MAJOR ARTICLE

Vaccine-Induced Gag-Specific T Cells Are Associated With Reduced Viremia After HIV-1 Infection

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The contribution of host T-cell immunity and HLA class I alleles to the control of human immunodeficiency virus (HIV-1) replication in natural infection is widely recognized. We assessed whether vaccine-induced T-cell immunity, or expression of certain HLA alleles, impacted HIV-1 control after infection in the Step MRKAd5/HIV-1 *gag/pol/nef* study. Vaccine-induced T cells were associated with reduced plasma viremia, with subjects targeting ≥3 gag peptides presenting with half-log lower mean viral loads than subjects without Gag responses. This effect was stronger in participants infected proximal to vaccination and was independent of our observed association of HLA-B*27, –B*57 and –B*58:01 alleles with lower HIV-1 viremia. These findings support the ability of vaccine-induced T-cell responses to influence postinfection outcome and provide a rationale for the generation of T-cell responses by vaccination to reduce viremia if protection from acquisition is not achieved. Clinical trials identifier: NCT00095576.

Keywords. HIV-1 vaccine; Step study; Gag-specific T cells; HLA class I alleles.

In recent years, landmark investigations showed that several prevention modalities offer promise in reducing human immunodeficiency virus type 1 (HIV-1) acquisition in adults living in regions of endemicity [1–6]. Nevertheless, a highly effective prophylactic HIV-1 vaccine providing long-term protective immunity remains a major global health goal.

The Step study, a phase IIb HIV-1 vaccine efficacy trial evaluating the MRKAd5 HIV-1 *gag/pol/nef* vaccine, was the first test-of-concept study to use a T-cell–based vaccine to protect against HIV-1 infection [7]. The vaccine was shown to be highly effective at inducing CD8$^+$ T cells [8], but did not lower the frequency of HIV-1 acquisition or post-infection viremia in vaccine recipients relative to placebo recipients [7, 9]. Nevertheless, the sieve analysis of the Step study comparing breakthrough HIV-1 sequences for vaccine and placebo recipients found that the vaccine impacted founding virus populations [10, 11], suggesting that T-cell responses exerted pressure on incoming viruses.

The importance of T-cell responses for the control of virus replication is supported by the temporal association between the early decline in HIV-1 viremia and the appearance of HIV-1-specific T cells [12] and by the failure of nonhuman primates to control simian immunodeficiency virus (SIV) replication upon depletion of CD8$^+$ T cells [13]. In addition, CD8$^+$ T-cell–mediated immune pressure commonly leads to escape within the recognized epitope [14, 15], resulting in spikes of viremia [16]. Several vaccine strategies aimed at inducing virus-specific T cells were evaluated in nonhuman primate models and led to the control of viremia and

Received 13 January 2013; accepted 26 March 2013; electronically published 21 July 2013.

Presented in part: 2012 Keystone Symposia Meeting on HIV Vaccines, Keystone, Colorado, 21–26 March 2012.

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The Journal of Infectious Diseases 2013;208:1231–9

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DOI: 10.1093/infdis/jit322

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an attenuated disease course [17–20] and to the prevention of productive SIV infection [21]. Whether CD8+ T cells are as effective in containing HIV-1 replication in humans has been less clear [22–25], but a number of studies have observed preferential targeting of Gag epitopes in association with control of viral replication in subjects infected with clade B [26, 27] and clade C [28, 29] HIV-1.

The marked diversity of HLA genes has long been recognized to influence HIV-1 pathogenesis and disease progression [30], and HLA-B*57, HLA-B*27, and HLA-B*58:01 have been commonly associated with better disease outcomes [31]. A possible explanation for this association is the ability of these alleles to present conserved Gag epitopes [32, 33]; escape from these immune responses is associated with a high fitness cost [34–36], resulting in delayed escape for HLA-B*27 [16]. Conversely, expression of some HLA alleles, most prominently HLA-B*35Px, as well as reduced HLA diversity though homozygosity, have been associated with accelerated HIV-1 disease progression [37]. Evidence of the significance of HLA allele expression in determining clinical outcomes continues to grow [31, 38–40], and these effects have been recapitulated in rhesus macaques [41, 42]. HLA-B*57 alleles have most notably been associated with HIV-1 control in 2 genome-wide association studies [43, 44], one of which implicated peptide binding to the HLA class I groove in determining control [44]. An additional genome-wide association study identified HLA as the major determinant of the magnitude of Gag-specific T-cell responses after vaccination in the Step study [45], supporting the hypothesis that the beneficial effects of HLA alleles are mediated through Gag-specific T cells. Step study participants provide a unique population in which to study the interplay between HLA and vaccine-induced Gag-specific immune responses in determining viral load after HIV-1 infection. This article examines the association between HIV-1 Viral load, expression of established protective or unfavorable HLA class I alleles or allele combinations, and vaccine-induced T-cell responses in Step study participants who acquired HIV-1 infection.

METHODS

Study Population

The Step study (Merck V520–023/HVTN 502) was a multicenter, double-blind, randomized, placebo-controlled phase IIb proof-of-concept study to evaluate the safety and efficacy of the MRKAd5 HIV-1 *gag/pol/nef* trivalent vaccine in 3000 HIV-1-negative adults with a high risk for HIV-1 infection (clinical trials identifier: NCT00095576). Vaccine recipients were given 3 doses of 1.5×10^{10} virus genomes at weeks 0, 4, and 26. We identified 157 Step study participants who acquired HIV-1 infection (95 vaccine recipients and 62 placebo recipients); the median time between the last vaccination and the diagnosis of infection was 12 months (range, 0–42 months). The median duration of follow-up after infection was 19 months (range, 1–51 months). Three placebo recipients were excluded from the analysis because of missing covariate data, resulting in a total of 154 subjects for analysis (95 vaccine recipients and 59 placebo recipients). Longitudinal immune responses were evaluated in 74 vaccine recipients who did not become HIV-1 infected. The institutional human subjects review committee at each clinical site approved the protocol before study initiation, and all participants provided written, informed consent.

