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# Control of Human Immunodeficiency Virus Type 1 Is Associated with HLA-B*13 and Targeting of Multiple Gag-Specific CD8$^+$ T-Cell Epitopes$^\nabla$

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**To better understand relationships between CD8$^+$ T-cell specificity and the immune control of human immunodeficiency virus type 1 (HIV-1), we analyzed the role of HLA-B*13, an allele associated with low viremia, in a cohort of 578 C clade-infected individuals in Durban, South Africa. Six novel B*13-restricted cytotoxic T lymphocyte epitopes were defined from analyses of 37 B*13-positive subjects, including three Gag epitopes. These B*13-restricted epitopes contribute to a broad Gag-specific CD8$^+$ response that is associated with the control of viremia. These data are consistent with data from studies of other HLA-class I alleles associated with HIV control that have shown that the targeting of multiple Gag epitopes is associated with relative suppression of viremia.**

Human immunodeficiency virus (HIV)-specific CD8$^+$ T-cell responses play a central role in the immune control of HIV (6, 23, 33, 34). However, high-frequency CD8$^+$ T-cell responses are also detectable in individuals progressing to AIDS (1, 5). Thus, there are qualitative differences in HIV-specific CD8$^+$ T cells that are likely to be critical in achieving immune control of HIV. A strong clue to the nature of these qualitative differences arises from immunogenetic studies which have shown associations between expression of particular HLA class I molecules, such as HLA-B*57, B*5801, and B*27, with successful control of HIV (20, 28, 31) and associations between expression of other class I alleles, such as HLA-B*5802, B*18, and B*3502/03, with unsuccessful control of HIV (7, 13, 20). These HLA associations imply that the specificity of the CD8$^+$ T-cell response is linked to the outcome of HIV infections, a hypothesis supported by studies of both HIV-infected persons (11, 15, 19, 25, 27) and the simian immunodeficiency virus macaque model (4, 12).

To further evaluate the relationship between the specificity of CD8$^+$ T-cell responses and immune control, we investigated HLA-B*13, a class I allele previously linked with successful immune control (16, 36) but for which comprehensive analyses of the CD8$^+$ T-cell response had not been undertaken. The initial study cohort comprised 1212 antiretroviral therapy-naïve adults from Durban, South Africa, with chronic HIV-1 C clade infections. The phenotypic frequency of HLA-B*13 was 3.9% in this cohort, and the viral load (taken from a single time point) of HLA-B*13-positive subjects ($n = 47$) was significantly lower than that of B*13-negative subjects (median, 10,800 HIV RNA copies/ml versus 40,900 copies/ml; $P = 0.009$ by the two-tailed Mann-Whitney test). Although >25% of the B*13-positive subjects had a viral load of <1,000 (28%; 9% for the B*13-negative subjects), the same proportion of B*13-positive subjects versus B*13-negative subjects also had a viral load of >150,000 (26% in each case; interquartile range, 789 to 151,000 for HLA-B*13-positive subjects and 7,547 to 154,000 for HLA-B*13-negative subjects) (data not shown). Thus, while a substantial proportion of the B*13-positive study subjects controlled viremia very effectively, an equally substantial proportion achieved no better control than the cohort as a whole. Therefore, the aim of this study was to determine whether differences in HLA-B*13-restricted CD8$^+$ T-cell responses could explain, at least in part, differential outcomes of disease progression in B*13-positive individuals.

Comprehensive analyses of CD8$^+$ T-cell responses were undertaken for 578 subjects, which included 27 C clade-infected B*13-positive individuals, by testing recognition of a panel of 410 overlapping 18-mer peptides spanning the consensus C clade proteome by gamma interferon (IFN-γ) enzyme-linked immunospot (ELISPOT) assays (20). Three novel HLA-B*13-restricted epitopes were identified within Gag (Fig. 1A to C). A previously described B clade epitope in Nef from C clade-

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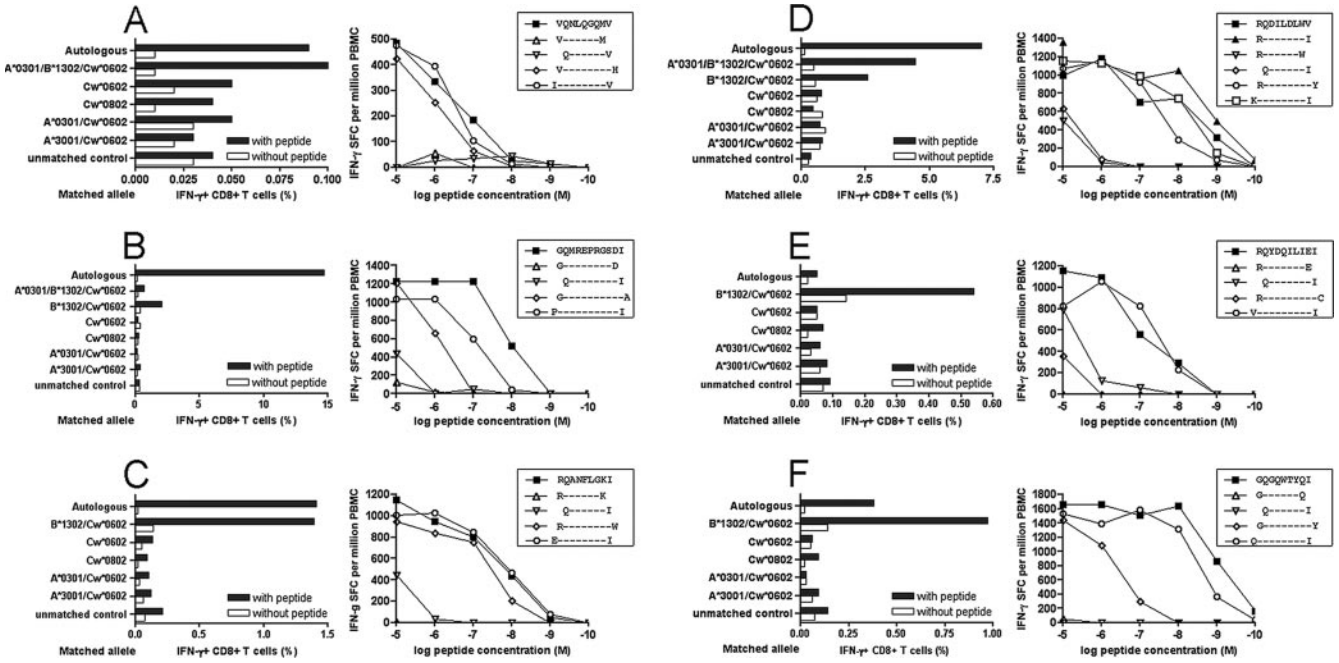



