Differential Characteristics of Cytotoxic T Lymphocytes Restricted by the Protective HLA Alleles B*27 and B*57 in HIV-1 Infection

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Objective: HLA-B*27 and B*57 are associated with relatively slow progression to AIDS. Mechanisms held responsible for this protective effect include the immunodominance and high magnitude, breadth, and affinity of the cytotoxic T lymphocytes (CTL) response restricted by these HLA molecules, as well as superior maintenance of CTL responses during HIV-1 disease progression.

Design: We examined CTL responses from HIV-1–infected individuals restricted through protective and nonprotective HLA alleles within the same host, thereby excluding any effects of slow or rapid progression on the CTL response.

Results: We found that neither immunodominance, nor high magnitude and breadth, nor affinity of the CTL response are general mechanisms of protection against disease progression. HLA-B*57-restricted CTL responses were of exceptionally high affinity and dominated the HLA-A*02-restricted CTL response in individuals coexpressing these HLA alleles. In contrast, HLA-B*27-restricted CTL responses were not of particularly high affinity and did not dominate the response in individuals coexpressing HLA-B*27 and HLA-A*02. Instead, in individuals expressing HLA-B*27, the CTL response restricted by nonprotective HLA alleles was significantly higher and broader, and of higher affinity than in individuals expressing these alleles without HLA-B*27. Although HLA-B*27 and B*57 are thought to target the most conserved parts of HIV, during disease progression, CTL responses restricted by HLA-B*27 and B*57 were lost at least as fast as CTL responses restricted by HLA-A*02.

Conclusions: Our data show that many of the mechanisms of CTL that are generally held responsible for slowing down HIV-1 disease progression hold for HLA-B*57 but do not hold for HLA-B*27.

Key Words: HIV, HLA-B*27, HLA-B*57, protection against progression to AIDS, T cells, CTL responses

(*J Acquir Immune Defic Syndr* 2014;67:236–245)

INTRODUCTION

It has consistently been shown that certain HLA class I alleles (eg, HLA-B*27 and HLA-B*57) are associated with relatively slow progression to AIDS (reviewed in Carrington and O'Brien[1]). The mechanism behind these associations is not fully understood but most likely involves both virologic and immunological mechanisms. Several studies have shown that the cytotoxic T lymphocytes (CTL) restricted by these HLA molecules play an important role. A recent study suggested that the actual presence of certain HLA-B*57 or B*27 HIV-specific $CD8^+$ T-cell responses during primary HIV infection better defines HIV-disease progression than the HLA genotype alone.[2] Moreover, conformational differences in peptide presentation due to polymorphisms in amino acids located in the HLA-peptide binding groove have been implicated in control of HIV infection.[3]

Viral escape from CTL responses targeting immunodominant CTL epitopes restricted by HLA-B*27 and B*57 often requires the accumulation of several mutations, including mutations that can compensate for the loss of viral fitness caused by the actual CTL escape mutation(s).[4–6] The immunological mechanisms involved in the protective effect of

Received for publication January 28, 2014; accepted July 15, 2014.

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Supported by a grant from the Landsteiner foundation for Blood transfusion research (LSBR), Grant No. 0317. The Amsterdam Cohort Studies on HIV infection and AIDS, a collaboration between the Amsterdam Health Service, the Academic Medical Center of the University of Amsterdam, Sanquin Blood Supply Foundation, the University Medical Center Utrecht, and the Jan van Goyen Clinic are part of the Netherlands HIV Monitoring Foundation and financially supported by the Center for Infectious Disease Control of the Netherlands National Institute for Public Health and the Environment.

Meetings where (part of) these data have been presented. Fifth Netherlands Conference on HIV Pathogenesis, Prevention and Treatment (Amsterdam, 2011); Keystone Symposium Viral Immunity (Keystone, 2008); AIDS vaccine (Montreal, 2005); HIV dynamics and evolution (Utrecht, 2013).

The authors have no conflicts of interest to disclose.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

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HLA-B*27 and B*57 are less well understood. It is even unknown if the mechanism of protection is similar for both HLA molecules. HLA-B*57-restricted CTL are known to be immunodominant and of high functionality, features frequently proposed to be correlates of protection against disease progression.[7–9] It has, therefore, been suggested that $CD8^+$ T cells restricted by protective HLA alleles in general may confer protection because they are superior in terms of, for example, magnitude, frequency, or functional avidity.[10–12]

HLA alleles associated with slow disease progression are known to have an intrinsic preference to present Gag p24-derived epitopes.[13–16] P24 is one of the most conserved regions of the HIV genome,[17] and higher order constraints on viral evolution are present.[18] Persons who durably control HIV spontaneously often target multidimensionally constrained regions of p24.[18] Therefore, the protective HLA alleles B*27 and B*57 might be associated with slow disease progression because the CTL responses against epitopes restricted through these alleles may be better preserved throughout the course of infection than CTL responses restricted through other HLA alleles.

Here, we present a comprehensive study comparing CTL responses restricted through protective and nonprotective HLA molecules within the same host. By analyzing HLA-B*27 and B*57 separately, we reveal important insights in differences and similarities in their potential mechanism of protection.

