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Correlates of spontaneous viral control among long-term survivors of perinatal HIV-1 infection expressing HLA-B57

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Abstract

Objective—We sought to identify immunologic and virologic correlates of spontaneous viral control among long-term survivors of perinatal HIV infection expressing the protective HLA-B57 allele.

Design—The frequency, epitope specificity, and functional attributes of HIV-specific T cells and sequence variation within B57-restricted epitopes were compared between “spontaneous controllers” who maintained normal CD4 percentages and viral loads <3000 copies/ml without antiretroviral therapy, and “treated progressors” who had initiated HAART.

Methods—Recognition of HIV optimal epitopes was assessed by IFN γ Elispot. Functional characterization of CD8 cells targeting B57 epitopes was performed by staining for cytokine production (intracellular IFN γ , IL-2, TNF α) and degranulation. Sequencing of autologous RNA was performed to determine the prevalence of viral escape mutations within B57-restricted epitopes and associated compensatory mutations.

Results—HLA-B57 remained immunodominant during chronic infection in both controllers and progressors, but controllers recognized fewer epitopes and targeted epitopes within Gag and RT only, whereas progressors demonstrated a broader response targeting additional proteins. No individual epitope was targeted more frequently by spontaneous controllers. CD8 cytokine production patterns were heterogeneous among individuals and even among different epitopes in the same individual, and did not correlate with spontaneous viral control. Extensive sequence variation within B57 epitopes was observed in both groups, but only progressors displayed additional capsid mutations that are known to offset the viral fitness cost of B57-driven immune escape.

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Conclusions—Among HLA-B57+ long-term survivors, spontaneous control of viremia is not associated with a qualitatively or quantitatively superior T cell response, but with uncompensated fitness-attenuating mutations in the viral capsid.

Keywords

HIV-specific T cells; long-term nonprogressors; perinatal HIV infection; HLA-B57; viral escape; pediatrics

Introduction

Expression of particular class I HLA alleles, primarily those at the HLA-B locus[1], exerts a strong influence on the rate of progression to AIDS that is consistent across multiple cohorts [2–7]. The strongest and most reproducible of these associations is with HLA-B57, which is present in 44–85% of “elite controllers” who spontaneously control plasma HIV RNA levels to <50 copies/ml in the absence of antiviral therapy[8,9]. Yet this allele is also present in 10% of adults with progressive HIV infection[8,9], indicating that expression of B57 alone is not sufficient for elite control. Despite extensive studies, the molecular mechanism by which HLA-B57 confers the ability to spontaneously control viremia in some, but not all, HIV-infected individuals remain unclear.

HLA-B57-restricted CD8 T cell responses are highly immunodominant[10,11], but the frequency of HIV-specific cytotoxic T lymphocytes restricted by this allele does not differ between adults who spontaneously control viremia and those with high viral loads and progressive infection[12]. Extensive characterization of CD8 responses to HIV in adult controllers has revealed CD8 subpopulations displaying functional attributes, such as preserved proliferative capacity[12], simultaneous production of multiple cytokines[13], and enhanced effector molecule loading[14] that are not observed among individuals with higher levels of viremia. However it can be difficult to discern from such studies whether this functional superiority is a cause or consequence of restricted viral replication. Additional studies have detailed viral sequences changes selected by B57-mediated immune pressure that incur a cost to viral replicative capacity[15–18]. Nonetheless, progression to AIDS occurs in many B57+ individuals in whom these fitness-attenuating viral mutations are observed.

We examined a unique cohort of B57+ long-term survivors – children and adolescents who have lived >10 years following perinatal HIV infection – to identify viral and immunologic factors that are associated with sustained and spontaneous control of HIV viremia to low levels in the absence of antiretroviral therapy.

