

HIV-1 Specific CD8+ T Cell Responses from HLA-B*57 Positive Adolescents Preferentially Target the Gag Protein

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ABSTRACT

Background: HIV-infected subjects expressing the class I allele, HLA-B*57, are among those who progress to AIDS more slowly when compared to other individuals. However, no data exist in infected subjects to suggest that favorable alleles are associated with different HIV-specific CD8+ T cell responses when compared to individuals with unfavorable alleles.

Methods: Chronically infected subjects were recruited from the REACH clinical sites and were comprised of a group of minority adolescents. We measured HIV-specific gamma interferon (IFN- γ) responses using the ELISPOT assay in PBMC taken from subjects with HLA-B*57 (7 subjects) and compared them to subjects with alleles associated with rapid progression of disease (HLA-B*35 and B*53; 16 total subjects). PBMC were stimulated overnight with overlapping HIV-1 peptides spanning the Gag, Pol, Env, and Nef proteins. Positive T cell responses were then mapped to the 20mer peptide. Statistical analysis was performed using Wilcoxon signed-rank and Fischer exact test.

Results: HLA-B*57 subjects had lower plasma viral loads (VL) and higher CD4+ T cell counts when compared with HLA-B*35 and B*53 subjects (median VL, 170 vs 4700 copies/ml; median CD4, 705 vs 613/ μ l, respectively). A higher proportion of HLA-B*57 subjects had Gag-specific responses when compared with the HLA-B*35 or B*53 individuals (86% vs 56%, $p = 0.09$). Response to Gag was immunodominant in 6/7 (86%) of the HLA-B*57 subjects while it was dominant in only 3/16 (19%) of subjects in the HLA-B*35 and B*53 groups ($p = 0.01$) and in only 16/49 (33%) of the subjects that did not express HLA-B*57 ($p = 0.01$). All but one of the HLA-B*57 subjects had immunodominant responses to Gag that mapped to either of two highly conserved HLA-B*57-restricted epitopes in p24 (ISPRTLNAW; aa147-155 or KAFSPEVPMF; aa 162-172).

Conclusions: Our data support the hypothesis that immunodominant responses to highly conserved epitopes protect against disease progression seen in HLA-B*57 positive individuals. While other factors are likely to also be important, these findings have important implications for HIV pathogenesis and could have an impact on the design of future HIV-specific treatment.

INTRODUCTION

Several studies have implicated HLA in playing an important role in HIV-1 pathogenesis. Among HLA class I genes, B*27 and B*57 have been consistently associated with a slower progression to AIDS compared to individuals with other haplotypes. In contrast, class I alleles such as B*08, B*35 and B*53 have been shown repeatedly to confer a rapid progression to AIDS. Several class I alleles are widely shared among ethnically distinct populations whereas some are limited in their distribution. For e.g. B*5701 is found in most human populations and its prevalence is about two-fold higher in Caucasians than African-Americans and Hispanics. In contrast, B*5702 and B*5703 are considered unique alleles to populations with African ancestry. B*5702 allele is rarely detected or is absent in Caucasians. A number of B*57 restricted CD8+ CTLs have been mapped to HIV-1 Gag, Nef and Reverse transcriptase. In a study of HIV-1 recombinant canary-pox vaccine recipients, HIV-1 specific CD8+ CTLs were found in significantly higher proportion in vaccinees carrying favorable alleles like B*27 and B*57 compared to others. In another Caucasian study of Long term non progressors (LTNP) and progressors, it was found that LTNP group was over represented by B*57 class I alleles. In both the LTNPs and progressors, the predominant CTL activity was directed towards Gag. In LTNPs, the T cell responses were highly focused to 4 Gag peptides: QMVFHQAI^{**SPRTLNAW**}VKVE (aa 141-160), EKAF^{**SPEVPMF**}SALSEGAT (aa 161-180), PRGSDIAGT^{**STLQEQIGWM**} (aa 231-250), YKTLRAE^{**QASQEVK**}NWMTET (aa 301-320). These peptides contain previously described, highly conserved HLA-B*57 epitopes shown in bold. The breadth of the Gag specific CD8+ responses in progressors however exceeded those seen in LTNPs as the T cell responses in this group were restricted by other MHC class I alleles besides B*57. In this study we sought to characterize the dominant HLA-B*57 restricted responses at the epitope level. Characterization of CTL responses directed by HLA-B*57 and other protective alleles may contribute to the development of an AIDS vaccine.