MATERIALS AND METHODS

Patient Selection

Twenty-four HIV-1–infected individuals from the Amsterdam Cohort Studies on HIV-1 infection and AIDS (ACS) with a known date of seroconversion were included based on HLA expression. Eighteen individuals expressed HLA-A*02, of which 3 coexpressed HLA-B*57 and 8 coexpressed HLA-B*27. Six individuals expressed HLA-B*57 or B*27 without coexpressing HLA-A*02 (see Table 1 for more details). Additionally, 8 HIV-1 seroprevalent individuals (5 coexpressing HLA-A*02 and B*27 and 3 coexpressing HLA-A*02 and B*57) from the ACS were included during asymptomatic chronic infection. All individuals were treatment naive at the time of the analysis. Two-digit genotyping of the HLA class I loci was performed as described elsewhere.[19] Informed written consent was obtained from all participants, and the study was approved by the Medical Ethical Committee of the Academic Medical Center, Amsterdam. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

TABLE 1. Patient Characteristics Per HLA Expression Group

HLA Group	n*	CD4 Counts†	CD8 Counts‡	Viral Load§
HLA-A*02	7	350	800	76.000
HLA-B*08	5	330	780	52.000
HLA-B*27	1	600	nd‖	<1.000
HLA-B*57	5	580	960	<1.000
HLA-A*02 + B*27	13	430	1.000	4.800
HLA-B*08 + B*27	5	470	920	4.800
HLA-A*02 + B*57	6	690	920	2.550

*Number of included individuals who express the specific HLA class I allele(s).
†$CD4^{++}$ T-cell counts ($\times 10^6$ cells/μL).
‡$CD8^{++}$ T-cell counts ($\times 10^6$ cells/μL).
§HIV-1 RNA load (copies/mL), detection limit 1.000 copies/mL.
‖Not determined.

Interferon-γ Enzyme-Linked Immunospot Assay

IFN-γ-producing antigen-specific $CD8^+$ T cells were measured using the IFN-γ ELISpot assay as described previously.[20] To avoid a bias in peptide selection, we included known HIV-1 peptides published in the Los Alamos Database, and epitopes predicted to be presented through these HLA alleles,[21] see Table S1 (see **Supplemental Digital Content,** http://links.lww.com/QAI/A560) and Schellens et al.[20] Phytohemagglutinin (PHA) stimulation served as a positive control, and medium without peptide or PHA served as a negative control. IFN-γ-producing cells were detected as dark spots and counted using an AELVIS EliScan (EliAnalyse Software version 4, Hanover, Germany). The number of IFN-γ-producing cells was calculated by subtracting the negative control value and was reported as number of spot-forming units per 10^6 PBMC. Samples with >100 spots per million PBMC after subtraction of the negative control values were considered positive.

Tetramer Dissociation Assay

PBMC were thawed and washed twice. Subsequently, 3×10^6 cells were stained for 60 minutes at 4°C with mAb for CD8 (PerCP) and one of the following PE labeled tetramers: A*02-tetramers SLYNTVATL (SL9) or ILKEPVHGV (IV9), B*27-tetramers KRWIILGLNK (KK10) or KRKGGIGGY (KY9), B*57-tetramers KAFSPEVIPMF (KF11) or IATESIVIW (IW9). Cells were washed and resuspended in PBA. After removing 500,000 cells for T_0, the remaining cells were incubated with a 5 times excess APC labeled tetramer for 90 minutes. After 5, 10, 15, 30, 60, and 90 minutes, 500,000 cells were removed, washed, and fixed. Per sample 200,000 events were acquired using a FACS LSRII (BD). Data were analyzed using BD FACSDiva software. The natural log (LN2) of the geometric mean fluorescent intensity of the PE-labeled tetramer was plotted against time. The half-life of the T-cell receptor (TCR)-tetramer interaction was derived from the slope of this curve ($T_{1/2}$ = LN2/slope).

PCR Amplification and Sequencing

Clonal HIV-1 variants were obtained by cocultivation of increasing numbers of patient PBMC with 2–3 day PHA-stimulated PBMC from a healthy donor (PHA-PBMC) as described.[22,23] Total DNA was isolated using the L6 isolation method,[24] and DNA was amplified as described.[25] PCR products were purified using High Pure PCR product purification kits (Roche Diagnostics, Basel, Switzerland) and sequenced using ABI Prism Big Dye Terminator v1.1/3.1 Cycle sequencing kits (Applied Biosystems, Basel, Switzerland) with nested PCR primers. Sequences were analyzed on an Applied Biosystems/Hitachi 3130 xl Genetic Analyzer.

Prediction of CTL Epitopes

Epitopes were predicted using the proteasomal cleavage/TAP transport/MHC class I–combined predictor available at http://tools.immuneepitope.org, using the most abundant 4-digit HLA type of each HLA serotype. Cutoff values used were 1.135 for proteasomal cleavage, −0.56 for TAP transport, and −2.7 for MHC binding.[26]

Statistical Analysis

Data were analyzed using SPSS 15.0 software (SPSS, Chicago, IL). Groups were compared using Wilcoxon signed ranks, χ^2, or Mann–Whitney *U* tests. Correlations were tested using Spearman's correlation test. A *P* value ≤ 0.05 was considered statistically significant.

RESULTS

Magnitude and Breadth of CTL Responses Restricted by Protective and Nonprotective HLA Alleles

It is well known that HLA-B*57 dominates the total HIV-specific CTL response during both acute and chronic infection.[7,8] For HLA-B*27, most studies focused only on the dominant p24 Gag-derived KK10 epitope (eg, Refs. 5, 6, 27, and 28). To investigate whether a high magnitude and/or breadth of the CTL response is a general mechanism for protection against disease progression, we here performed a detailed analysis of the CTL responses restricted by HLA-B*27 and B*57. To this end, we measured CTL responses against 78 peptides derived from the entire HIV-1 subtype B genome (30 HLA-A*02-, 29 HLA-B*57-, and 19 HLA-B*27-restricted peptides, respectively, see **Table S1, Supplemental Digital Content,** http://links.lww.com/QAI/A560) using the IFN-γ enzyme-linked immunospot (ELISpot) assay. HIV-1–infected individuals were selected from the Amsterdam Cohort Studies on HIV-1 infection and AIDS (Table 1) on the basis of expression of one of the protective HLA alleles HLA-B*27 (RH = 0.43, $P = 0.001$) or HLA-B*57 (RH = 0.55, $P = 0.04$) and/or a nonprotective HLA allele [either HLA-A*02 (RH = 0.91, $P = 0.41$) or HLA-B*08 (RH=0.97, $P = 0.82$)].[29]

