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Immunological control of chronic HIV-1 infection: HLA-mediated immune function and viral evolution in adolescents

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Abstract

Background—Differential protein targeting by HIV-specific CD8 T cells is associated with disparate plasma viral loads; however, it is unclear if the quality of these responses differs depending upon the specificity of the targeted epitopes.

Methods—We examined HIV-specific CD8 T-cell responses in HIV-infected adolescents carrying either an HLA class I allele associated with a favorable prognosis (HLA-B*57) or an allele associated with usual disease progression (HLA-B*35 or HLA-B*53) using interferon- γ ELISpot and ICS assays.

Results—In an interferon- γ ELISpot assay, p24 was the dominant protein targeted by B*57 carriers while responses to Nef dominated in B*35 or B*53 positive carriers. This differential protein targeting did not change during 4 years of follow-up. In these chronically infected adolescents, there were no significant differences in the quality of the immunodominant T-cell responses between the B*57 and B*35/B*53 carriers as measured by peptide avidity, degranulation, and immune memory markers. There was a trend towards higher expression of interleukin-2 from B*57-KF11 restricted CD8 T cells although this difference was not significant. Nevertheless both B*57 and B*35/53-restricted responses were relatively potent as reflected by the propensity of CD8 T cells to escape in p24 and Nef, respectively.

Conclusions—Differential protein targeting rather than the quality of T-cell responses appears to be a major distinguishing feature of HIV-specific CD8 T cells induced in B*57 carriers. These data suggest that viral fitness costs associated with CD8 T-cell pressure is an important factor determining differences in the viral load among HIV-infected patients.

Keywords

adolescents; CD8 T-cells; Gag; HIV-1; HLA-Class I; Nef

Introduction

Although HIV-1 infection invariably results in progression to AIDS in the vast majority of patients, host genetic factors impact the relative rates of disease progression. Among HLA class I alleles, HLA-B*57 and B*27 have consistently been associated with slower rates of disease progression, while HLA-B*35 and B*53 have been associated with more rapid development of AIDS [1–6]. The absolute necessity of the HLA class I system for elicitation of CD8 T-cell responses combined with the importance of the latter responses in the control of HIV replication [7] strongly support the hypothesis that the quality of CD8 T-cell responses differ depending on the specificity of epitope targeting and/or HLA class I restriction.

The targeting of the Gag protein by CD8 T cells has been shown to correlate with favorable makers of disease progression [8–14]. More recent work has shown that the lower viral loads associated with HLA-B*57 and other favorable HLA class I carriers is due in part to targeting of the Gag protein [15]. Only a few studies have been able to demonstrate qualitative differences among CD8 T cells restricted by distinct HLA class I alleles even when the cytotoxic T lymphocytes (CTL) target identical epitopes [16]. HIV vaccination in seronegative vaccinees carrying HLA-B*57 or B*27 was found to have greater antigen specific CD8 T-cell responses when compared to those without such alleles [17]. In addition, Migueles and colleagues demonstrated that HIV-infected long-term nonprogressors (LTNP; mainly HLA-B*57 carriers) had HIV-specific CD8 T cells that were able to proliferate more efficiently compared with the usual progressors [18]. HIV-specific polyfunctional CD8 T cells were also shown to be associated with lower plasma viral loads and found to be independent of HLA-B*57 carriage [19].

We hypothesized that the relative lack of immune control in HLA-B*35/B*53 positive patients is due to targeting outside of the Gag protein by a functionally distinct phenotype of T cells when compared to HLA-B*57. Using multiple assays to analyze the quality of these immune responses in subjects selected from an adolescent cohort, we found that the quality of the epitope specific responses did not differ depending upon HLA class I restriction. Rather the preferential targeting of Gag versus Nef proteins in patients with HLA class I alleles associated with favorable and poor prognosis, respectively was the only discernable feature.

Materials and methods

Study subjects

T-cell responses to the entire HIV-1 expressed genome were measured in HIV-1 clade B infected adolescents from the REACH cohort of US Adolescent Medicine HIV-1/AIDS Research Network (AMHARN) [20]. Selected subjects from the REACH cohort with HLA class I alleles B*35 (n = 23), B*53 (n = 31) and B*57 (n = 15) were then enrolled and followed longitudinally for an additional 2 years in a new study (Adolescent Medicine Trials Network for HIV/AIDS Interventions that is ATN 026). For all 69 subjects who enrolled into ATN 026, peripheral blood mononuclear cells (PBMC) and clinical data including viral load, CD4 cell counts, and treatment history were obtained every 6 months. To evaluate the T-cell responses and analyze the sequence changes, PBMC from the REACH and ATN026 studies were utilized allowing for a longitudinal follow-up period of approximately 4 years. Eighteen patients (7 B*57, 5 B*35, and six B*53 carriers) were off antiretroviral therapy (ART) for at least two time points during this 4-year follow up with available PBMC for comprehensive interferon (INF)- γ ELISpot T-cell mapping. All subjects consented to participate in this study with Institutional Review Board approval obtained at each of the participating sites.