FIG. 1. Definition and optimization of six HLA-B*1302 epitopes. HLA restriction in each case was determined using intracellular IFN-γ-staining assays, as previously described (14). The HLA types on the *y* axis correspond to the Epstein-Barr virus transformed B-cell lines used as antigen-presenting cells. These match the HLA type of the effectors from subject 9308024 (HLA type, A*0301/3001, B*1302/1402, Cw*0602/0802). Epitope optimization was determined by IFN-γ enzyme-linked immunospot assays (14). (A to D) Epitopes from B and C clade-infected individuals. (E and F) Epitopes from B clade-infected individuals only. (A) Epitope VQNLQGQMV (Gag residues 135 to 143). (B) Epitope GQMREPRGSDI (Gag residues 226 to 236). (C) Epitope RQANFLGKI (Gag residues 429 to 437). (D) Epitope RQDILDLWV (Nef residues 106 to 114). (E) Epitope RQYDQILIEI (Pol residues 113 to 122). (F) Epitope GQGQWTYQI (Pol residues 488 to 496). SFC, spot-forming cells; PBMC, peripheral blood mononuclear cells.

infected subjects was confirmed (Fig. 1D) (16, 37), and an additional novel C clade HLA-B*13-restricted epitope was identified in Rev, within the overlapping peptide RPAEPV PLQLPPIERL (Rev residues 66 to 78). Targeting of this peptide was strongly associated with HLA-B*13 ($P = 2.17 \times 10^{-6}$ by Fisher's exact test); however, the optimal epitope was not confirmed in this case (Fig. 2). Additional analyses undertaken for 10 HIV-1 B-clade-infected subjects expressing B*13 revealed two additional novel B*13-restricted epitopes within protease and reverse transcriptase in epitopes that do not arise in the respective C clade proteins due to sequence variations (Fig. 1E to F).

Previous studies have suggested that Gag-specific $CD8^+$ T-cell responses may be critical in the immune control of HIV (10, 21, 22, 32, 35, 40). Therefore, we hypothesized that HLA-B*13 is associated with lower levels of viremia because of the number of HLA-B*13-restricted Gag epitopes targeted. We observed a trend that supported this hypothesis, with ≤1 B*13-restricted Gag response associated with a median viral load of 14,650 versus a median viral load of 1,525 for ≥2 responses ($P = 0.084$ by the two-tailed Mann-Whitney test) (data not shown). A consistent trend was also seen for the HLA-B*13-positive subjects for p17 Gag (the median viral load of subjects having ≤1 response was 12,700 versus 811 for those with ≥2