In individuals coexpressing HLA-A*02 and HLA-B*57, the CTL response indeed tended to be dominated by HLA-B*57-restricted CTL, although this difference was not statistically significant (Fig. 1A, C). In contrast, in individuals expressing both HLA-A*02 and HLA-B*27, the magnitude (Fig. 1B) and breadth (Fig. 1D) of the CTL response restricted by HLA-A*02 and HLA-B*27 were similar. Because none of our patients expressed both HLA-B*27 and B*57, responses restricted by these HLA molecules could not be compared within the same individual. Because viral loads in individuals expressing B*27 or B*57 were very similar, however, we did compare these CTL responses and found that the magnitude and breadth of HLA-B*27- and B*57-restricted responses were not significantly different ($P = 0.113$ and 0.979, respectively). Thus, although both protective HLA alleles induce CTL responses of comparable magnitude (Figure 1A vs. Figure 1B) and breadth (Figure 1C vs. Figure 1D), HLA-B*57-restricted responses are, but B*27-restricted responses are not, immunodominant in individuals coexpressing HLA-A*02 and one of the protective HLA class I alleles.

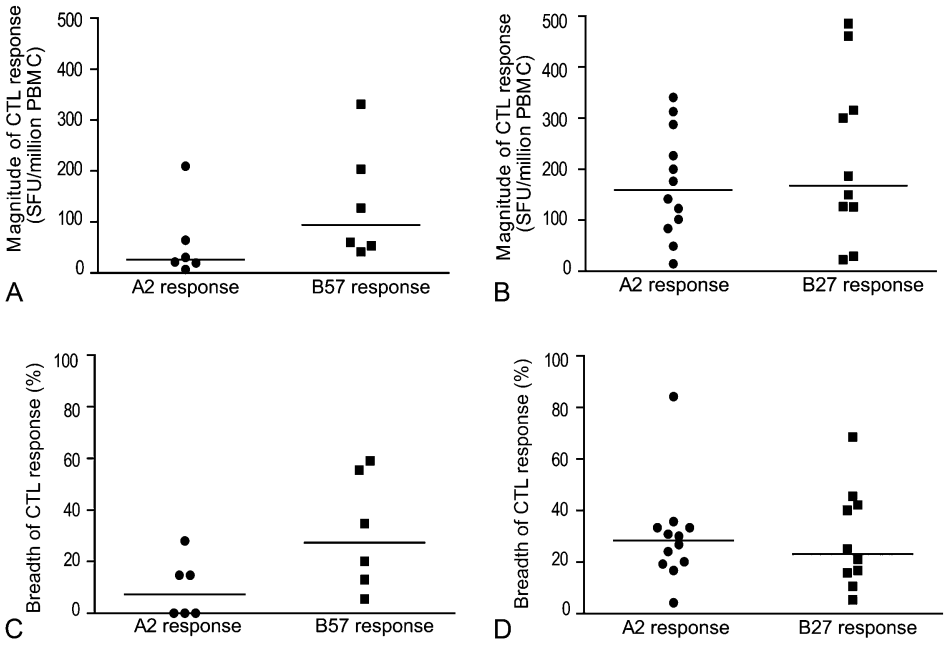

FIGURE 1. Comparison of the magnitude and breadth of CTL responses restricted by protective and nonprotective HLA molecules. The magnitude and breadth of the HLA-A*02-, B*27-, and B*57-restricted CTL response were analyzed based on IFN-γ ELISpot assays during chronic HIV-1 infection. The breadth was determined as the fraction of tested peptides that elicited a positive CTL response. The left panels show the magnitude (A) and breadth (C) of the HLA-A*02- and B*57-restricted CTL response in individuals coexpressing these HLA alleles in spot forming units per million PBMC. The right panels show the magnitude (B) and breadth (D) of the HLA-A*02- and B*27-restricted CTL response in individuals coexpressing these HLA alleles. Each dot represents 1 individual, and the black line represents the median value for the whole group. No differences were observed in individuals with a known date of seroconversion and seroprevalent individuals.

Differences in Affinity of the TCR for Different Peptide–MHC Complexes

We next investigated whether the affinity of the TCRs for their cognate peptide–HLA (pHLA) complexes is associated with HIV-1 disease progression rates by performing tetramer decay assays (see Fig. 2A for an example). In line with our previous findings,[30] we observed a stronger interaction for HLA-B*57-restricted peptides (median half-life: 444 minutes; range: 307–735 minutes) compared with HLA-A*02–peptide complexes (median: 184; range: 91–255; $P = 0.001$; Fig. 2B). In contrast, the half-life of the interaction between HLA-B*27–peptide complexes and the TCRs was not significantly higher than that observed for HLA-A*02–peptide complexes (Fig. 2B). Even when comparing protective and nonprotective HLA alleles within the same individual, no difference was observed in the affinity of the TCR for the HLA-A*02–peptide complexes and the HLA-B*27–peptide complexes (data not shown).

These data indicate that a low relative hazard of disease progression of an HLA molecule can (HLA-B*57) but is not always (HLA-B*27) associated with a stronger interaction between the pHLA complex and the TCRs.

CTL Responses Restricted by Protective HLA Alleles Are Not Better Preserved

Another mechanism that is thought to play a role in the protective effect of HLA-B*27 and B*57 is the maintenance of the CTL response throughout the course of infection. To investigate this, we analyzed HIV-1–specific CTL responses