METHODS

A total of 37 HIV-1 chronically infected subjects were recruited from the REACH study of the Adolescent Medicine HIV/AIDS Research Network and from the AIDS clinic at University of Alabama at Birmingham (CMI cohort). The study participants in the REACH cohort were primarily females and African-American. Subjects from the AIDS clinic at UAB used in this study were mostly Caucasian males. Absolute CD4+ counts and plasma vRNA of enrolled subjects were determined using standard assays. HLA class I alleles were typed by PCR using sequence-specific primers. Fresh or thawed PBMC were stimulated overnight with HIV-1 clade B peptides in an IFN- γ ELISPOT assay. The peptides used were mainly 20 mers overlapping by 10 which encompassed HIV-1 Gag (HXB2); Pol (HXB2); Env (MN) and Nef (BRU) proteins. We measured and quantitated HIV-1 specific gamma interferon (IFN- γ) responses to HIV-1 peptides in PBMC taken from selected subjects in both cohorts.

CONCLUSIONS

- The current study demonstrates that HIV-1 infected patients in the REACH cohort who are HLA-B*57, preferentially target a highly conserved region located in p24 Gag.
- The dominant Gag specific T cell responses targeted by HLA-B*57 individuals were directed towards two p24 peptides: QMVFHQAI^{**SPRTLNAW**}VKVE (aa 141-160) and EKAF^{**SPEVPMF**}SALSEGAT (aa 161-180). These peptides contain highly conserved, B*57 restricted CTL epitopes (shown in bold).
- In the CMI cohort, the individuals who targeted EKAF^{**SPEVPMF**}SALSEGAT (aa 161-180) or the highly conserved flanking 20mer region (MFSALSEGAT^{**PDQLNTMLNT**}, aa 171-190) also had favorable markers for disease progression. The latter peptide was targeted by HLA-B*07, B*42, or B*8101.
- The precise mechanism by which HLA-B*57 mediates a protective effect in terms of HIV-1 pathogenesis remains unclear. However, our data suggests that this highly focused targeting of responses to conserved p24 regions may be playing a role in controlling progression to AIDS in HLA-B*57 individuals infected with HIV-1.