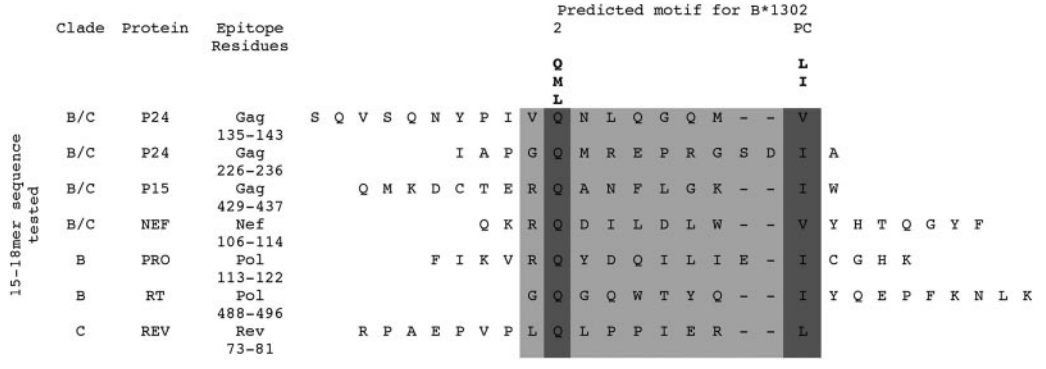
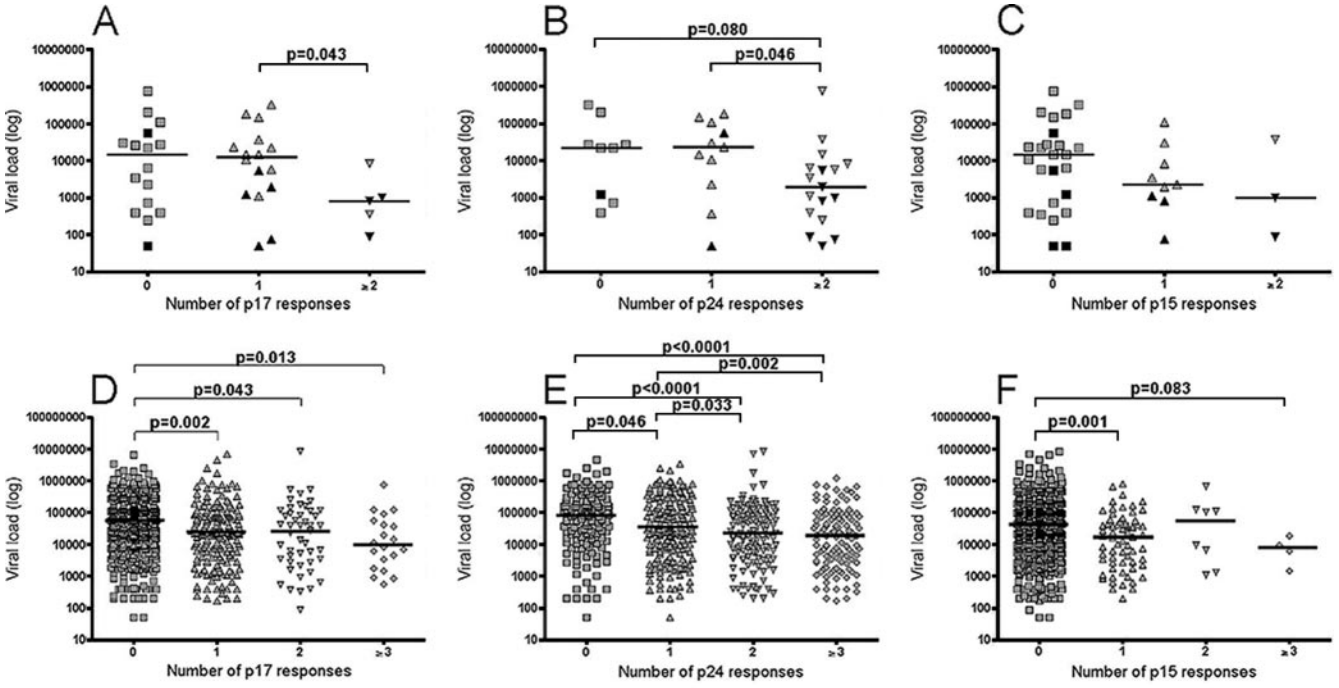



FIG. 2. Confirmation of the predicted motif for B*1302 with six defined epitopes and one predicted epitope. The B*1302 motif is inferred for position 2 and the carboxy terminus (PC) of the epitope.




G

| Protein in Gag | Median ≤1 response | Median ≥2 responses | p value |
|---|---|---|---|
| p17 | 12700 | 811 | 0.053 |
| p24 | 23050 | 1950 | 0.021 |
| p15 | 7420 | 1010 | ns |

FIG. 3. Comparison of numbers of p17, p24, and p15 Gag responses to viral load. (A to C) Results for B*13-positive individuals (black and gray symbols represent clade B- and C-infected individuals, respectively). (D to F) Results for whole cohort of 578 individuals. (G) Association of ≤1 and ≥2 p17, p24, and p15 Gag responses to viral load for HLA-B*13-infected individuals.

responses; $P = 0.053$), p24 Gag (median viral load, 23,050 versus 1,950; $P = 0.021$), and p15 Gag (median viral load, 7,420 versus 1,010; $P > 0.05$) (Fig. 3).

Overall, $CD8^+$ T-cell responses in the cohort revealed a strong association between the increasing Gag-specific breadth of the response and the decreasing viral load (21). Here, using multiple regression analyses, we analyzed the contributions of the responses toward the individual Gag proteins, p17 Gag, p24 Gag, and p15 Gag, in the cohort overall ($n = 578$) (data not shown). These data show that the breadth of p24 Gag-specific responses is associated significantly with decreasing viremia, each additional p24 Gag-specific response targeted being associated with a 0.114 $\log_{10}$ reduction in viral load after adjustments for the effects of both p15 and p17 Gag responses ($P < 0.001$) (data not shown). The breadth of p17 Gag- and p15 Gag-specific responses, however, was not significantly associated with decreasing viral load in this study (data not shown).

