controllers. Taken together, these data are consistent with our hypothesis that HIV-specific CD4 T cell responses are likely to be beneficial in the control of HIV during chronic infection. In contrast, although it has been suggested that HIV-specific CD8 T cell responses are responsible for the control of HIV replication, it is important to note that a significant association between the breadth of total HIV-specific CD8 T cell responses and control of viremia has not yet been demonstrated in chronic infection (1, 10).

We next characterized the epitope specificity of HIV-specific CD4 T cell responses to individual peptides. Interestingly, while previous reports utilizing peptide pools have only reported narrow targeting of Gag and Nef (2, 9), we observed a tight clustering of CD4 T cell responses in three regions of the HIV proteome (Fig. 2). The most dominantly targeted region was Gag, particularly the N terminus of p17 and a 20-amino-acid region within the p24 protein, which have been shown to be essential for the formation of the matrix protein and capsid dimerization (7). These Gag epitopes overlap with defined immunodominant HIV-specific CD8 T cell responses that have been previously associated with

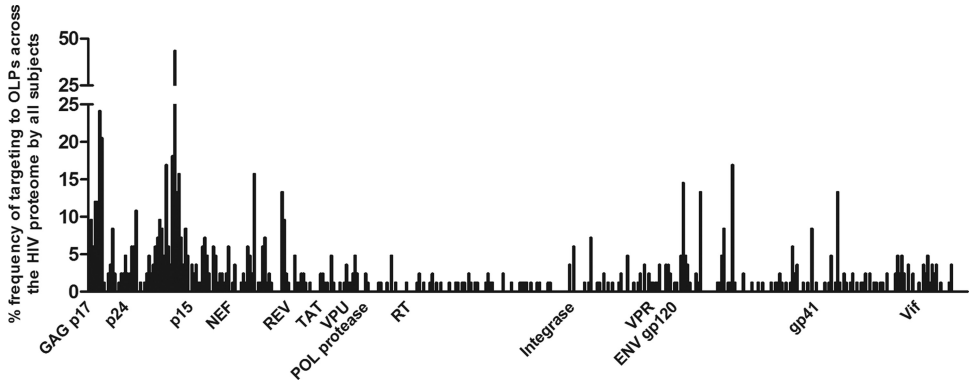

FIG 2 HIV-specific CD4$^+$ T cell responses dominantly target epitopes within Gag, Nef, and Env gp120. The percentage of treatment-naïve subjects demonstrating detectable HIV-specific CD4 T cell recognition to each of 410 overlapping peptides (OLPs) spanning the entire HIV proteome is shown.

viral control (24), raising the possibility that dual dominant targeting by CD4 and CD8 T cells may enhance HIV control. Additional immunodominant epitopes were located within glycoprotein gp120 of the envelope (Env) region, predominantly in conserved domains and to a lesser extent in variable loops, as well as the accessory protein Nef. Thus, our study highlights an abundance of detectable HIV-specific CD4 T cell responses across the HIV proteome, dominantly targeting Gag and Nef, in addition to previously underestimated yet immunodominant Env responses (Table 2).

Importantly, we observed remarkable differences in epitope specificity in subjects able to spontaneously control viral replication and in high-viremia progressors. HIV-specific CD4 T cell responses in HIV controllers dominantly targeted epitopes against the p24 capsid of Gag, while nearly no responses against Env were detectable. In stark contrast, high-viremia progressors showed virtually no HIV-specific CD4 T cell responses against the p24 region of Gag, and yet they demonstrated a notable dominance of HIV-specific responses against gp120 (Fig. 3A and B). Interestingly, this shift in CD4 T cell dominance not only differentiated HIV controllers from progressors but also distinguished elite controllers from viremic controllers and high-viremia progressors from other noncontroller subgroups (as defined in Table 1). In particular, while elite and viremic controllers demonstrated significantly greater breadth of p24 epitope targeting than high-viremic progressors ($P \leq 0.0001$), the targeting of p24 was also significantly different in high-viremic progressors and the low-/intermediate-viremic progressors ($P \leq 0.05$). Differential targeting of proteins between subgroups was also observed for p17 ($P = 0.018$), pol ($P \leq 0.0001$), and gp120 ($P = 0.007$) when assessed by ANOVA.

