Impact of Select Immunologic and Virologic Biomarkers on CD4 Cell Count Decrease in Patients with Chronic HIV-1 Subtype C Infection: Results from Sinikithemba Cohort, Durban, South Africa

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Background. The extent to which immunologic and clinical biomarkers influence human immunodeficiency virus type 1 (HIV-1) infection outcomes remains incompletely characterized, particularly for non-B subtypes. On the basis of data supporting in vitro HIV-1 protein–specific CD8 T lymphocyte responses as correlates of immune control in cross-sectional studies, we assessed the relationship of these responses, along with established HIV-1 biomarkers, with rates of CD4 cell count decrease in individuals infected with HIV-1 subtype C.

Methods. Bivariate and multivariate mixed-effects models were used to assess the relationship of baseline CD4 cell count, plasma viral load, human leukocyte antigen (HLA) class I alleles, and HIV-1 protein–specific CD8 T cell responses with the rate of CD4 cell count decrease in a longitudinal population-based cohort of 300 therapy-naive, chronically infected adults with baseline CD4 cell counts >200 cells/mm^3 and plasma viral loads >500 copies/mL over a median of 25 months of follow-up.

Results. In bivariate analyses, baseline CD4 cell count, plasma viral load, and possession of a protective HLA allele correlated significantly with the rate of CD4 cell count decrease. No relationship was observed between HIV-1 protein–specific CD8 T cell responses and CD4 cell count decrease. Results from multivariate models incorporating baseline CD4 cell counts (201–350 vs >350 cells/mm^3), plasma viral load ($\leqslant$100,000 vs >100,000 copies/mL), and HLA (protective vs not protective) yielded the ability to discriminate CD4 cell count decreases over a 10-fold range. The fastest decrease was observed among individuals with CD4 cell counts >350 cells/mm^3 and plasma viral loads >100,000 copies/mL with no protective HLA alleles (-59 cells/mm^3 per year), whereas the slowest decrease was observed among individuals with CD4 cell counts 201–350 cells/mm^3, plasma viral loads $\leqslant$100,000 copies/mL, and a protective HLA allele (-6 cells/mm^3 per year).

Conclusions. The combination of plasma viral load and HLA class I type, but not in vitro HIV-1 protein–specific CD8 T cell responses, differentiates rates of CD4 cell count decrease in patients with chronic subtype-C infection better than either marker alone.

Rates of CD4 cell count decrease vary widely among human immunodeficiency virus type 1 (HIV-1)–infected individuals; however, the factors influencing these differences remain incompletely characterized. Determinants of HIV-1 infection outcomes include plasma viral load, CD4 cell count [1], and to a lesser extent, human leukocyte antigen (HLA) class I profile

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[2–4], as well as other human genetic polymorphisms [5]. However, less than one-half of the population variation in rates of CD4 cell count decrease can be explained by differences in plasma viral load [1, 6], and only a minor portion of plasma viral load set-point variation is explained by expression of HLA-B*57 and other key polymorphisms in the major histocompatibility complex region [7, 8]. Investigation of additional correlates of CD4 cell count decrease is clearly necessary. Moreover, given the global HIV-1 subtype distribution, there is a critical need to expand outcomes research for non-B subtypes.

Cross-sectional studies have reported associations between in vitro HIV-1 protein–specific CD8 T lymphocyte responses and clinical disease markers in patients with untreated chronic infection. In particular, detection of Gag (most notably p24)–specific responses have been associated with lower plasma viral loads and/or higher CD4 cell counts [9–15], whereas detection of responses to envelope and/or accessory proteins have been associated with higher plasma viral loads [10, 11]. Although these observations suggest that CD8 T cell responses against certain viral targets may be more beneficial than others, the cross-sectional nature of these studies precludes inference of cause and effect. Smaller observational studies have reported links between Gag-specific responses and slow or nonprogressive infection [16–19]; however, the relationship between CD8 T cell responses and HIV-1 infection outcomes has rarely been investigated in a population-based setting [20] and, thus, remains unclear. We therefore sought to evaluate whether in vitro HIV-1 protein–specific CD8 T cell responses, along with plasma viral load and HLA class I types, correlate with rates of CD4 cell count decrease in patients with chronic untreated HIV-1 subtype C infection.