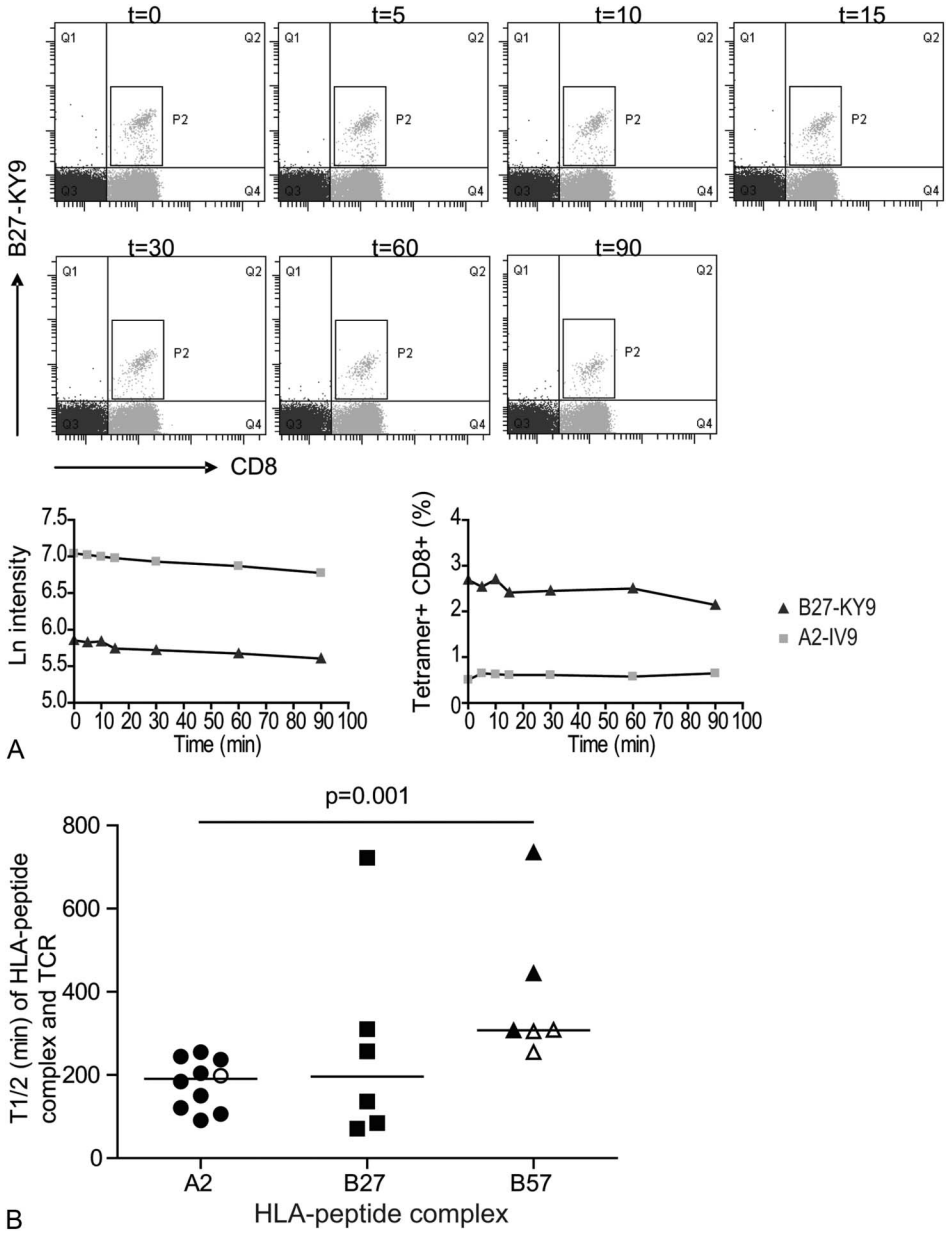

FIGURE 2. Comparison of the half-life of HLA-peptide complexes and the TCRs for protective and non-protective HLA molecules. In (A), an example of the tetramer decay assay is shown for the B*27-KY9 tetramer. The mean fluorescent intensity of the tetramer$^+$ CD8$^+$ T cells (P2) decreases over time. An example of a dissociation graph of the B*27-KY9 (black triangles) and A*02-IV9 (gray squares) tetramers is shown (left panel, patient 8, $t_{1/2}$ KY9 257 minutes, $t_{1/2}$ IV9 244 minutes), as well as the percentage of tetramer positive cells (right panel), which remains relatively constant throughout the experiment. B, The half-lives of the interaction between the HLA–peptide complexes and the TCR for peptides presented through HLA-A*02 (n = 9, black dots; the white dot depicts data from our previous study[30]), B*27 (n = 6, black squares), and B*57 (n = 3, black triangles; the white triangles depict data from our previous study[30]). Some individuals did not have high enough frequencies of the specific CTL to perform our analyses and were therefore not included. Significant differences between the groups ($P \leq 0.05$, Mann–Whitney *U* test) are depicted when applicable.

TABLE 2. Patient Characteristics

		CD4 Counts*		CD8 Counts†		Viral Load‡	
Subject	**HLA Type**	**Early**	**Late**	**Early**	**Late**	**Early**	**Late**
1	**A2**, A32, **B27**, B40	0.43	0.28	0.70	0.80	<1.000	5.800
2	**A2**, A30, **B27**, B40	nd§	0.30	nd	1.00	32.000	<1.000
3	**A2**, A1, **B27**, B8	0.67	0.57	0.81	1.25	13.000	nd
4	**A2**, A68, **B27**, B7	0.54	0.38	0.60	0.60	8.600	27.000
5	**A2**, **B27**, B15	1.06	0.58	1.30	1.75	12.000	1.700
6	**A2**, **B27**, B8	0.84	0.45	0.50	0.90	3.100.000	4.800
7	**A2**, **B27**, B8	nd	0.43	nd	1.30	12.000	4.800
8	**A2**, A31, **B27**, B35	1.39	0.82	2.10	3.03	11.000	<1.000
	Median	0.76	0.44	0.76	1.13	12.000	4.800
9	**A2**, A1, **B57**, B41	0.74	1.56	0.50	1.00	<1.000	<1.000
10	**A2**, B37, **B57**	1.13	1.19	0.50	0.64	<1.000	3.100
11	**A2**, A32, B14, **B57**	0.53	0.45	0.70	0.71	33.000	16.937
	Median	0.72	0.87	0.55	0.81	<1.000	9.050
12	**A2**, A3, B7, B40	0.58	0.37	0.80	0.80	37.000	74.000
13	**A2**, A1, B8, B35	0.6	0.24	0.60	0.30	31.000	110.000
14	**A2**, A1, B8, B40	0.81	0.69	0.40	1.10	64.000	<1.000
15	**A2**, A1, B7, B15	0.58	0.35	0.50	0.70	220.000	90.000
16	**A2**, A11, B40	0.72	0.60	1.00	0.90	<1.000	60.000
17	**A2**, B18, B44	0.47	0.15	1.60	1.10	770.000	59.000
18	**A2**, A1, B8, B38	1.38	0.30	0.90	0.60	15.000	nd
	Median	0.60	0.35	0.80	0.80	37.000	67.000
19	A24, A26, B8, **B27**	nd	0.60	nd	nd	33.000	<1.000
20	A1, A11, B8, **B57**	0.56	0.58	0.50	1.17	8.800	5.450
21	A1, A68, B14, **B57**	1.11	0.74	0.70	0.40	<1.000	<1.000
22	A1, A32, B15, **B57**	1.08	0.81	0.80	1.02	<1.000	<1.000
23	A1, A24, B35, **B57**	0.36	0.29	1.00	0.96	19.000	<1.000
24	A3, A68, B35, **B57**	0.42	0.46	0.50	0.80	15.000	72.000
	Median	0.56	0.58	0.70	0.96	8.800	<1.000