Quantitative immunophenotyping of CD4 T-cells and HIV-1 plasma RNA viral load were measured by standard assays as described previously [21]. HLA class I alleles were typed to

two- or four-digit specificity by PCR amplification using sequence-specific primers (Pel-Freez Clinical Systems, Brown Deer, Wisconsin, USA) as described before [21].

Entropy

Entropy Year 2003 genome and by-protein alignments in the Los Alamos HIV Database (www.hiv.lanl.gov) were used to obtain a Shannon entropy score for each position in the protein alignment. Entropy is a measure of the amino-acid variability at a given position that takes into account both the number of possible amino acids allowed and their frequency [22]. In each patient, an entropy score of every positive T-cell response was calculated. The entropy score of a specific peptide was calculated as an average of entropy scores of each amino acid included in the peptide.

Peptides

HIV-1 peptides used in this study were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. The peptides used in this study were 15-mers overlapping by 11 encompassing Gag (#5107), Pol (#6208), Env (#6451), Nef (#5189) and Accessory proteins of HIV-1 (# 5138, 6444, 6445, 6446, 6447). Each peptide was used at was 2 µg/ml. All HLA-B*57 and B*35/B*53-restricted epitopes (9–11-mers) described in the Los Alamos database were synthesized (Polymx, Huntsville, Alabama, USA) and tested in an IFN-γ ELISpot assay.

IFN-γ ELISpot assay

An IFN-γ ELISpot assay was performed as described in prior studies [20,23]. In brief, Millipore plates (MAHAN# 4550) were coated overnight with anti-IFN-γ mAb (Mabtech #1DIK). The following day, the cells were plated at 1×10^5 cells/well and incubated with peptide pools and optimized peptides for 20–24 h. For spot detection, biotinylated anti-IFN-γ (Mabtech #7B61) followed by streptavidin-AP and BCIP substrate (Southern Biotech #s 7100-04 and 3032-01 respectively) were used. Spots were counted using a CTL ‘Immunospot’ plate reader and expressed as SFC (spot forming cells)/ 10^6 PBMC. In this study, breadth of response is defined by the total number of peptides pools/peptides recognized. Magnitude is defined as the reactivity either to the sum total of all the peptides in a pool or to a single peptide.

HIV-1 sequencing

Genomic DNA was extracted from the PBMC of HIV-positive patients using QIAamp DNA Blood mini kit (Qiagen, Valencia, California, USA). HIV-1 genomic sequence was amplified using FAST Start High Fidelity PCR System (Roche, Nutley, New Jersey, USA) kit. The Outer PCR primers used in this study to amplify full-length Nef sequences (8329–9548 on pNL4.3) were: Nef-For: 5′-AATAGAGTTAGGCAGGGATAC-3′ and Nef-Rev: 5′-TGGTCTAACCAGAGAGAC-3′. The inner Nef primers were: For: 5′-TAAGACAGGGCTTGGAAAGG-3′ and Rev 5′-AAAGTCCCAGCGGAAAGTC-3′. The PCR primer pair for the amplification of the full-length gag sequences (664–2509 on pNL4,3) were Gag-outer-For: 5′-AAGTAAGACCAGAGGAGATCTCTCGAC-3′; Gag-outer-Rev: 5′-CC AATTATGTTGACAGGTGTAGGTCCTAC-3′ and Gag-inner-For: 5′-TTTGACTAGCGGAGGCTAGAAGGA-3′; Gag-inner-Rev: 5′-GTATCATCTGCTCCTGTGTCTAAGAGAGAC-3′. The selected primers amplified the entire *gag* and *nef* regions for sequencing. All PCR products were purified either by QIAquik PCR Purification kit or QIAquik gel Extraction kit (Qiagen) and sequenced using the Big-Dye chemistry (Applied Biosystems, Foster City, California, USA).

