MAJOR ARTICLE

Antiviral Inhibitory Capacity of CD8+ T cells Predicts the Rate of CD4+ T-Cell Decline in HIV-1 Infection

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Background. Rare human immunodeficiency virus type 1 (HIV-1)–infected individuals who maintain control of viremia without therapy show potent CD8+ T-cell–mediated suppression of viral replication in vitro. Whether this is a determinant of the rate of disease progression in viremic individuals is unknown.

Methods. We measured CD8+ T-cell–mediated inhibition of a heterologous HIV-1 isolate in 50 HIV-1–seropositive adults with diverse progression rates. Linear mixed models were used to determine whether CD8+ T-cell function could explain variation in the rate of CD4+ T-cell decline.

Results. There was a significant interaction between CD8+ T-cell antiviral activity in vitro and the rate of CD4 + T-cell decline in chronically infected individuals ($P < .0001$). In a second prospective analysis of recently infected subjects followed for up to 3 years, CD8+ T-cell antiviral activity strongly predicted subsequent CD4+ T-cell decline ($P < .0001$) and explained up to 73% of the interindividual variation in the CD4+ T-cell slope. In addition, it was inversely associated with viral load set point ($r = -0.68$ and $P = .002$).

Conclusions. The antiviral inhibitory capacity of CD8+ T cells is highly predictive of CD4+ T-cell loss in early HIV-1 infection. It has potential as a benchmark of effective immunity in vaccine evaluation.

CD8+ T-cell–based vaccines against human immunodeficiency virus type 1 (HIV-1) aim to induce responses that abort or limit early viral replication and thus delay disease progression and reduce transmission risk. Recombinant DNA and attenuated viral vector vaccines showed promise because of their capacity to induce high frequencies of interferon γ (IFN-γ)–secreting and/or polyfunctional T cells in healthy volunteers [1, 2]. However, the disappointing results of the Step Study, in which there was no effect of vaccine-induced T-cell responses on viral replication after seroconversion, highlighted the need for a reliable immunological correlate of virus control [3]. CD8+ T-cell depletion experiments in macaques with simian immunodeficiency virus (SIV) infection and vaccine strategies to induce T-cell responses against SIV have demonstrated unequivocally that CD8+ T cells suppress AIDS virus replication, with the most promising results to date being achieved by vaccination with a rhesus cytomegalovirus–vectored vaccine [4–8]. CD8+ T cells are induced early during primary HIV-1 infection, in tandem with peak viremia and prior to the appearance of neutralizing antibodies, but in the majority of cases these early CD8+ T cells fail to attenuate viral replication [9, 10]. Patients who spontaneously control HIV-1 (ie, long-term nonprogressors [LTNPs] and/or controllers), who represent <5% of all infected individuals, differ from typical

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progressors in functional aspects of their antiviral CD8+ T-cell responses, which suggests that the quality of these responses may influence the course of HIV-1 infection [11–13]. However, these observations are derived largely from cross-sectional studies. Thus, it is unclear whether preserved CD8+ T-cell function is a consequence rather than a cause of effectively suppressed viral replication. In support of the former notion, viremic individuals followed longitudinally showed loss of CD8 + T-cell functions over time [14].

Assessment of CD8+ T-cell–mediated inhibition of viral replication in vitro may provide a more accurate indication of immune control in vivo than other, more established measures of HIV-specific T-cell function, as it relies on the recognition of endogenously generated viral peptides within CD4+ T cells, in contrast to the loading of target cells with exogenous peptides at nonphysiological concentrations [15]. This is supported by studies showing that CD8+ T cells from HIV-1 LTNP/controllers show efficient inhibition of HIV-1 replication in vitro [16–18]. However, interpretation of these data is also limited by the cross-sectional study design. To address this, we measured the antiviral inhibitory capacity of ex vivo CD8+ T cells from 50 HIV-1–seropositive individuals and investigated its relationship to the rate of CD4+ T-cell decline in retrospective and prospective cohorts.

Table 1. Characteristics of Patients With Chronic or Acute Human Immunodeficiency Virus Type 1 Infection

Patient cohort	Chronic (n=30)	Acute (n=20)
Male sex	66	100
Age, years	34 (29–49)	28 (27–33)
Known duration of infection[a]	3 (1.25–5)	198 (133–230)
CD4+ T-cell count, cells/µL	425 (345–610)	470 (432–563)
Plasma viral load, $\log_{10}$ copies/mL	4.23 (3.78–4.67)	3.95 (3.78–4.5)
CDC clinical stage, patients, no.		
A	28	20
B	2	0
C	0	0
Subject with known protective HLA class I allele[b]	6 (20)	6 (30)

Data were recorded at the time of sampling for the viral suppression assay and are no. (%) of patients or median (interquartile range), unless otherwise indicated.

Abbreviation: CDC, Centers for Disease Control and Prevention.

[a] Data for patients with chronic infection are in years, and data for patients with acute infection are in days.