The median of each subgroup is indicated in bold.
*$CD4^+$ T-cell counts ($\times 10^6$ cells/μL).
†$CD8^+$ T-cell counts ($\times 10^6$ cells/μL).
‡HIV-1 RNA load (copies/mL), detection limit 1.000 copies/mL.
§Not determined.

at 2 sequential time points, within 6 months after seroconversion (early infection) and approximately 5 years later (during asymptomatic chronic infection). Table 2 shows detailed characteristics of the individuals included for this analysis. In line with previous studies,[31–34] the CTL response in chronic infection was broader than early after seroconversion (Fig. 3A, B). Remarkably, this broadening of the CTL response was observed for HLA-A*02-restricted responses (Fig. 3A; $P = 0.002$) but not for HLA-B*27- and HLA-B*57-restricted responses (Fig. 3B; $P = 0.155$, Wilcoxon signed rank test), which were already relatively broad during early HIV-1 infection, in line with previous results.[8]

Because the maintenance of CTL responses may be affected by disease progression itself,[35,36] we next confined our analysis to patients who coexpressed HLA-A*02 and a protective HLA allele [either HLA-B*27 (n = 7) or B*57 (n = 3)], allowing us to compare the evolution of CTL responses restricted by both types of HLA within the same host. We included only CTL responses that were present during early HIV-1 infection and analyzed whether these responses were still present 5 years later. Fig. 3C shows that within an individual, CTL responses toward peptides presented through the protective HLA allele B*27 were not better (if not even worse) preserved during disease progression (median: 50% of CTL responses is preserved; range: 40%–100%) than CTL responses restricted by the nonprotective HLA-A*02 (median: 87.5%, range: 33%–100%). Individuals coexpressing HLA-A*02 and HLA-B*57 hardly showed responses toward peptides presented through HLA-A*02 (Fig. 3A), making it impossible to compare the maintenance of both responses. Therefore, we additionally used peptide prediction programs (http://immuneepitope.org)[21] to reveal the number of potential CTL epitopes present in HIV sequences obtained during early and chronic infection, which resulted in similar findings. On average, 91% (range: 80%–100%) of the HLA-A*02-restricted epitopes predicted to be present early are still present during chronic infection, which is again comparable with (or even higher than) the 85% (range: 73%–100%) and 74% (range: 70%–78%) for HLA-B*27- and B*57-restricted CTL epitopes, respectively (data not shown).