METHODS

Patient selection. The study group was comprised of antiretroviral naive, chronically HIV-1 subtype C–infected adults from the Sinikithemba cohort based at McCord Hospital (Durban, South Africa), enrolled from August 2003 through June 2006 [10, 21]. Sociodemographic characteristics, plasma viral load measurements, and CD4 cell counts were obtained at baseline. Follow-up CD4 cell counts and plasma viral load measurements were performed at 3- and 6-month intervals, respectively. Of the 449 original participants, 68 were excluded because of a lack of CD8 T cell response data. An additional 7 were excluded because of missing age data, 1 was excluded because of pediatric infection, and 3 were excluded because of missing baseline clinical data. Because our objective was to characterize typical rates of CD4 cell count decrease in patients with chronic infection, spontaneous HIV-1 controllers (plasma viral load, <500 copies/mL; $n = 10$) and individuals with advanced disease who were eligible for antiretroviral treatment (baseline CD4 cell count, <200 cells/mm^3; $n = 60$) were excluded, leaving a final study population of 300 individuals. Informed consent was obtained from all patients, and approval was obtained from the appropriate institutional review boards.

Laboratory methods. In vitro CD8 T cell responses were measured by interferon (IFN)-γ enzyme-linked immunosorbent spot (ELISpot) assays with use of a set of 410 18-mer overlapping peptides spanning the HIV-1 subtype C proteome [10]. Initially, responses were assessed to pools of 11 or 12 peptides, after which responses to individual peptides were confirmed independently. Negative control experiments were performed in quadruplicate. Responses of >100 spot-forming cells per 1 million cells, after subtraction of average background, were considered to be positive. Responses were classified by protein as follows: Gag, Pol, Env, and accessory/regulatory (including Tat, Rev, Vpr, Vpu, Vif, and Nef) [10]. In the primary analysis, individuals were classified as "nonresponders" or "responders" if they exhibited positive responses to 0 or $\geqslant$1 peptides within each protein, respectively.

High-resolution HLA class I typing was performed using molecular methods [21]. In the primary analysis, "protective" alleles were defined as B*57, B*5801, and B*4201, because these alleles were significantly associated with lower plasma viral load in patients with subtype C infection, after correction for HLA linkage disequilibrium [21].

Statistical analysis. Bivariate and multivariate mixed-effects models were constructed to assess the relationship between immunologic and clinical biomarkers (independent variables) and rate of CD4 cell count decrease (dependent variable). To enhance the transparency of interpretation, all primary analyses featured the following biomarkers as binary variables: baseline CD4 cell count (201–350 vs >350 cells/mm^3), plasma viral load (mean of all measurements obtained during untreated follow-up; $\leqslant$100,000 vs >100,000 copies/mL), HLA class I (possession of a protective allele vs no protective allele), and HIV-1 protein–specific CD8 T cell activity (responders vs nonresponders). Secondary analyses employing alternative binary classifiers and treating biomarkers as continuous variables were also performed. Biomarkers exhibiting associations with CD4 cell count decrease with $P < .1$ in bivariate analyses were advanced in the multivariate model. Individuals were censored at their last visit up to 15 April 2008. Individuals who initiated highly active antiretroviral therapy ($n = 33$) were censored on their treatment initiation date. P values <.05 were considered to be statistically significant. Analyses were performed using SAS, version 9.1 (SAS Institute).

RESULTS

Baseline characteristics. The cohort was stratified by baseline CD4 cell count (201–350 vs >350 cells/mm^3) (table 1). Age, sex, and HLA distributions did not differ significantly between these strata. No significant differences in the frequency of CD8

Table 1. Baseline Characteristics of the Study Group

Characteristic	Overall (n = 300)	Baseline CD4 cell count, cells/mm^3: 201–350 (n = 114)	>350 (n = 186)	P
Age, median years (IQR)	31 (27–37)	32 (27–38)	31 (26–36)	.25
Female sex	245 (82)	90 (79)	155 (83)	.34
Plasma viral load >100,000 copies/mL	107 (36)	57 (50)	50 (27)	<.001
CD8 T lymphocyte response				
p24^{Gag}	232 (77)	82 (72)	150 (81)	.08
Pol	244 (81)	98 (86)	146 (78)	.11
Env	127 (42)	52 (46)	75 (40)	.37
Accessory/regulatory	263 (88)	97 (85)	166 (89)	.29
Protective HLA allele	116 (39)	49 (43)	67 (36)	.23
No. of CD4 cell count measurements per person	8 (4–11)	7 (3–9)	9 (6–12)	<.001
Follow-up duration, median months (IQR)	25 (15–41)	21 (10–28)	27 (20–45)	<.001

NOTE. Data are no. (%) of persons, unless otherwise indicated. IQR, interquartile range.