Materials and Methods

Study subjects

Eleven HLA-B57-positive long-term survivors of perinatal infection with clade B HIV-1 were recruited at Children’s Hospital in Boston, Boston Medical Center, the National Cancer Institute, Queen Elizabeth Hospital in Barbados, and University of the West Indies in Jamaica. Clinical, CD4, and HIV viral load data at the time of evaluation are displayed in Table 1. High-resolution class I HLA typing was performed by sequencing. This study was approved by the Institutional Review Boards at Massachusetts General Hospital and at all collaborating institutions. All subjects and/or guardians signed written informed consent prior to participation.

HIV-1 Peptides corresponding to all known HIV-1 optimal epitopes in the Los Alamos HIV Molecular Immunology Database (with an expanded list of 16 HLA-B57-restricted epitopes

[19]) were synthesized on an automated synthesizer at the Massachusetts General Hospital Peptide Core Facility using 9-fluorenylmethoxy carbonyl chemistry. Subjects were screened for recognition of optimal epitopes restricted by HLA-B57 and their other HLA alleles (range 3–24 non-B57 optimals tested per subject). Peptides corresponding to autologous variant sequences within B57-restricted Gag epitopes (TW10, KF11, QW9, and ISW9) were also assessed using progressive dilutions of the consensus and autologous variant peptide in Elispot assays.

IFN γ Elispot assay

Peripheral blood mononuclear cells were isolated from whole blood by Ficoll-Hypaque density gradient centrifugation. Fresh PBMC were plated at 50,000–100,000 cells/well in 96-well polyvinylidene difluoride backed plates (Millipore, Bedford, MA) pre-coated with 2 μ g/ml anti-IFN γ monoclonal antibody (Mabtech, Stockholm, Sweden). Peptides were added at a final concentration of 20 μ g/ml, and plates were incubated overnight and processed by standard methods. Three negative control wells contained cells and media alone, and a positive control well contained phytohaemagglutinin. Individual IFN γ -secreting cells were counted using the AID Elispot Reader System (Cell Technology, Inc.). Results were calculated as the number of spot-forming cells (SFC) per million input cells after subtraction of the background response (mean SFC of all no-antigen wells; in all cases ≤ 20 SFC/million). A response was considered positive if it was both >50 SFC/million PBMC and >3 standard deviations above the average of the negative control wells.

Multiparameter intracellular cytokine staining assay

Cryopreserved PBMCs were thawed, rested overnight, examined for viability by trypan blue exclusion, and aliquoted at 10^6 viable PBMC per tube with anti-CD28 and CD49d (1 μ g/ml each) and FITC-conjugated anti-CD107a antibodies. Cells were stimulated with HIV peptides (2 μ g/ml) for 6 hours at 37°C. Brefeldin A (1 μ g) and monensin (0.6 μ g) were added after the first hour. Following stimulation, samples were washed and stained with blue Cell Viability Dye (Invitrogen/Molecular Probes). Cells were then stained with fluorescently labeled antibodies to CD3 (Pacific Blue), CD4 (allophycocyanin; APC), and CD8 (APC-Cy7). After surface staining, cells were fixed and permeabilized (Fix & Perm Medium A and B, CALTAG/Invitrogen) and then stained with antibodies against intracellular cytokines IFN γ (PE-Cy7), TNF α (Alexa Fluor 700), and IL-2 phycoerythrin (PE). Each assay included a positive control containing phytohemagglutinin (1 μ g/ml) and a negative control with no peptide. Antibodies and protein transport inhibitors were obtained from BD Biosciences.

Flow cytometry acquisition and analysis

Data were acquired within 6 hours of staining on an LSR II cytometer using FACSDiva software (BD Immunocytometry Systems, v4.1.2), with bead compensation (Becton Dickinson) and analyzed using FlowJo (Tree Star, CA). Pie charts were generated using SPICE 4.1.6 and Pestle *f* software (kindly provided by Mario Roederer, Vaccine Research Center, NIH). A lymphocyte gate was set based on forward- and side-scatter, and CD3+ events were gated to remove dead cells stained with the blue Cell Viability Dye. The frequency of CD8+ T cells producing each cytokine (or degranulating) was determined by subtracting the percentage of cytokine-positive cells in the unstimulated CD28/49d control tube from that of each peptide-stimulated tube. Boolean gating was used to create the full array of 16 possible response patterns.