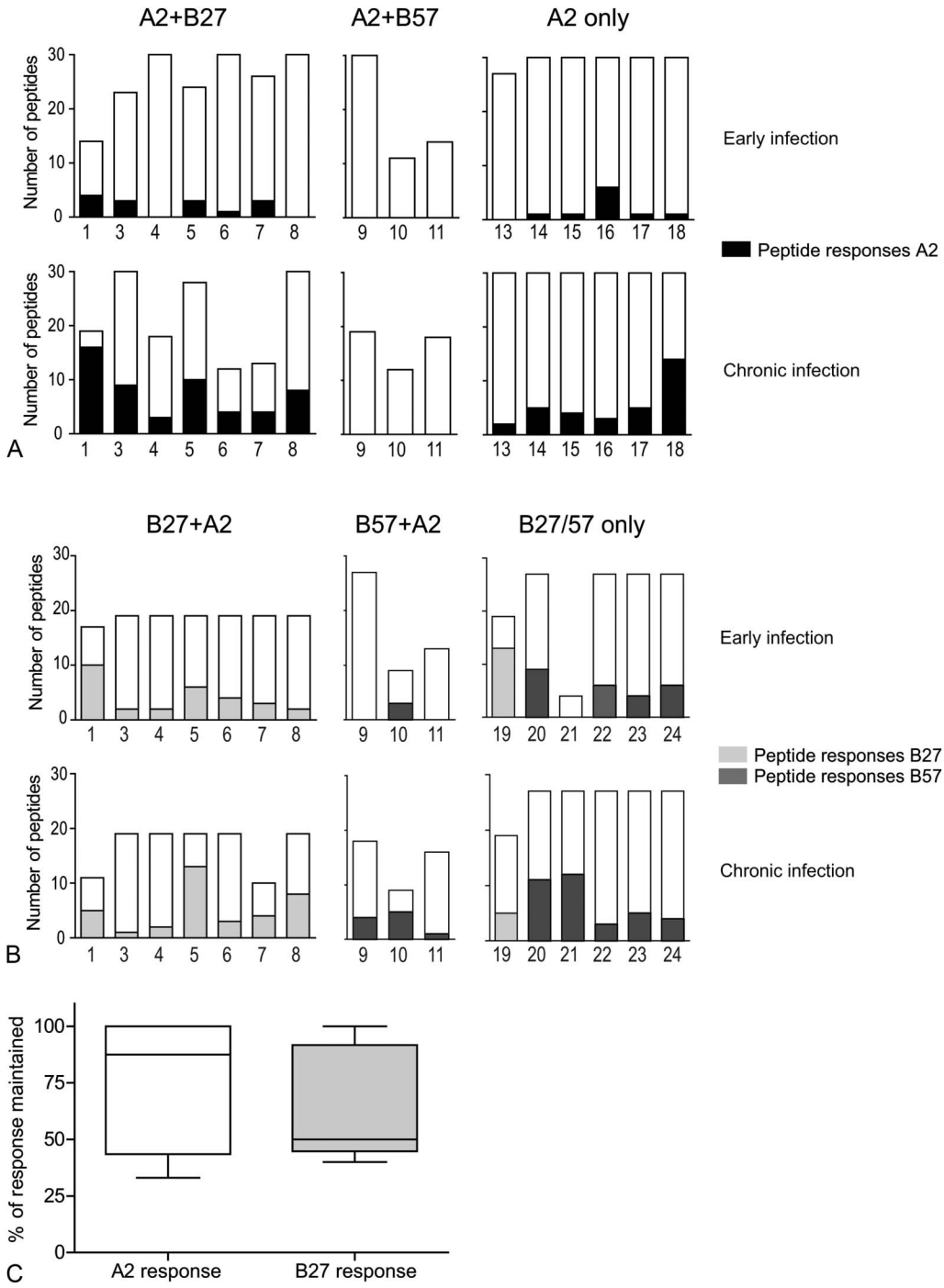

FIGURE 3. Changes in CTL response between early and chronic HIV-1 infection. CTL responses toward a maximum of 30 HLA-A*02-, 19 HLA-B*27-, and 29 HLA-B*57-restricted peptides were measured within 6 months after seroconversion (early infection) and 5 years later (chronic infection). A, Shows the HLA-A*02-restricted CTL response per patient for patients coexpressing HLA-A*02 and B*27 (A2 + B27), patients coexpressing HLA-A*02 and B*57 (A2 + B57), and patients expressing HLA-A*02 without any of the protective HLA alleles (A2 only). Likewise, (B) shows the HLA-B*27- or B*57-restricted CTL response per patient for patients coexpressing HLA-B*27 and A*02 (B27 + A2), patients coexpressing HLA-B*57 and A*02 (B57 + A2), and patients expressing HLA-B*27 or B*57 without HLA-A*02 (B27/B57 only). The *y* axis represents the number of tested peptides; the whole bar shows the total number of tested peptides, filled bars show the number of peptides to which a CTL response was observed (black: HLA-A*02-binding peptides, light gray: HLA-B*27-binding peptides, dark gray: HLA-B*57-binding peptides). Two patients (2 and 12) were not included because of a lack of PBMC from the early infection time point. Samples with >100 spots per million PBMC, after subtraction of the negative control values, were considered

(*continued on next page*)

Coexpression of HLA-B∗27, But Not B∗57, Results in Enhanced Responsiveness of HIV-Specific T Cells Restricted Through HLA-A∗02

It has previously been shown that HLA alleles might have an impact on CTL responses restricted by other HLA alleles expressed by individuals.[37,38] Indeed, when we compared the HLA-A*02-restricted CTL response in individuals expressing either HLA-A*02 alone or both HLA-A*02 and HLA-B*27 or B*57, we found that individuals coexpressing HLA-A*02 and B*27 responded to more HLA-A*02-restricted peptides (Fig. 4A; $P = 0.045$) and with higher magnitude (Fig. 4B; $P = 0.022$) compared with individuals without HLA-B*27. Individuals coexpressing HLA-A*02 and B*57, however, responded to fewer HLA-A*02-restricted peptides (Fig. 4A; $P = 0.007$) and with lower magnitude (Fig. 4B; $P = 0.032$). Also, the half-life of the HLA-A*02-peptide–TCR interaction was significantly higher in individuals coexpressing HLA-B*27 compared with individuals expressing HLA-A*02 without B*27 ($P = 0.037$; Fig. 4C). These data indicate that the HLA-A*02-restricted CTL response is differentially influenced by the presence of the different protective HLA alleles.

When we plotted the magnitude of the HLA-A*02-restricted CTL response against HIV-1 viral load, including individuals who did and did not coexpress HLA-B*27, a negative correlation was observed ($r = -0.750$, $P = 0.003$; Fig. 4D). Because the presence of HLA-B*27 results in a low viral load, we hypothesized that proper viral suppression, because of the presence of HLA-B*27, preserves CTL function including CTL restricted by nonprotective HLA alleles. To substantiate this finding, we repeated our analysis for another nonprotective HLA molecule. We selected 10 patients expressing HLA-B*08, which is not associated with delayed disease progression (RH = 0.97, $P = 0.82$),[29] 5 of which coexpressed HLA-B*27. Again, the magnitude of the CTL response restricted by the nonprotective HLA allele B*08 was negatively correlated with HIV-1 RNA load ($r = -0.833$, $P = 0.005$; Fig. 4E), with individuals coexpressing HLA-B*27 showing the highest magnitude. This shows that expression of HLA-B*27 also leads to preservation of HLA-B*08-restricted CTL responses.

Taken together, our data suggest that although HLA-B*57-restricted responses are of exceptionally high affinity and downregulate CTL responses restricted through other HLA molecules, HLA-B*27-restricted responses are not exceptionally high, broad, or of high affinity but have a beneficial effect on CTL responses restricted by other HLA molecules of the host. Moreover, although protective HLA alleles are thought to target the most conserved parts of HIV, we found that CTL responses restricted by HLA-B*27 and B*57 were lost at least as fast during the course of infection as CTL responses restricted by a nonprotective HLA allele.