HLA Typing

HLA class I typing was performed by sequence-based typing (Supplementary Materials). HLA groups were defined on the basis of previous studies as "protective" (HLA-B*27, HLA-B*57, and HLA-B*58:01 [31, 40]), "unfavorable" (HLA-B*35:02, HLA-B*35:03, HLA-B*35:04, HLA-B*53:01, or homozygous in at least one locus [37, 46]), or "neutral" (all other HLA class I types). The distribution of HLA groups is shown in Supplementary Table 1.

Immunogenicity

The magnitude of the preinfection HIV-1-specific T-cell response was assessed by interferon γ (IFN-γ) enzyme-linked immuno spot (ELISpot) analysis in 85 vaccine recipients who later became HIV-1 infected, using preinfection samples from week 8 (4 weeks after the second vaccination) and vaccine-matched Gag, Pol, and Nef peptide pools. Magnitudes were reported as spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMC). Assays were run at Merck Research Laboratories (n = 56) or at the Fred Hutchinson HVTN laboratory (n = 29). Magnitudes were grouped according to quartile of IFN-γ–secreting cells (SFC/million PBMC; Table 1).

The breadth of HIV-1-specific T-cell responses at week 8 was assessed in 72 vaccine recipients who became HIV-1 infected, by mapping responses to single 15-mer vaccine-matched peptides as described in the Supplementary Materials and elsewhere [47]. Individuals who had no positive responses to pools were assumed to have zero breadth. Breadth was categorized as 0, 1–2, or ≥3 measured responses (Table 2).

Intracellular cytokine staining was used to study the decline in the preinfection vaccine-induced T-cell responses over time as previously described [8, 48]. We evaluated 74 vaccine recipients who did not become HIV-1 infected for the duration of the trial at weeks 30, 52 and 104. Response magnitudes were reported as the percentage of CD4+ or CD8+ T cells expressing IFN-γ and/or IL-2 when stimulated with vaccine-insert matched Gag, Pol, or Nef peptide pools, minus the same percentage for unstimulated cells.

Plasma HIV-1 RNA Determination

Plasma HIV-1 RNA levels were determined using the Roche Amplicor assay at weeks 1, 2, 8, 12, 26 post-infection, and every 6 months thereafter. Only values before antiretroviral therapy

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(ART) initiation were included. Plasma viral load is reported as $\log_{10}$ HIV-1 RNA copies per milliliter.

Statistical Analysis

The mean $\log_{10}$ magnitude of the immune response to peptide pools was modeled using linear regression. The mean breadth of responses was modeled using overdispersed Poisson regression. Because differences in the magnitude of responses were found between the Merck and Fred Hutchinson laboratories, response magnitude was modeled using quartiles of response, and quartiles were determined for each laboratory separately.

The longitudinal mean $\log_{10}$ viral load was modeled using weighted generalized estimating equations (GEE) models with independence working correlation. Generalized Wald tests were used for inference. Only viral loads before ART initiation were used. To account for missing viral load data, observations were weighted with respect to the inverse probability of missing data (Supplementary Materials). Models included week postinfection and treatment assignment (vaccine vs placebo) as covariates, where appropriate.

The following baseline participant characteristics were considered: adenovirus 5 (Ad5) seropositivity, region of residence (North America vs other), race (white vs other), self-reported circumcision status, age (≤30 vs >30 years), HLA group, and herpes simplex virus type 2 (HSV-2) serostatus. Two subjects living in the United States who were missing data on circumcision status were assumed to be circumcised. One subject missing data on HLA type and 2 subjects missing data on HSV-2 status were excluded from analyses involving these covariates. Analyses among vaccine and placebo recipients adjusted for these baseline characteristics, but analyses among vaccine recipients alone did not adjust for these characteristics, because of the small sample size. However, conclusions were the same based on the unadjusted and adjusted analyses.

Data were analyzed using R, version 2.9.2. All *P* values are 2 sided.

RESULTS

Expression of Protective HLA Class I Alleles Is Associated With a Lower Viral Load After Infection

We evaluated the effect of HLA class I alleles on plasma viremia in 154 Step study participants who acquired HIV-1 infection (95 vaccine recipients and 59 placebo recipients). We compared mean longitudinal pre-ART $\log_{10}$ plasma viral loads between subjects expressing protective, unfavorable, or neutral HLA class I alleles. A weighted GEE model that included baseline participant characteristics (see Methods) and time since infection was used. Compared with subjects with neutral HLA alleles, the estimated viral load was 0.81 logs lower (95% confidence interval [CI], .49–1.12 logs lower) among subjects with protective HLA alleles ($P < .0001$) and 0.21 logs higher (95% CI, .04 logs lower to .45 logs higher) among those with unfavorable HLA alleles ($P = .1$).

To assess whether vaccination modified the effect of HLA class I alleles on host control of HIV-1 RNA levels, we added an interaction between HLA group and treatment assignment to the regression model. While there was some suggestion that the effect of HLA on viral load was more pronounced in the vaccine arm (Figure 1), this effect was not significant ($P = .15$).

Increased Breadth but Not Magnitude of Vaccine-Induced Gag-Specific T-Cell Responses Is Associated With a Lower Viral Load

The trend toward a reduction in viral load among vaccine recipients as compared to placebo recipients with protective HLA alleles suggested that vaccine-induced T-cell responses may influence the control of viral replication in some participants.