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HLA*B57 POSITIVE SUBJECTS IN REACH COHORT PREFERENTIALLY TARGET p24

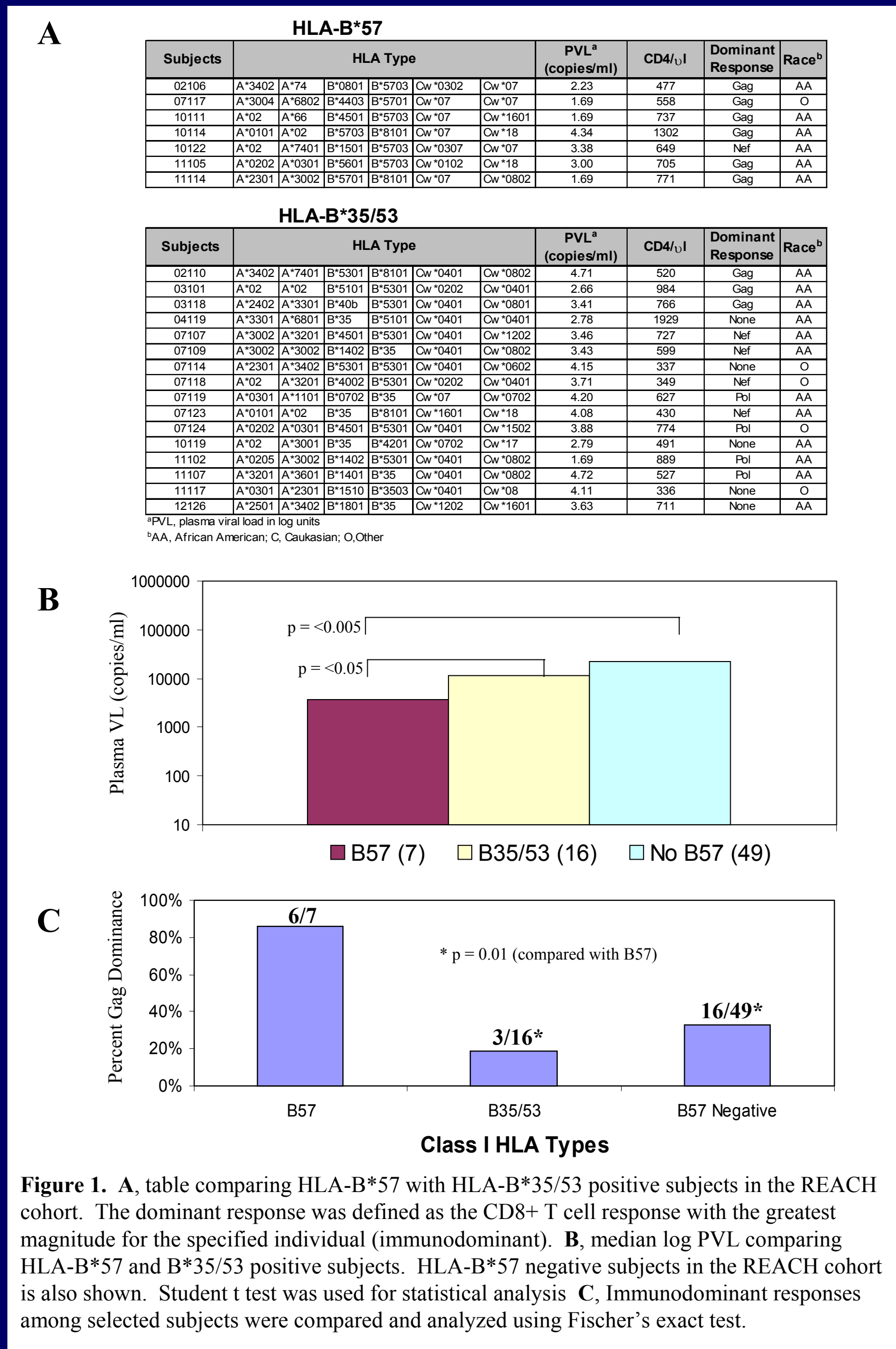


Figure 1. A, table comparing HLA-B*57 with HLA-B*35/53 positive subjects in the REACH cohort. The dominant response was defined as the CD8+ T cell response with the greatest magnitude for the specified individual (immunodominant). **B**, median log PVL comparing HLA-B*57 and B*35/53 positive subjects. HLA-B*57 negative subjects in the REACH cohort is also shown. Student t test was used for statistical analysis. **C**, Immunodominant responses among selected subjects were compared and analyzed using Fischer's exact test.