[b] HLA-B*5701/03, B*27, B*51, B*5801, and B*8101.

METHODS

Study Subjects

Chronic HIV-1–infected, asymptomatic, antiretroviral therapy (ART)–naive adult patients were recruited from clinics in the United Kingdom. All had CD4+ T-cell counts of >250 cells/µL at study entry. Twenty adults with acute HIV-1 infection were recruited from the Beijing PRIMO cohort, YouAn Hospital (Beijing, People's Republic of China). Acute infection was defined as described previously [19]. Plasma viral loads (pVLs) in Beijing subjects were measured at Duke University (Durham, NC), using a Roche Amplicor test. Demographic and clinical characteristics are shown in Table 1. Control subjects were healthy HIV-seronegative volunteers. Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood samples by density centrifugation and stored in vapor-phase liquid nitrogen until use. All subjects were HLA typed by amplification refractory mutation system–polymerase chain reaction, using sequence-specific primers. This study was approved by local research ethics committees (Mid & South Buckinghamshire Research Ethics Committee, United Kingdom, and YouAn Hospital, Capital University, People's Republic of China). Each participant provided written informed consent.

CD8+ T-Cell Antiviral Suppression Assay

HIV-1 inhibition by CD8+ T cells was measured using a previously described assay, with modifications [20, 21]. Cryopreserved PBMCs were thawed and depleted of CD8+ cells by magnetic bead separation (MACS, Milteny-Biotec). CD8-depleted cells (hereafter referred to as CD4+ T cells) were stimulated with PHA (5 µg/mL) in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal calf serum (R10) for 3 days, washed, and infected with HIV-1_{BaL} (National Institute for Biological Standards and Control, United Kingdom) at a multiplicity of infection (MOI) of 0.01. To confirm that this MOI achieved optimal levels of productive infection, we cultured HIV-1_{BaL}–superinfected CD4+ T cells (5×10^4/well, obtained from a PBMC panel of 6 donors, kindly provided by the Comprehensive T cell Vaccine Immune Monitoring Consortium, courtesy of Dr Richard Koup) without autologous CD8+ T cells for 5 days and quantified free p24 antigen (Ag) in culture supernatants by p24 enzyme-linked immunosorbent assay (Cambridge Biosciences). Cumulative p24 Ag concentrations were 6.4–7.3 $\log_{10}$ pg/mL under these conditions, which was consistent with other studies [17, 20]. To assess viral inhibition, HIV-superinfected CD4+ T cells (5×10^4) were cultured in triplicate in R10 with interleukin 2 (20 IU/mL) in 96-well round-bottomed plates, alone or together with unstimulated ex vivo CD8+ T cells. The latter were obtained by positive bead selection of PBMCs from a freshly thawed vial on day 3. CD8+ T cells were confirmed as >98% pure by staining for CD3, CD8, and CD56 immediately after selection and at various times during culture. For

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assays in chronically infected subjects, CD8+ and CD4+ T cells were cultured at a ratio of 2:1 and harvested on days 5 and 7; for the Beijing PRIMO cohort, 3 CD8+ /CD4+ T-cell ratios were tested, and cultures were harvested on day 6 only, as PBMC stocks were limited. Cells were stained first with Aqua Live/Dead Fixable stain (Invitrogen), fixed with 1% paraformaldehyde/20 µg/mL lysolecithin at RT, permeabilized with cold 50% methanol followed by 0.1% Nonidet P-40, and finally stained with p24 antibody (KC-57-FITC; Beckman Coulter) and antibodies to CD3, CD4, and CD8 (conjugated to APC-Cy7, PerCP, and APC, respectively; BD Biosciences). Samples were acquired on a CyAn flow cytometer. Data were analyzed using Summit software. Antiviral suppressive activity was expressed as percentage inhibition and determined as follows: [(fraction of p24 + cells in $CD4^+$ T cells cultured alone) − (fraction of p24 + in CD4+ T cells cultured with CD8+ cells)]/(fraction of p24 + cells in $CD4^+$ T cells cultured alone) × 100.

In selected experiments, we assessed requirements for HLA class I–restricted T-cell recognition in the viral suppression assay, using HIV-infected allogeneic CD4+ T cells that were HLA mismatched or matched for a single HLA class I allele as targets.

IFN-γ Elispot Assay

The frequency of circulating IFN-γ–secreting HIV-specific T cells in chronically infected subjects was determined by an IFN-γ Elispot assay, using unfractionated PBMCs (10^5 cells/well) stimulated with pools of overlapping 15-mer peptides (4 µg/mL), based on the HIV-1 consensus clade B proteome (NIH AIDS Reagent Program), as described previously [22].