Table 1. Difference in Viral Load (VL), by Quartile (Q), in the Total or Gag-Specific Magnitude of the Immune Response Among Vaccine Recipients Who Acquired Human Immunodeficiency Virus, Overall and Given Infection Within 1 Year of the Last Vaccination

Group	Δ $\log_{10}$ VL, Mean (95% CI)[a]	*P*
Overall (n=72)		
Total magnitude[b]		.06
Q1	0	
Q2	−0.39 (−.83 to .05)	.08
Q3	−0.27 (−.85 to .31)	.36
Q4	0.07 (−.32 to .45)	.74
Gag magnitude[b]		.38
Q1	0	
Q2	0.14 (−.37 to .65)	.59
Q3	−0.22 (−.75 to .30)	.40
Q4	−0.10 (−.60 to .40)	.69
Infected within 1 year (n=36)		
Total magnitude[b]		.01
Q1	0	
Q2	−0.540 (−1.08 to −.002)	.05
Q3	0.015 (−.750 to .720)	.97
Q4	0.125 (−.384 to .633)	.63
Gag magnitude[b]		.20
Q1	0	
Q2	−0.102 (−.817 to .613)	.78
Q3	−0.600 (−1.293 to .093)	.09
Q4	−0.310 (−.985 to .364)	.37

Abbreviation: CI, confidence interval.

[a] Difference in mean log viral load between each magnitude quartile and the first quartile. Estimates are adjusted for time since HIV-1 infection and HLA group.

[b] Quartiles are defined separately for assays run at Merck and Fred Hutchinson (FH) laboratories. Cutoffs defining Merck (and FH) quartiles for $\log_{10}$ total magnitude are 430 (390), 886 (625), and 2130 (1014) SFC/10^6 PBMC; for Gag magnitude, cutoffs are 157 (107), 216 (163), and 441 (284) SFC/10^6 PBMC.

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Table 2. **Difference in Viral Load (VL), by Total or Gag-Specific Breadth of the Immune Response, Among Vaccine Recipients Who Acquired Human Immunodeficiency Virus, Overall and Given Infection Within 1 Year of the Last Vaccination**

Group	No. Recipients	Δ Log_{10} VL, Mean (95% CI)[a]	*P*
Overall (n=72)			
Total breadth, responses, no.			.21
0	23	0	
1–2	21	−0.32 (−.73 to .09)	.13
≥3	28	−0.33 (−.75 to .09)	.13
Gag breadth, responses, no.			.02
0	46	0	
1–2	21	−0.38 (−.71 to .05)	.02
≥3	5	−0.53 (−.98 to −.09)	.02
Infected within 1 year (n=36)			
Total breadth, responses, no.			.03
0	10	0	
1–2	8	−0.512 (−.974 to −.050)	.03
≥3	18	−0.529 (−.969 to −.09)	.02
Gag breadth, responses, no.			<.001
0 responses	22	0	
1–2 responses	11	−0.607 (−1.041 to −.174)	.006
≥3 responses	3	−0.768 (−1.241 to −.293)	.002

Abbreviation: CI, confidence interval.

[a] Difference in mean log_{10} viral load between each breadth group and the zero breadth group. Estimates are adjusted for time since HIV-1 infection and HLA group.

Therefore, we evaluated whether vaccine-induced T-cell responses before infection predicted postinfection HIV-1 RNA levels among vaccine recipients. The breadth and magnitude of preinfection T-cell responses were measured by IFN-γ ELISpot at week 8 in 72 of the vaccine recipients who later acquired HIV-1 infection [8, 45]. Sixty-eight percent of vaccinees (49/72) recognized 1–15 15-mers (Figure 2). The median number of 15-mers recognized in the vaccine group overall was 1, with higher baseline Ad5 neutralizing antibody titers associated with a lower mean breadth (*P* = .03, overdispersed Poisson regression model). The median magnitude of the total response (Gag + Pol + Nef) was 625 SFC/10^6 PBMC at the Fred Hutchinson laboratory and 886 SFC/10^6 PBMC at the Merck laboratory (Supplementary Figure 1).

To evaluate whether vaccine-induced T-cell responses contributed to the control of viral replication after infection, we used the weighted GEE model for viral load described above, with additional adjustment for HLA group and time since infection (see Methods). Given that Gag-specific T-cell responses have been most consistently found to be associated with control of viral replication in unvaccinated HIV-1-infected cohorts, we studied Gag-specific and total T-cell responses. The magnitude of the response was grouped by quartile (Table 1), while the breadth was categorized as 0, 1–2, or ≥3 measured responses (Table 2). These analyses did not adjust for baseline characteristics, because of the small sample size.

We found no significant association between the magnitude of the total (*P* = .06) or Gag-specific T-cell responses (*P* = .38) and plasma HIV-1 RNA loads (Table 1). Similarly, no significant association was detected between the total breadth of responses and viral loads (Table 2).

Interestingly, vaccine recipients who targeted a higher number of Gag 15-mers showed increased control of HIV-1 replication (*P* = .02), with the mean viral load among subjects targeting ≥3 Gag 15-mers more than half a log lower than that among subjects not targeting Gag (Table 2). Results adjusted for baseline characteristics were similar (data not shown).

Vaccine-Induced T-Cell Immune Responses Wane Over Time

Given that 44% of the subjects studied here had HIV-1 infection diagnosed >1 year after the last vaccination, we evaluated whether preinfection vaccine-induced responses remained stable over time. HIV-1-specific T-cell responses were measured in 74 male vaccine recipients who did not acquire HIV-1 infection, by intracellular cytokine staining at peak (week 30 [ie, 4 weeks after the third vaccination]) and during the early memory and late memory phase (weeks 52 and 104 [ie, 1 and 2 years, respectively, after the first vaccination]).