THE TARGETING OF KEY p24 EPITOPES IN THE CMI COHORT CORRELATES WITH FAVORABLE MARKERS OF HIV DISEASE PROGRESSION

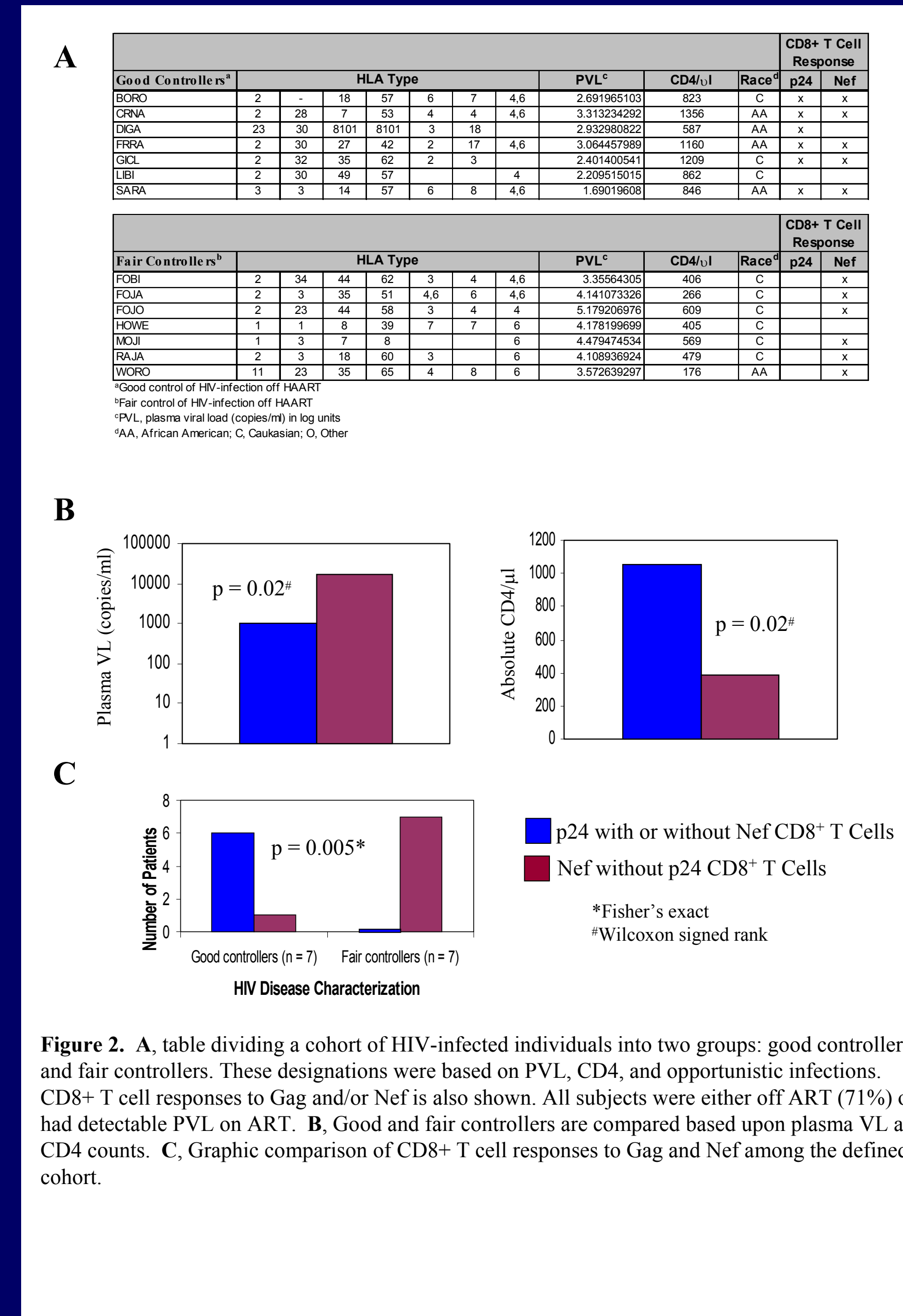


Figure 2. A, table dividing a cohort of HIV-infected individuals into two groups: good controllers and fair controllers. These designations were based on PVL, CD4, and opportunistic infections. CD8+ T cell responses to Gag and/or Nef is also shown. All subjects were either off ART (71%) or had detectable PVL on ART. **B**, Good and fair controllers are compared based upon plasma VL and CD4 counts. **C**, Graphic comparison of CD8+ T cell responses to Gag and Nef among the defined cohort.