Statistical Methods

The Mann-Whitney *U* test, the Spearman rank correlation test, Kaplan-Meier survival curves, and the log-rank test were performed using GraphPad Prism 4. *P* values of < .05 were considered statistically significant. A linear mixed model method was used to analyze CD4+ T-cell count data. All analyses were carried out using SAS, version 9.1. The dependent variable was the square root of the CD4+ T-cell count. All available CD4+ T-cell counts for each subject were used. Time was measured from the first positive antibody test for the cohort with chronic HIV-1 infection; for the cohort with acute infection, it was measured from the date of infection, which was estimated as described previously [23]. Change in the square root of the CD4+ T-cell count over time was modeled using a linear regression equation. Random effects, an intercept and gradient, were calculated for each subject, each parameter a random deviation from the population regression model. Person-level covariates were included in the model, as main effects and as an interaction with time, as the fixed effects. We investigated the extent to which these covariates could explain variation between subjects in the intercepts and gradients, focusing on the effect of the interaction of the covariate with time and the effect on the rates of change in the square root of the CD4+ T-cell count.

RESULTS

CD8+ T-Cell Antiviral Activity Is Expressed on a Continuum in Chronic HIV-1 Infection and Is Strongly Associated With the Rate of CD4+ T-Cell Decline

We first measured CD8+ T-cell antiviral activity in 30 ART-naive individuals who were diverse with respect to CD4+ T-cell counts, pVLs, and durations of infection. This cohort included 6 subjects who were classified as viremic controllers (pVL consistently <5000 copies/mL for >1 year) (Table 1). Antiviral activity was determined at a CD8+ /CD4+ T-cell ratio of 2:1 and was specific to HIV-1–infected individuals, in whom the median inhibition value was 78%, while the median response in HIV-uninfected controls was 0% ($P < .0001$) (Figure 1*A* and 1*B*). Median inhibition values were significantly higher in viremic controllers than in patients with viral loads of >5000 copies/mL (90% vs 74%; $P = .04$), consistent with previous reports [16–18]. We confirmed that CD8+ T-cell antiviral activity was dependent on recognition of HLA class I–bound viral peptides by measuring inhibition of HIV-1 replication by CD8+ T cells when cultured with allogeneic CD4+ T cells that were matched for a single HLA class I allele or completely HLA mismatched. Antiviral activity was only detected when targets shared an HLA class I allele with the CD8+ T-cell donor (Supplementary Figure 1).

Depletion of CD4+ T cells is the hallmark of HIV-1 disease progression. Therefore, we next used linear mixed models to investigate the extent to which CD8+ T-cell antiviral suppressive activity in chronic infection could explain variation in the rate of CD4+ T-cell decline. CD4+ T-cell slopes were determined from a median of 9 CD4+ T-cell counts (interquartile range [IQR], 7–19) over a median of 4.5 years (IQR, 2–6 years) (Figure 1*C*). The interaction between CD8+ T-cell antiviral suppressive capacity and the CD4+ T-cell gradient was highly significant ($P < .0001$), with greater inhibition values associated with slower CD4+ T-cell loss (Figure 1*D*, Supplementary Table 1). From this model, estimates of mean CD4+ T-cell loss were 20 cells/µL of blood per year for an inhibition value of 90%, assuming a baseline CD4+ T-cell count of 500 cells/µL, and 85 cells/µL of blood per year for an inhibition value of 50% (Supplementary Results). Of note, neither age at baseline nor sex explained any of the variation in CD4+ T-cell slope. Furthermore, neither the CD4+ T-cell count ($r = 0.3$ and $P = .1$) nor the plasma viral load ($r = -0.16$ and $P = .39$) at the time of sampling for the assay showed a significant correlation with the level of CD8+ T cell antiviral suppressive activity.

We also investigated the relationship between CD8+ T-cell antiviral suppression and the frequency of circulating