As shown in Figure 3, $CD8^+$ T-cell response rates to the vaccine HIV-1 inserts started waning at the early memory time point. The magnitude among responders remained stable for the first year but then declined as well. Similar trends were observed for $CD4^+$ T-cell responses (data not shown).

The Beneficial Effect of the Breadth of Gag-Specific T-Cell Responses Is More Pronounced in Vaccine Recipients Infected Within a Year of the Last Vaccination

Since vaccine-induced immune responses declined over time, their ability to influence viral load after infection may have been diminished in vaccine recipients infected long after the last vaccination. Therefore, we performed a sensitivity analysis whereby the analyses linking immune responses and viral load were repeated in the subset of subjects infected with HIV-1 within a year of the last vaccination. The association between HLA and viral load in this subgroup of 86 subjects was similar to that seen in the overall cohort (data not shown).

The association between the breadth of vaccine-induced preinfection T-cell responses and viral load after infection was stronger among subjects infected within a year of the last vaccination (36 vaccine recipients with immunogenicity data). Compared with vaccine recipients who had no Gag responses, the

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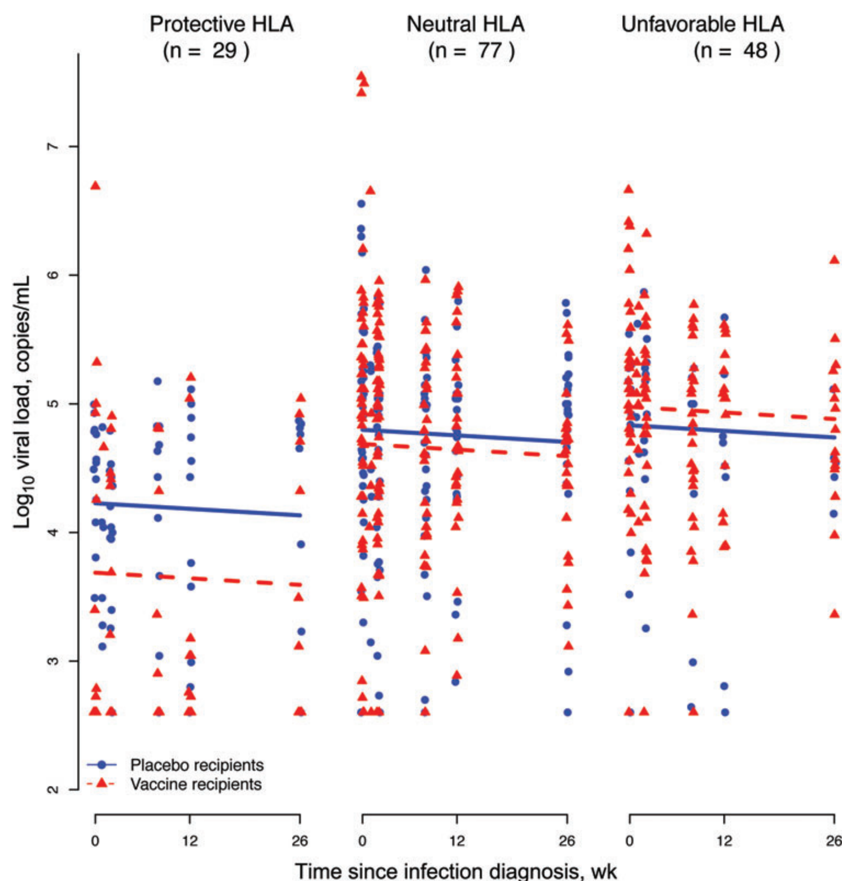

Figure 1. Pre–antiretroviral therapy $\log_{10}$ HIV-1 RNA copies/ml over time for vaccine and placebo recipients in each HLA group among 154 HIV-1-infected participants. Lines represent estimated means at each week postinfection, based on a weighted generalized estimating equations model that adjusts for baseline participant characteristics and time since HIV-1 infection. There was some indication of a stronger HLA effect in vaccine (red symbols) versus placebo recipients (blue symbols), but this difference was not statistically significant ($P = .15$). Among placebo recipients, the mean viral load was 0.57 logs lower (95% confidence interval [CI], .19–.95 logs lower) among those with protective HLA alleles and 0.035 logs higher (95% CI, .35 logs lower to .42 logs higher) among those with unfavorable HLA alleles, compared with those with neutral HLA alleles. In the vaccine arm, the mean viral load was 1.00 log lower (95% CI, .55–1.46 logs lower) among those with protective HLA alleles and 0.29 logs higher (95% CI, .02 logs lower to .60 logs higher) among those with unfavorable HLA alleles, compared to those with neutral HLA alleles.

mean viral load among vaccine recipients with 1–2 Gag responses was 0.61 logs lower ($P = .006$), and the mean viral load among those with ≥3 Gag responses was 0.77 logs lower ($P = .002$; Table 2 and Figure 4).

Whereas the association between the total breadth of the response and the viral load was not significant in the overall cohort, among subjects infected with a year of the last vaccination, an increased total breadth was a significant predictor of a lower mean viral load ($P = .03$; Table 1). Also in contrast to the results in the entire cohort, the increased total magnitude of the response was associated with a significant reduction in the mean log viral load ($P = .01$; Table 1). These models did not adjust for participants' baseline characteristics, because of the small sample size, but covariate-adjusted results were similar.