Given the discordant association of Gag- and Env-specific CD4 T cell responses with viral load and clinical outcome, we found that the mean ratio of the magnitudes of Env and Gag responses (Env/Gag ratio) was a robust marker of viral control (Fig. 3C). While the lowest mean Env/Gag ratio was found in elite controllers (0.20), the highest mean ratio was recorded in high-viremia progressors (2.63), with concordant results also observed for the frequency of these responses (Fig. 3D). Overall, the ratios of Env/Gag magnitudes ($P = 0.017$) and frequencies ($P = 0.021$) were significantly different among the subgroups (ANOVA). This analysis illustrates that while Env-specific CD4 T cell responses may coexist with Gag, a higher frequency and magnitude of Gag responses and dampened targeting of Env are associated with viral control within a given HIV-infected individual. Indeed, the frequent targeting of Gag protein identified among HIV controllers

TABLE 2 Immunodominant peptides targeted by treatment-naïve subjects[a]

% of subjects responding	Protein	HXB2 position	OLP	Peptide sequence
40	Gag-p24	164–181	41	YVDRFYKTLRAEQASQEV
18	Gag-p17	37–51	6	ASRELERFAVNPGLL
16	Nef	81–97	78	YKAAVDLSHFLKEKGGL
16	Env-gp120	207–224	316	KVSFEPIPIHYCAPAGFA
14	Gag-p24	133–150	37	WIILGLNKIVRMYSPTSI
14	Env-gp120	30–46	293	AAEQLWVTVYYGVPVWK
13	Env-gp120	88–105	301	NVTENFNMWKNNMVEQMH
12	Env-gp41	61–77	365	GIKQLQARVLAVERYLK
11	Gag-p17	25–41	4	GKKKYKLKHIVWASREL
11	Gag-p24	31–47	23	AFSPEVIPMFSALSEGA
11	Gag-p24	180–193	43	EVKNWMTETLLVQNA
11	Nef	176–193	91	PEKEVLVWKFDSRLAFHH
10	Gag-p17	9–26	2	SGGELDRWEKIRLRPGGK
10	Gag-p17	42–58	7	ERFAVNPGLLETSEGCR

[a] An immunodominant response is defined herein as the most frequently detected peptide-specific CD4 T cell response within the study population. The frequencies of subjects responding to immunodominant peptides are shown as percentages.