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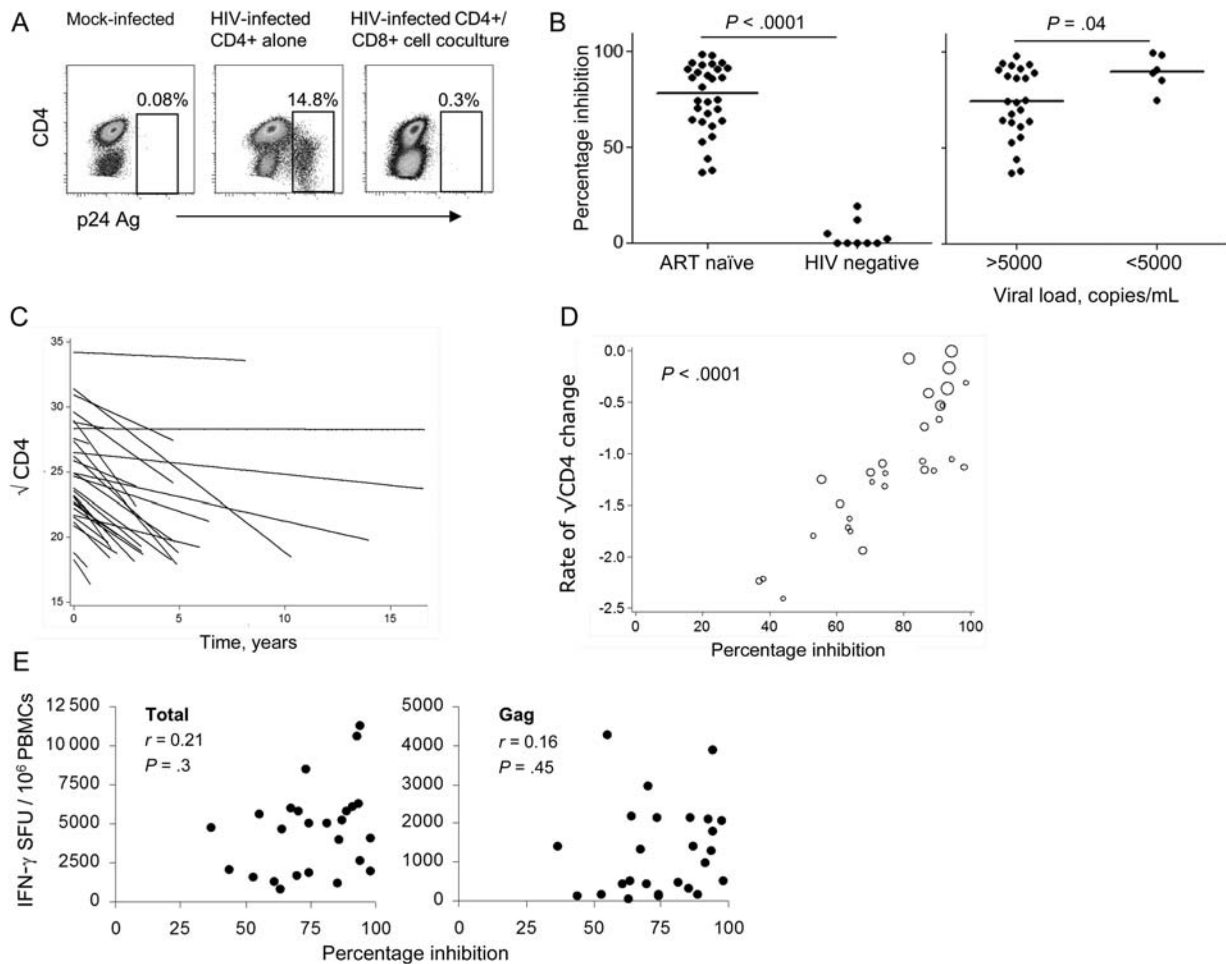

Figure 1. *A*, Representative flow cytometry plots showing detection of human immunodeficiency virus type 1 (HIV-1)–infected (p24 antigen–positive) cells within the CD4+ T cell population after gating on live CD3 + $CD8^{neg}$ lymphocytes. Mock-infection (*left*), HIV-1_{BaL}–superinfected CD4+ T cells cultured alone (*middle*) or with autologous ex vivo unstimulated CD8+ cells (*right*). As HIV-1 infection induces CD4 downregulation, the infected cell population includes CD4-low/negative p24 Ag–positive cells. *B*, CD8+ T cell suppression of HIV-1 replication in HIV-1_{BaL}–superinfected autologous CD4+ T cells in 30 antiretroviral therapy (ART)–naive patients with chronic HIV-1 infection and 9 HIV-uninfected/low-risk healthy volunteers (*left panel*). Patients were then stratified according to plasma viral loads, either above or consistently below 5000 copies/mL (*right panel*). Data shown are the mean of percentage inhibition values obtained on days 5 and 7 of coculture. Horizontal lines indicate medians. *C*, Fitted lines for CD4+ T-cell count trajectories, modeled from square root–transformed values over time in years in 30 HIV-1 chronically infected ART-naive patients. *D*, Interaction between CD8+ T-cell antiviral activity (percentage inhibition) and rate of CD4+ T-cell decline (modeled from the square root of the CD4+ T-cell count over time) in the 30 chronically infected subjects shown in *B* and *C*. Percentage inhibition values are the mean of day 5 and day 7 samples as in (*B*), at a CD8/CD4+ T-cell ratio of 2:1. The area of each circle is proportional to the weight of the point, which is defined as 1/[variance of estimate of rate of change]. The weight is related to the number of assessments per patient. *E*, Correlation (Spearman *r*) between the magnitude of HIV-specific T-cell responses in 26 HIV-1 chronically infected ART-naive subjects, as determined in ex vivo interferon γ (IFN-γ) Elispot assays, and CD8+ T-cell antiviral activity (CD8+ CD4+ T-cell ratio = 2:1). The 2 assays were performed using contemporary samples. *Left,* summed responses to pools of overlapping peptides (OLPs), based on the HIV-1 clade B consensus proteome, after subtraction of mock-stimulated responses. *Right,* responses to the HIV-1 gag OLP pool. Abbreviation: PBMC, peripheral blood mononuclear cell.