Polychromatic flow cytometry

Co-stimulatory monoclonal antibodies (anti-CD28 and anti-CD49d; each at 1 µg/ml) and CD107-FITC (a/b) (Becton Dickinson, San Jose, California, USA) were added to 1×10^6 PBMC. Cells were pulsed with the appropriate peptide (10 µM) for 1 h before Monensin (10 µg/ml) was added, followed by a 5h incubation at 37°C in the presence of 5% CO₂. For a positive control, Staphylococcus enterotoxin B at 1 µg/ml (Sigma-Aldrich, St Louis, Missouri, USA) was used. The cells were washed twice in phosphate-buffered saline and permeabilized with the Cytotfix/cytoperm reagent (Becton Dickinson) for 20 min. at room temperature in the dark. The permeabilized cells were washed twice with the perm/wash buffer. The cells were labeled in a single step with the surface and intracellular antibodies. For the surface phenotype, the samples were stained with anti-CD3-Pacific Blue and anti-CD8-PercpCy5.5 (Becton Dickinson). The fluorochromes used for the intracellular cytokine staining were anti-interleukin (IL)2-APC, anti-tumor necrosis factor (TNF)α-PE Cy7, and anti-IFN-γ Alexa Fluor 700 conjugated antibodies (Becton Dickinson). For data analysis, at least 100 000 CD3 events were acquired on an LSR II flow cytometer (Becton Dickinson). Data was analyzed using FlowJo Version 8.1.1 software (TreeStar, San Carlos, California, USA). Lymphocytes were gated using forward scatter (FSC) and side scatter (SSC) and cytokine production was quantified using data gated on CD3 cells. CD127 and CD107 expression were analyzed on CD3CD8 T cells. Four functions (IFN-γ, IL-2, TNF-α and/or CD107) analysis of CD3CD8 T-cells was performed using Boolean gating. A positive response was defined as ≥ 3 standard deviations of the media (unstimulated) control and a value that was at least twice the media control in the individual assay.

Statistical analyses

Differences in the immune responses among the patients with selected HLA class I types were compared using the nonparametric Mann-Whitney U test. Plasma viral load and absolute CD4 T-cell counts changes over time were compared using the Wilcoxon signed rank test.

Results

Significant differences in epitope targeting by HLA B*57 and B*35/B*53 carriers in a cross-sectional study

Our initial analysis drew upon a population of young, predominantly African-American females described previously [20]. We compared the IFN-γ ELISpot responses to the entirety of the expressed HIV-1 genome, mapped down to individual 20-mers, and separated the results based on HLA class I allele carriage. T-cell responses in 23 subjects with HLA-B*57 (n = 7), HLA-B*35 (n = 7) or HLA-B*53 (n = 9) were compared with 29 subjects with other HLA class I alleles (total n = 52). The clinical parameters of these study subjects including the number of subjects on ART is given in Table 1. We analyzed B*35/53 carriers jointly since they have nearly identical predicted HLA class I binding motifs, have been shown to bind identical HIV-1 optimized epitopes [23], have both been repeatedly associated with disease progression [2], and showed similar patterns of peptide recognition in this study. We did not sort the subjects into the B*35-Px and B*35-PY groups [24] due to the fact that studies in this US adolescent [21], or other populations [25] did not support clinical differences when comparing these two HLA-I alleles.

B*57 carriers had a lower magnitude of CD8 T-cell responses when compared to B*35/53 carriers or to the rest of the cohort ($P \leq 0.02$). This finding also extended to the breadth of responses since B*57 carriers recognized fewer individual peptides (mean, 2.7) when compared with B*35/53 carriers (mean, 7.3; $P \leq 0.03$). B*57 carriers predominantly targeted p24 while B*35/53 carriers targeted Nef ($P = 0.005$ and 0.03 , respectively; Fig. 1). We also calculated the entropy score of each HIV-1 protein [22] and compared it to the percentage of

total T-cell response. The entropy of the individual targeted peptides were also the lowest (most conserved) in B*57 carriers compared with either the B*35/53 carriers or the rest of the cohort (data not shown).

B*57 and B*35/B*53 carriers persistently targeted p24 and Nef respectively during longitudinal follow-up

In order to look in more detail at the potential changes and quality of CD8 T-cell responses, we enrolled 69 participants into protocol ATN 026 who carried HLA-B*35, B*53, and/or B*57. In the 45 participants who were off ART for at least one time point, the median plasma viral load (VL) was significantly different in B*35/B*53 (n=34) compared with B*57 (n=11) carriers; VL, 10 310 versus 222 copies/ml respectively, ($P < 0.0001$). We analyzed immune responses in 18 patients over a 4-year period who remained off ART or whose plasma VL did not decrease despite ART initiation (seven HLA-carriers and 11 B*35/53 carriers). The absolute CD4 cell counts remained stable over this period of time for both groups of patients; however, the VL significantly increased in the B*35/53 carriers (13793 to 23907; $P = 0.01$), whereas it remained unchanged (170–222 RNA copies/ml) in the B*57 patients. Only four of 18 patients (all B*53 carriers) initiated ART during the longitudinal follow-up, and the plasma VL did not change with ART in any of them. In the B*57 group, >40% of the T-cell response was directed against Gag at baseline and this specificity was maintained (Fig. 2a). The mean breadth of the peptide recognition increased from 2.6 (baseline) to 3.3 (year 4) when 12 B*57 restricted epitopes were tested in an IFN- γ ELISpot assay (data not shown). During follow-up, only the immunodominant B*57 restricted Gag-KF11 responses increased in magnitude (median 180 to 750 SFC/10⁶ PBMC; Fig. 2b). Median responses towards other HLA-B*57 restricted Gag and Nef epitopes declined or remained unchanged over time.