HLA*B57 POSITIVE SUBJECTS PREFERENTIALLY TARGET A HIGHLY CONSERVED REGION OF p24

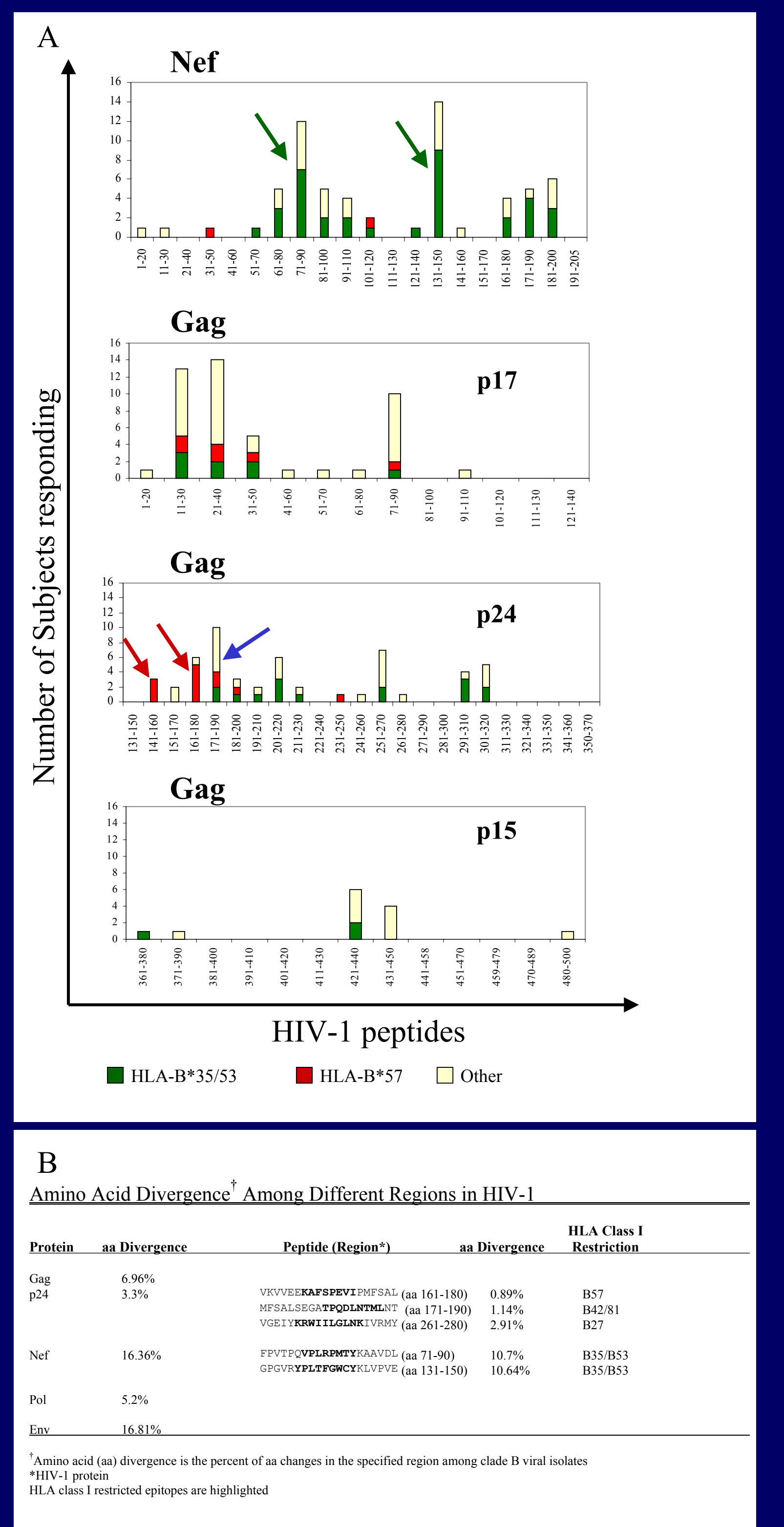


Figure 3. A, epitope mapping of the Gag and Nef proteins performed on PBMC obtained from the REACH cohort. Responses of the HLA-B*35/53 and B*57 positive individuals in the cohort are highlighted. Immunodominant peptides recognized by HLA-B*35/53 (green arrow), B*57 (red arrow), and HLA-B*42/81 (blue arrow) positive subjects is also shown. **B**, the amino acid diversity of the major HIV-1 proteins and selected epitopes is shown.