Viral sequencing

Population sequencing of the full HIV-1 genome was performed from plasma using the QIAamp Viral RNA Mini Kit (Qiagen) following amplification by nested PCR (inner and outer

primer sets and PCR conditions as previously described)[20]. The PCR products of the secondary reaction were purified and sequenced directly using the ABI 3100 DNA Analyzer from Applied Biosystems. The HXB2 numbering system is used to specify individual amino acid residues. Viral sequences could not be amplified from progressor CH-14.

Statistical analysis was performed using Stata Statistical Software: Release 8.0 (StataCorp; College Station, TX). Two-group comparisons were performed using the Wilcoxon Rank-Sum Test and categorical outcomes were compared using Fisher's Exact Test. All tests were two-tailed, with $p < 0.05$ considered significant.

RESULTS

Long term survival among B57+ children following perinatal HIV infection

Eleven perinatally HIV-infected HLA-B57-positive subjects ranging in age from 10–21 years were identified. Four subjects, termed “spontaneous controllers”, were clinically asymptomatic and maintained HIV RNA levels < 3000 copies/ml in the absence of therapy. Two of these spontaneous controllers (CH-04 and CH-22) were naïve to antiretroviral therapy. Subject CH-13 had briefly received didanosine monotherapy during infancy before being lost to follow-up for 6 years, and subsequently presented with an HIV RNA level of 620 copies/ml at 9 years of age. Subject CH-03 discontinued dual nucleoside reverse transcriptase inhibitor (NRTI) therapy with azidothymidine and didanosine at age 13 and subsequently maintained a viral load < 1000 copies/ml for more than 5 years. Each of these subjects maintained a normal CD4 percentage of $> 25\%$ [21]. The remaining 7 subjects, termed “treated progressors”, had begun antiretroviral therapy due to clinical disease progression and/or elevated viremia at a median age of 8 years (range 7–13). All had documented HIV RNA levels $> 10,000$ copies/ml before starting antiviral therapy, but comprehensive data regarding the early clinical course was not available for all subjects and it is possible that some may have fit the spontaneous controller definition at an early stage of their infection. In response to antiviral therapy, HIV RNA levels were low in all treated progressors at the time of study ($< 50 - 4017$ copies/ml, Table 1). Spontaneous controllers and treated progressors did not statistically differ with respect to age ($p = 0.34$).

Sustained spontaneous control of viremia is characterized by a narrower T cell response

Subjects were tested by IFN γ Elispot for recognition of all optimal epitopes restricted by their class I HLA alleles (both B57 and non-B57 epitopes) that were listed in the 2006 HIV Molecular Immunology Database. Despite longstanding infection, the response to B57-restricted epitopes dominated the total T cell response to HIV among both spontaneous controllers and treated progressors. Among the entire cohort, 67% of HIV epitopes targeted, and 74% of HIV-specific cells, were restricted by HLA-B57. While this dominance was slightly more pronounced among spontaneous controllers than treated progressors, the difference did not achieve statistical significance (Fig. 1a). Spontaneous controllers recognized significantly fewer of the 16 B57-restricted HIV-1 epitopes than progressors (median 2.5 vs. 7; $p = 0.044$) and the frequency of T cells specific for B57-restricted epitopes was also lower among controllers, although this difference did not achieve statistical significance (median 2350 vs. 6750 SFC/million PBMC; $p = 0.163$). When responses to optimal epitopes restricted by all HLA alleles were compared, a similar pattern was observed, with controllers exhibiting a reduced magnitude and breadth of response relative to treated progressors, despite the very low levels of HIV viremia in both groups (Fig. 1b and Supplemental Table 1).