We conducted additional analyses of an alternative end point, set point viral load, defined as an average of week 8 and week 12 values (Supplementary Materials). This was the protocol-specified primary viral load end point for the Step study and constitutes a subset of the viral load data analyzed above by use of GEE modeling. The results for this end point were largely consistent, with the exception that higher total and Gag-specific magnitude of T-cell responses were significant predictors of lower set point viral load in the full cohort of 72 vaccine recipients ($P < .001$ and $P = .002$, respectively; data not shown).

HLA Class I Allele Expression Is Not Significantly Associated With Breadth or Magnitude of the Vaccine-Induced T-Cell Responses

To investigate whether the induction of vaccine-induced responses could be modulated by HLA class I haplotypes, we compared the magnitude and breadth of vaccine-induced responses between HLA groups, using regression models (85 subjects for

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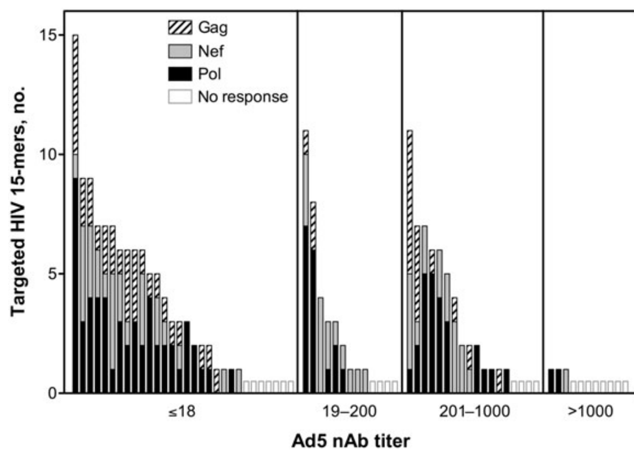

Figure 2. Number of insert-specific T-cell responses by Ad5 titer stratum. Each bar represents one of 72 participants. Responses were measured pre-infection. Panels show responses by baseline adenovirus 5 (Ad5) neutralizing antibody (nAb) titer: ≤18: n = 31; 19–200: n = 13; 201–1000: n = 18; and >1000: n = 11. The height of each bar represents the number of targeted 15-mer peptides for Gag, Nef, and Pol. Participants with no response to single 15-mers are represented with open grey bars.

magnitude analyses and 72 subjects for breadth analyses; analyses were unadjusted for baseline participant characteristics, because of the small sample size; see Methods). The presence of protective or unfavorable HLA alleles was not significantly associated with the magnitude of the total ($P = .34$) or Gag-specific ($P = .10$) T-cell responses (Supplementary Figure 1) nor did HLA alleles correlate with the breadth of vaccine-induced T-cell responses ($P = .45$ for total breadth; $P = .59$ for Gag breadth; data not shown). Results adjusted for baseline characteristics were similar (data not shown).

T-Cell Responses Do Not Mediate the Effect of Protective HLA Class I Alleles on Viral Load

The finding that vaccine-induced T-cell responses were associated with viral load in Step study participants raises the hypothesis that the association between HLA and viral load was mediated through vaccine-induced T-cell responses. We addressed this question by studying whether the association between HLA and viral load changed after adjustment for immunogenicity, using all 72 vaccine recipients with immunogenicity data. Adjustment for the total or Gag-specific magnitude or breadth in the viral load model had a very minor impact on the estimated HLA effect (Supplementary Table 2). Therefore, these results provide no evidence that T-cell responses mediate the HLA effect on HIV-1 RNA levels and suggest that HLA and Gag breadth act independently on viral load. Similarly consistent HLA effects were seen when comparing immunogenicity-adjusted and unadjusted analyses in the subset of 36 vaccine recipients infected within 1 year of the last vaccination (data not shown).

DISCUSSION

Expression of HLA-B*57 is the single most dominant predictor of the control of HIV-1 replication in natural history cohorts [43]. The mechanism of action for this protective effect is not fully understood, but binding of epitopes to the HLA groove seems to play an important role in mediating control [44]. There are several HLA-B*57–restricted epitopes in HIV-1 Gag, which could be acting in concert to explain the observed inverse association between Gag-specific T-cell responses and viral load [26, 28, 29]. Nonetheless, HLA allele expression and targeting of specific epitopes are not redundant measures of HIV-1 control [33]. The Step study, the first HIV-1 vaccine trial to test whether vaccine-induced T-cell responses could mediate protection, provided a unique opportunity to assess whether vaccination would alter the effect of HLA on disease progression, potentially through the induction of protective immune responses before infection.

We found that expression of protective HLA class I alleles was clearly associated with reduced viral load in Step study participants. This effect was slightly more pronounced in vaccine recipients, compared with placebo recipients, consistent with an earlier analysis including fewer HIV-1-infected participants [9], although the difference between treatment groups was not statistically significant ($P = .15$). However, our analysis failed to identify that the effect of protective HLA class I alleles on viral load is mediated through an increased breadth or magnitude of the T-cell response to vaccination. Rather, the slight benefit observed in vaccine recipients as compared to placebo recipients could stem from the observation that significantly higher cytotoxic responses were generated in vaccine recipients carrying the HLA class I alleles B*27, B*57, or B*58 [49].

There is modest evidence that the breadth of vaccine-induced Gag-specific but not total T-cell responses contributed to the control of viral replication independent of HLA. The fact that the association between Gag response and viral load was stronger when analyses were restricted to subjects infected within the first year after the last vaccination suggests that vaccine-induced T-cell responses may significantly impact postinfection outcome if they are still present at the time of HIV-1 acquisition. Our evidence that vaccine-induced responses wane beyond the first year after the last vaccination highlights the importance of vaccines that induce long-lasting memory responses.