DISCUSSION

Functional studies focusing on those relatively rare patients able to spontaneously control HIV-disease progression are key to obtaining insights into the characteristics of CTL responses needed to delay HIV-disease progression. Such studies have previously pinpointed differences in CTL functions between HIV controllers and patients with progressive disease (eg, Refs. 10, 39–46). Our study uniquely compared CTL responses restricted by nonprotective and protective HLA alleles within the same host, such that the effects of slow or rapid progression are not interfering with our readout. Moreover, we analyzed the 2 HLA alleles most convincingly associated with slow disease progression separately, to reveal potential different and/or shared mechanisms of protection. In concordance with previous studies,[7,8] we found that the CTL response in individuals coexpressing HLA-B*57 was dominated by CTL against HLA-B*57-binding peptides. In contrast, the CTL response in B*27+ patients was not dominated by CTL against HLA-B*27-binding peptides. In fact, CTL responses restricted by HLA-B*27 and HLA-A*02 were indistinguishable in height and breadth. Moreover, although it has convincingly been shown that CTL specific for the dominant HLA-B*27-restricted epitope KK10 are highly polyfunctional and have a superior functional capacity compared with other HIV-1–specific CTL,[10] our data suggest that this is not because of an increased half-life of the interaction between the TCRs and the HLA-B*27–KK10 complex, or the pHLA–B*27 complex in general, indicating that a strong interaction between pHLA complexes and the TCR is not a prerequisite for a protective T-cell response. Thus, although immunodominance, breadth, magnitude, and affinity of the T-cell response might be associated with protection against progression to AIDS in HLA-B*57 expressing individuals, our data show that this is not the case for HLA-B*27.

Additionally, we found that CTL responses restricted by the protective HLA alleles HLA-B*27 and B*57 were lost at least as fast as CTL responses restricted through the nonprotective HLA allele HLA-A*02. Even at the HIV-sequence level, we found no evidence that HLA-B*27 or B*57-binding epitopes are more conserved during disease progression than HLA-A*02-binding epitopes. Maintenance of CTL responses per se is, therefore, also not a main determinant of protection against progression to AIDS. The finding that protective HLA alleles contribute strongly to the total CTL response during primary infection, as was shown before,[8] might however certainly add to their protection.

Our data clearly show that T-cell responses restricted by different HLA molecules influence each other differently, which has also been described for other viruses.[37,38] Although the mechanism behind these observed associations is not well

FIGURE 3 (*Continued*). positive. In (C), we analyzed whether the responses that were present during early HIV-1 infection were still present during chronic infection in individuals coexpressing HLA-A*02 and one of the protective HLA alleles. Within each box, the median is indicated by a horizontal line, the bar represents the 25%–75% interval, and whiskers represent minimum and maximum values. D, The pie charts depict the fraction of MHC-binding epitopes that were predicted to be present during early infection and maintained (gray) or lost (white) during chronic infection based on HIV sequences derived from individuals carrying the respective HLA molecule.