Responses to stimulation by optimal peptides may not accurately represent the virus-specific response in individual subjects due to amino acid sequence mismatches between the peptides used for stimulation and the virus replicating in the host. We have previously reported that T

cell responses to autologous variant epitopes may be detectable when a response to the “consensus” epitope is not [22]. Therefore, we also assessed recognition of peptides corresponding to autologous sequence variants of the four B57-restricted Gag epitopes. None of these chronically infected children recognized the consensus version of epitope TW10-Gag, which is immunodominant during acute infection [10], but responses to the autologous TW10-Gag variant were present in the majority of both spontaneous controllers and treated progressors and exceeded the consensus optimal peptide response in magnitude, as previously described [22]. KF11 peptide variants were recognized less well than the corresponding optimal peptide in the 2 subjects exhibiting mutations in this epitope, with >1 log increase in the peptide concentration required to elicit the half-maximal response (Fig 2a). Among the four subjects who recognized QW9-Gag, responses to the common E180D variant peptide were similar in magnitude, and nonresponders to the consensus optimal failed to recognize the variant as well (Fig 2b). Similarly, the I147L substitution in ISW-Gag did not appreciably impact recognition, although the upstream antigen processing mutation A146P may diminish recognition of this epitope in vivo, an effect that is not detected by peptide-stimulation assays (Fig 2c) [23]. Thus, among all 4 B57-restricted epitopes in Gag, only TW10 variants were recognized in the absence of a response to the corresponding optimal epitope. Taking into account these variant-specific responses, both the breadth of the B57-restricted CD8 response remained significantly lower in spontaneous controllers.

Narrow response to conserved regions of Gag and RT is associated with spontaneous control of viremia

All 4 B57+ spontaneous controllers recognized only a limited number of epitopes in Gag and Reverse Transcriptase (RT). Each of these epitopes was targeted frequently by progressors as well; thus durable viral control in the absence of antiviral therapy could not be attributed to recognition of any single critical antigenic determinant. Interestingly, epitopes within other HIV proteins (Nef, Integrase, Vif, Vpr, and Rev) were targeted exclusively by treated progressors (Fig 1d). Overall, these data support prior observations from adult studies suggesting that spontaneous controllers preferentially target a limited number of epitopes within conserved regions of the virus [8]. Because samples from early in the disease course were available for only a limited number of subjects, we were unable to explore the hypothesis that treated progressors had experienced a broadening of responses coincident with rising viremia, as has been described following viral escape from the immunodominant HLA-B27 epitope KK10-Gag [24].

CD8 T cell cytokine production patterns vary in response to different B57-restricted epitopes and do not correlate with spontaneous viral control

As recognition of particular B57-restricted epitope(s) was not associated with spontaneous control of viremia, we explored the hypothesis that the functional capacity of B57-restricted CD8 T cells, rather than their epitope specificity, enables B57+ subjects to spontaneously control viremia. Previous studies have concluded that “polyfunctional” CD8 cells capable of producing multiple cytokines are preferentially maintained in HIV+ controllers [13,14]. We therefore assessed the ability of CD8 T cells to degranulate and to secrete multiple cytokines (IFN γ , TNF α , and IL-2) following stimulation with peptides corresponding to B57-restricted optimal epitopes [25]. This analysis revealed marked differences in the profile of cytokines produced by CD8 T cells targeting different B57-restricted epitopes, even within the same subject (Fig 3a). For instance, subject CH-11 exhibited a highly polyfunctional response to KF11, with degranulation and co-production of IFN γ and TNF α , whereas the response to QW9 in the same assay was markedly dysfunctional with little cytokine production by degranulating cells.

To minimize heterogeneity, we focused our further analyses on the most frequently recognized epitopes, KF11 and ISW9. Nonetheless, marked differences in the functional response profile of KF11-specific and ISW9-specific CD8 cells were observed among subjects. Although antigen-specific cells exhibiting 3 or 4 simultaneous effector functions were observed, such cells were not detected more frequently among spontaneous controllers than treated progressors. Interestingly, the subject with the most polyfunctional CD8 T cells and the highest mean fluorescence intensity of cytokine staining was a progressor who had been on suppressive antiviral therapy for four years (Fig 3b). Together, these data indicate that with respect to cytokine production, B57-restricted HIV-specific CD8 cells were not functionally superior in long-term survivors with spontaneous control relative to those whose viremia is controlled by HAART.