T cell responses to different HIV-1 proteins were observed; however, there was a trend towards more frequent p24^{Gag} responses in individuals in the upper versus the lower CD4 cell count stratum ($P = .08$). Individuals in the upper CD4 cell count stratum had lower plasma viral loads and longer follow-up durations, compared with those in the lower CD4 cell count stratum ($P < .001$).

Correlation between biomarkers and CD4 cell count decrease: bivariate analysis. In bivariate analyses, individuals with baseline CD4 cell counts >350 cells/mm^3 exhibited 2 times greater rates of CD4 cell count decrease, compared with individuals with baseline CD4 cell counts of 201–350 cells/mm^3 (table 2). Similarly, rates of CD4 cell count decrease among individuals with plasma viral loads >100,000 copies/mL were nearly twice as high as those for individuals with plasma viral loads ⩽100,000 HIV-1 RNA copies/mL. Expressed as a continuous variable, every 100-cell increment in baseline CD4 cell count resulted in the loss of an additional 9.1 cells/mm^3 per year (standard error, 0.89 cells/mm^3 per year; $P < .001$). Similarly, every $\log_{10}$ increment in plasma viral load resulted in the loss of an additional 11.0 cells/mm^3 per year (standard error, 2.37 cells/mm^3 per year; $P < .001$). A modest but significantly lower rate of CD4 cell count decrease was observed among individuals who possessed at least 1 subtype-C–specific protective HLA allele (B*57, B*5801, or B*4201), compared with individuals who did not possess a protective allele (table 2).

The data, however, provided insufficient statistical evidence to support an association between in vitro HIV-1 protein–specific CD8 T cell responses and rates of CD4 cell count decrease when the cohort was divided into "nonresponders" versus "responders" to each individual HIV-1 protein (table 3). Moreover, the magnitude of the differences in CD4 cell count decrease between responders versus nonresponders revealed no trends suggestive of clinical importance.

Secondary analyses featuring alternative binary classifiers of absolute breadth (⩽1 vs ⩾2 responses), relative breadth (proportion of total responses directed against each HIV-1 protein, <25% vs ⩾25%), and relative magnitude (proportion of the total magnitude of responses against each HIV-1 protein, <25% vs ⩾25%) also failed to reveal significant correlations with CD4 cell count decrease (data not shown). Similarly, treatment of absolute breadth, relative breadth, and magnitude as continuous variables also failed to reveal significant correlations with CD4 cell count decrease (table 4), with the exception of a weak association between higher-magnitude p24^{Gag} responses and slower CD4 cell count decrease. The magnitude of this association, however, was extremely modest (every 100 spot-forming cells per 1 million cells increment was associated with a difference in the rate of CD4 cell count decrease of +0.2 cells/mm^3 per year) and did not remain statistically significant after consideration for multiple tests. The evaluation of responses to the entire Gag protein (as opposed to p24 only) yielded comparable results in all analyses (data not shown).

On the basis of data suggesting that the ability to target specific functionally-constrained regions in Gag may be associated with protective effects mediated by the expression of specific HLA alleles [22, 23], we investigated whether CD8 T cell responses to these specific regions may be associated with the rate of CD4 cell count decrease in our cohort. We thus defined "functionally-constrained" peptides as those that include ⩾1 of the following Gag residues, as described elsewhere [22]: 146, 147, 177, 182, 186, 242, 247, 302, 310, and 456.