In the B*35/B*53 group, nearly 40% of the T-cell response targeted Nef and this responder frequency persisted (Fig. 2c). There was also an increase in Nef specific T-cell responses largely targeting the HLA B*35/B*53-restricted YY9 epitope (median 150–500 SFC/10⁶ PBMC; Fig. 2d). The median magnitude of other B*35/B*53 restricted Gag and Nef epitope responses remained unchanged. Of the 17 B*35/B*53 restricted peptides tested, the mean breadth of the response increased from 2.1 to 4.4 peptides over the 4-year time period (data not shown). Responses towards other HIV specific responses for both B*57 and B*35/B*53 responses were either negative or low in magnitude (data not shown).

The quality of the immunodominant CD8 T-cell responses were similar among B*57 and B*35/B*53 carriers

Using PBMC from four (B*57) and six (B*35/B*53) carriers and serial-fold peptide titrations in an IFN- γ ELISpot assay, we measured 'peptide avidity' or the lowest concentration of peptide needed to stimulate a CD8 T-cell response. The mean YY9 peptide avidity was about one log higher when compared to KF11 (data not shown). Next, we analyzed cytokine production (IFN- γ , IL-2 and TNF- α), degranulation (CD107a/b) and memory marker (CD127) in T-cells in response to the Gag pool and KF11 (n = 10) B*57 subjects; Nef pool and YY9 peptides (n = 8) B*35/B*53 subjects in an ICS assay. A representative example is shown in Fig. 3. There were no significant differences in median responses between the groups in terms of either cytokine(s) production, degranulation or memory phenotype from either the protein pools or the single peptide.

Boolean gating was performed to evaluate the percentage of CD8 T-cells responding with 1–4 functions (IFN- γ , IL-2, TNF- α and/or CD107). The responses to both the YY9 and the KF11 epitope were predominantly monofunctional (mean magnitude of 0.28 and 0.13 for KF11 and YY9, respectively). A much smaller percentage of responses to either epitope were bi-functional (mean magnitude of 0.04 and 0.02; KF11 and YY9, respectively), and 3% of the

KF11-specific CD8 T cells secreted three cytokines including IFN- γ , IL-2 and TNF- α (mean magnitude 0.01) (Fig. 4a and 4b). While the bi-functional cells produced CD107 and IFN- γ (0.02 for KF11 and YY9), monofunctional cells were largely CD107-positive followed by IFN- γ -positive and/or IL-2-positive (Fig. 4c and 4d). There was a trend for KF11 specific CD8 T cells to produce more IL-2 than YY9 specific CD8 T-cells ($P = 0.056$). The one-four function responses were also evaluated for the sub-dominant Gag (B*57) and sub-dominant Nef (B*35/B*53) responses. The poly-functionality of these sub-dominant responses was similar in quality to the immunodominant KF11(B*57) or the YY9 (B*35/B*53) specific responses (data not shown). Therefore, while some differences did exist between KF11 and YY9 CD8 T cells in the quality of their responses, overall these differences were extremely small.

CTL escape in p24 Gag and Nef is a common feature in B*57 and B*35/B*53 carriers, respectively

To determine if CTL escape was playing a role in the differences noted in viral control, we sequenced Gag and Nef from four B*57 and seven B*35/B*53 longitudinal pairs. In three of the B*57 subjects, CTL escape was observed in the Gag-IW9 [26] and Gag-TW10 [27] epitopes (Table 2). Two subjects had an escape mutation in the B*57 restricted Nef-HQ10 and Nef-KF9 epitopes [28–30] (Table 2). Among the B*35/B*53 carriers, very few mutations were observed in Gag; the E to D change in QW9 epitope has been described as a common variant and not a selection driven escape mutation [6]. In contrast, several mutations (Table 2) were observed in Nef, of which the Y81F has been shown to be an escape mutation [31]. Mutations in the YY9 epitope were observed in five subjects: Y135F in subjects 5 and 6, F139L in subject 7, and T138C in subjects 9 and 11. Peptides corresponding to the sequence changes in YY9 epitope at amino acids 138 and 139 were synthesized, tested in a peptide titration ELISpot assay and their poor recognition indicated CTL escape (data not shown).

Discussion

Our prior findings led us to the hypothesis that the viral control seen in HLA-B*57 carriers is associated with CD8 T-cell targeting of Gag [8]. A corollary to this hypothesis would predict that HLA class I alleles not associated with improved viral control would mediate CD8 T-cell responses skewed away from this protein. As anticipated, the current study demonstrated that HIV-specific CD8 T cells in B*57 carriers were focused on lower entropy epitopes in p24 while B*35/53 targeted higher entropy epitopes in Nef. These findings are consistent with the recent demonstration that viral control in HLA-B*5703 carriers was associated with targeting of Gag [15], whereas targeting of envelope and accessory proteins was associated with poor viral control. One possible explanation for the observed differences in protein targeting would be that the peptides with the capacity to bind different HLA class I molecules are unevenly distributed throughout the HIV-1 genome. If that were the case, B*57 would preferentially target p24 because this protein coincidentally contained more B*57 epitopes. However, the 'Motif Scan' program for predicting HLA class I bound peptides (Los Alamos National Laboratory), identified 49 HLA-B*5301 restricted Gag epitopes and only 15 B*57 restricted ones (data not shown).

