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Emergence of Individual HIV-Specific CD8 T Cell Responses during Primary HIV-1 Infection Can Determine Long-Term Disease Outcome

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ABSTRACT

Events during primary HIV-1 infection have been shown to be critical for the subsequent rate of disease progression. Early control of viral replication, resolution of clinical symptoms and development of a viral set point have been associated with the emergence of HIV-specific CD8 T cell responses. Here we assessed which particular HIV-specific CD8 T cell responses contribute to long-term control of HIV-1. A total of 620 individuals with primary HIV-1 infection were screened by gamma interferon (IFN-γ) enzyme-linked immunospot (ELISPOT) assay for HLA class I-restricted, epitope-specific CD8 T cell responses using optimally defined epitopes approximately 2 months after initial presentation. The cohort was predominantly male (97%) and Caucasian (83%) (Fiebig stages II/III [$n = 157$], IV [$n = 64$], V [$n = 286$], and VI [$n = 88$] and Fiebig stage not determined [$n = 25$]). Longitudinal viral loads, CD4 count, and time to ART were collected for all patients. We observed strong associations between viral load at baseline (initial viremia) and the established early viral set points ($P < 0.0001$). Both were significantly associated with HLA class I genotypes ($P = 0.0009$). While neither the breadth nor the magnitude of HIV-specific CD8 T cell responses showed an influence on the early viral set point, a broader HIV-specific CD8 T cell response targeting epitopes within HIV-1 Gag during primary HIV-1 infection was associated with slower disease progression. Moreover, the induction of certain HIV-specific CD8 T cell responses—but not others—significantly influenced the time to ART initiation. Individual epitope-specific CD8 T cell responses contribute significantly to HIV-1 disease control, demonstrating that the specificity of the initial HIV-specific CD8 T cell response rather than the restricting HLA class I molecule alone is a critical determinant of antiviral function.

IMPORTANCE

Understanding which factors are involved in the control of HIV-1 infection is critical for the design of therapeutic strategies for patients living with HIV/AIDS. Here, using a cohort of over 600 individuals with acute and early HIV-1 infection, we assessed in unprecedented detail the individual contribution of epitope-specific CD8 T cell responses directed against HIV-1 to control of viremia and their impact on the overall course of disease progression.

Events occurring during acute human immunodeficiency virus type 1 (HIV-1) infection appear to be critical for the subsequent speed of disease progression, as indicated by the strong association between the duration and intensity of the acute infection syndrome and the time to AIDS (1–5). Moreover, antiretroviral treatment (ART) studies initiated during primary HIV-1 infection have shown that some rare individuals can gain spontaneous control over viral replication after treatment cessation (6), supporting the notion that changes in the earliest immunobiology may influence long-term viral control.

During natural acute HIV-1 infection, viremia peaks to exceedingly high levels, followed by a decline that is temporally associated with the emergence of HIV-specific CD8 T cell responses (7, 8) and certain HIV-specific CD4 T cell responses (9, 10). These T cell responses control viral replication to a semistable viral set point that has been shown to be highly predictive of long-term disease outcome (11), supporting a role for the earliest HIV-specific T cell responses in long-term control of viral replication. While the first HIV-specific CD8 T cell responses are narrowly directed against a limited number of epitopes (12), they are able to potently suppress viral replication. Indeed, recent proof-of-concept vaccine studies in nonhuman primates demonstrated that while single epitope-specific CD8 T cell responses are not sufficient to prevent simian immunodeficiency virus (SIV) infection, they can be significantly associated with long-term control of viremia (13). However, HIV-1 can escape from this CD8 T cell-mediated immune control through the selection of mutations

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within targeted CD8 T cell epitopes (14, 15), ultimately leading to a loss of viral control and subsequent disease progression (16).

One of the strongest factors associated with viral control is the expression of particular HLA class I molecules that restrict HIV-specific CD8 T cell responses (17). Interestingly, with the knowledge of the HLA class I allele, the first emerging CD8 T cell responses can be to some degree successfully predicted (12). Thus, while overall HIV-specific CD8 T cells are most likely the strongest contributor to control of primary HIV-1 viremia, it has not been possible to date to dissect which specific CD8 T cell responses directed against HIV-1 epitopes have the strongest influence on viral control. Moreover, significant virus-specific CD8 T cells are detectable both in chronically infected individuals that progress rapidly to AIDS and in those who do not experience HIV-1 disease progression for decades. In particular, the level of control over viral replication is not predicted by the overall breadth, magnitude, or function of virus-specific CD8 T cell responses in chronic HIV-1 infection (18–21).

Here we assessed in unprecedented detail the individual contribution of epitope-specific CD8 T cell responses directed against HIV-1 to control of viremia in the largest cohort of acutely and early HIV-1-infected individuals studied to date. We demonstrated that within individuals encoding protective and nonprotective HLA class I alleles, certain epitope-specific CD8 T cell responses, but not others restricted by the same HLA class I molecule, significantly contribute to long-term control. Thus, these data demonstrate that the specificity of the initial CD8 T cell response to HIV-1 is critical for the subsequent control of viremia.

MATERIALS AND METHODS

Patient characteristics. A total of 620 subjects with acute or early HIV-1 infection participated in this study. The patients were recruited from primary infection cohorts in North America, Germany, and Australia (Massachusetts General Hospital, Boston, MA; Fenway Community Health Center, Boston, MA; AIDS Research Institute, University of California, San Francisco, CA; McGill University, Montreal, Canada; Kirby Institute, University of New South Wales, Sydney, Australia; Jessen-Praxis, Berlin, Germany; and University of California, San Diego, CA). The cohort was predominantly homogenous in terms of demographics and was predominantly a male (96.7%) Caucasian (82.9%) population. Primary HIV-1 was classified using Fiebig staging as previously described (22): 157 (25.3%) of the HIV-1-infected individuals were identified during Fiebig stage II/III, 64 (10.3%) in Fiebig IV, 286 (46%) in Fiebig V, and 88 (14%) in Fiebig VI. For 25 (4%) individuals, Fiebig staging was not possible due to lack of data. Detailed demographic and clinical characteristics are shown in Table 1.