There are several limitations to the analyses presented here. First, the association we detected between Gag response and viral load may not be causal. Although we attempted to control for confounding by adjusting for baseline characteristics that are plausibly associated with T-cell response and viral load, there may be other unmeasured factors that impact the Gag response that actually control viral load. In addition, the vaccine-induced T-cell responses we measured used vaccine-matched peptides, since the data were generated for the purposes of

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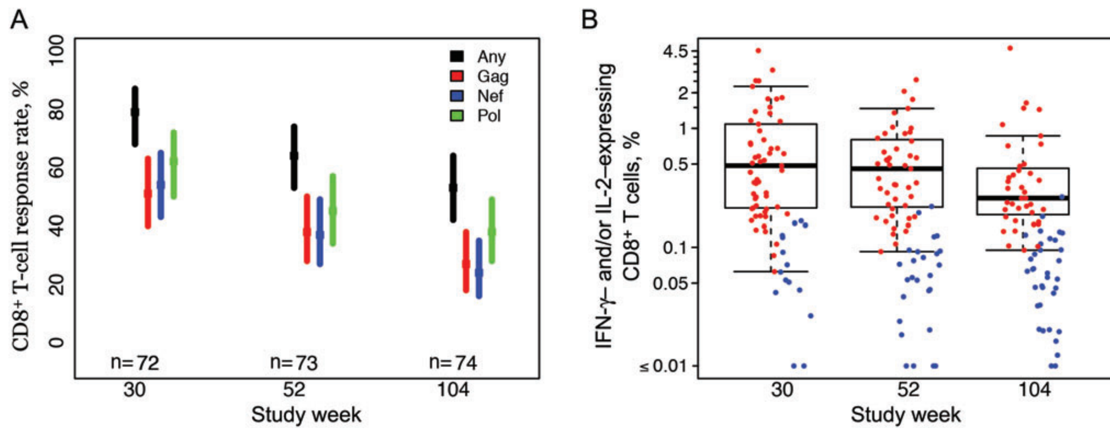

Figure 3. Rate and magnitude of the HIV-1–specific CD8⁺ T-cell response over time among 74 vaccine recipients who remained HIV-1 uninfected. HIV-1-specific CD8⁺ T-cell responses were measured by intracellular cytokine staining using Gag, Nef, and Pol peptide pools. *A*, Response rates were determined for each protein pool separately (Gag: red; Nef: blue; Pol: green), as well as for any pool (black) using established positivity criteria. Response rates and 95% confidence intervals are shown. *B*, The total magnitude of response across peptide pools for positive (red symbols) and negative (blue symbols) CD8⁺ T-cell responders. Box plots show medians and interquartile ranges for positive responders; whiskers extend from the upper and lower quartiles to the furthest point within 1.5 times the interquartile range.

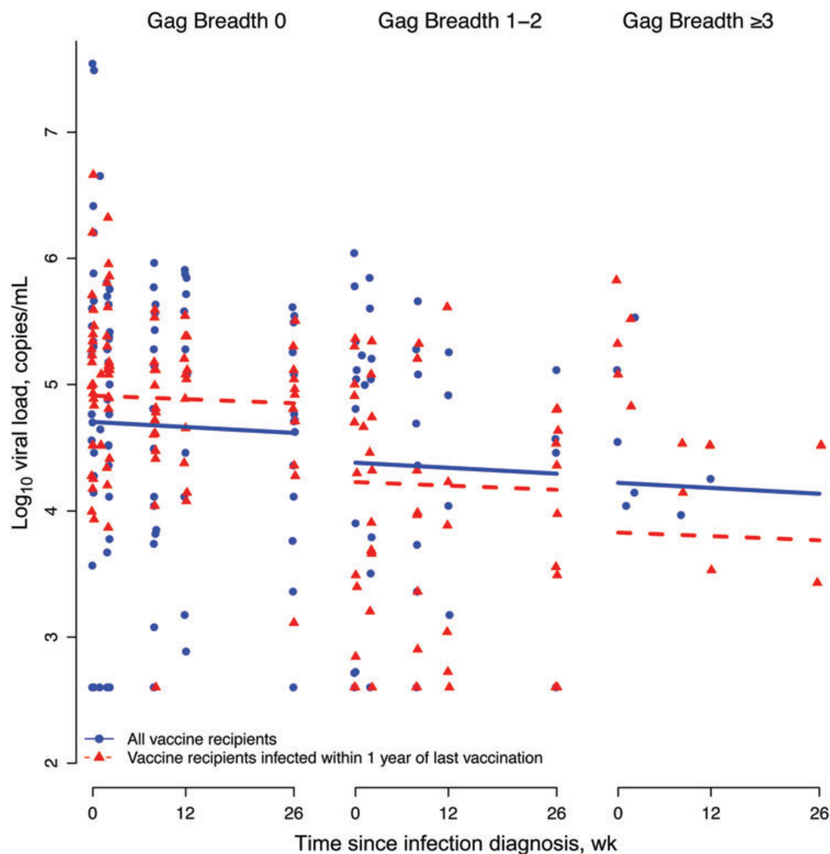

Figure 4. Pre–antiretroviral therapy $\log_{10}$ viral loads over time among HIV-1–infected vaccine recipients, by breadth of the vaccine-induced Gag response (0 vs 1–2 vs ≥3 reactive 15-mers). Lines represent estimated means at each week postinfection, based on a weighted generalized estimating equations model that adjusts for time since HIV-1 infection and HLA group. Estimates are shown based on the 72 vaccine recipients with breadth data (blue) and the subset of 36 vaccine recipients who were infected within 1 year of the last vaccination (red).