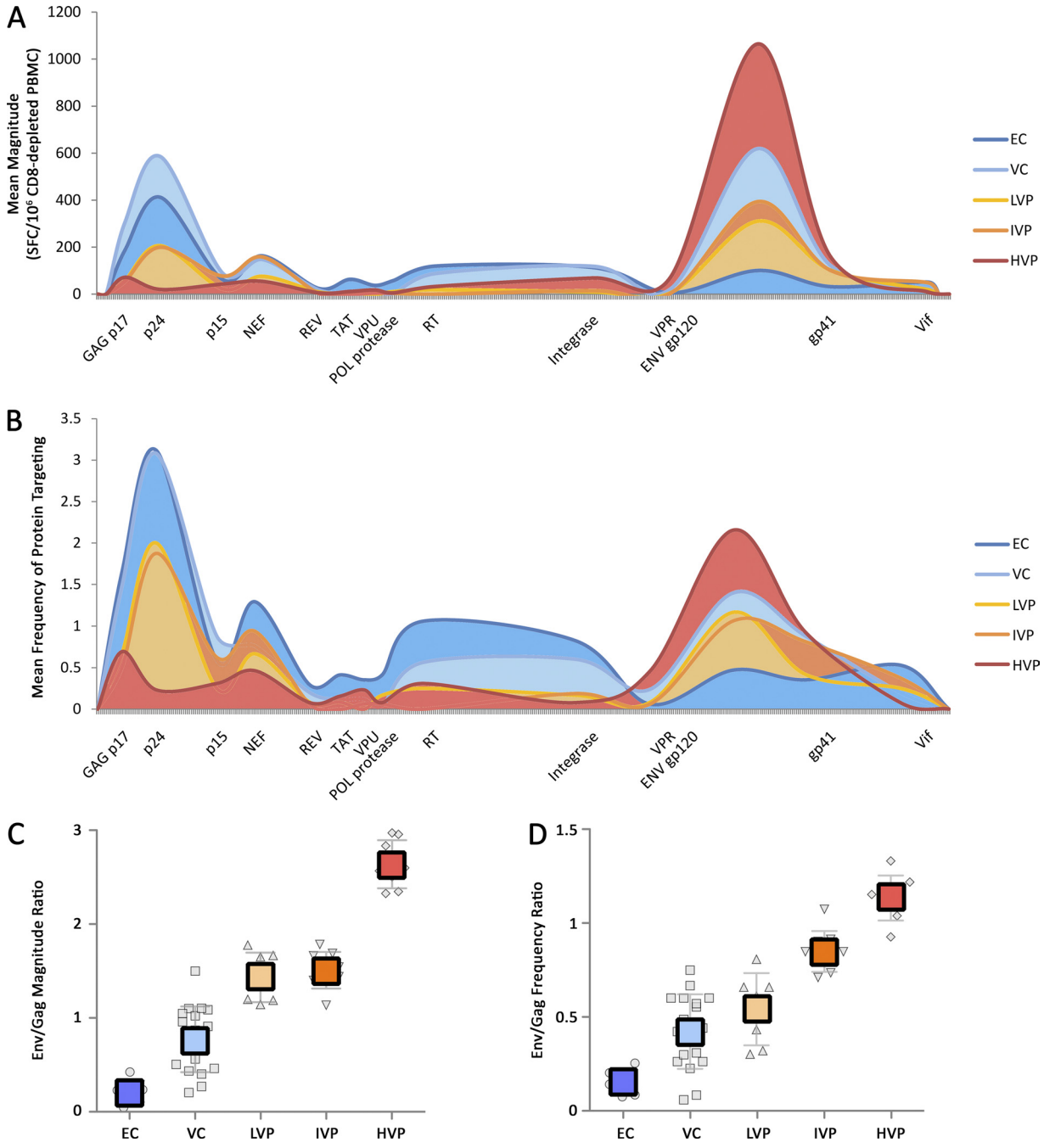

FIG 3 HIV-specific CD4 T cell responses targeting different protein subunits have discordant associations with clinical outcome. (A) The mean magnitudes of protein-specific CD4 T cell responses vary between HIV controller and progressor subgroups. The sum of responses measured for each individual epitope was corrected for the number of subjects and protein length to calculate the mean magnitude of responses per protein. Significant differences in magnitude were observed for responses to p17 ($P = 0.019$), p24 ($P \leq 0.0001$), p15 ($P = 0.022$), pol ($P = 0.0004$), and gp120 ($P = 0.002$) based on ANOVA. (B) The frequencies of protein-specific CD4 T cell responses show striking differences in the immunodominance profiles of HIV controllers and progressors, with Gag dominantly targeted by HIV controllers. The sum of IFN-γ-positive responses measured for individual epitopes was corrected for the number of subjects and protein length to show the mean frequency of protein targeting. Significant differences in the mean frequency of targeting were observed for p17 ($P = 0.018$), p24 ($P \leq 0.0001$), pol ($P \leq 0.0001$), and gp120 ($P = 0.007$) based on ANOVA. The ratio of the Env/Gag mean magnitude (C) or mean frequency (D) of HIV-specific CD4 T cell responses stratifies HIV controller and progressor subgroups. Significant differences were observed in the magnitudes ($P = 0.017$) and frequencies ($P = 0.021$) of Env/Gag ratios based on ANOVA. Error bars represent standard deviation.