Previous studies of HLA-B*57, B*5801, and B*2705 HLA class I alleles associated with successful control of HIV have identified epitope-specific mutations within p24 Gag that result in a fitness cost to the virus (15, 19, 24, 27, 30). To investigate whether characteristic Gag sequence variants are also associated with the HLA-B*13 Gag-specific epitopes defined, autologous virus sequences from DNA and RNA were determined as previously described (25) (Fig. 4A). p24 Gag sequences from 562 C clade-infected individuals in Durban, including 38 B*13-positive subjects, were analyzed, and significant variations from the consensus in association with the B*13 p24 Gag epitope VQNLQGQMV (Gag residues 135 to 143) at Gag residue 147 ($P = 1.23 \times 10^{-9}$ by Fisher's exact test) (Fig. 4A) were identified. Variations at Gag residues 146 and 147 have previously been identified in association with expression of B*57/5801 (25), the A146P mutation at 146 being an endoplasmic reticulum amino peptidase I-processing mutation for the B*57/5801-restricted ISPRTLNAW epitope (Gag residues 147 to 155) (9). Exclusion of viral sequences from B*57/5801-positive individuals strengthened the association between HLA-B*13 and the viral sequence variation at Gag residue 147 and indicated a B*13 association at Gag residue 146 ($P = 0.032$) (Fig. 4A). A third epitope lies within the same region of p24 Gag, the HLA-B*1510-restricted epitope VHQAISPRTL (Gag residues 143 to 152), and there is also a B*1510-associated sequence variation at Gag residue 146 ($P = 0.008$; with all sequences, excluding those from B*57/5801-positive subjects, $P = 6.7 \times 10^{-5}$). Thus, selection pressure associated with the B*13, B*57/5801, and B*1510 p24 Gag epitopes driving sequence change occurs at residues downstream of the epitope,

**A**

| C-Clade consensus | | Mutation | Number of Sequences | | | | Fishers Exact test All sequences p value | Fishers Exact test Excluding B*57/5801 p value |
|---|---|---|---|---|---|---|---|---|
| | | | B*13 +ve WT | B*13 +ve Mutation | B*13 –ve WT | B*13 –ve Mutation | | |
| 130 | Q | X | 38 | 0 | 517 | 7 | NS | NS |
| 131 | N | X | 38 | 0 | 524 | 0 | NS | NS |
| 132 | Y | X | 38 | 0 | 528 | 6 | NS | NS |
| 133 | P | X | 38 | 0 | 523 | 1 | NS | NS |
| 134 | I | X | 37 | 1 | 518 | 6 | NS | NS |
| 135 | V | X | 38 | 0 | 522 | 2 | NS | NS |
| 136 | Q | X | 38 | 0 | 522 | 2 | NS | NS |
| 137 | N | X | 38 | 0 | 522 | 2 | NS | NS |
| 138 | L | X | 35 | 3 | 460 | 64 | NS | NS |
| 139 | Q | X | 38 | 0 | 524 | 0 | NS | NS |
| 140 | G | X | 38 | 0 | 523 | 1 | NS | NS |
| 141 | Q | X | 37 | 1 | 522 | 2 | NS | NS |
| 142 | M | X | 38 | 0 | 515 | 9 | NS | NS |
| 143 | V | X | 36 | 2 | 518 | 6 | NS | NS |
| 144 | H | X | 38 | 0 | 522 | 2 | NS | NS |
| 145 | Q | X | 38 | 0 | 520 | 4 | NS | NS |
| 146 | A | X | 24 | 14 | 392 | 132 | NS | 0.032 |
| 147 | I | LM | 8 | 30 | 372 | 152 | $1.23 \times 10^{-9}$ | $2.50 \times 10^{-10}$ |
| 148 | S | X | 38 | 0 | 519 | 5 | NS | NS |

**B**

| C-Clade consensus | | Mutation | Number of sequences | | | | Fishers Exact test All sequences p value |
|---|---|---|---|---|---|---|---|
| | | | B*13 +ve WT | B*13+ve Mutation | B*13 –ve WT | B*13 –ve Mutation | |
| 424 | K | X | 45 | 0 | 300 | 2 | NS |
| 425 | D | X | 42 | 3 | 290 | 12 | NS |
| 426 | C | X | 45 | 0 | 302 | 0 | NS |
| 427 | T | X | 42 | 3 | 284 | 18 | NS |
| 428 | E | X | 42 | 3 | 293 | 9 | NS |
| 429 | R | X | 44 | 1 | 302 | 0 | NS |
| 430 | Q | X | 45 | 0 | 302 | 0 | NS |
| 431 | A | X | 45 | 0 | 301 | 1 | NS |
| 432 | N | X | 45 | 0 | 302 | 0 | NS |
| 433 | F | X | 45 | 0 | 302 | 0 | NS |
| 434 | L | X | 45 | 0 | 301 | 1 | NS |
| 435 | G | X | 45 | 0 | 300 | 2 | NS |
| 436 | K | R | 35 | 10 | 282 | 20 | 0.002 |
| 437 | I | VLM | 32 | 13 | 292 | 10 | $2.41 \times 10^{-7}$ |
| 438 | W | X | 45 | 0 | 302 | 0 | NS |
| 439 | P | X | 44 | 1 | 301 | 1 | NS |
| 440 | S | X | 45 | 0 | 302 | 0 | NS |
| 441 | H | X | 35 | 10 | 219 | 83 | NS |
| 442 | K | X | 45 | 0 | 296 | 6 | NS |