HLA typing. High- and intermediate-resolution HLA class I typing was performed by sequence-specific PCR according to standard procedures.

IFN-γ enzyme-linked immunosorbent spot assay. HIV-1-specific CD8 T cell responses were assessed on frozen peripheral blood mononuclear cell (PBMC) samples collected during untreated infection for chronically infected individuals, or 8 weeks (±10 days) following diagnosis with primary HIV-1 infection. HIV-1-specific CD8 T cell responses were quantified by gamma interferon (IFN-γ) enzyme-linked immunosorbent spot (ELISPOT) assay, using a panel of 286 HLA class I-matched optimal epitope responses as previously described (12, 23). A response was considered positive only if there were ≥55 spot-forming cells (SFCs)/10^6 PBMCs and it was at least three times greater than the mean background in the negative wells and three times greater than the standard deviation within the negative controls.

Statistical analysis. Statistical analysis and graphical presentation were done using GraphPad Prism 5.0, Microsoft Excel, and SAS 9.1 (SAS Institute). Results are given as means ± standard deviation (SD) or medians with ranges. Correlations were assessed by Spearman rank analysis. Statistical analysis of significance (*P* values) was based on two-tailed *t* tests and linear regression analysis. Survival Kaplan-Meyer analysis was performed using a log-rank test. For each epitope, we first computed a two-sided *P* value for the correlation between the presence or absence of a CD8 T cell response and viral load using the Mann-Whitney test. Next, we computed a two-sided *P* value for the correlation between the response magnitude and viral load by computing the Spearman rank correlation and then applying the standard test for significance. For both tests, we determined *q* values from the *P* values using the approach described in reference 24.

RESULTS

The mean viral load of all enrolled study subjects at baseline visit was 1.40×10^6 HIV RNA copies/ml (SD, 6.47×10^6). Thirteen percent of all subjects with peak viral load data had an initial viremia above the upper cutoff of >750,000 HIV RNA copies/ml, and for those patients, no viral load dilution data were available. Seven enrolled subjects had an initial viremia below the cutoff of <50 HIV RNA copies/ml (Fig. 1A). The average viral load was significantly different at baseline between Fiebig stages (Fiebig II/III, $3.5 \times 10^6 \pm 9.67 \times 10^6$; Fiebig IV, $828{,}032 \times 10^6 \pm 1.25 \times 10^6$; Fiebig V, $867{,}661 \pm 5.98 \times 10^6$; Fiebig VI, 159,387 ± 478,164; $P <$ 0.0001 one-way analysis of variance [ANOVA]). The median time to antiretroviral treatment initiation was 154 days (range, 7 to 3,052) after diagnosis with primary HIV-1 infection. Time to treatment initiation differed significantly between sites. Subjects in Boston initiated therapy more rapidly, while subjects in Berlin were put on highly active antiretroviral therapy (HAART) more slowly than patients at the other sites. The decision regarding treatment initiation was based on viral load and CD4 count by the individual treating physician. Out of all enrolled individuals, 271 subjects remained treatment naive until a viral set point was established. The early viral set point was determined following a previously described algorithm (12). The average viral set point was with 58,290 ± 107,280 HIV RNA copies/ml, significantly ($P <$ 0.0001) lower than the viral load measured at enrollment. Initial viremia at baseline and at the viral set point was highly correlated ($R = 0.46$; $P < 0.0001$) (Fig. 1A and B), as previously described (25).

Interestingly, when we stratified baseline viremia by HLA class I alleles, we found that viral loads at baseline already differed significantly between individuals expressing different HLA class I alleles (Fig. 1C) at their first study visit. While HLA-B*57-positive individuals had an average of 4.13 $\log_{10}$ HIV RNA copies/ml, individuals carrying HLA-B*8 had, on average, 10-fold-higher viral loads (5.12 $\log_{10}$ HIV RNA copies/ml). The significant differences in baseline viral loads by HLA class I alleles ($P = 0.0003$, one-way ANOVA) were not driven by differences in Fiebig stages, and the frequency of the respective HLA class I alleles in this primary HIV-1 infection cohort did not differ from the HLA class I frequency in the overall North American population. Moreover, HLA frequencies of patients within the respective Fiebig stages were also not different (data not shown). Thus, these data suggest that HLA class I had no impact on HIV-1 acquisition, but during primary HIV-1 infection, the well-described HLA class I effects associated with viral control were already being observed. We next stratified the viral set points according to HLA class I alleles in all 271 individuals for which a viral set point could be determined (Fig. 1D). Indeed, the same significant HLA class I effects as de-