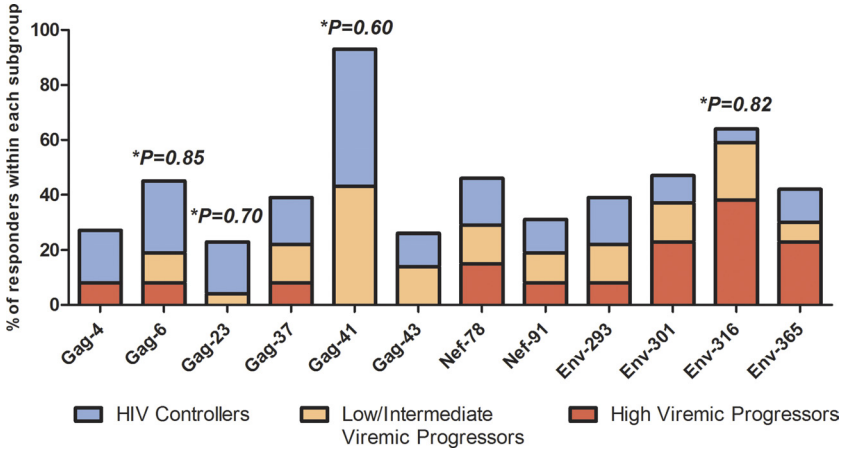

FIG 4 Three Gag peptides are strongly associated with spontaneous HIV control in the absence of antiretroviral therapy. Immunodominant peptides within the cohort were differentially targeted by HIV controllers, low-/intermediate-viremia progressors, and high-viremia progressors. The percentage of patients within each of the three subgroups that showed a IFN-γ-positive CD4 T cell response to each immunodominant peptide is shown in stacked bars. Peptides Gag-23, Gag-41, and Gag-43 were targeted by 0% of high-viremia progressors. Multivariate bootstrap analysis of HIV-specific CD4 T cell responses in HIV controllers and progressors further identified three Gag epitopes that were predicted to associate with spontaneous control ($P = 0.60–0.85$) and one Env gp120 peptide associated with high viremia ($P = 0.82$). All peptide specificities were tested, and a probability threshold of >0.5 was used to infer the likelihood that a peptide response was statistically associated with loss or gain of HIV control.

in this analysis was confirmed to be significantly associated with low viremia in a multivariate bootstrap analysis ($P = 0.001$). In particular, control of viral replication was positively associated with a high frequency of responses to Gag p24, with medians of 3.3 responses in controllers and only 1.6 responses in noncontrollers ($P = 0.002$).

Finally, we assessed whether the HIV-specific CD4 T cell response to any of the 410 individual HIV peptides was associated with spontaneous control. We noted that the targeting of several individual peptides across the HIV proteome (Table 2) differed markedly between the patient groups (Fig. 4). A multivariate bootstrap analysis identified three distinct Gag-specific CD4 T cell epitopes that were linked to spontaneous HIV control, ASRELERFA VNPGLL$_{37–51}$ ($P = 0.85$) in p17 and AFSPEVIPMFSALSEGA$_{31–47}$ ($P = 0.70$) and YVDRFYKTLRAEQASQEV$_{164–181}$ ($P = 0.60$) in p24. These three immunodominant Gag peptides were associated with spontaneous control in our cohort, independent of the vast variation in patient HLA haplotypes. Interestingly, the most immunodominant peptide lies within a 20-amino-acid sequence known as the major homology region (MHR), which may be especially important for CD4 T cell targeting. This sequence is absolutely conserved among all studied lentiviruses due to its role in p24 capsid dimerization (7), and escape within this region is known to impose a detrimental impact on viral fitness and replication capacity (24). Furthermore, it has been demonstrated that sequence conservation of the MHR results in a high degree of cross-clade CD4 T cell recognition across multiple HIV-1 and HIV-2 isolates (15, 18) and elicits high-avidity CD4 T cells (27). Thus, it is likely that the functional and structural constraints imposed upon these peptides, together with highly avid CD4 T cell targeting, act in conjunction to limit viral escape within these Gag peptides.