**C**

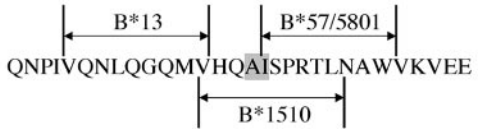



FIG. 4. Mutations in Gag associated with HLA-B*13. (A) Comparisons of numbers of variations from the consensus for p24 VQN LQGQMV (Gag residues 135 to 143; numbering corresponds to that for the HXB2 reference strain) with those for five residues beyond the N and C termini of the epitope. A total of 562 C clade RNA and DNA sequences were compared. Comparisons were also made for subjects not carrying B*57/5801. (B) Comparisons of numbers of variations from the consensus for p15 RQANFLGKI (Gag residues 429 to 437; numbering corresponds to that for the HXB2 reference strain) with those for five residues beyond the N and C termini of the epitope. A total of 347 C clade RNA and DNA sequences were compared. (C) Three epitopes restricted by B*13, B*1510, and B*57/5801 and their relations to the mutation at position 146, which is significantly associated with B*13, B*1510, and B*57/5801, and to the mutation at position 147, which is significantly associated with B*13 and B*57/5801 (shaded residues). +ve, positive; −ve, negative; WT, wild type.

upstream of the epitope, and within the epitope, respectively (Fig. 4C), but in each case involves the same Gag residues, 146 and 147. HLA-B*57, B*5801, and B*1510 were the only alleles in addition to B*13 that showed evidence of selection pressure on the virus in this region of p24 Gag (data not shown). This convergence of sequence variation supports the hypothesis that epitope mutation within p24 Gag is severely limited by viral fitness constraints and that the identities of the escape mutations may be stereotypic not only for a given allele (2) but also for several alleles targeting epitopes in the same region (27). These data, supported by earlier published work in relation to the B*57/5801-associated A146P mutation (9), suggest that variations at Gag residues 146 and 147 may arise at relatively little cost to viral fitness, whereas variations arising at residues in the immediate vicinity of Gag residues 146 and 147 may significantly reduce viral fitness.

Analysis of 347 p15 Gag sequences in the region of the HLA-B*13-restricted Gag epitope RQANFLGKI (residues 429 to 437) also revealed B*13-associated mutations K436R and I437VLM ($P = 0.002$ and $2.41 \times 10^{-7}$, respectively) (Fig. 4B). The mutations are situated at the C terminus of the HLA-B*13 epitope. The epitope exactly maps the cleavage recognition site for protease, between p7 (nucleocapsid protein) and p1. Notably, of the seven B*13 individuals targeting this epitope for whom sequence and response data were available, none had the I437VLM mutation. The consequences of the mutations driven by the HLA-B*13-restricted p15 Gag-specific response are unknown, but these data support the hypothesis that viral fitness may be affected as a result of interference with protease cleavage at this site.

These studies of the HIV-specific $CD8^+$ T-cell responses restricted by HLA-B*13-positive individuals demonstrated the existence of three novel Gag-specific responses, the targeting of which is associated with lower viral loads. These data are thus consistent with data from studies of other HLA class I alleles associated with the control of HIV infection that have shown that targeting of multiple Gag epitopes is associated with relative suppression of viremia (10, 21, 22, 32, 35, 40). Only the breadth of the $CD8^+$ T-cell responses targeted to p24 Gag was associated significantly with decreased viral load.

The mutations within Gag associated with B*13-restricted alleles and overlapping Gag epitopes restricted by other class I alleles identified here support accumulating data from other studies that viral escape from p24 Gag-specific $CD8^+$ T-cell responses is severely limited by viral fitness constraints (15, 19, 24, 27). Lack of longitudinal data available from the study subjects precluded direct assessment of whether reduced control of viremia is precipitated by HLA-B*13-associated escape mutations, and the impact of the particular mutations identified here on viral fitness and $CD8^+$ T-cell recognition were not evaluated in this study. However, it is noteworthy that a potential B*13-associated processing mutation could arise four amino acids downstream (at Gag residue 147) of the C terminus of the epitope, in this case VQNLQGQMV (Gag residues 135 to 143). Processing escape mutations downstream of HIV-specific epitopes at two, five, and eight residues downstream of the C terminus have been described (3, 29, 39), and viral nucleoprotein mutations introduced experimentally in the mouse influenza model have shown that changes four amino acids downstream of the C terminus of the epitope can abrogate (or restore) presentation of that epitope (38). Thus, there is a precedent for processing escape mutations to be selected four amino acids or more downstream of the epitope C terminus.

The seven HLA-B*13-restricted HIV-specific epitopes shown

in Fig. 2 suggest a peptide-binding motif for HLA-B*13 that has not previously been defined. Characteristically, glutamine is at position 2, and a medium-to-small hydrophobic amino acid (leucine/isoleucine/valine) is at the C terminus. The residues forming the B pocket of B*13 are identical with those of HLA-B*1501 (HLA-B62), for which the peptide binding motif has been determined, and in this case the motif has Gln, Met, or Leu at P2 (8, 17, 18). In addition, it is noteworthy that three of the seven HLA-B*13 epitopes defined have Arg at position 1. Comparison of the HLA-B*13 A pocket with that of B*2705, which also has a preference for Lys or Arg at position 1, shows that they are identical in the constituent residues important for binding (26).

In conclusion, these data show that carrying the HLA-B*13 allele is associated with an advantage for HIV-1-infected individuals but that this association with low viremia depends to a great extent on the targeting of Gag epitopes, a proportion of which may be B*13 restricted. The reasons for the importance of Gag as a target for successful control of HIV remain unknown. The hypothesis that Gag escape variants often incur a significant cost to viral fitness needs to be further evaluated.

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The authors declare that they have no competing financial interests.