In bivariate analyses, we observed no statistically significant or clinically relevant differences in rates of CD4 cell count

Table 2. Clinical and Immunogenetic Biomarkers and Rates of CD4 Cell Count Decrease: Primary Bivariate Analysis

Biomarker, group	Frequency, no. of persons (%)	CD4 cell count decrease, cells/mm^3 per year	P
Baseline CD4 cell count, cells/mm^3			
>350	186 (62)	−39.9	<.001
201–350	114 (38)	−19.3	
Plasma viral load, copies/mL			
>100,000	107 (36)	−51.8	<.001
⩽100,000	193 (64)	−28.1	
Human leukocyte antigen class I allele type			
Nonprotective	184 (61)	−40	<.001
Protective	116 (39)	−27.2	

decrease between individuals who did not respond to any functionally-constrained Gag region ($n = 112$; −36.1 cells/mm^3 per year), compared with those who responded to at least 1 ($n = 188$, −33.9 cells/mm^3 per year; $P = .56$). Similarly, restricting this analysis to individuals expressing at least 1 protective HLA allele failed to yield statistically significant differences in rates of CD4 cell count decrease between responders and nonresponders to these regions ($P = .42$; data not shown). Finally, no statistically significant relationship between the absolute breadth of proteome-wide CD8 T cell responses and CD4 cell count decrease was observed ($P > .29$; data not shown). On the basis of a lack of evidence supporting a clear relationship between HIV-1 protein–specific CD8 T cell responses and CD4 cell count decrease in bivariate analyses, CD8 T cell responses were not advanced to the multivariate model.

Correlation between biomarkers and CD4 cell count decrease: multivariate analysis. In multivariate analyses including baseline CD4 cell counts, plasma viral load, and HLA as binary variables (as in the primary analysis), the mean rate of CD4 cell count decrease in the cohort was −32.6 cells/mm^3 per year (figure 1*A*). In a subsequent model, stratification by baseline CD4 cell counts yielded mean decreases of −41.0 and −11.9 cells/mm^3 per year in individuals with baseline CD4 cell counts of >350 and 201–350 cells/mm^3, respectively ($P < .001$; figure 1*B*). Further stratification by plasma viral load revealed faster CD4 cell count decreases among individuals with a plasma viral load >100,000 copies/mL, compared with those with plasma viral load ⩽100,000 copies/mL, in both CD4 cell count strata; mean absolute rates of decrease were −57.8 and −36.2 cells/mm^3 per year, respectively, for those with baseline CD4 cell counts >350 cells/mm^3 and −17.4 and −6.5 cells/mm^3 per year for those with baseline CD4 cell counts of 201–350 cells/mm^3 (overall, $P < .001$; figure 1*C*). In pair-wise comparisons designating individuals with CD4 cell counts of 201–350 cells/mm^3 and plasma viral loads ⩽100,000 copies/mL as the reference group, all differences in rates of CD4 cell count decrease were statistically significant with $P < .05$.

Addition of HLA to the model further discriminated rates of decrease in all groups ($P < .001$; figure 1*D*), although the magnitude of separation between the group of individuals with CD4 cell counts of 201–350 cells/mm^3 and plasma viral loads ⩽100,000 copies/mL was relatively minor. Among the individuals with a CD4 cell count >350 cells/mm^3, the fastest decrease was observed in patients with a plasma viral load >100,000 copies/mL with no protective HLA alleles (−59.6 cells/mm^3 per year), whereas the slowest decrease was observed in patients with a plasma viral load ⩽100,000 copies/mL and a protective HLA allele (−33.9 cells/mm^3 per year). Among the individuals with a CD4 cell count of 201–350 cells/mm^3, corresponding rates of CD4 cell count decrease were −24.8 and −5.5 cells/mm^3 per year, respectively. In pair-wise comparisons designating individuals with CD4 cell counts of 201–350 cells/mm^3, plasma viral loads ⩽100,000, and a protective HLA allele as the reference group, all comparisons were statistically significant ($P < .01$), except those comparisons with persons with CD4 cell counts of 201–350 cells/mm^3, plasma viral load ⩽100,000 copies/mL, and no protective HLA allele and with persons with CD4 cell counts of 201–350 cells/mm^3, plasma viral load >100,000 cells/mL, and a protective HLA allele.