TABLE 1 Patient characteristics

		Fiebig	2/3	4	5	6	not done
		%	(25.3%)	(10.3%)	(46%)	(14%)	(4%)
male	600	96.7	152	61	277	87	23
female	18	2.9	5	3	9	1	-
unknown	2	0.3	-	-	-	-	2
Caucasian	514	82.9	133	51	233	76	21
African	28	4.5	10	6	10	2	0
Asian	17	2.7	5	1	10	1	0
Pacific Islander	5	0.8	2	1	1	0	1
Latino	12	1.9	1	0	7	4	0
Other/mixed	36	5.8	6	4	23	2	1
unknown	8	1.2	-	1	2.00	3	2
Viral load [HIV RNA copies/ml]							**viral set point**
n=	615		156	63	284	80	271
Minimum	49		49	49	49	63	40
25% Percentile	17,615		424,000	144,543	11,050	6,262	7,202
Median	**153,000**		**750,000**	**629,000**	**66,794**	**29,393**	**23,961**
75% Percentile	750,000		1,248,000	750,001	368,000	124,806	54,599
Maximum	95,000,000		84,200,000	7,440,000	95,000,000	3,918,000	794,800
Mean	**1,404,000**		**3,496,000**	**828,032**	**867,661**	**159,387**	**58,290**
Std. Deviation	6,471,000		9,669,000	1,255,000	5,988,000	478,164	107,280
CD4 count [cells/μl]							***1 year follow up***
n=	561		151	63	265	80	184
Minimum	42		42	124	210	99	138
25% Percentile	384		314	360	407	439	432
Median	**506**		**434**	**488**	**540**	**529**	**579**
75% Percentile	645		595	613	672	669	791
Maximum	1404		1290	1334	1404	1192	1958
Mean	**540**		**474**	**516**	**569**	**586**	**646**
Std. Deviation	224		217	236	221	215	294
time to treatment							
median (days)	**154**	*(range: 7-3052)*					

scribed for the viral loads at baseline were observed ($P = 0.0009$, one-way ANOVA), and HLA-B*57-positive individuals showed a mean viral set point of 3.67 $\log_{10}$ HIV RNA copies/ml, again the lowest viral set point.

Despite differences in the early viral set point between HLA class I alleles, it is important to note that within a group of individuals carrying the same HLA class I allele, the early viral set point also differed substantially. For example, viral set points within HLA-B*57$^+$ individuals ranged from 263 to 683,400 HIV RNA copies/ml, and those within HLA-B*8$^+$ individuals ranged from 98 to 511,583 HIV RNA copies/ml (Fig. 1D). We therefore wondered whether the differences in the early viral set points within individuals carrying the same HLA class I allele were driven by the presence or absence of certain epitope-specific CD8 T cell responses directed against HIV-1. We first determined whether CD8 T cell targeting of the most frequently recognized (immunodominant) HIV-1 epitopes restricted by the respective HLA class I molecules during primary HIV-1 infection had an impact on the early viral set point (Fig. 2A). However, neither the presence nor absence of the most commonly targeted epitope-specific CD8 T cell responses was associated with a higher or lower viral set point. The largest difference in viral load was observed for the immunodominant HIV-specific CD8 T cell response directed against the HLA-B*57-restricted Gag epitope TW10, which was previously associated with control of HIV-1 viremia (26). Surprising, however, the presence (average, 54,462 HIV RNA copies/ml) of the HLA-B*57-TW10 CD8 T cell response was associated with a higher viral set point than the absence of the TW10 response (average, 7,969 HIV RNA copies/ml), though not significantly (Fig. 2A). We therefore assessed whether, globally, the presence or absence of any of the epitope-specific CD8 T cell responses studied had an impact on the early viral set point (Fig. 2B). Unexpectedly, all HIV-specific CD8 T cell responses with the exception of one were significantly associated with higher viral load when present during primary HIV-1 infection compared to an absence of the respective epitope-specific CD8 T cell response. In particular, CD8 T cell responses directed against A*3RK10(Vif) ($P = 0.004$), B*51RL9(Gp41) ($P = 0.0005$), and Cw*3RL9(Gp41) ($P = 0.006$) were significantly associated with higher viral load (Fig. 2B). This association was not driven by Fiebig stages and thus not associated with time of emergence of HIV-specific CD8 T cell responses after primary HIV-1 infection ($P = 1$, Fisher's exact test). Only the presence of HIV-specific CD8 T cell responses directed against the epitope B*15-FY10 in Tat showed a marginal association with a lower viral load ($P = 0.03$) compared to the absence of this particular epitope-specific CD8 T cell response. Nonetheless, none of