Despite the fact that numerous studies have demonstrated the importance of Gag-specific CD8 T-cell responses, the mechanism underlying this viral control benefit remains elusive. We hypothesized that B*57 restricted responses might be qualitatively superior to those restricted by other alleles. In that case, B*57 restricted CD8 T cells would respond to lower concentrations of peptides; however, the opposite was observed as B*35/53-restricted YY9 CD8 T cells tended to respond to a lower peptide concentration. Another recently suggested possibility is that LTNP exert better control of HIV because their CD8 T cells were able to secrete multiple cytokines and degranulate [19]. We therefore determined if these polyfunctional CD8 T cells

were seen more commonly in our cohort of HLA-B*57 carriers. Although a small fraction (3%) of the B*57 restricted CD8 T cells were polyfunctional, it is important to note that this marginal increase in polyfunctional response may not be responsible for effective viral control. Rather, it is possible that effective HIV control results in a polyfunctional response.

We also reasoned that the targeting of low entropy epitopes as seen in B*57 carriers would make it more difficult for the virus to escape away from these responses. However, three of the four B*57 carriers had escape mutations present in p24 epitopes. While escape from these epitopes is clearly associated with decreased viral control [26], it is not an absolute association as several LTNP showed evidence of escape mutations in B*57-restricted epitopes [6]. We also noted numerous escape mutations in the Nef epitopes of B*35/53 carriers. In view of the fact that not all CD8 T-cell responses select for escape variants [32], our data indicate that even the B*35/53 responses are relatively superior in quality. This statement is supported by the fact that we were not able to demonstrate significant differences in the quality of B*57 and B*35/53 responses indicating that any variances may be quite subtle.

In total, we demonstrate that B*57 restricted responses induced viral escape mutations in Gag and Nef, while B*35/53 restricted responses were only able to induce escape mutations in Nef. These data are consistent with the hypothesis that mutations in Gag incur a high fitness cost to the virus [27,33–37] while those in Nef incur very little fitness cost [30,38]. Interestingly, we have also recently demonstrated that recipients of certain HLA class I associated p24 mutations are associated with a lower plasma viral load set point in newly infected individuals while Nef mutations were not associated with a decreased VL set point (P.A. Goepfert, *et al.*, unpublished data).

Taken together, these findings demonstrate an important mechanism whereby carriers of certain HLA class I alleles may experience significantly different disease outcomes. Since many of the current HIV vaccine strategies aim at protecting more against disease progression than against HIV infection [39–41], it would be important to include Gag in any of these vaccines. This recommendation is based not only on the fact that Gag mutations induce a viral fitness cost but also because the targeting of Gag may occur very early [42] even prior to the Nef-induced down-modulation of MHC class I expression [43,44]. Although our findings as well as those of other groups do not demonstrate viral control benefits with CD8 T-cell targeting of Nef in chronic infection [13,15], it should not be concluded that these types of responses would be deleterious in a preventive vaccine. Including immunogens like Nef that can be targeted relatively early by the immune system may well be beneficial [45]. A better understanding of immune correlates of protection is needed for the optimal design of a preventive HIV vaccine.

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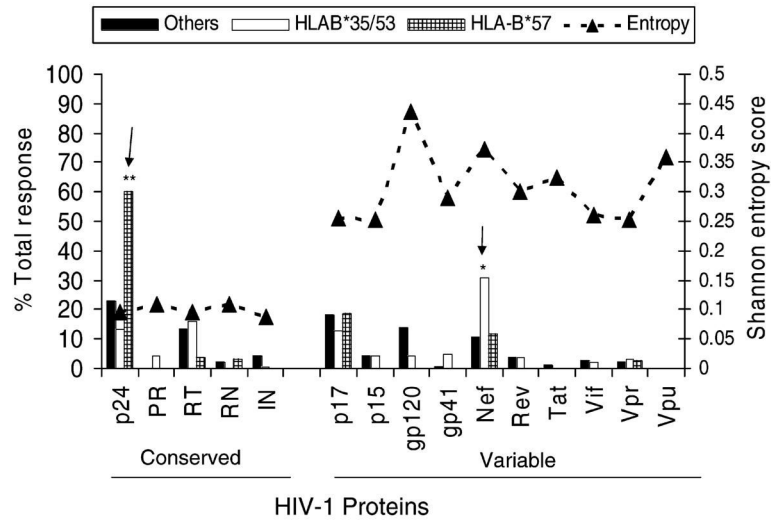


Fig. 1. Comparison of T-cell responses with amino acid variability

REACH subjects were sorted into three groups on the basis of HLA class I, i.e. HLA B*57, B*35 and B*53 and others alleles. The average percentage of the total response to each protein in the three patient groups is shown in relation to the average entropy for each protein. All the T-cell responses at the 15–20-mer peptide level were pooled to represent each protein: **B*57 response compared with B*35/53 ($P=0.005$) or others ($P=0.02$); *B*35/53 compared with B*57 ($P=0.03$) or others ($P=0.001$) using Mann-Whitney U test.