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characterizing the vaccine's immunogenicity. Immune responses to peptide antigens representing actual infecting HIV-1 strains may be more predictive of control of viral replication; however, testing autologous peptide sets for 72 subjects would have been prohibitively expensive. The apparent lack of mediation of the HLA effect by the T-cell response could also be specific to our measures of immune response: the magnitude and number of reactive 15-mers or the breadth. A more refined measure of immune response that takes into account which 15-mers or, ideally, epitopes elicited a response might serve as a better mediator.

Alternative measures of HIV-1 disease progression may also have been interesting to evaluate. The Step study provided no evidence that the vaccine modified the HLA effect on $CD4^+$ T-cell count [9]. The time to ART initiation was not evaluated here because, since ART initiation guidelines were not uniform across study sites (ie, guidelines were not prescribed by the study) and showed dramatic regional variation, there was concern about the relevance of this end point. Acute viral load, measured early in infection before viral escape occurs, would also have been interesting to study and might perhaps have been better correlated with T-cell immune responses. However, this was not possible to evaluate in the Step study since very few participants had samples obtained during the acute infection period as a consequence of 6-month intervals between study visits [11]. In general, our analyses were somewhat limited by the sample size and by missing viral load data; this influences the power of the study and makes it difficult to assess and adjust for confounding.

In summary, data from the Step vaccine trial further support the importance of HLA class I alleles in controlling HIV-1 viral replication after infection and suggest that T-cell responses to a vaccine may also play a role in controlling viral load, particularly before their decline due to insufficient memory formation. If an increased Gag breadth is indeed beneficial, the fact that the Step study found no overall vaccine effect on viral load [9] suggests that the MRKAd5 vaccine induced responses of insufficient breadth. In part this may be the result of including subjects who were Ad5 seropositive at baseline and our finding of reduced breadth in these participants. The waning of immune responses may also explain the lack of an overall effect of the MRKAd5 vaccine on viral load. Our findings support the strategy of designing future vaccines to generate long-lasting T-cell responses and support the inclusion of Gag to reduce viral load should breakthrough infection occur.

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

Acknowledgments. We thank the Step study volunteers; the staff and community members at each of the Step study sites; the staff at the HVTN Administrative Core and SCHARP Statistical Center; the staff at Merck, including Clinical Research Specialist Organization, Worldwide Clinical Data Management Operations, Clinical Research Operations, and Serology Laboratory; Margaret Johnston, Carl Dieffenbach, Alan Fix, and Jorge Flores at the Division of AIDS in the National Institute of Allergy and Infectious Diseases, for their facilitation of the study design, conduct, and release of study results from this study; and Stephen Voght, for technical editing of the manuscript.

This research was supported in part by the Intramural Research Program of the NIH, Frederick National Lab, Center for Cancer Research.

Disclaimer. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Financial support. This work was supported by the National Institutes of Health Public Health Service (grants UM1 AI068618 and AI068635) and the Frederick National Laboratory for Cancer Research (contract HHSN261200800001E).

Potential conflicts of interest. D. R. C. is an employee and shareholder of Merck. M. J. M. has served as investigator on Merck-funded research. All other authors report no potential 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.

References

1. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Eng J Med **2012**; 366:1275–86.
2. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Eng J Med **2012**; 367:399–410.
3. Bailey RC, Moses S, Parker CB, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. Lancet **2007**; 369:643–56.
4. Abdool Karim Q, Abdool Karim SS, Frohlich JA, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science **2010**; 329:1168–74.
5. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Eng J Med **2010**; 363:2587–99.
6. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Eng J Med **2011**; 365:493–505.
7. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet **2008**; 372(9653): 1881–93.
8. McElrath MJ, De Rosa SC, Moodie Z, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. Lancet **2008**; 372(9653):1894–1905.
9. Fitzgerald DW, Janes H, Robertson M, et al. An Ad5-vectored HIV-1 vaccine elicits cell-mediated immunity but does not affect disease progression in HIV-1-infected male subjects: results from a randomized placebo-controlled trial (the Step study). J Infect Dis **2011**; 203:765–72.
10. Rolland M, Tovanabutra S, de Camp AC, et al. Genetic impact of vaccination on breakthrough HIV-1 sequences from the STEP trial. Nat Med **2011**; 17:366–71.
11. Janes H, Frahm N, DeCamp A, et al. MRKAd5 HIV-1 Gag/Pol/Nef vaccine-induced T-cell responses inadequately predict distance of breakthrough HIV-1 sequences to the vaccine or viral load. PLoS One **2012**; 7:e43396.
12. Koup RA, Safrit JT, Cao Y, et al. Temporal association of cellular immune responses with the initial control of viremia in primary