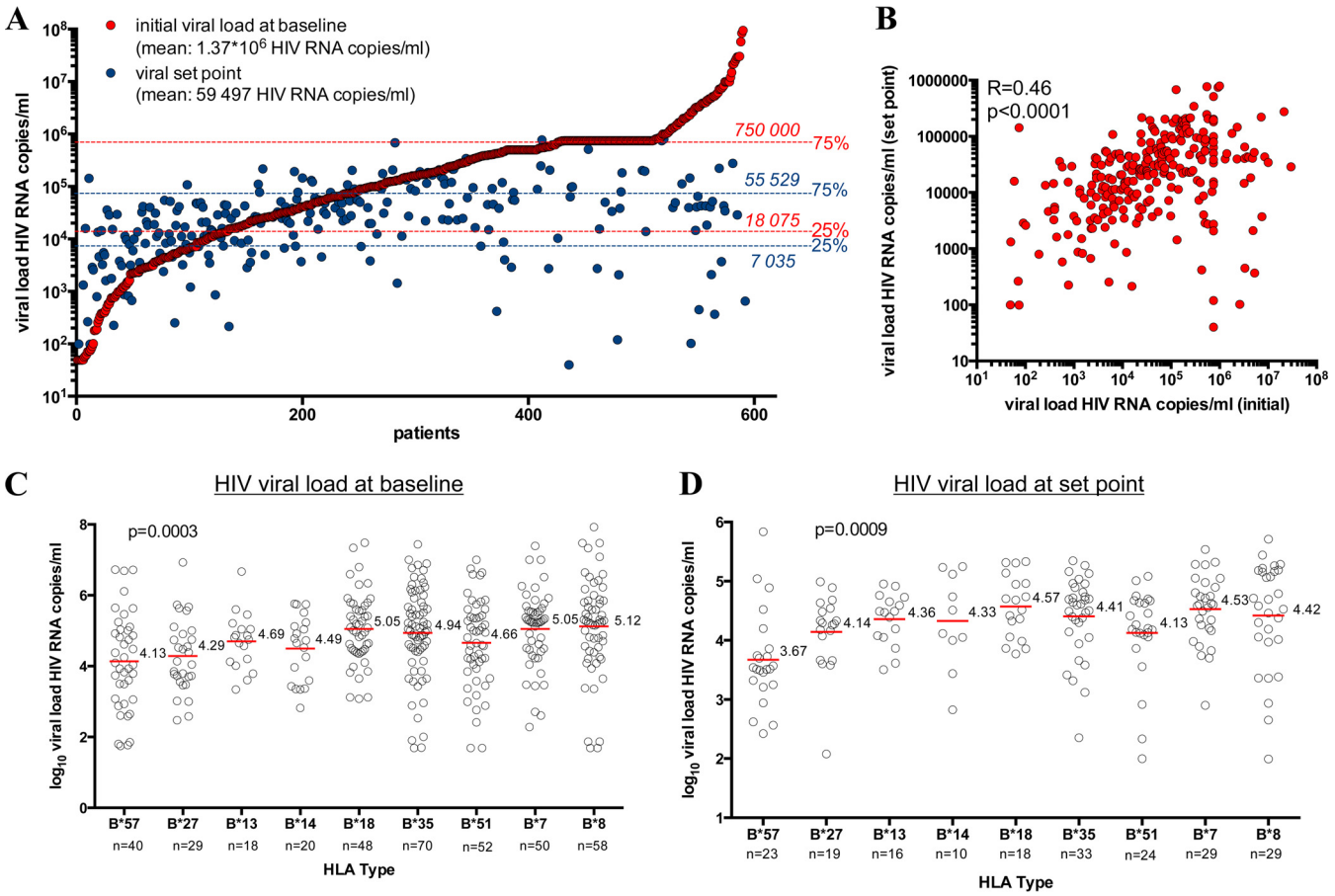

FIG 1 Association between baseline viral load, viral set point, and HLA class I allele. (A) Viral loads of all 620 HIV$^+$ individuals (x axis) at baseline (red) and corresponding viral set point (blue) when available. (B) Strong association between initial viral load measured at baseline and viral set point ($R = 0.46$; $P < 0.0001$, Spearman rank test). (C and D) Association of the expression of selected HLA class I alleles with viral loads showed significant differences between HLA class I alleles and the respective viral loads measured at baseline ($P = 0.0003$, one-way ANOVA) and viral set point ($P = 0.0009$, one-way ANOVA) (D).

these associations remained significant after correction for multiple comparisons (q value > 0.2). Thus, the simple presence or absence of an epitope-specific CD8 T cell response directed against HIV-1 did not show any associations with early control of viral replication.

To investigate whether the magnitude, rather than the simple presence, of the respective HIV-1-specific CD8 T cell responses influenced the level of the early viral set point, we determined Spearman rank associations for the magnitude of individual epitope-specific CD8 T cell responses (measured as spot-forming cells/million PBMCs [SFC/M]) and respective viral set points. We observed that the magnitudes of 12 epitope-specific CD8 T cell responses directed against HIV-1 were significantly associated with set point viremia, and three remained marginally significant after corrections for multiple comparisons ($q < 0.2$) (Table 2). However, with the exception of two epitopes [A*2-AL9(Vpr) and B*8-RL8(gp41)], the magnitudes of all HIV-specific CD8 T cell responses against the respective epitopes were again significantly associated with a higher and not a lower viral set point. Moreover, one of those HIV-specific CD8 T cell responses that was associated with a lower viral set point was the HLA-A*2-restricted epitope AL9 (Vpr). A previous study suggested that this epitope is present as an escape sequence in the majority of HIV-1 circulating strains (referred to as a "negatope"), and thus reversion to wild-type sequence may rather represent a gain in viral fitness (27). Thus, the association with lower viral load may be seen in this context as somewhat less beneficial. Overall, only the HIV-specific CD8 T cell response directed against B*8RL9 (gp41) showed marginal nominal association with a lower viral set point (Fig. 3) ($R = -0.3$; $P = 0.04$). However, this epitope was only targeted in 47% of the HLA-B*8$^+$ individuals during primary HIV-1 infection. The most frequently recognized HLA-B*8-restricted epitope was B8*FL8 in Nef (72% of the HLA-B*8$^+$ individuals), a response to which was associated with a higher viral set point ($R = 0.34$; $P = 0.02$). Thus, in this particular case, the subdominant HLA-B*8-restricted CD8 T cell responses against the RL9(Gp41) epitope may play a more prominent role in viral control than the immunodominant FL8 response restricted by the same HLA class I molecule.