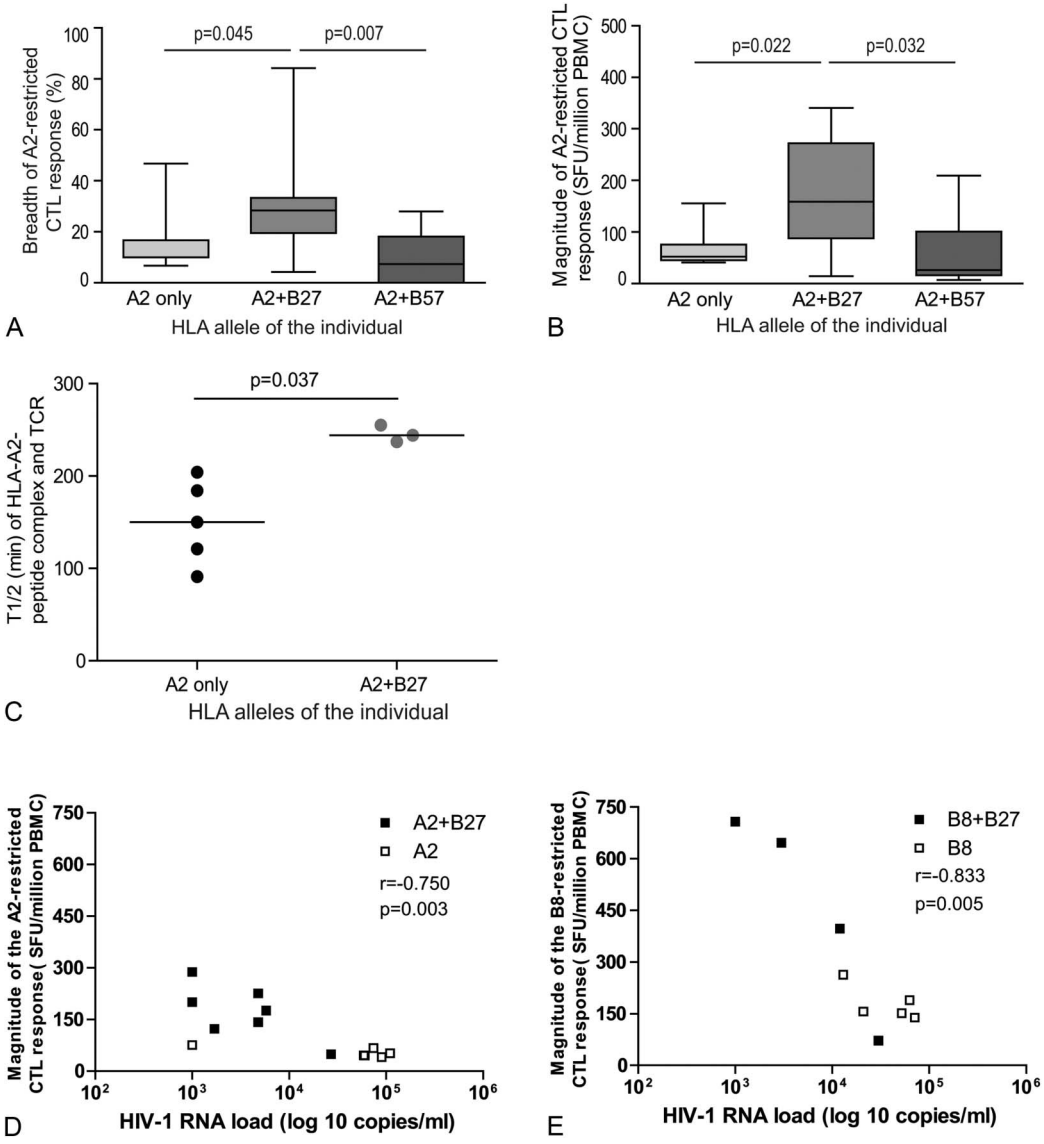

FIGURE 4. The influence of coexpression of protective HLA alleles on the CTL response restricted by nonprotective HLA alleles. The breadth (A) or magnitude (B) of the HLA-A*02-restricted CTL response in individuals expressing HLA-A*02 without any of the protective HLA alleles (A2 only) or in combination with HLA-B*27 (A2 + B27) or HLA-B*57 (A2 + B57) is shown. An additional 8 HIV-1 seroprevalent individuals (3 individuals coexpressing HLA-A*02 and B*57 and 5 individuals coexpressing HLA-A*02 and B*27) were included to increase the sample size. Within each box, the median is indicated by a horizontal line, the bar represents the 25%–75% interval, and whiskers represent minimum and maximum values. C, The half-lives of the interaction between the HLA-A*02-peptide complexes and the TCRs of the responding CTL for individuals expressing HLA-A*02 alone (black dots) and individuals coexpressing HLA-A*02 and B*27 (gray dots). Significant differences between the groups ($P \leq 0.05$, Mann–Whitney U test) are depicted when applicable. HIV-1 RNA load was plotted against the magnitude of the HLA-A*02-restricted CTL response (D) or HLA-B*08-restricted CTL response (E) in individuals expressing HLA-A*02 or B*08 (open squares) and individuals coexpressing HLA-B*27 and HLA-A*02 or B*08 (black squares). Each square represents the mean HLA-A*02-restricted CTL response (D) or B*08-restricted CTL response (E) of 1 individual. Correlations were tested using Spearman's correlation test.

understood, this phenomenon has implications for the design of epitope-specific vaccines. In our case, it seems that the effect of HLA-B*27 on the magnitude and breadth of responses restricted through other HLA alleles is because of the beneficial effect of HLA-B*27 on HIV viral load. The low HIV viral load and activation level in patients coexpressing HLA-B*27 might result in a decreased level of exhaustion of all HIV-specific $CD8^+$ T cells, hence also the ones restricted by HLA-A*02 or HLA-B*08. Our findings thereby also illustrate the difficulty in interpreting the quality of CTL responses, as a high and broad (HLA-A*02 or B*08 restricted) CTL response apparently not necessarily means

that the HLA in question is driving the favorable clinical outcome.

HLA-B*27 is also associated with beneficial outcome of hepatitis C virus (HCV) infection. A recent study showed that functional avidity, the functional profile, antiviral efficacy, or naive precursor frequency of the immunodominant HLA-B*27-restricted HCV-specific $CD8^+$ T-cell epitope was not superior to T-cells targeting epitopes restricted by HLA-A*02,[47] in line with our current observations. However, epitope generation was much more efficient for this B*27-restricted peptide compared with the A*02-restricted peptides, indicating that kinetics of antigen processing might be associated with HLA-B*27-mediated protection in HCV infection.[47] Such a mechanism might also play a role in HIV infection.

Our data indicate that there are at least 2 different strategies through which HLA class I alleles can be protective, which include (1) inducing a very dominant CTL response (eg, HLA-B*57) and (2) preservation of total T-cell responses (as observed for HLA-B*27). The marked differences between the 2 protective HLA alleles that we observed are an important new insight, as previous studies often did not distinguish between HLA-B*27 and B*57 when investigating HIV control (eg, Refs. 41, 44–46). Box 1 depicts the observed similarities and discrepancies between HLA-B*27 and B*57, which may contribute to their protective effect. The observation that HLA-B*27 and B*57 exert their protective effect at distinct moments after HIV infection[48] suggests the existence of a different mechanism of protection. The effect of HLA-B*57 already occurs early after infection, before the $CD4^+$ T-cell count drops below 200 cells per microliter, whereas HLA-B*27 delays progression to AIDS-defining illnesses when the $CD4^+$ T-cell counts have already dropped below 200 cells per microliter.[48] This fits with our observation that the beneficial effect of HLA-B*27 is evident during chronic infection but not early during infection (within 6 months after seroconversion, data not shown).

In conclusion, the actual mechanism(s) of protection offered by an HLA molecule involve both virologic and immunological features. Several virologic features are known,[4–6] but the immunological mechanisms are less well understood. We here show that certain mechanisms at least are not required for protection against disease progression. Our data indicate that although HLA-B*57-restricted responses are more likely to be of exceptionally high affinity and downregulate CTL responses restricted through other HLA molecules, HLA-B*27-restricted responses are of moderate affinity, but have a clear beneficial effect on CTL responses restricted by other HLA molecules of the host.

Box 1. Similarities and Differences Between HLA-B*27 and B*57 Revealed in This Study

SIMILARITIES

- CTL responses restricted through both HLA molecules are already broad during early HIV infection
- Both HLA molecules induce CTL responses that are not better maintained during disease progression than those restricted by nonprotective HLA alleles
- During chronic HIV infection, CTL responses restricted through both HLA molecules are comparable in breadth and magnitude

DIFFERENCES

- HLA-B*57-restricted responses are of exceptionally high affinity, which is not observed for HLA-B*27-restricted responses
- Although HLA-B*57-restricted responses seem to downregulate CTL responses restricted through other HLA molecules, HLA-B*27-restricted responses have a clear beneficial effect on CTL responses restricted by other HLA molecules of the host

ACKNOWLEDGMENT

The authors thank Philip Davies for linguistic advice.

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