## REFERENCES

1. **Addo, M. M., X. G. Yu, A. Rathod, D. Cohen, R. L. Eldridge, D. Strick, M. N. Johnston, C. Corcoran, A. G. Wurcel, C. A. Fitzpatrick, M. E. Feeney, W. R. Rodriguez, N. Basgoz, R. Draenert, D. R. Stone, C. Brander, P. J. Goulder, E. S. Rosenberg, M. Altfeld, and B. D. Walker.** 2003. Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. J. Virol. **77:**2081–2092.
2. **Allen, T. M., M. Altfeld, S. C. Geer, E. T. Kalife, C. Moore, K. M. O'Sullivan, I. DeSouza, M. E. Feeney, R. L. Eldridge, E. L. Maier, D. E. Kaufmann, M. P. Lahaie, L. Reyor, G. Tanzi, M. N. Johnston, C. Brander, R. Draenert, J. K. Rockstroh, H. Jessen, E. S. Rosenberg, S. A. Mallal, and B. D. Walker.** 2005. Selective escape from $CD8^+$ T-cell responses represents a major driving force of human immunodeficiency virus type 1 (HIV-1) sequence diversity and reveals constraints on HIV-1 evolution. J. Virol. **79:**13239–13249.
3. **Allen, T. M., M. Altfeld, X. G. Yu, K. M. O'Sullivan, M. Lichterfeld, S. Le Gall, M. John, B. R. Mothe, P. K. Lee, E. T. Kalife, D. E. Cohen, K. A. Freedberg, D. A. Strick, M. N. Johnston, A. Sette, E. S. Rosenberg, S. A. Mallal, P. J. R. Goulder, C. Brander, and B. D. Walker.** 2004. Selection, transmission, and reversion of an antigen-processing cytotoxic T-lymphocyte escape mutation in human immunodeficiency virus type 1 infection. J. Virol. **78:**7069–7078.
4. **Barouch, D. H., J. Kunstman, M. J. Kuroda, J. E. Schmitz, S. Santra, F. W. Peyerl, G. R. Krivulka, K. Beaudry, M. A. Lifton, D. A. Gorgone, D. C. Montefiori, M. G. Lewis, S. M. Wolinsky, and N. L. Letvin.** 2002. Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature **415:**335–339.
5. **Betts, M. R., D. R. Ambrozak, D. C. Douek, S. Bonhoeffer, J. M. Brenchley, J. P. Casazza, R. A. Koup, and L. J. Picker.** 2001. Analysis of total human immunodeficiency virus (HIV)-specific $CD4^+$ and $CD8^+$ T-cell responses: relationship to viral load in untreated HIV infection. J. Virol. **75:**11983–11991.
6. **Borrow, P., H. Lewicki, X. Wei, M. S. Horwitz, N. Peffer, H. Meyers, J. A. Nelson, J. E. Gairin, B. H. Hahn, M. B. Oldstone, and G. M. Shaw.** 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat. Med. **3:**205–211.
7. **Carrington, M., G. W. Nelson, M. P. Martin, T. Kissner, D. Vlahov, J. J. Goedert, R. Kaslow, S. Buchbinder, K. Hoots, and S. J. O'Brien.** 1999. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science **283:**1748–1752.
8. **Choo, S. Y., L. A. Fan, and J. A. Hansen.** 1993. Allelic variations clustered in the antigen binding sites of HLA-Bw62 molecules. Immunogenetics **37:**108–113.
9. **Draenert, R., S. Le Gall, K. J. Pfafferott, A. J. Leslie, P. Chetty, C. Brander, E. C. Holmes, S. C. Chang, M. E. Feeney, M. M. Addo, L. Ruiz, D. Ramduth, P. Jeena, M. Altfeld, S. Thomas, Y. Tang, C. L. Verrill, C. Dixon, J. G. Prado, P. Kiepiela, J. Martinez-Picado, B. D. Walker, and P. J. Goulder.** 2004. Immune selection for altered antigen processing leads to cytotoxic T lymphocyte escape in chronic HIV-1 infection. J. Exp. Med. **199:**905–915.
10. **Edwards, B. H., A. Bansal, S. Sabbaj, J. Bakari, M. J. Mulligan, and P. A. Goepfert.** 2002. Magnitude of functional $CD8^+$ T-cell responses to the Gag protein of human immunodeficiency virus type 1 correlates inversely with viral load in plasma. J. Virol. **76:**2298–2305.
11. **Frahm, N., S. Adams, P. Kiepiela, C. H. Linde, H. S. Hewitt, M. Lichterfeld, K. Sango, N. V. Brown, E. Pae, A. G. Wurcel, M. Altfeld, M. E. Feeney, T. M. Allen, T. Roach, M. A. St. John, E. S. Daar, E. Rosenberg, B. Korber, F. Marincola, B. D. Walker, P. J. R. Goulder, and C. Brander.** 2005. HLA-B63 presents HLA-B57/B58-restricted cytotoxic T-lymphocyte epitopes and is associated with low human immunodeficiency virus load. J. Virol. **79:**10218–10225.