Given the predominantly positive associations between epitope recognition of HIV-specific CD8 T cell responses and early viral set point, we reasoned that the detectability and level of an HIV-specific CD8 T cell response may also be driven by viremia, and therefore, the early viral set point may not be the best readout for disease progression. We therefore determined the time from presentation with primary HIV-1 infection until individuals initiated antiretroviral therapy as an additional parameter for HIV-1 disease progression. We first stratified time to treatment initiation based on HLA class I allele and found that there were significant

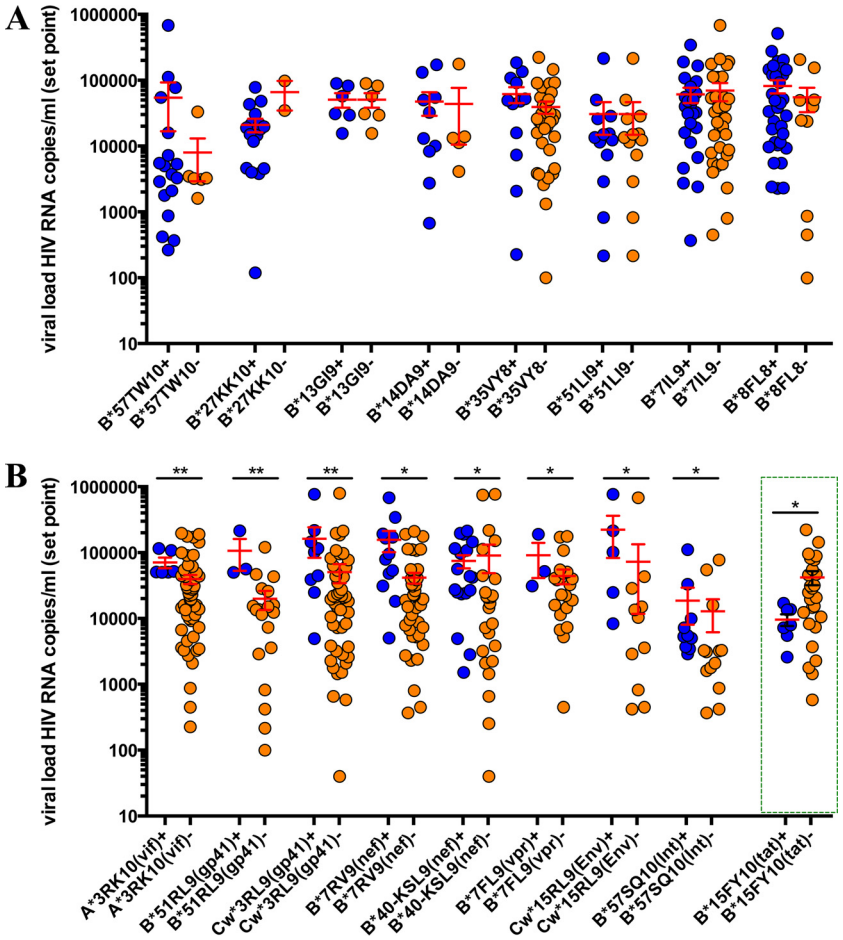

FIG 2 Association of the presence or absence of HIV-specific CD8 T cell responses with viral set point. (A) Presence (blue) or absence (orange) of the most frequently targeted HIV-specific CD8 T cell responses restricted by the respective HLA class I alleles showed no association with the viral set point. (B) Presence (blue) or absence (orange) of HIV-specific CD8 T cell responses that showed a nominal significant association with viral set point (association was not significant after correction for multiple comparison [$q > 0.2$]). The green box highlights the only HIV-specific CD8 T cell response that was nominal significantly associated with lower viral load.

differences (Fig. 4A). The median times to treatment initiation for HLAB*57$^+$ and HLAB*13$^+$ subjects were 1,111 and 992 days, respectively, significantly longer than those for subjects with all other HLA class I alleles ($P = 0.0001$, log rank test). The shortest time to ART initiation was observed in individuals carrying the HLA-B*8 allele (median, 145 days), which was also associated with a high viral set point, as shown above. Thus, time to treatment initiation reflected the overall HLA class I associations that were also observed for initial viremia and viral set point.

TABLE 2 Association of magnitude of epitope-specific CD8 T cell responses with set point viremia

optimal epitope	association with viral load	p-value	q-value	rho
A2*AL9 (Vpr)	**lower**	**0.001**	**0.19**	**-0.28**
B*7-TM9 (Nef)	higher	0.002	0.19	0.39
B*7-RV9 (Nef)	higher	0.003	0.19	0.39
B*51-IL10 (Vif)	higher	0.006	0.32	0.6
B*8-RL9 (gp41)	**lower**	**0.008**	**0.32**	**-0.39**
B*14-SL9 (Rev)	higher	0.009	0.32	0.68
B*57-SW10 (Int)	higher	0.009	0.32	0.5
A*30-KQY9 (Env	higher	0.01	0.41	0.65
B*13-GI9 (RT)	higher	0.02	0.45	0.73
B*8-FL8 (Nef)	higher	0.02	0.51	0.3
B*13-RI9 (p15)	higher	0.02	0.46	0.69
B*57-KF11 (p24)	higher	0.03	0.46	0.43

We next determined whether the breadth of HIV-specific CD8 T cell responses mounted during primary HIV-1 infection had an influence on long-term disease outcome (Fig. 4B). Interestingly, we observed that individuals with the largest breadth of measured HIV-specific CD8 T cell responses during primary HIV-1 infection also showed the slowest disease progression, measured as the time the individuals remained off ART ($P = 0.003$, log rank). Individuals that had no detectable HIV-specific CD8 T cell responses during primary infection initiated antiretroviral therapy within a median of 107 days, while in contrast, individuals with 6 to 10 detectable HIV-specific CD8 T cell responses initiated treatment after a median of 691 days and 670 days with more than 10 responses. While the presence of more broadly directed HIV-specific CD8 T cell responses during primary acute HIV-1 infection showed no association with early viral set point ($R = 0.07$; $P = 0.19$; data not shown), we observed a significant impact on long-