Sensitivity analyses. Several post-hoc sensitivity analyses were performed. First, data were reanalyzed in terms of relative CD4 cell count decrease (percentage decrease from baseline). In bivariate analyses, plasma viral load and HLA type but not baseline CD4 cell count or CD8 T cell responses correlated significantly with percentage CD4 cell count decrease per year (data not shown). In multivariate model A adjusting for baseline CD4 cell count, plasma viral load, and HLA type, the relative rate of CD4 cell count decrease in the cohort was 6.4% per year. Stratification by baseline CD4 cell count revealed decreases of 7.2% and 4.2% per year in the groups with CD4 cell

Table 3. Human Immunodeficiency Virus Type 1 Protein–Specific CD8 T cell Responses and Rates of CD4 Cell Count Decrease: Primary Bivariate Analysis

Biomarker, group	Frequency, no. of persons (%)	CD4 cell count decrease, cells/mm^3 per year	P
p24^{Gag}			
Nonresponder	68 (23)	−33.3	.77
Responder	232 (77)	−34.7	
Pol			
Nonresponder	56 (19)	−36.4	.65
Responder	244 (81)	−34.1	
Env			
Nonresponder	173 (58)	−36.6	.18
Responder	127 (42)	−32.1	
Accessory/regulatory			
Nonresponder	37 (12)	−35.4	.84
Responder	263 (88)	−34.3	

NOTE. Individuals were classified as nonresponders or responders if they demonstrated a CD8 T cell response to 0 or ⩾1 peptide, respectively, within the indicated protein.

counts >350 and 201–350 cells/mm^3, respectively, a difference which was statistically significant (multivariate model B, $P < .001$). Addition of plasma viral load data revealed greater relative CD4 cell count decreases among individuals with plasma viral load >100,000 copies/mL, compared with those with plasma viral load ⩽100,000 copies/mL (10.5% vs 6.3% per year for the CD4 cell count >350 cells/mm^3 stratum, compared with 6.0% vs 2.4% per year in the CD4 cell count 201–350 cells/mm^3 stratum). Multivariate model C was significant with $P < .001$; all pair-wise comparisons were statistically significant with individuals with CD4 cell counts of 201–350 cells/mm^3 and plasma viral loads <100,000 copies/mL used as a reference group ($P < .001$). However, adding HLA type to this model further discriminated rates of CD4 cell count decrease for the group with CD4 cell counts of 201–350 cells/mm^3 and plasma viral loads >100,000 copies/mL only; previously the decrease was 6.0% year in model C, whereas rates in this model were now 8.2% and 2.4% per year among individuals lacking or having a protective HLA allele, respectively (model D; overall, $P < .001$).

Second, on the basis of data from cohorts of individuals infected with HIV-1 subtype C in Zambia [24] and South Africa [25] (P.G., unpublished data), we expanded the definition of "protective" HLA alleles to include B*3910, B*8101, and B*13. Possession of a protective allele ($n = 134$; 45%) was associated with slower CD4 cell count decrease (bivariate analysis, $P < .001$). Consistent with the primary analysis, sequential addition of CD4 cell count, plasma viral load, and HLA type to multivariate models A-D resulted in the incremental ability to discriminate rates of CD4 cell count decrease in all resulting groups ($P < .001$).

Third, to examine potential biases attributable to informative censoring, we restricted the analysis to the first 7 clinic visits only (18 months) in individuals with a minimum of 7 visits. Of the original 300 patients, 193 (64.3%) met these criteria (136 and 57 individuals with baseline CD4 cell counts >350 and 201–350 cells/mm^3, respectively). In multivariate analyses adjusting for baseline CD4 cell count, plasma viral load, and HLA type, faster CD4 cell count decreases were observed among individuals with baseline CD4 cell counts >350 cells/mm^3 (−22.7 cells/mm^3 per year), compared with those with CD4 cell counts of 201–350 cells/mm^3 (−10.1 cells/mm^3 per year) (model B, $P = .003$). Further stratification by plasma viral load in model C revealed a faster CD4 cell count decrease among those with plasma viral loads >100,000 copies/mL, compared with those with plasma viral loads ⩽100,000 copies/mL, regardless of baseline CD4 cell counts; rates were −30.1, −20.8, −15.5, and −6.2 cells/mm^3 per year for individuals with CD4 cell counts >350 cells/mm^3 and plasma viral loads >100,000 copies/mL ($n = 30$), those with CD4 cell counts >350 cells/mm^3 and plasma viral loads ⩽100,000 copies/mL ($n = 106$), those with CD4 cell counts of 201–350 cells/mm^3 and plasma viral loads >100,000 copies/mL ($n = 23$), and those with CD4 cell counts of 201–350 cells/mm^3 and plasma viral loads ⩽100,000 copies/mL ($n = 34$), respectively (overall, $P = .005$). However, further stratification by HLA type in model D did not yield significantly improved ability to discriminate rates