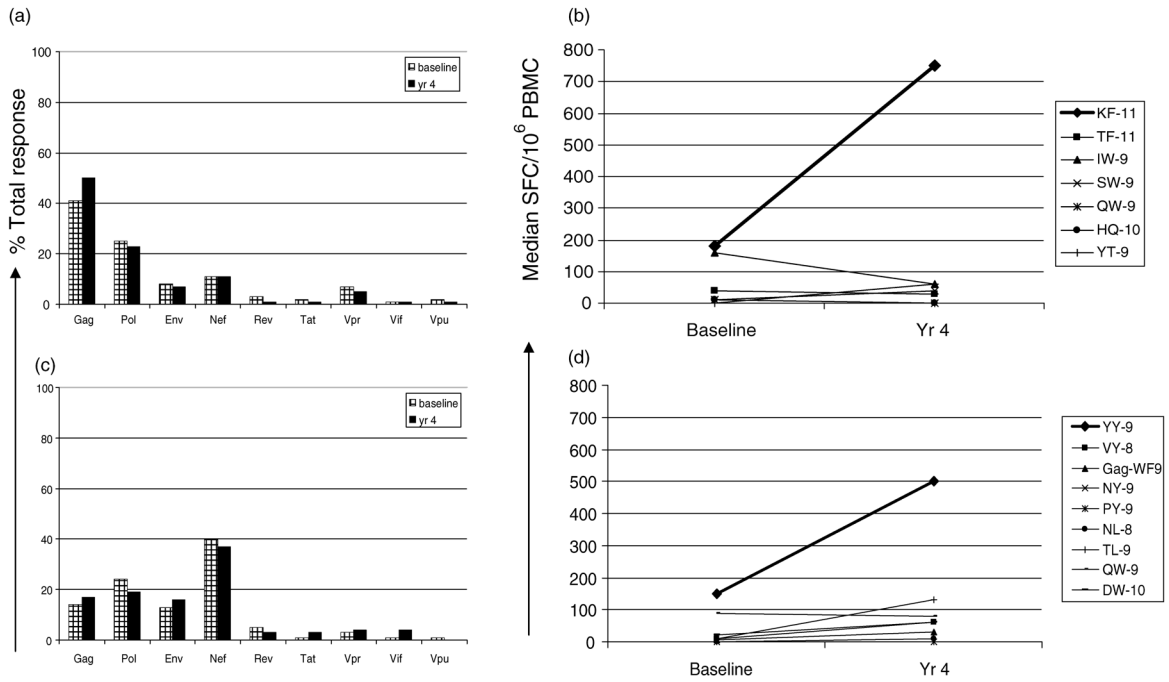


Fig. 2. Longitudinal analysis of T-cell responses in B*57 and B*35/53 subjects

IFN- γ ELISpot T-cell responses in HLA-B*57 (n = 7) and HLA-B*35/B*53 (n = 11) subjects over a 4-year period are shown. The average percentage of the total response is shown for each targeted HIV-1 protein in (a) B*57 and (c) B*35/B*53. The median magnitude of response (SFC/10⁶ PBMC) to all the optimized HLA-B*57 restricted HIV-1 Gag and Nef epitopes (9-11-mer) is shown in (b). The B*57 restricted peptides tested were: Gag 162–172: KAFSPEVIPMF (KF 11); Gag 240–250: TSTLQEQIGWF (TF 11); Gag 147–155: ISPRTLNAW (IW 9); Gag 241–249: STLQEQIGW (SW 9); Gag 308–316: QASQEVKNW (QW 9); Nef 116–125: HTQGYFPDWQ (HQ 10) and Nef 120–128: YFPDWQNYT (YT 9). (d) B*35/B*53 restricted peptides that were tested: Nef 135–143: YPLTFGWY (YY 9); Nef 74–81: VPLRPMTY (VY 8); Gag 36–44: WASRELERF (WF 9); Gag 124–142: NSSKVSQNY (NY 9); Gag 254–262: PPIPVGDIY (PY 9); Gag 327–334: NPCKTIL (NL 8); Gag 180–188: TPQDLNTML; Gag 308–316: QASQEVKNW (QW 9) and Gag 203–212: DTINEEAAEW (DW10).