HIV-specific IFN-γ–producing T cells, since this is a widely used measure of HIV-specific cellular immunity. CD8+ T-cell antiviral activity did not show a significant correlation with HIV-specific T-cell IFN-γ responses to gag, other proteins, or the entire proteome (Figure 1*E*; data not shown). In addition, the magnitude of HIV-1 gag-specific IFN-γ responses had no effect on the CD4+ T-cell slopes when examined as a covariate in the model.

Antiviral CD8+ T-Cell Suppressive Capacity Predicts the Rate of CD4+ T-Cell Decline in Early HIV-1 Infection

To determine whether CD8+ T-cell antiviral function could predict the rate of CD4+ T-cell decline, we analyzed CD8+ T-cell antiviral suppressive capacity prospectively in 20 patients who had enrolled in the Beijing PRIMO study, a prospective cohort of primary HIV-1–infected individuals. The median duration of infection at enrollment was 30 days (IQR,

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25–33 days) (Table 1). However, because of limited sample availability, we assessed CD8+ T-cell suppressive activity, using PBMCs sampled after the pVL set point had been attained (median duration of infection, 198 days). All subjects were asymptomatic at this time. We tested CD8+ T cells at 3 CD8+ /CD4+ T-cell ratios (2:1, 1:10, and 1:100) because efficient antiviral inhibition at low ratios was previously reported to distinguish HIV-1 controllers and progressors [16]. All but one patient had detectable suppressive activity at the highest CD8+ /CD4+ T-cell ratio and all showed a reduction in activity with titration of the ratio (Figure 2A). Next, we modeled CD4+ T-cell decline from all the available CD4+ T-cell counts obtained during follow-up. Slopes were derived from a median of 13.5 CD4+ T-cell counts (IQR, 11–16 counts) obtained during a median follow-up period of 895 days (IQR, 727–1039 days) (Supplementary Table 2). We used linear mixed models to investigate whether the patient-level covariates CD8+ T cell antiviral activity (percentage inhibition), viral load set point, and viral subtype explained any of the variation between patients in the rate of change of the square root of the CD4+ T-cell count when added to the model separately, and whether this interpretation changed when covariates were included together in the model. Age at baseline had no effect when added as a covariate and therefore was not included in any analysis. The antiviral suppressive capacity of CD8+ T cells measured in early HIV-1 infection was highly predictive of subsequent CD4+ T-cell gradients at all 3 CD8+ /CD4+ T-cell ratios tested (Table 2). The effect was strongest at the ratios of 1:10 and 1:100 ($P < .0001$), indicating that titration of CD8+ T cells to suboptimal levels improved the capacity of the assay to distinguish fast and slow progressors. Percentage inhibition alone explained 40%, 73%, and 56% of the variance in CD4+ T-cell slopes, at CD8+ /CD4+ T-cell ratios of 2:1, 1:10, and 1:100, respectively (Table 2).

Viral load set point was determined as described by Fellay et al for 18 of 20 subjects; there were insufficient data to