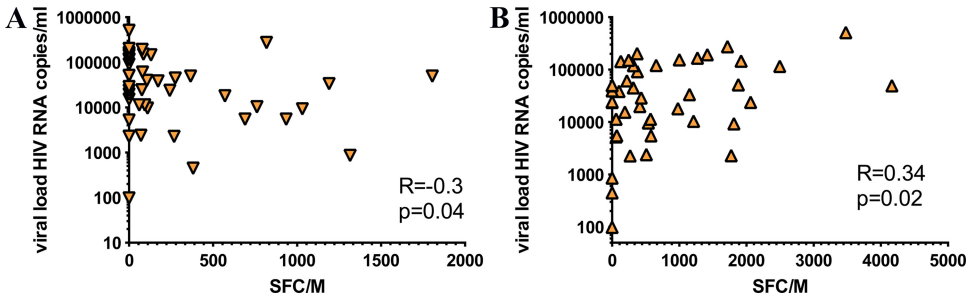

FIG 3 Association of the magnitude of individual HIV-specific CD8 T cell responses and viral set point. (A) The HIV-specific CD8 T cell response directed against RL9 (gp41) showed a nominally significant association of stronger CD8 T cell response magnitude (measured as SFC/M) and lower viral set point ($R = -0.3$, $P = 0.03$). (B) The magnitude (in SFC/M) of the most frequently targeted HLA-B8-restricted CD8 T cell response B8FL8 in Nef showed a nominally significant association of stronger CD8 T cell response with a slightly higher viral set point ($R = 0.34$, $P = 0.02$).

term disease outcome, measured by time to treatment initiation. We next wondered whether the breadth also determined long-term outcome within individual HLA class I alleles (Fig. 4C). However, while a larger breadth of HIV-1-specific CD8 T cell responses within the same HLA class I allele seemed to trend toward slower disease progression for some HLA class I alleles, there was no statistical significant difference (Fig. 4C). Taken together, our data demonstrate that a broader HIV-specific CD8 T cell re-

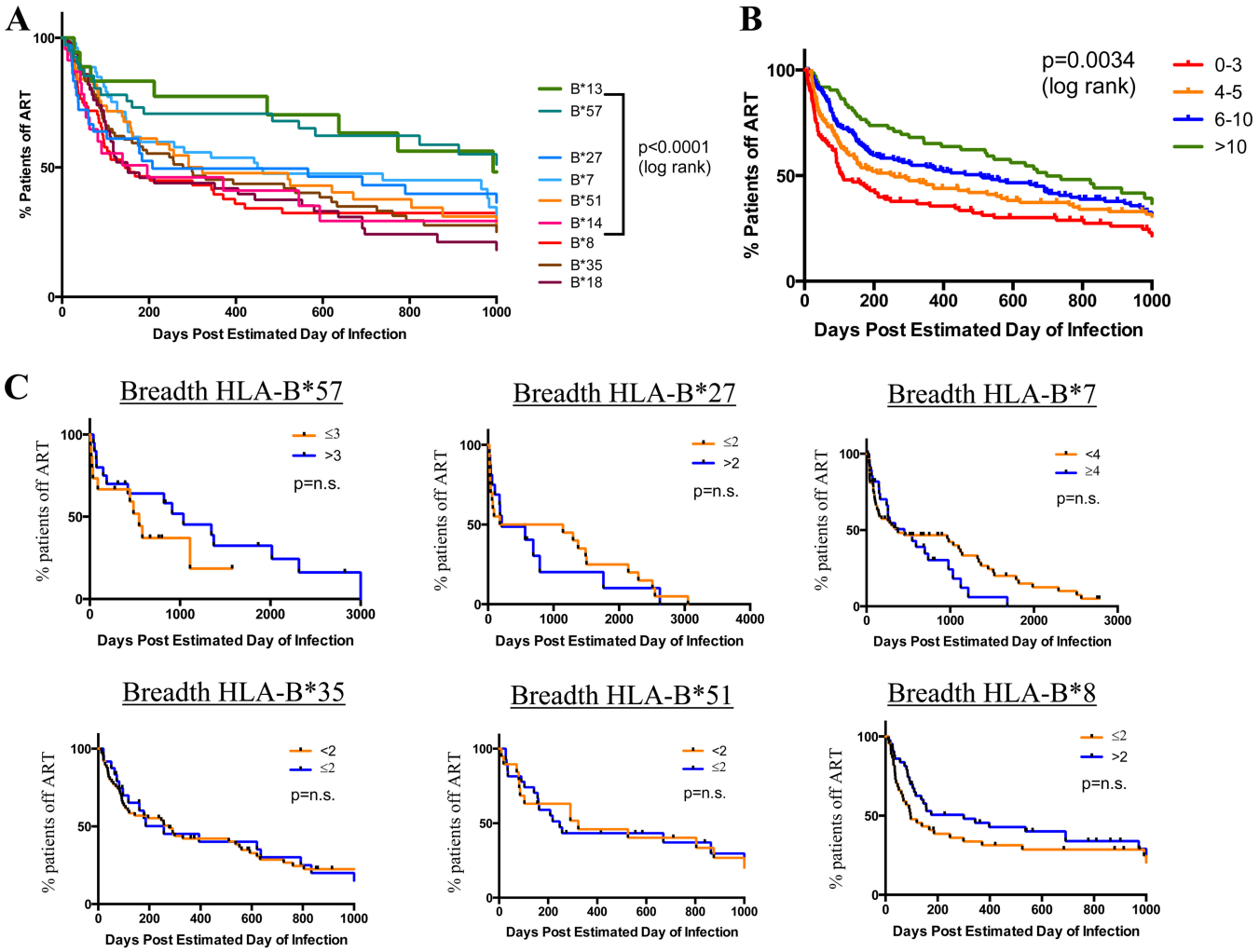

FIG 4 Association of breadth of HIV-specific CD8 T cell responses with time patients remained off antiretroviral therapy (ART). (A) Significant association of time patients remained off ART with HLA class I expression highlights the protective effect by HLA-B57 and HLA-B13, with significant slower progression. (B) Larger breadth of HIV-specific CD8 T cell responses during primary HIV infection was significantly associated with time patients remained off ART. (C) Association of the breadth of HIV-specific CD8 T cell responses within the same HLA class I allele showed trends but no significant association.