Table 4. Human Immunodeficiency Virus Type 1 Protein–Specific CD8 T cell Responses and Rates of CD4 Cell Count Decrease: Secondary Continuous Analyses

Analysis, biomarker	Difference in rate of CD4 cell count decrease, per indicated increment, cells/mm^3 per year (standard error)	*P*
Absolute breadth, per unit increment		
p24^{Gag}	0.44 (1.0)	.65
Pol	0.98 (0.8)	.24
Env	0.98 (1.8)	.58
Accessory/regulatory	0.42 (0.8)	.61
Relative Breadth, per 10% increment		
p24^{Gag}	−0.01 (0.83)	.99
Pol	1.0 (0.9)	.26
Env	2.2 (1.6)	.2
Accessory/regulatory	0.2 (1.0)	.84
Magnitude, per increment of 100 spot-forming cells per 1 million cells		
p24^{Gag}	0.2 (0.1)	.05
Pol	0.1 (0.1)	.09
Env	0.3 (0.2)	.29
Accessory/regulatory	0.1 (0.1)	.15

NOTE. In the secondary analyses, biomarkers were treated as continuous variables, and results are expressed in terms of the difference in the rate of CD4 cell count decrease (in cells/mm^3 per year) per each indicated increment for the biomarker. For example, every per-unit increment in the absolute breadth of Gag p24 responses is associated with a difference in rate of CD4 cell count decrease of +0.44 cells/mm^3 per year.

of CD4 cell count decrease, possibly because of diminished statistical power in this subanalysis.

Finally, a mixed-effects model treating baseline CD4 cell count and plasma viral load as continuous variables and expressing CD4 cell count decrease in absolute terms was performed using a backwards selection procedure. In this analysis, expression of nonprotective HLA alleles was associated with the loss of an additional 6.6 cells/mm^3 per year, compared with expression of a protective allele ($P = .04$). Moreover, the interaction between $\log_{10}$ plasma viral load and baseline CD4 cell count was significantly associated with differences in the rate of CD4 cell count decrease. Specifically, every 100-unit increase in the product of $\log_{10}$ plasma viral load multiplied by baseline CD4 cell count resulted in the loss of an additional 3.2 cells/mm^3 per year (standard error, 1.2 cells/mm^3 per year; $P = .002$).

DISCUSSION

Elucidating the relationship between HIV-1–specific CD8 T cell responses and disease outcomes has important implications for the design of vaccines and immunotherapeutic strategies aimed at stimulating these responses. Indeed, consistent correlations between Gag-specific CD8 T cell responses and favorable clinical profiles in cross-sectional studies of individuals with chronic infection [9, 11, 12], including an expanded version of the present cohort [10], suggest that strategies aimed at stimulating Gag-specific responses may be beneficial. Cross-sectional studies, however, do not address whether Gag-specific responses in chronic infection are predictive of subsequent disease outcomes—a critical question addressed by few population-based longitudinal studies. In the present study, we observed neither statistically significant nor clinically relevant evidence of an association between either the magnitude or frequency (absolute or relative) of in vitro CD8 T cell responses to any HIV-1 protein and the subsequent rate (either absolute or relative) of CD4 cell count decrease in patients with chronic untreated subtype C infection. A subanalysis investigating the relationship between CD8 T cell responses to key functionally-constrained regions in Gag also failed to reveal any significant association with rates of CD4 cell count decrease.