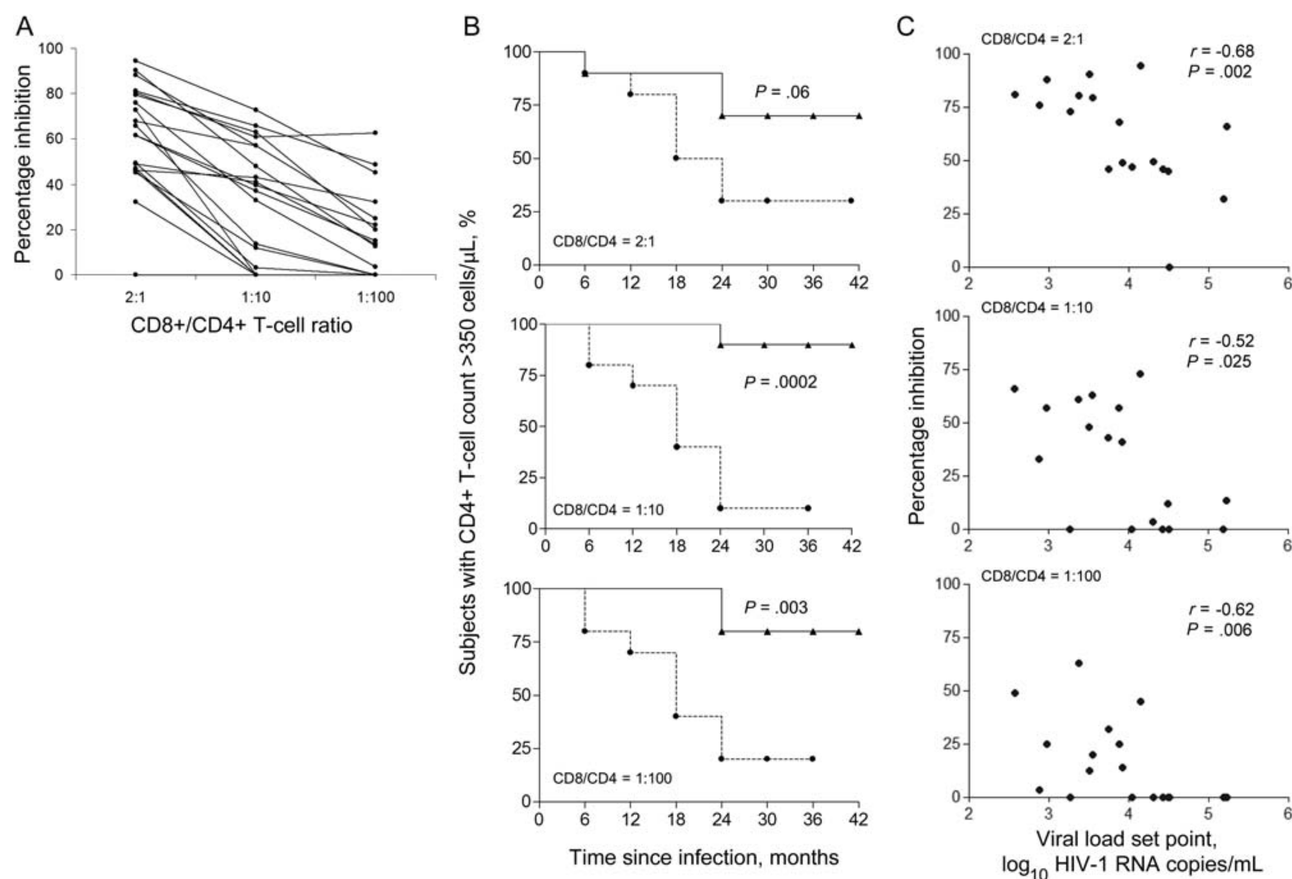

Figure 2. *A*, CD8+ T-cell suppression of human immunodeficiency virus type 1 $(HIV-1)_{BaL}$ replication in superinfected autologous CD4+ T cells was tested on day 6 of coculture at CD8+ /CD4+ T-cell ratios of 2:1, 1:10, and 1:100 in 20 recently infected subjects. Each line represents 1 subject. *B*, Kaplan-Meier curves showing the proportion of recently infected subjects maintaining CD4+ T-cell counts of >350 cells/µL after stratification by CD8+ T-cell antiviral suppressive capacity above (*solid lines*, *triangles*) or below (*broken lines*, *circles*) the median percentage inhibition values obtained at each CD8+ /CD4+ T-cell ratio (10 subjects per group). *C*, Correlation (Spearman *r*) between viral load set point after acute HIV-1 infection and CD8+ T-cell antiviral suppression values for the CD8+ /CD4+ T-cell ratios shown.

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Table 2. **Analysis of Square Root–Transformed CD4+ T-Cell Count Scale Over Time in 20 Subjects with Recent Human Immunodeficiency Virus Type 1 Infection**

	Model 1			Model 2			Model 3			Model 4		
	No covariates			Percentage inhibition at CD8/CD4 2:1			Percentage inhibition at CD8/CD4 1:10			Percentage inhibition at CD8/CD4 1:100		
Variable	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*
Fixed effects												
Constant baseline	23.65	22.47–24.82		22.21	18.82–25.61		22.74	20.94–24.53		22.97	21.48–24.46	
Time (in years)	−2.12	−3.01 to −1.22	<.0001	−5.19	−7.36 to −3.01	<.0001	−4.01	−4.95 to −3.06	<.0001	−3.18	−4.09 to −2.28	<.0001
Percentage inhibition	…	…		0.023	−.028–.075		0.029	−.014–.072		.043	−.017–.103	
Time × percentage inhibition	…	…		0.050	.018–.083	.002	0.057	.036–.079	<.0001	0.064	.031–.098	<.0001
Random effects												
Variance between slopes	3.3426	…		2.0140	…		0.9058	…		1.4649	…	
Variance between intercepts	6.3023	…		6.1000	…		5.5852	…		5.6461	…	
Correlation (slope × intercept)	0.63	…		0.63	…		0.84	…		0.68	…	
Residual variance	3.5314	…		3.5040	…		3.5117	…		3.5518	…	
Proportion of variance between slopes accounted for with reference to model 1	…	…		0.40	…		0.73	…		0.56	…	

The time at baseline was defined as the date of infection, estimated as described in Methods. Models were generated from 260 observations.

Abbreviation: CI, confidence interval.