In contrast, the epitope KVSFEPIPIHYCAPAGFA$_{206–220}$, located in the C2 domain of gp120, was associated with high viremia ($P = 0.82$) and was almost exclusively recognized by HIV progressors, including nearly half of all high-viremic progressors (46%). This region is highly conserved between viral isolates since residues within this peptide represent a contact site with gp41 that is critical for CD4 binding (4). However, studies have shown that modifications in N-glycan sugar residues flanking KVSFEPIPIHY CAPAGFA can modulate epitope processing and radically alter T helper responses (13). Thus, high-viremic progressors may exhibit a CD4 T cell response specific to viruses that are escaping through mutations in the glycan shield.

Taken together, our data suggest a fine balance between Gag-specific CD4 T cell responses that are associated with effective immune control and Env-specific CD4 T cell responses that are linked with predominantly unfavorable clinical outcomes. Interestingly, a similar association has been previously observed for HIV-specific CD8 T cell responses at the population level, suggesting that targeting of the Gag protein may be inherently superior in HIV control (10). In fact, the epitope-specific CD4 T cell responses identified as being associated with slow protection lie within the same regions of Gag that have been previously associated with protection in subjects expressing protective HLA class I alleles such as HLA-B57 or -B27 (24). Previous data have suggested that, in some instances, the recognition of a protein by CD4 T cells and CD8 T cells in tandem might be beneficial, as CD4 T cells can directly provide help to these responses (3, 25). Moreover, although HIV-specific CD8 T cell responses are considered to be responsible for control of viral replication, it is important to note that correlations between the breadth or magnitude of total HIV-specific CD8 T cell responses and the level of viral control have not been detected (1). Only the increasing breadth of Gag-specific CD8 T cell targeting has been shown to be significantly associated with lower viremia (10). This is in contrast to the findings in our study, in which the breadth of total HIV-specific CD4 T cell responses and, also, the breadth and magnitude of Gag-specific CD4 T cell targeting were significantly correlated with viral load. Although the cause versus consequence of these associations cannot be conclusively answered, this study advances our current understanding of which CD4 T cell responses are present

during an efficacious immune response and supports the hypothesis that HIV-specific CD4 T cell responses contribute to viral control during chronic HIV infection.

In conclusion, while HIV-specific CD4 T cells were detected in all patient groups, our results reveal that the breadth of these responses is inversely correlated with viral load, particularly for the Gag protein. Furthermore, shifts in the immunodominance profile were observed between subjects who control viral replication and subjects with chronic progressive infection. Interestingly, the protein targeting of HIV-specific CD4 T cell responses distinguishes not only elite controller from high-viremia progressor but also elite controller from viremic controller. Moreover, a strong marker of viral control for all groups was the ratio of Env/Gag responses. The Env/Gag ratio reflects a fine balance between beneficial Gag responses that are significantly associated with low viral load and Env responses that are possibly linked with unfavorable clinical outcome and higher viral load. In a multivariate predictive model, we refined this association to three highly conserved Gag epitopes that were linked to spontaneous HIV control and one Env epitope that was associated with high viremia, independent of HLA associations. Together, the identification of highly immunogenic regions and the characterization of HIV-specific CD4 T cell responses present during an efficacious immune response are likely to have important implications for HIV vaccines, since their inclusion in future vaccine constructs may enhance beneficial T cell responses.