Sequence mutations within B57-restricted HIV epitopes

To determine the relationship between HIV sequence variation, epitope recognition, and spontaneous control of viremia, the *gag*, *nef*, and *pol* genes were sequenced from plasma RNA. As has been observed in adult studies, sequence variation within B57-restricted epitopes was common, with a median of 9 mutations per subject (Table 2). Treated progressors and spontaneous controllers did not differ with regard to the total number of mutations observed within epitopes in *Gag* (median 4 vs. 4.5; $p=0.82$), *nef*, (2.5 vs. 3.5; $p=0.23$), *RT* (1 vs. 1; $p=1.00$), or all regions combined (8.5 vs. 9.5; $p=0.28$). All intra-epitope sequence variants that were commonly observed in treated progressors were observed in the controller group as well. Moreover, no epitope was observed in “consensus” form more frequently in controllers than in progressors, and the total number of B57-restricted epitopes whose sequence remained “consensus” did not differ between the two groups. Thus it is unlikely that the shift toward recognition of CD8 T cell targets within accessory and regulatory proteins observed in treated progressors can be attributed to viral escape alone.

Well-characterized mutations conferring escape from B57-restricted *Gag* epitopes TW10 and ISW9 were present in both spontaneous controllers and treated progressors. All subjects except for a single controller exhibited the T242N mutation in p24-*Gag*, which is consistently selected for during early infection and has been demonstrated to attenuate viral fitness [10,15,16,25]. This subject (CH-13) exhibited an uncommon mutation within the TW10 epitope (248D) that is associated with a marked reduction in the viral replication rate [26] and likely represents an alternative pathway to escape from this epitope. Numerous studies have shown that following selection for T242N, additional mutations at interacting sites in p24 (residues 219, 223, and 228) accumulate and serve to “compensate” for the fitness-reducing impact of this epitope [15–17,25]. We examined these residues to determine whether the accumulation of such “compensatory” mutations was associated with loss of spontaneous viral control in our cohort (Table 2). Strikingly, all treated progressors demonstrated the amino acid substitutions H219Q and M228I/L and most demonstrated I223V as well, while none of these mutations were observed in any controller. Sequence variation within the immunodominant epitope KF11, which rarely escape in clade B infection [27], was observed in 2 controllers but no treated progressors. Subject CH-22 exhibited the A163X mutation that has been shown to confer immune escape and a subtle fitness cost [27], but this subject lacked the S165X mutation which partially compensates for this fitness defect [18]. Sequences from CH-13 – the child with the rare TW10 mutation – displayed an unusual substitution within KF11 as well, suggesting that mutational pathways to escape from these two epitopes may be interdependent. Mutations within or adjacent to the ISW9 epitope were observed in all subjects; the 146P processing escape mutation was more common in controllers while the intra-epitope A147L mutation was more common in progressors, although these differences did not achieve statistical significance ($p=0.191$ and $p=0.133$, respectively). When p24 compensatory mutations were taken into account, both the number of total B57-associated mutations ($p=0.048$) and the number of B57-

associated *gag* mutations in ($p=0.03$) were significantly higher in treated progressors. Together these cross-sectional data indicate that extensive variation with B57-restricted epitopes accumulates in both spontaneous controllers and progressors, but in the latter group additional mutations have emerged that compensate for the fitness defect induced by TW10 escape.