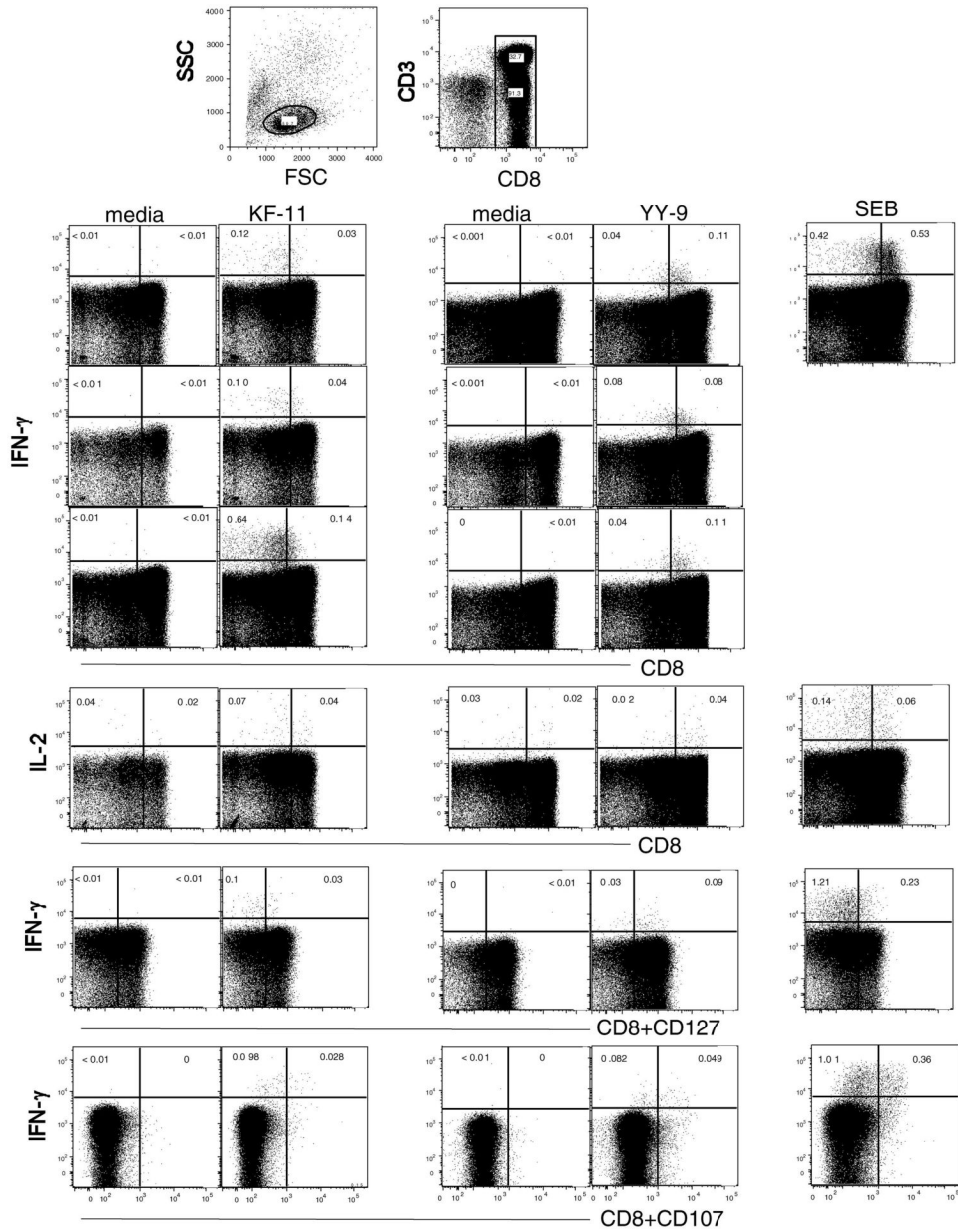


Fig. 3. Cytokine production, degranulation and memory phenotype of B*57–KF11 and B*35/B*53-YY9 restricted CD8 T-cell responses
 PMBC were stimulated with peptides encompassing the KF11 peptide (B*57 subjects) or YY9 peptide (B*35/B*53 subjects) or SEB in an ICS assay as described. The production of IFN-γ, IL-2 and TNF-α by CD8 T-cells and the upregulation of CD127 and CD107 by CD3CD8 are shown. The top panel shows representative CD8 IFN-γ data from three subjects in each group. The bottom three panels show a representative data for CD8-IL2, CD8CD127 IFN-γ and CD8CD107 IFN-γ. The gates were drawn based upon density plots (not shown) using FlowJo software where a clear distinction was observed between CD8 positive and negative populations.