TABLE 3 Association of epitope-specific CD8 T cell responses with delayed initiation of ART[a]

Epitope name	Protein	sequence	p value	association
B*57-IW9(p24)	Gag*	ISPRTLNAW	0.0044	slow
A*11-QVK9 (RT)	Pol	QIYAGIKVK	0.0145	slow
Cw*5-AM12 (p24)	Gag	AEQASQEVKNWM	0.0165	slow
A*2-FK10 (p15)	Gag	FLGKIWPSYK	0.0166	slow
B*44-EW9 (protease)	Pol	EEMNLPGRW	0.0287	slow
B*40-GI9 (p17)	Gag	GELDRWEKI	0.031	slow
B*27-KK10(p24)	Gag	KRWIILGLNK	0.0396	slow
B*57-KV8 (protease)	Pol	KAIGTVLV	0.0002	rapid
B*57-LL9 (nef)	Nef	LTFGWCFKL	0.0002	rapid
A*3-TK10 (gp120)	Env	TVYYGVPVWK	0.0015	rapid
B*35-VL11(gp120)	Env	VPVWKEATTTL	0.0019	rapid
B*18-FK10(p24)	Gag	FRDYVDRFYK	0.0025	rapid
B*13-GI11(P24)	Gag	GQMREPRGSDI	0.0176	rapid
A*3-ATK9 (RT)	Pol	AIFQSSMTK	0.0269	rapid
B*57-YT9(nef)	Nef	YFPDWQNYT	0.027	rapid
A*1-RY9 (gp41)	Env	RRGWEVLKY	0.0271	rapid
B*57-KF9(Int)	Pol	KTAVQMAVF	0.036	rapid
B*57-TW10(p24)	Gag	TSTLQEQIGW	0.0385	rapid
B*51-LI9 (INT)	Pol	LPPVVAKEI	0.0469	rapid

[a] Fisher's exact test for Gag versus other proteins, $P = 0.02$.

sponse is associated with a delayed onset to ART initiation. While we found a similar trend for some single HLA class I alleles, the data suggest that restrictions by several HLA class I alleles may be involved in the overall control of acute viremia.

Based on the above results, we assessed whether the presence or absence of individual HIV-specific CD8 T cell responses also impacted disease progression, measured as the time to treatment initiation. Interestingly, out of all measured HIV-specific CD8 T cell responses, we identified seven epitope-specific CD8 T cell responses that were significantly associated with delayed initiation of ART (Table 3). Among those, the epitopes HLA-B*57IW9 (p24) ($P = 0.003$), HLA-B*27KK10(p24) ($P = 0.039$), HLA-A*2FK10 (p24) ($P = 0.016$), and HLA-CW5*AM12 (p24) ($P = 0.016$) showed the strongest association (Fig. 5). Moreover, we also identified 12 epitope-specific CD8 T cell responses that were significantly associated with a shorter time to initiation of therapy (Table 3). These included some extensively studied HIV-1 epitopes that have been previously associated with HIV-1 control, such as the HLA-B*57-restricted epitope TW10 within HIV-1 Gag (26). Unfortunately, we were not able to determine escape mutations in the individual viral sequences in this large study cohort. Thus, it is possible that the lack of HIV-specific CD8 T cell responses in those instances is reflective of viral escape within the individual subjects. Nevertheless, we also noted that there were significantly more HIV-specific CD8 T cell responses associated with delayed initiation of antiretroviral therapy targeted epitopes in Gag than there were epitope-specific CD8 T cell responses associated with earlier initiation of treatment ($P = 0.02$, Fisher's exact test), suggesting a particular role for Gag-specific CD8 T cell responses mounted during primary HIV-1 infection and long-term control of HIV-1 viremia.

DISCUSSION

The role of HIV-specific CD8 T cell responses in control of HIV-1 infection has been well established. One of the most remarkable pieces of evidence is the strong association of HLA class I alleles with disease outcome, suggesting that HIV-specific CD8 T cell responses restricted by those alleles have better qualities for controlling viremia. However, one inherent problem is to dissect which individual epitope-specific CD8 T cell responses have the strongest influence on HIV-1 control and which specific CD8 T cell responses control initial HIV-1 viremia. In the largest cohort of individuals with primary HIV-1 infection studied to date, we