DISCUSSION

In this cross-sectional study, we examined viral and immunologic factors associated with sustained and spontaneous control of HIV viremia in the absence of antiviral therapy, which is an exceptionally rare outcome in pediatric infection. The overall rate of progression to AIDS is more rapid following perinatal HIV infection. Without antiviral therapy, <8% of HIV+ children survive to age 8 without significant immunocompromise [28]. Thus the 4 B57+ spontaneous controllers described here are highly unusual as they maintained low levels of viremia as well as high CD4 percentages despite 10–17 years of infection. We have previously shown that most HLA-B57+ infants control viremia to low levels during infancy and early childhood[25]. In this study we examined correlates of spontaneous viral control after 10 or more years of infection in B57+ children, two-thirds of whom had experienced disease progression. It is important to note that the terms “spontaneous controllers” and “treated progressors” may denote two different stages of HIV infection rather than two distinct populations of individuals. Indeed the relatively late age at which most progressors in this study initiated HAART suggests that these children had a benign early clinical course, and some may have met the “spontaneous controller” definition at an earlier stage.

Our results indicate that neither the specificity of epitopes targeted nor the functional qualities of the responding CD8 T cells differ between spontaneous controllers and treated progressors by the assays employed. In agreement with prior studies of B57+ adults, the CD8 response of spontaneous controllers was notable for its narrow focus on a small number of epitopes in conserved regions of p24-Gag [8] and RT. The frequency of HIV-specific cells, both B57-restricted and overall, was higher among progressors, despite the fact that most progressors had undetectable viremia on HAART. Because antiviral therapy is known to diminish the magnitude and breadth of virus-specific T cell responses [14,20,29], the use of HAART by progressors would be expected to bias our analysis toward the null hypothesis. While all subjects in the current study had low levels of viremia, HIV RNA levels were >50 copies/ml in all spontaneous controllers but only a minority of treated progressors. Therefore the low frequency of HIV-specific cells observed in spontaneous controllers cannot be readily attributed to a lack antigenic stimulation, and was more likely related to the restricted breadth of response, as epitope-specific cell frequencies were similar between the groups. The association of spontaneous viral control with a relatively narrow breadth of targeted epitopes has been convincingly established in both B57+ subjects[8] and the general population of HIV controllers[9]. We have previously shown in the setting of HLA-B27 expression that escape from the immunodominant CD8 response is associated with both loss of spontaneous viral control and a coincident broadening of the T cell epitopes targeted[24]. Longitudinal studies are needed to determine whether a progressive broadening of responses accompanies loss of spontaneous viral control in B57+ individuals.

A population of “polyfunctional” HIV-specific T cells, capable of degranulation and simultaneous production of multiple cytokines, has been described in some adult HIV controllers but not in those with untreated progressive infection[30]. Using similar assays, we observed significant functional heterogeneity between subjects, as well as heterogeneity in the response to distinct epitopes within the same subject. Overall, however, the cytokine profile of epitope-specific CD8 T cells was not superior among spontaneous controllers, although the small number of subjects studied limits the power of this analysis. Intra-subject heterogeneity in the response to different viral epitopes has been previously described in a study by Streeck

[31], which found progressive loss of CD8 cell function to correlate with persistent antigen exposure. However in our cohort the degree of polyfunctionality did not correlate with levels of residual viremia nor with sequence variation in the targeted epitope. As reported by Streeck, we observed polyfunctional CD8 populations among subjects with prolonged HAART-mediated viral suppression, raising the question of whether polyfunctional responses that have been observed in elite controllers are a cause or a consequence of restricted viral replication [13]. Additional parameters of CD8 T cell function that were not assessed in this study, such as proliferative capacity[12], effector molecule content, and per-cell cytotoxicity[14], might be better able to discriminate CD8 populations that contribute to sustained and effective control of viremia.