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calculate the set point for 2 patients [24]. When analyzed alone, it had a significant effect on the rate of CD4+ T-cell decline at all 3 CD8+ /CD4+ T-cell ratios ($P < .05$) and explained 59% of the variance in CD4+ T-cell slopes (Table 3), consistent with findings from previous cohort studies [25, 26]. When both pVL set point and percentage inhibition were included in the model, they had a statistically significant effect at ratios of 1:10 and 1:100 ($P < .001$ and $P = .02$, respectively) and together explained up to 91% of the variance in CD4+ T-cell slope (Table 3). There is controversy as to whether viral subtype impacts the rate of CD4+ T-cell decline and clinical outcomes [27–29]. Ten of the 20 patients in the Beijing PRIMO cohort were infected with subtype B virus and 9 with CRF01_AE virus (1 subtype was not determined). Viral subtype was therefore included as a covariate in the model with and without including percentage inhibition. Its interaction with time was not significant ($P = .7$), indicating that HIV-1 subtype explained none of the variance in CD4+ T-cell slope.

We also performed a survival analysis to determine whether CD8+ T-cell antiviral suppressive capacity could influence the time to reach a CD4+ T-cell count of <350 cells/μL, a widely used threshold for initiation of antiretroviral therapy. We compared the duration for which CD4+ T-cell counts were maintained >350 cells/μL after stratifying patients according to "strong" (ie, above the median) or "weak" (ie, below the median) CD8+ T-cell inhibition values. Patients with weak antiviral suppressive activity reached a CD4+ T-cell count of <350 cells/μL in a significantly shorter time than patients showing strong antiviral activity (64%, 38%, and 13% suppression; P (by the log-rank test) .06, .0002, and .003 for CD8/CD4+ T-cell ratios of 2:1, 1:10, and 1:100, respectively) (Figure 2*B*).

Finally, we investigated the relationship between CD8+ T-cell antiviral suppressive capacity and pVL set point, as the latter is a known predictor of the rate of progression to AIDS, and both variables together explained most of variation in the rate of CD4+ T-cell decline in this cohort. A significant inverse correlation was observed for all 3 CD8+ /CD4+ T-cell ratios ($r = -0.68$ and $P = .002$ at the 2:1 ratio) (Figure 2*C*).

DISCUSSION

A critical role for CD8+ T cells in the control of HIV-1 has been inferred from observations on the selection of viral escape mutants by evolving HIV-1–specific CD8+ T-cell responses, from HLA class I associations with viremia control in vivo, from studies of transmission risk, and from experimental depletion of CD8+ T cells in SIV-infected macaques [4, 9, 24, 30–33]. The efficient inhibition of viral replication by CD8+ T cells from HIV-1 controllers/LTNPs provides compelling evidence that the quality of CD8+ T cells influences the rate of HIV-1 progression, but this has not been demonstrated in a longitudinal study [16–18]. Here, we provide the first report that the antiviral activity of CD8+ T cells is expressed as a continuous variable throughout HIV-1 infection and is strongly predictive of the rate of CD4+ T-cell decline, explaining up to 73% of interindividual variance in CD4+ T-cell slope during the first 3 years of infection. Of note, CD8+ T-cell antiviral activity was inversely related to set-point viremia in recently infected individuals, and together these 2 variables explained almost all of the variance in CD4+ T-cell slope in this cohort.

Although recent studies have elucidated some of the distinguishing features of HIV-specific immune responses in rare HIV-1 controllers, heterogeneity in progression rate among the majority of infected individuals is less well understood [11, 13, 16]. Virus-specific IFN-γ–producing CD8+ T cells are readily detectable in viremic individuals, yet neither the magnitude nor the breadth of these responses correlated with CD4+ T-cell count or viral load in cross-sectional studies [34, 35]. It is perhaps unsurprising that the magnitude of gag-specific IFN-γ responses failed to explain variation in the rate of CD4+ T-cell decline in our cohort and did not correlate with CD8+ T-cell antiviral activity. This suggests that the latter is a more direct measure of immune control in vivo.

The predictive value of CD8+ T-cell antiviral suppressive capacity in early HIV-1 infection has implications for therapeutic intervention in early infection and for the evaluation of HIV-1 vaccine candidates in phase I clinical trials.

Adult treatment guidelines in many settings recommend that asymptomatic individuals without comorbidities defer antiretroviral therapy until a CD4+ T-cell count of 350 cells/μL is reached [36]. Immediate antiretroviral therapy reduces transmission risk and may preserve immune function, but it is not currently a realistic option for the majority of infected individuals worldwide [37]. At the population level, pVL in early HIV-1 infection is predictive of the rate of progression to AIDS and death; however, the presenting pVL is a poor predictor of the rate of CD4+ T-cell decline in chronic infection, when most patients receive their diagnosis [25, 26, 38]. Our findings suggest that assessment of CD8+ T-cell antiviral activity in patients with CD4+ T-cell counts above current thresholds for ART initiation could identify individuals who are at high risk of rapid progression and thus might benefit from earlier intervention. This requires confirmation in larger cohorts but nevertheless indicates a possible approach to targeting therapy.