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REFERENCES

1. **Addo MM, et al.** 2003. Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. J. Virol. **77**:2081–2092.
2. **Betts MR, et al.** 2001. Analysis of total human immunodeficiency virus (HIV)-specific CD4(+) and CD8(+) T-cell responses: relationship to viral load in untreated HIV infection. J. Virol. **75**:11983–11991.
3. **Carreno BM, Turner RV, Biddison WE, Coligan JE.** 1992. Overlapping epitopes that are recognized by CD8+ HLA class I-restricted and CD4+ class II-restricted cytotoxic T lymphocytes are contained within an influenza nucleoprotein peptide. J. Immunol. **148**:894–899.
4. **Chen B, et al.** 2005. Structure of an unliganded simian immunodeficiency virus gp120 core. Nature **433**:834–841.
5. **Chevalier MF, et al.** 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.
6. **Douek DC, et al.** 2002. HIV preferentially infects HIV-specific CD4+ T cells. Nature **417**:95–98.
7. **Gamble TR, et al.** 1997. Structure of the carboxyl-terminal dimerization domain of the HIV-1 capsid protein. Science **278**:849–853.
8. **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.
9. **Kaufmann DE, et al.** 2004. Comprehensive analysis of human immunodeficiency virus type 1-specific CD4 responses reveals marked immunodominance of gag and nef and the presence of broadly recognized peptides. J. Virol. **78**:4463–4477.
10. **Kiepiela P, et al.** 2007. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat. Med. **13**:46–53.
11. **Kim JH, Rerks-Ngarm S, Excler JL, Michael NL.** 2010. HIV vaccines: lessons learned and the way forward. Curr. Opin. HIV AIDS **5**:428–434.
12. **Lagrelius M, Franck JPK, 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.
13. **Li H, et al.** 2009. Proximal glycans outside of the epitopes regulate the presentation of HIV-1 envelope gp120 helper epitopes. J. Immunol. **182**:6369–6378.
14. **Norris PJ, et al.** 2004. Beyond help: direct effector functions of human immunodeficiency virus type 1-specific CD4(+) T cells. J. Virol. **78**:8844–8851.
15. **Ondondo BO, et al.** 2008. Comprehensive analysis of HIV Gag-specific IFN-gamma response in HIV-1- and HIV-2-infected asymptomatic patients from a clinical cohort in The Gambia. Eur. J. Immunol. **38**:3549–3560.
16. **Pitcher CJ, et al.** 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.
17. **Porichis F, et al.** 2011. Responsiveness of HIV-specific CD4 T cells to PD-1 blockade. Blood **118**:965–974.
18. **Ramduth D, et al.** 2009. Immunodominant HIV-1 Cd4+ T cell epitopes in chronic untreated clade C HIV-1 infection. PLoS One **4**:e5013.
19. **Rerks-Ngarm S, et al.** 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N. Engl. J. Med. **361**:2209–2220.
20. **Ritchie AJ, et al.** 2011. Differences in HIV-specific T cell responses between HIV-exposed and -unexposed HIV-seronegative individuals. J. Virol. **85**:3507–3516.
21. **Rosenberg ES, et al.** 1997. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science **278**:1447–1450.
22. **Soghoian DZ, Streeck H.** 2010. Cytolytic CD4(+) T cells in viral immunity. Expert Rev. Vaccines **9**:1453–1463.
23. **Streeck H, Frahm N, Walker BD.** 2009. The role of IFN-gamma Elispot assay in HIV vaccine research. Nat. Protoc. **4**:461–469.
24. **Streeck H, et al.** 2007. Recognition of a defined region within p24 gag by CD8+ T cells during primary human immunodeficiency virus type 1 infection in individuals expressing protective HLA class I alleles. J. Virol. **81**:7725–7731.
25. **Takahashi H, Germain RN, Moss B, Berzofsky JA.** 1990. An immunodominant class I-restricted cytotoxic T lymphocyte determinant of human immunodeficiency virus type 1 induces CD4 class II-restricted help for itself. J. Exp. Med. **171**:571–576.
26. **Tibshirani R.** 1997. The lasso method for variable selection in the Cox model. Stat. Med. **16**:385–395.
27. **Vingert B, et al.** 2010. HIV controller CD4+ T cells respond to minimal amounts of Gag antigen due to high TCR avidity. PLoS Pathog. **6**:e1000780.
28. **Virgin HW, Wherry EJ, Ahmed R.** 2009. Redefining chronic viral infection. Cell **138**:30–50.