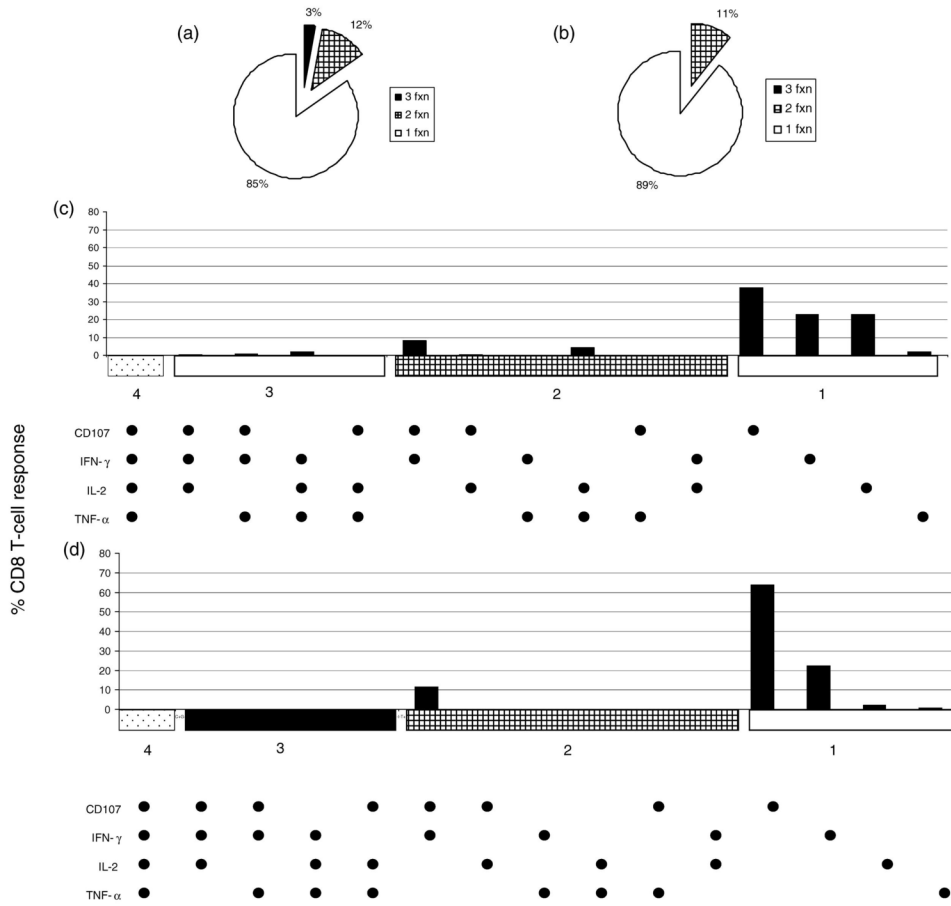


Fig. 4. Functional response patterns for KF11 and YY9 specific CD8 T-cells
 T-cell responses specific for B*57-KF11 (a) or B*35/B*53-YY9 (b) expressed as a mean of the CD8 T-cell response in polychromatic flow cytometry are shown in a pie chart based on their functional capacity (single, dual, triple, and quadruple function). Boolean gating was performed to determine the contributions of one to four functions measured from CD3CD8 cells that produced IFN- γ , IL-2, TNF- α and/or CD107. The responder frequency (% mean) of each of the 15 functional subpopulations is shown for B*57-KF11 (c) and B*35/B*53-YY9 (d).

Table 1

Clinical and laboratory characteristics of the study participants.

	<i>N</i>	Race n/N (%) ^a	CD4 ^b	VL ^c	ART (%)
HLA-B*57	7	6/7 (86)	634	2	3 (43)
HLA-B*35/53	16	13/16 (81)	629	3.3	10 (63)
Other HLA	29	18/29 (62)	558	3.6	23 (79)

^a African-American;

^b absolute CD4 cell count ($\times 10^6/l$);

^c log₁₀ viral load (RNA copies/ml).

(8)	T	L	Q	E	Q	I	G	W	M	Q	A	S	Q	E	V	K	N	W	
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	N	-	-	-	-	-	A	-	-	-	-	-	-	D	-	-	-	-	-
	N	-	-	-	-	-	A	-	-	-	-	-	-	D	-	-	-	-	-
	N	P	-	-	-	-	T	-	-	-	-	-	-	D	-	-	-	-	-
	N	-	-	-	-	-	T	-	-	-	-	-	-	D	-	-	-	-	-

(8)	P	D	W	Q	N	Y	T													
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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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(8)

(0)	I	P	V	G	E	I	Y	Q	A	S	Q	E	V	K	N	W	N	P	D	C	K	T	I	L
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(aa 135-143)

(aa 166-177)

(7)

(7)	G	P	G	I	R	Y	P	L	Y	P	L	T	F	G	W	C	F	H	P	M	S	L	H	G	M	D	D	P	E
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	T	-	F	-	-	F	-	-	-	-	-	-	-	-	-	-	V	C	Q	-	-	-	-	-	-	
	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	Q	-	-	-	-	-	-	
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