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human immunodeficiency virus type 1 syndrome. J Virol **1994**; 68: 4650–55.
13. Schmitz J, Kuroda M, Santra S, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science **1999**; 277:333–8.
14. Cao J, McNevin J, Malhotra U, McElrath MJ. Evolution of CD8+ T cell immunity and viral escape following acute HIV-1 infection. J Immunol **2003**; 171:3837–46.
15. Allen TM, O'Connor DH, Jing P, et al. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. Nature **2000**; 407:386–90.
16. Goulder PJ, Phillips RE, Colbert RA, et al. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. Nat Med **1997**; 3:212–7.
17. Barouch DH, Santra S, Schmitz JE, et al. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science **2000**; 290:486–92.
18. Amara RR, Villinger F, Altman JD, et al. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Science **2001**; 292:69–74.
19. Shiver JW, Fu TM, Chen L, et al. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Nature **2002**; 415:331–5.
20. Wilson NA, Keele BF, Reed JS, et al. Vaccine-induced cellular responses control simian immunodeficiency virus replication after heterologous challenge. J Virol **2009**; 83:6508–21.
21. Hansen SG, Vieville C, Whizin N, et al. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. Nat Med **2009**; 15:293–9.
22. Betts MR, Ambrozak DR, Douek DC, et al. Analysis of total human immunodeficiency virus (HIV)-specific CD4(+) and CD8(+) T-cell responses: relationship to viral load in untreated HIV infection. J Virol **2001**; 75:11983–91.
23. Ogg GS, Jin X, Bonhoeffer S, et al. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science **1998**; 279:2103–6.
24. Addo MM, Yu XG, Rathod A, et al. Comprehensive epitope analysis of HIV-1-specific T cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. J Virol **2003**; 77:2081–92.
25. Cao J, McNevin J, Holte S, Fink L, Corey L, McElrath MJ. Comprehensive analysis of human immunodeficiency virus type 1 (HIV-1)-specific gamma interferon-secreting CD8+ T cells in primary HIV-1 infection. J Virol **2003**; 77:6867–78.
26. Zuniga R, Lucchetti A, Galvan P, et al. Relative Dominance of Gag p24-specific cytotoxic T lymphocytes is associated with human immunodeficiency virus control. J Virol **2006**; 80:3122–5.
27. Ranasinghe S, Flanders M, Cutler S, et al. HIV-specific CD4 T cell responses to different viral proteins have discordant associations with viral load and clinical outcome. J Virol **2012**; 86:277–83.
28. Kiepiela P, Ngumbela K, Thobakgale C, et al. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med **2007**; 13:46–53.
29. Masemola A, Mashishi T, Khoury G, et al. Hierarchical targeting of subtype C human immunodeficiency virus type 1 proteins by CD8+ T cells: correlation with viral load. J Virol **2004**; 78:3233–43.
30. Steel CM, Ludlam CA, Beatson D, et al. HLA haplotype A1 B8 DR3 as a risk factor for HIV-related disease. Lancet **1988**; 1:1185–8.
31. O'Brien SJ, Gao X, Carrington M. HLA and AIDS: a cautionary tale. Trends Mol Med **2001**; 7:379–81.
32. Frahm N, Linde CH, Brander C. Identification of HIV-derived, HLA class I restricted CTL epitopes: insights into TCR repertoire, CTL escape and viral fitness. In: Korber BTM, Brander C, Haynes BF, et al. eds. HIV immunology and HIV/SIV vaccine databases 2004. Los Alamos, NM: Los Alamos National Laboratory, Theoretical Biology and Biophysics, **2006/2007**:3–28.
33. Dinges WL, Richardt J, Friedrich D, et al. Virus-specific CD8+ T-cell responses better define HIV disease progression than HLA genotype. J Virol **2010**; 84:4461–8.
34. Martinez-Picado J, Prado JG, Fry EE, et al. Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. J Virol **2006**; 80:3617–23.
35. Brockman MA, Schneidewind A, Lahaie M, et al. Escape and compensation from early HLA-B57-mediated cytotoxic T-lymphocyte pressure on human immunodeficiency virus type 1 Gag alter capsid interactions with cyclophilin A. J Virol **2007**; 81:12608–18.
36. Schneidewind A, Brockman MA, Yang R, et al. Escape from the dominant HLA-B27-restricted cytotoxic T-lymphocyte response in Gag is associated with a dramatic reduction in human immunodeficiency virus type 1 replication. J Virol **2007**; 81:12382–93.
37. Carrington M, Nelson GW, Martin MP, et al. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science **1999**; 283: 1748–52.
38. Kaslow RA, Carrington M, Apple R, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. Nat Med **1996**; 2:405–11.
39. Carrington M, Walker BD. Immunogenetics of spontaneous control of HIV. Annu Rev Med **2012**; 63:131–45.
40. Carrington M, O'Brien SJ. The influence of HLA genotype on AIDS. Annu Rev Med **2003**; 54:535–51.
41. Mothe BR, Weinfurter J, Wang C, et al. Expression of the major histocompatibility complex class I molecule Mamu-A*01 is associated with control of simian immunodeficiency virus SIVmac239 replication. J Virol **2003**; 77:2736–40.
42. Yant LJ, Friedrich TC, Johnson RC, et al. The high-frequency major histocompatibility complex class I allele Mamu-B*17 is associated with control of simian immunodeficiency virus SIVmac239 replication. J Virol **2006**; 80:5074–7.
43. Fellay J, Shianna KV, Ge D, et al. A whole-genome association study of major determinants for host control of HIV-1. Science **2007**; 317:944–7.
44. Pereyra F, Jia X, McLaren PJ, et al. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. Science **2010**; 330:1551–7.
45. Fellay J, Frahm N, Shianna KV, et al. Host genetic determinants of T cell responses to the MRKAd5 HIV-1 gag/pol/nef vaccine in the step trial. J Infect Dis **2011**; 203:773–9.
46. Gao X, Nelson GW, Karacki P, et al. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. N Eng J Med **2001**; 344:1668–75.
47. Frahm N, DeCamp AC, Friedrich DP, et al. Human adenovirus-specific T cells modulate HIV-specific T cell responses to an Ad5-vectored HIV-1 vaccine. J Clin Invest **2012**; 122:359–67.
48. Horton H, Thomas EP, Stucky JA, et al. Optimization and validation of an 8-color intracellular cytokine staining (ICS) assay to quantify antigen-specific T cells induced by vaccination. J Immunol Methods **2007**; 323:39–54.
49. Migueles SA, Rood JE, Berkley AM, et al. Trivalent adenovirus type 5 HIV recombinant vaccine primes for modest cytotoxic capacity that is greatest in humans with protective HLA class I alleles. PLoS Pathog **2011**; 7:e1002002.

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