Viral sequence analysis revealed clear differences between treated progressors and spontaneous controllers. While mutations accumulated within B57-restricted epitopes in both groups, p24 compensatory mutations that mitigate the fitness-attenuating impact of The T242N mutation were observed only in treated progressors. Prior studies have demonstrated that biologically cloned HIV-1 variants derived from LTNP and treated progressors differ in their replication capacity in vitro[32], that stereotypic B57-driven p24 escape mutations (including T242N) reduce viral replication rates in an additive fashion[33], and that an associated cluster of mutations in p24 partially offset the fitness deficit incurred by T242N[17]. In the present study, we observed that accumulation of these compensatory mutations during natural infection is associated with loss of spontaneous control of viremia. This difference is not readily attributable to antiviral treatment in the progressor group, as HAART decreases viral replication and hence would be expected to minimize rather than increase the accumulation of viral mutations. Our findings support a prior report that the presence of two or more compensatory mutations is associated with HIV progression[32]. We have also observed that transmitted compensatory mutations are associated with levels of viremia during early infection and may influence the propensity of T242N to revert following transmission to B57-negative hosts[25]. Together these data indicate that T242N-associated compensatory mutations can nullify the protective impact of HLA-B57 expression whether they are transmitted or are selected during chronic infection.

Longitudinal studies are needed to examine the temporal relationship between emergence of B57-associated mutations and loss of spontaneous viral control. Our data are consistent with the hypothesis that superior control of viremia in B57+ subjects is facilitated by escape mutations within Gag epitopes, particularly T242N, and that control persists until the subsequent emergence of compensatory mutations that restore fitness, eventuating in increased viral replication and disease progression.

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Figure 1. Comparison of the HIV-specific T cell response in B57+ controllers and progressors by IFN γ Elispot

All responses are expressed as spot-forming cells per million PBMC (SFC/million). **A.** In both spontaneous controllers and treated progressors, a high proportion of HIV-specific T cells targeted B57-restricted epitopes (SFC/million recognizing B57 optimals divided by SFC/million recognizing all HLA-matched optimals; median plus interquartile range is shown).

B. Both the breadth (upper panel) and the magnitude (lower panel) of response were higher among progressors, following stimulation with both B57 optimal epitopes (left half of each panel) and all HLA-matched optimals (right half of each panel). **C.** Among controllers, the B57-restricted optimal response exclusively targeted epitopes in Gag and RT, whereas progressors exhibited a broader response, which targeted Nef and other proteins in addition to Gag and RT. **D.** No individual B57-restricted epitope was targeted more frequently by controllers than progressors.

Figure 2. Recognition of autologous variant Gag peptides restricted by HLA-B57

Recognition of defined optimal epitopes (a) KF11, (b) QW9, and (c) ISW9 (solid lines) and peptides corresponding to the autologous viral variant present in each subject (dotted lines) were compared using progressive titrations of the peptides in an IFN γ Elispot assay. (Data regarding recognition of TW10 variants has been previously published[22]). Variants of KF11 were recognized with lower avidity than the wild-type epitope, but no consistent impact of sequence variation within ISW9 or QW9 was noted.

Figure 3. Functional assessment of CD8 T cells specific for B57-restricted HIV epitopes
 Degranulation (assessed by CD107a staining) and production of 3 cytokines (IL-2, IFN- γ or TNF- α) by individual CD8 T cells was measured in a multiparameter flow assay. All flow plots are gated on CD8+ T cells; NA= CD28/49d-only negative control. **A.** Functional profiles of CD8+ T cells targeting distinct B57-restricted epitopes differ markedly within a single individual, as demonstrated by the response of CH-11, a representative HIV-infected progressor who recognized 5 B57-restricted epitopes. The flow plots highlight the divergent response pattern to epitopes KF11 and QW9. The pie charts depict the proportion of CD8 cells exhibiting each distinct functional profile in response to all 5 epitopes. **B.** Functional profiles of CD8 T cells targeting a single epitope (KF11-Gag) differed markedly among subjects, but

did not correlate with disease control. Pie charts depict the proportion of KF11-specific CD8 T cells exhibiting 1, 2, 3, or 4 functions.