12. **Friedrich, T. C., E. J. Dodds, L. J. Yant, L. Vojnov, R. Rudersdorf, C. Cullen, D. T. Evans, R. C. Desrosiers, B. R. Mothe, J. Sidney, A. Sette, K. Kunstman, S. Wolinsky, M. Piatak, J. Lifson, A. L. Hughes, N. Wilson, D. H. O'Connor, and D. I. Watkins.** 2004. Reversion of CTL escape-variant immunodeficiency viruses in vivo. Nat. Med. **10:**275–281.
13. **Gao, X., G. W. Nelson, P. Karacki, M. P. Martin, J. Phair, R. Kaslow, J. J. Goedert, S. Buchbinder, K. Hoots, D. Vlahov, S. J. O'Brien, and M. Carrington.** 2001. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. N. Engl. J. Med. **344:**1668–1675.
14. **Goulder, P. J., C. Brander, K. Annamalai, N. Mngqundaniso, U. Govender, Y. Tang, S. He, K. E. Hartman, C. A. O'Callaghan, G. S. Ogg, M. A. Altfeld, E. S. Rosenberg, H. Cao, S. A. Kalams, M. Hammond, M. Bunce, S. I. Pelton, S. A. Burchett, K. McIntosh, H. M. Coovadia, and B. D. Walker.** 2000. Differential narrow focusing of immunodominant human immunodeficiency virus Gag-specific cytotoxic T-lymphocyte responses in infected African and Caucasoid adults and children. J. Virol. **74:**5679–5690.
15. **Goulder, P. J., R. E. Phillips, R. A. Colbert, S. McAdam, G. Ogg, M. A. Nowak, P. Giangrande, G. Luzzi, B. Morgan, A. Edwards, A. J. McMichael, and S. Rowland-Jones.** 1997. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. Nat. Med. **3:**212–217.
16. **Harrer, E. G., S. Bergmann, K. Eismann, M. Rittmaier, A. Goldwich, S. M. Muller, B. M. Spriewald, and T. Harrer.** 2005. A conserved HLA B13-restricted cytotoxic T lymphocyte epitope in Nef is a dominant epitope in HLA B13-positive HIV-1-infected patients. AIDS **19:**734–735.
17. **Hildebrand, W. H., J. D. Domena, S. Y. Shen, M. Lau, P. I. Terasaki, M. Bunce, S. G. Marsh, M. G. Guttridge, W. B. Bias, and P. Parham.** 1994. HLA-B15: a widespread and diverse family of HLA-B alleles. Tissue Antigens **43:**209–218.
18. **Honeyborne, I., A. Rathod, R. Buchli, D. Ramduth, E. Moodley, P. Rathnavalu, S. Chetty, C. Day, C. Brander, W. Hildebrand, B. D. Walker, P. Kiepiela, and P. J. Goulder.** 2006. Motif inference reveals optimal CTL epitopes presented by HLA class I alleles highly prevalent in southern Africa. J. Immunol. **176:**4699–4705.
19. **Kelleher, A. D., C. Long, E. C. Holmes, R. L. Allen, J. Wilson, C. Conlon, C. Workman, S. Shaunak, K. Olson, P. Goulder, C. Brander, G. Ogg, J. S. Sullivan, W. Dyer, I. Jones, A. J. McMichael, S. Rowland-Jones, and R. E. Phillips.** 2001. Clustered mutations in HIV-1 Gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses. J. Exp. Med. **193:**375–386.
20. **Kiepiela, P., A. J. Leslie, I. Honeyborne, D. Ramduth, C. Thobakgale, S. Chetty, P. Rathnavalu, C. Moore, K. J. Pfafferott, L. Hilton, P. Zimbwa, S. Moore, T. Allen, C. Brander, M. M. Addo, M. Altfeld, I. James, S. Mallal, M. Bunce, L. D. Barber, J. Szinger, C. Day, P. Klenerman, J. Mullins, B. Korber, H. M. Coovadia, B. D. Walker, and P. J. Goulder.** 2004. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature **432:**769–775.
21. **Kiepiela, P., K. Ngumbela, C. Thobakgale, D. Ramduth, I. Honeyborne, E. Moodley, S. Reddy, C. de Pierres, Z. Mncube, N. Mkhwanazi, K. Bishop, M. van der Stok, K. Nair, N. Khan, H. Crawford, R. Payne, A. Leslie, J. Prado, A. Prendergast, J. Frater, N. McCarthy, C. Brander, G. H. Learn, D. Nickle, C. Rousseau, H. Coovadia, J. I. Mullins, D. Heckerman, B. D. Walker, and P. Goulder.** 2007. $CD8^+$ T-cell responses to different HIV proteins have discordant associations with viral load. Nat. Med. **13:**46–53.
22. **Klein, M. R., C. A. van Baalen, A. M. Holwerda, S. R. Kerkhof Garde, R. J. Bende, I. P. Keet, J. K. Eeftinck-Schattenkerk, A. D. Osterhaus, H. Schuitemaker, and F. Miedema.** 1995. Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. J. Exp. Med. **181:**1365–1372.
23. **Koup, R. A., J. T. Safrit, Y. Cao, C. A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D. D. Ho.** 1994. Temporal association of cellular immune

responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J. Virol. **68:**4650–4655.

24. **Leslie, A., D. Kavanagh, I. Honeyborne, K. Pfafferott, C. Edwards, T. Pillay, L. Hilton, C. Thobakgale, D. Ramduth, R. Draenert, S. Le Gall, G. Luzzi, A. Edwards, C. Brander, A. K. Sewell, S. Moore, J. Mullins, C. Moore, S. Mallal, N. Bhardwaj, K. Yusim, R. Phillips, P. Klenerman, B. Korber, P. Kiepiela, B. Walker, and P. Goulder.** 2005. Transmission and accumulation of CTL escape variants drive negative associations between HIV polymorphisms and HLA. J. Exp. Med. **201:**891–902.
25. **Leslie, A. J., K. J. Pfafferott, P. Chetty, R. Draenert, M. M. Addo, M. Feeney, Y. Tang, E. C. Holmes, T. Allen, J. G. Prado, M. Altfeld, C. Brander, C. Dixon, D. Ramduth, P. Jeena, S. A. Thomas, A. St. John, T. A. Roach, B. Kupfer, G. Luzzi, A. Edwards, G. Taylor, H. Lyall, G. Tudor-Williams, V. Novelli, J. Martinez-Picado, P. Kiepiela, B. D. Walker, and P. J. Goulder.** 2004. HIV evolution: CTL escape mutation and reversion after transmission. Nat. Med. **10:**282–289.
26. **Marsh, S. G. E., P. Parham, and L. D. Barber.** 2000. The HLA facts book. Academic Press, San Diego, CA.
27. **Martinez-Picado, J., J. G. Prado, E. E. Fry, K. Pfafferott, A. Leslie, S. Chetty, C. Thobakgale, I. Honeyborne, H. Crawford, P. Matthews, T. Pillay, C. Rousseau, J. I. Mullins, C. Brander, B. D. Walker, D. I. Stuart, P. Kiepiela, and P. Goulder.** 2006. Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. J. Virol. **80:**3617–3623.
28. **Migueles, S. A., M. S. Sabbaghian, W. L. Shupert, M. P. Bettinotti, F. M. Marincola, L. Martino, C. W. Hallahan, S. M. Selig, D. Schwartz, J. Sullivan, and M. Connors.** 2000. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc. Natl. Acad. Sci. USA **97:**2709–2714.
29. **Milicic, A., D. A. Price, P. Zimbwa, B. L. Booth, H. L. Brown, P. J. Easterbrook, K. Olsen, N. Robinson, U. Gileadi, A. K. Sewell, V. Cerundolo, and R. E. Phillips.** 2005. CD8+ T cell epitope-flanking mutations disrupt proteasomal processing of HIV-1 Nef. J. Immunol. **175:**4618–4626.
30. **Nietfield, W., M. Bauer, M. Fevrier, R. Maier, B. Holzwarth, R. Frank, B. Maier, Y. Riviere, and A. Meyerhans.** 1995. Sequence constraints and recognition by CTL of an HLA-B27-restricted HIV-1 gag epitope. J. Immunol. **154:**2189–2197.
31. **O'Brien, S. J., X. Gao, and M. Carrington.** 2001. HLA and AIDS: a cautionary tale. Trends Mol. Med. **7:**379–381.
32. **Ogg, G. S., X. Jin, S. Bonhoeffer, P. R. Dunbar, M. A. Nowak, S. Monard, J. P. Segal, Y. Cao, S. L. Rowland-Jones, V. Cerundolo, A. Hurley, M. Markowitz, D. D. Ho, D. F. Nixon, and A. J. McMichael.** 1998. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science **279:**2103–2106.
33. **Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. M. Bangham, C. R. Rizza, and A. J. McMichael.** 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature **354:**453–459.
34. **Price, D. A., P. J. Goulder, P. Klenerman, A. K. Sewell, P. J. Easterbrook, M. Troop, C. R. Bangham, and R. E. Phillips.** 1997. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. Proc. Natl. Acad. Sci. USA **94:**1890–1895.
35. **Rivière, Y., M. B. McChesney, F. Porrot, F. Tanneau-Salvadori, P. Sansonetti, O. Lopez, G. Pialoux, V. Feuillie, M. Mollereau, S. Chamaret, et al.** 1995. Gag-specific cytotoxic responses to HIV type 1 are associated with a decreased risk of progression to AIDS-related complex or AIDS. AIDS Res. Hum Retroviruses **11:**903–907.
36. **Tang, J., S. Tang, E. Lobashevsky, A. D. Myracle, U. Fideli, G. Aldrovandi, S. Allen, R. Musonda, R. A. Kaslow, and the Zambia-UAB HIV Research Project.** 2002. Favorable and unfavorable HLA class I alleles and haplotypes in Zambians predominantly infected with clade C human immunodeficiency virus type 1. J. Virol. **76:**8276–8284.
37. **Venet, A., and B. D. Walker.** 1993. Cytotoxic T-cell epitopes in HIV/SIV infection. AIDS **1**(Suppl. 7)**:**S117–S126.
38. **Yellen-Shaw, A. J., and L. C. Eisenlohr.** 1997. Regulation of class I-restricted epitope processing by local or distal flanking sequence. J. Immunol. **158:** 1727–1733.
39. **Zimbwa, P., A. Milicic, J. Frater, T. J. Scriba, A. Willis, P. J. R. Goulder, T. Pillay, H. Gunthard, J. N. Weber, H.-T. Zhang, and R. E. Phillips.** 2007. Precise identification of a human immunodeficiency virus type 1 antigen processing mutant. J. Virol. **81:**2031–2038.
40. **Zuñiga, R., A. Lucchetti, P. Galvan, S. Sanchez, C. Sanchez, A. Hernandez, H. Sanchez, N. Frahm, C. H. Linde, H. S. Hewitt, W. Hildebrand, M. Altfeld, T. M. Allen, B. D. Walker, B. T. Korber, T. Leitner, J. Sanchez, and C. Brander.** 2006. Relative dominance of Gag p24-specific cytotoxic T lymphocytes is associated with human immunodeficiency virus control. J. Virol. **80:**3122–3125.