These results contrast with those of a smaller subtype B study of 31 individuals that reported slower CD4 cell count decreases among individuals with ⩾50% (vs <50%) of CD8 T cell responses directed at Gag [19]. In the current study, grouping by these criteria yielded too few patients with ⩾50% Gag responses to robustly verify these findings. Note that, on average, the clade B cohort not only had more dominant Gag responses but also had higher CD4 cell counts and lower plasma viral loads [19], compared with the present cohort, possibly indicating earlier disease stage (dates of infection were unknown for both cohorts). If the predictive ability of Gag-specific responses differs throughout the disease course [26], this could

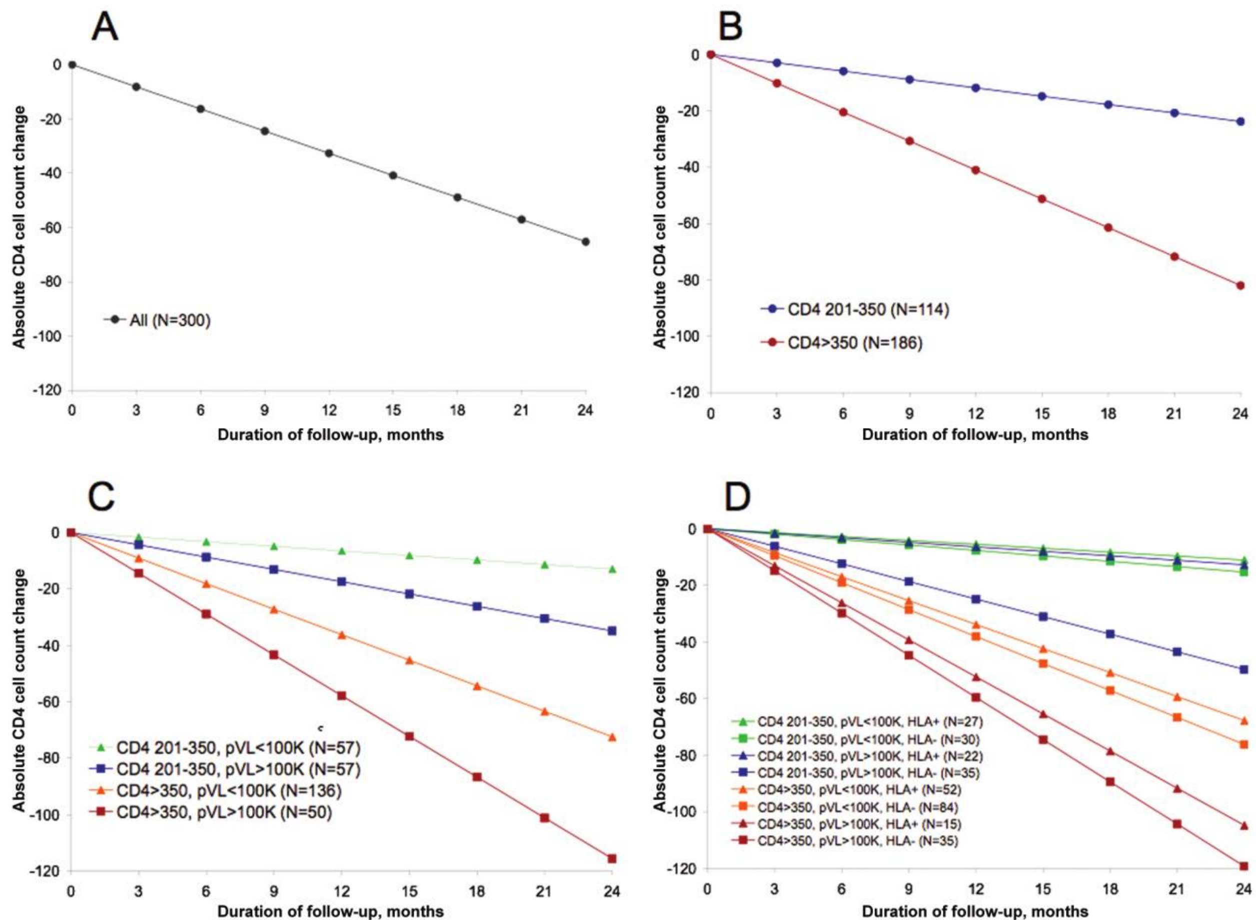

Figure 1. Absolute rates of CD4 cell count decrease, stratified by baseline CD4 cell count, plasma viral load, and human leukocyte antigen (HLA) class I alleles. Results of multivariate, sequentially built mixed-effects models demonstrate mean rates of CD4 cell count decrease in the overall cohort *(A)*; stratified by baseline CD4 cell count *(B)*; stratified by baseline CD4 cell count and plasma viral load (pVL) *(C)*; and stratified by baseline CD4 cell count, pVL, and the possession of a protective HLA allele *(D)*. Individuals in different subgroups within the same stratum can be distinguished by the use of different shades of the same color; for example, individuals with baseline CD4 cell counts of 201–350 (CD4 201–350) and >350 (CD4 >350) cells/mm^3 are represented by blue/green and red/orange lines, respectively, in panels *C* and *D*. pVL is stratified as ⩽100,000 (pVL<100K) versus >100,000 copies/mL (pVL>100K), and HLA class I alleles are classified as protective (HLA+) or nonprotective (HLA−).