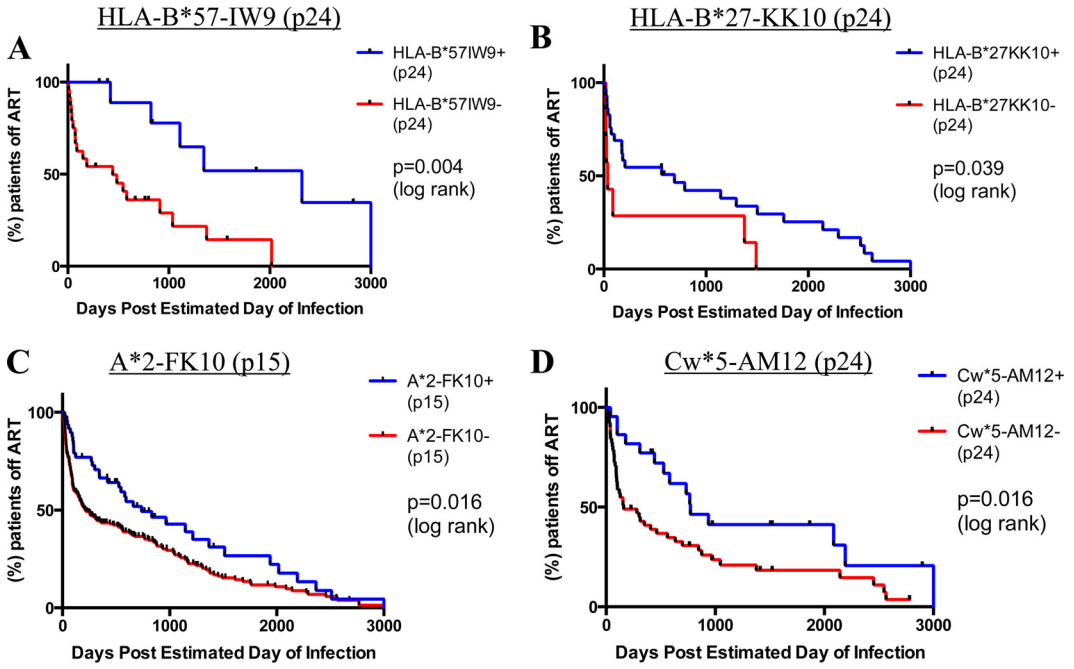

FIG 5 Association of individual HIV-specific CD8 T cell response with time patients remained off ART. (A to D) The presence of individual HIV-specific CD8 T cell responses is significantly associated with longer time patients remained off ART.

aimed to dissect the influence and contribution of individual epitope-specific CD8 T cell responses on peak viremia, viral set point, and time to initiation of antiretroviral therapy. We found strong associations between HLA class I genotypes and initial viremia as well as viral set point. Moreover, we observed that neither the presence/absence nor the magnitude of HIV-specific CD8 T cell responses showed an association with early viral set point. Intriguingly, however, we observed a relationship between the presence of individual HIV-specific CD8 T cell responses and the time until infected patients initiated antiretroviral therapy.

Previous studies have suggested that the emergence of HIV-specific CD8 T cell responses during acute HIV-1 infection is associated with control of viral replication and postpeak viral load decline. Unexpectedly, in this large cohort of individuals with primary infection, the presence of particular epitope-specific CD8 T cell responses was associated with a higher viral set point, in contrast to individuals expressing the same HLA class I alleles in whom the respective responses were absent. Similarly, a higher magnitude of HIV-specific CD8 T cell responses was associated with a higher viral load set point. While these data at first suggest that the presence of HIV-specific CD8 T cell responses may drive a higher set point, the underlying biology suggests that the detection of HIV-specific CD8 T cell responses in peripheral blood instead reflects the level of antigenemia and thus represents a consequence of the level of viremia. Furthermore, the paradoxical finding that lower viral set point is associated with better disease outcome (11, 28, 29), yet that the magnitude of the early HIV-specific CD8 T cell responses is associated with higher viral load, may be explained by the model that high viremia drives the proliferation of HIV-specific CD8 T cell responses and therefore rather reflects a secondary effect of increased viremia than the level of control mediated by HIV-specific CD8 T cell response. However, it is important to note that the strongest indicator for differences in the early viral set point remains the expression of HLA class I alleles and not the respective HIV-specific CD8 T cell responses. Whether this is due to other factors associated with HLA class I alleles (for example, expression levels of host restriction factors [30]) remains to be studied.

Time to initiation of antiretroviral treatment following infection represents a good indicator for long-term control of HIV-1 infection. In this large cohort of individuals identified during primary infection we observed a significant association between the presence/absence of individual HIV-specific CD8 T cell responses and the time individuals remained off antiretroviral therapy. While we found regional differences in the initiation of antiretroviral therapy, patients with protective alleles did better in all regions. We identified seven individual epitope-specific CD8 T cell responses that were associated with better outcome. To our knowledge, this is the first time that an association between an individual's HIV-specific CD8 T cell responses and HIV-1 disease outcome has been described.

However, it is important to note that the study was not able to take into account potential sequence variations and escape mutations in HIV-specific CD8 T cell-targeted epitopes and used consensus sequences of previously defined CD8 T cell epitopes to detect virus-specific immune responses. This limitation might be best reflected by the observed association between CD8 T cell responses directed against the HLA-B*57-restricted Gag epitope TW10 with higher viral load and more rapid initiation of antiretroviral therapy. Previous studies have suggested that viral escape from this particular response resulted in loss of viral replication fitness and thus might contribute to control of viremia (26). Autologous viruses from about 38% of HIV-infected HLA-B*57$^+$ individuals show escape mutations in the HLA-B*57TW10 epitope within the first year of infection (31). While this is speculative, the lack of escape in some individuals from this epitope may result in overall higher viremia and lack of control, and thus the persistent detection of this particular CD8 T cell response was associated with more rapid initiation of antiretroviral therapy.

To our knowledge, this is the first study that is able to pinpoint individual HIV-specific CD8 T cell responses and their association with slower disease progression. Furthermore, this study provides a potential link for some HLA class I alleles and their association with better disease outcome to a specific CD8 T cell response. Whether the specific induction of these responses may be able to guide long-term control through vaccination remains to be seen. However, it supports the notion that the specificity of individual HIV-specific CD8 T cell responses may drive long-term control.

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