The lack of a reliable immune correlate of HIV-1 control is a major roadblock in the identification of vaccine candidates that should be prioritized for clinical development beyond phase I trials. The frequency of HIV-specific IFN-γ–producing T cells remains a widely used criterion of immunogenicity, despite its limitations [3]. Induction of CD8+ T-cell antiviral

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Table 3. Analysis of Square Root–Transformed CD4+ T-Cell Count Scale Over Time in 18 Subjects with Recent Human Immunodeficiency Virus Type 1 Infection, After Inclusion of Viral Set-Point Data

	Model 1			Model 2			Model 3			Model 4		
	Reference			Percentage inhibition at CD8/CD4 2:1			Percentage inhibition at CD8/CD4 1:10			Percentage inhibition at CD8/CD4 1:100		
Variable	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*	Coefficient	95% CI	*P*
Fixed effects												
Constant baseline	23.13	21.69–24.58		23.12	21.67–24.58		23.25	21.80–24.70		23.22	21.65–24.80	
Time (in years)	−1.69	−2.35 to −1.02	<.0001	−1.69	−2.34 to −1.04	<.0001	−1.85	−2.39 to −1.30	<.0001	−1.82	−2.45 to −1.19	<.0001
Viral load set point	−2.23	−4.22 to −.23		−2.20	−4.69–.30		−1.98	−4.39–.43		−2.05	−4.53–.44	
Time × viral load set point	−1.71	−2.79 to −0.62	.002	−1.40	−2.69 to −.11	.03	−0.99	−1.91 to −.08	.03	−1.23	−2.29 to −.17	.02
Percentage inhibition[a]	…	…		0.002	−.076–.080		0.008	−.056–.073		0.012	−.08–.103	
Time × percentage inhibition[a]	…	…		0.013	−.021–.047	.45	0.036	.015–.056	.001	0.038	.005–.066	.02
Random effects												
Proportion of variance between slopes accounted for with reference to model 1[b]	0.59	…		0.635	…		0.91	…		0.73	…	

The time at baseline was defined as the date of infection, estimated as described in Methods. Models were generated from 154 observations.

Abbreviation: CI, confidence interval.

[a] Model coefficients are different from those in Table 2 because of different numbers of subjects and observations.

[b] The reference model as given is for a viral load set point of 3.8 $\log_{10}$ RNA copies/mL, percentage inhibition at 2:1 of 62, percentage inhibition at 1:10 of 32, and percentage inhibition at 1:100 of 16.

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suppressive responses in HIV-uninfected recipients of DNA and/or replication-defective Ad5-vectored HIV-1 vaccines has been assessed retrospectively: individuals carrying protective HLA class I alleles showed overlap with LTNPs in the magnitude of HIV-specific cytotoxic CD8+ T-cell responses [17, 18, 39–42]. These findings, when taken together with our observations, suggest that a new benchmark for phase I vaccine trials might be induction of CD8+ T cells with suppressive capacity comparable to that of HIV-1 LTNPs/controllers.

We have shown that CD8+ T-cell–mediated inhibition of a single viral isolate explained a significant proportion of the variation in the rate of CD4+ T-cell loss, whereas the patients' viral subtype did not, indicating that HIV-specific CD8+ T-cell responses measured in our assay were cross-reactive and/or specific for conserved viral epitopes. Inclusion of a panel of diverse viruses in the viral suppression assay may be more informative with regard to true breadth of CD8+ T-cell responses. However, this would increase the complexity of the assay and could limit its use in clinical trials. We speculate that the efficacy of T cell–based vaccines will depend in part on their capacity to elicit CD8+ T cells that recognize diverse HIV-1 strains. To this end, we are currently evaluating CD8+ T-cell responses to an HIV-1 immunogen comprising conserved viral sequences in phase I trials [43] ClinicalTrials.gov registration numbers NCT01024842 and NCT01151319).

Our study has several limitations. First, the models assume that CD8+ T-cell antiviral activity does not change over time; second, in the chronic infection cohort analysis, distinguishing cause and effect in the relationship between CD8+ T-cell antiviral activity and CD4+ T-cell decline is problematic; third, the analysis of recently infected subjects was limited to samples obtained in early chronic infection. These limitations could largely be addressed by a longitudinal study examining CD8+ T-cell antiviral function repeatedly from the earliest stages of infection. The role of CD4+ T-cell help in maintaining optimal CD8+ T-cell antiviral function also merits investigation.

In summary, we show that the efficacy of CD8+ T-cell inhibition of heterologous HIV-1 replication in vitro is a strong predictor of the rate of CD4+ T-cell loss in early HIV-1 infection. Quantification of CD8+ T-cell inhibitory activity has advantages over other assays of HIV-specific cellular immunity as it provides a composite measure of multiple components of the antiviral response. It has potential as a prognostic marker in early infection and could aid evaluation of T cell–based vaccine strategies against HIV-1.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online(http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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