help reconcile these findings. Likely because of a smaller sample size, the clade B study did not adjust for baseline CD4 cell count, plasma viral load, and HLA type, which may also affect interpretation.

Our finding that protective HLA allele expression but not CD8 T cell responses is associated with CD4 cell count decrease is somewhat surprising, because the most likely mechanism of HLA-mediated control of HIV-1 infection is through CD8 T cell responses. A possible explanation for this finding is that in vitro CD8 T cell responses measured during relatively advanced infection may be of reduced relevance to disease outcomes, even though these responses may have contributed to HIV-1 containment in earlier disease stages. This may be attributable in part to the decrease in immune function over the disease course or to the accumulation of escape mutations in the autologous virus that may compromise the ability to detect responses to the corresponding consensus peptide. Indeed, the use of consensus (instead of autologous) peptides may lead to an underestimation of response rates of up to 30% [27]. Note that our findings do not exclude the possibility that Gag-specific responses measured earlier in infection represent important and clinically relevant predictors of outcome. Indeed, there is some evidence supporting the relevance of acute or early CD8 T cell responses to disease outcomes [28–31], although this remains controversial [32–34].

In this cohort of chronically-infected patients, mean rates of CD4 cell count decrease were calculated based on initial CD4 cell counts, plasma viral loads, and HLA type. Analysis of this combination of biomarkers allowed discrimination of rates of CD4 cell count decrease better than each marker individually;

rates of CD4 cell count decrease ranged from −6 to −60 cells/mm^3 per year depending on the combination of biomarkers expressed. Of interest, among patients with CD4 cell counts of 201–350 cells/mm^3, comparable rates of CD4 cell count decrease were observed for those with plasma viral loads ⩽100,000 copies/mL (regardless of HLA type) and those with plasma viral loads >100,000 copies/mL with a protective HLA type, whereas those with plasma viral loads ⩽100,000 and no protective HLA allele experienced significantly faster CD4 cell count decreases, suggesting that having either lower plasma viral load or a protective allele may be beneficial at low CD4 cell counts. We suggest that population-level rates of CD4 cell count decrease could be useful in the clinical setting, for example, to estimate the time until antiretroviral treatment may be required in patients presenting with chronic infection.

Some limitations deserve to be mentioned. First, the restriction of the study to individuals with CD4 cell counts >200 cells/mm^3 with unknown dates of infection raises the possibility of a survivor bias. To address this, we stratified the cohort by baseline CD4 cell count and performed a sensitivity analysis by reporting CD4 cell count decrease on a relative scale (ie, by percentage), and we performed an "informative censoring" analysis by restricting the 2 CD4 cell count strata to equal follow-up durations, both of which yielded results consistent with the original findings. The IFN-γ ELISpot assay that was used to measure CD8 T cell responses also has some limitations [35], including its use of supraphysiological levels of synthetic HIV-1 peptides that bypass the normal intracellular antigen processing pathways, its use of consensus peptides that may not reflect immune responses to the autologous virus [27], and the fact that CD8 T cell function is measured indirectly (by quantification of a secreted cytokine) [27, 35, 36]. Despite these limitations, this assay is widely used to assess vaccine-induced and naturally occurring CD8 T cell responses [37–39]. Finally, because of the measurement of CD8 T cell responses at a single time point, we were unable to investigate the correlation between long-term stability of CD8 T cell responses and CD4 cell count decrease [17].

In conclusion, we observed neither statistically significant nor clinically important correlations between in vitro HIV-1–specific CD8 T cell responses and rates of CD4 cell count decrease among individuals with chronic HIV-1 subtype C infection. The combination of plasma viral load and HLA class I type, however, predict CD4 cell count decrease significantly better than either of these biomarkers alone.

PRESENT AFFILIATIONS

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