Table 1

HLA-B57+ long-term survivors of perinatal HIV infection: clinical and laboratory data

	Subject	Age	HLA class I			ARV (and duration)	VL	CD4 Abs (%)	B57 inheritance
			A	B	C				
Progressors	CH-02	10	2301, 6601	5703 , 5301	0401, 1801	HAART (1 year)	<50	886 (43%)	maternal
	CH-01	12	0201, 2301	5703 , 4403	0401, 0701	HAART (4 years)	<50	701 (28%)	maternal
	CH-08	13	0101, 0201	5701 , 1801	0602, 0701	HAART (5 years)	<50	1010 (33%)	paternal
	CH-11	16	2301, 2301	5701 , 4501	0602, 1601	HAART (9 years)	184	709 (20%)	unknown
	CH-10	17	0301, 0301	5703 , 4201	0701, 1701	3TC/D4T (10 years)	4017	418 (30%)	paternal
	CH-14	18	0101, 0201	5702 , 0702	0701, 1801	HAART (9 years)	<50	624 (33%)	unknown
	CH-12	21	0303, 0303	5701 , 3503	0401, 0602	HAART (8 years)	<50	741 (28%)	unknown
Controllers	CH-22	10	3101, 3301	5703 , 1402	0701, 0801	Naive	<400	740 (41%)	unknown
	CH-13	11	2301, 3301	5703 , 4403	0401, 1801	Off > 6 yrs	620	465 (32%)	unknown
	CH-04	15	6802, 6802	5703 , 5703	0701, 1801	Naive	2700	643 (36%)	both
	CH-03	17	3402, 6801	5703 , 1510	0304, 0701	Off > 5 yrs	170	484 (26%)	maternal

ARV= antiretroviral therapy. HAART= highly active antiretroviral therapy. VL= viral load (HIV RNA copies/ml). N/A=not available. CD4 data are provided as absolute number (cells/mm³) followed by percentage in parentheses.

Table 2

Sequence variation within B57-restricted HIV epitopes and T242N-associated compensatory mutations

		ISW9 [/]	KF11	QW9	TW10	219	223	228	HW9	KAF9	YY8	QF10	IVW9	IAW9
		(A)ISPRTLNAW	KAFSPEVPMF	QASQEVKNW	TSTLQEQIGW	H	I	M	HTQGYFPDW	KAAVDLSHF	YFPDWQNY	QATWIPWEF	IVLPEKDSW	IATESIVIW
Progressors	CH-02	-L-----	-----	-----S-	--N---Q-	Q	V	L	-----	-----	-----	-----	-K---E--	V-R-A-----
	CH-01	PL-----	-----	--T-----	--N---A-	Q	-	I	N-----	RG-V-----	-----	-----	-----	-----
	CH-08	-L-----	-----	-----	--N---T-	Q	V	I	N-----	-G-----	-----	-----	-M-----	-----
	CH-11	-L-----	-----	-----D-----	--N-A---A-	Q	V	L	N-----	--L-----	-----	-----	-K-----	-----
	CH-10	-L-----	-----	-----D-----	--N---A-	Q	V	L	N-----	-R-L-----	-----	-----	-----	-----
Controllers	CH-12	-L-----	-----	-----	--N---Q-	Q	-	I	N-----	-G-L-----	H-----	-----	-E-----	-S-C-----
	CH-22	P-----	-N-----	-----D-----	--N---A-	-	-	-	N-----	AG-LN-----	-----	-----	-----	-S-----
	CH-13	P-----	R-----I-----	-----D-----	-----D-	-	-	-	N-----	-G-L-----	-----	-----	-----	--L-----
	CH-04	PL-----	-----	-----D-----	--N-----	-	-	-	N-----	-G-L-----	-----	-----	-----	-----
	CH-03	-L-----	-----	-----	--N-----	-	-	-	N---F-----	-G-L-----	F-----	nd	-K---E--	--K-V-----

Epitopes in Gag, Nef, and RT are displayed; dashes represent preservation of the wild-type residue indicated in the column heading. Viral sequences could not be amplified from progressor CH-14.

[/]The amino acid residue preceding epitope B57-ISW9 is included in this table because substitution of proline (P) for the wild-type alanine (A) residue at this site has been shown to confer immune escape via altered antigen processing.