Compensatory mutation partially restores fitness and delays reversion of escape mutation within the immunodominant HLA-B*5703-restricted Gag epitope in chronic human immunodeficiency virus type 1 infection

Hayley Crawford, University of Oxford
Julia G. Prado, University of Oxford
Alasdair Leslie, University of Oxford
Stephane Hue, University of Oxford
Isobella Honeyborne, University of Oxford
Sharon Reddy, University of KwaZulu-Natal
Mary van der Stok, University of KwaZulu-Natal
Zenele Mncube, University of KwaZulu-Natal
Christian Brander, Massachusetts General Hospital
Christine Rousseau, University of Washington School of Medicine

Only first 10 authors above; see publication for full author list.

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Compensatory Mutation Partially Restores Fitness and Delays Reversion of Escape Mutation within the Immunodominant HLA-B*5703-Restricted Gag Epitope in Chronic Human Immunodeficiency Virus Type 1 Infection


Department of Pediatrics, University of Oxford, Peter Medawar Building for Pathogen Research, South Parks Rd., Oxford OX1 3SY, United Kingdom; HIV Pathogenesis Programme, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban 4013, South Africa; Partners AIDS Research Center, Massachusetts General Hospital, 13th St., Bldg. 149, Charlestown, Boston, Massachusetts 02129; Department of Microbiology, University of Washington School of Medicine, Seattle, Washington 98195-8070; University of Alabama at Birmingham, Birmingham, Alabama 35294-0022; Zambia-Emory HIV Research Group (ZEHRG) and Zambia Blood Transfusion Service, Lusaka, Zambia; Emory Vaccine Center, Emory University, Atlanta, Georgia 30329; and Howard Hughes Medical Institute, Chevy Chase, Maryland 20815

Volume 81, no. 15, p. 8346–8351, 2007. Page 8347, Table 1, column 3, patient SK233: All the AISPTNLNAW epitope sequences for patient SK233 should read “P------------.”
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HLA-B*5703 is associated with effective immune control in human immunodeficiency virus type 1 (HIV-1) infection. Here we describe an escape mutation within the immunodominant HLA-B*5703-restricted epitope in chronic HIV-1 infection, KAFSPEVIPMF (Gag 162-172), and demonstrate that this mutation reduces viral replicative capacity. Reversion of this mutation following transmission to HLA-B*5703-negative recipients was delayed by the compensatory mutation S165N within the same epitope. These data may help explain the observed association between HLA-B*5703 and long-term control of viremia.

Understanding the mechanisms by which successful immune control of human immunodeficiency virus (HIV) can be achieved in natural HIV infection is of critical importance to HIV vaccine design. HIV-specific CD8+ T-cell responses play a central role in immune control of HIV, but the majority of responses in chronic infection are not associated with effective suppression of viremia (10, 16). Gag-specific CD8+ T-cell responses are those most strongly associated with low viremia in chronic HIV infection (4, 8, 16, 17, 27, 28, 30). To further examine the central role of Gag-specific immune control of HIV, we addressed the hypothesis that a broad Gag-specific response is effective in part because of the immunogenicity of this viral protein but also because escape within conserved Gag epitopes may incur a significant reduction in viral replicative capacity, thereby facilitating subsequent control by other immune responses. Several recent studies in both HIV-infected humans and simian immunodeficiency virus (SIV)-infected macaques have demonstrated that viral escape within gag-encoded capsid protein epitopes significantly reduces viral replicative capacity and is associated with suppression of viremia (5, 7, 18, 21, 23, 25). Here we examined the impact of escape within KAFSPEVIPMF (KF11; Gag 162-172), the immunodominant CD8+ T-cell response in chronic infection restricted by HLA-B*5703 (16), an allele strongly associated with low viremia (15, 19, 33).

Three HLA-B*5703-restricted p24 Gag CD8+ T-cell epitopes have been well described. In one epitope, ISPRT (ISW9; Gag 147-155), early escape via an Ala→Pro substitution at residue 146 (A146P) affects endoplasmic reticulum aminopeptidase 1 processing but has little impact on viral replicative capacity (3). However, in the second epitope, TSTL QEQIAW (TW10; Gag 240-249), early predictable escape through the T242N substitution reduces viral fitness (21, 23). We hypothesized that escape within this third epitope, KF11, would similarly reduce viral replicative capacity, potentially contributing to the long-term slow progression exhibited by HLA-B*5703-positive individuals. Our study focused on a previously described (16) cohort of 520 treatment-naïve chronically infected Zulu and Xhosa individuals in Durban, South Africa, and was extended to include selected treatment-naïve Zambia-Emory HIV Research Group participants in Lusaka, Zambia.

We first analyzed gag sequences from 35 chronically infected HLA-B*5703-positive and 485 HLA-B*5703-negative individuals in Durban, South Africa (Fig. 1A). Nested PCR was performed on proviral DNA using previously described primers and conditions (15). The characteristic escape mutations selected within ISW9- and TW10-specific epitopes were present in all 35 individuals. The previously described escape mutation within KF11, A163X (principally A163G) (20, 34), strongly associated with HLA-B*5703 (P = 4.5 × 10-17; Fig. 1B), was present in 69% (24/35) of HLA-B*5703-positive individuals.
FIG. 1. Sequence variation with HLA-B*5703-restricted epitopes within p24 Gag. (A) Sequence variation in three HLA-B*5703-restricted epitopes in HLA-B*5703-positive individuals. X represents a change away from the consensus sequence. (B) Patterns of sequence variation in KF11 in the entire cohort. (C) Mutations in KF11 associated with a HLA-B*5703 footprint (mutations in ISW9 and TW10) in HLA-B*5703-negative individuals. Individuals expressing HLA-B*13, -B*1510, -B*5702, and -B*5801 were excluded from this analysis, as these alleles select for escape mutations at the same residues within ISW9 and TW10 (13, 21).

TABLE 1. Longitudinal sequence data from five HLA-B*5703-negative patients with mutations in KF11

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*pop.*, proviral DNA population sequence. The numbers indicate the number of clones in which virus was derived from plasma to the total number of clones.
This suggests that KF11 escape mutations occur subsequent to those in TW10 and ISW9, consistent with the observation that TW10 and then ISW9 responses dominate the HIV-specific response in acute infection in HLA-B*57-positive subjects (1, 7) and that KF11 is immunodominant only in chronic infection (9). Examination of sequence data from 16 HIV-infected HLA-B*5703-positive individuals in Lusaka, Zambia, revealed a similar pattern of polymorphisms (data available upon request).

Analysis of the sequence changes within KF11 in HLA-B*5703-positive subjects also suggests an order of appearance of the A163X and S165X polymorphisms (Fig. 1B). All 24 of the 35 HLA-B*5703-positive subjects whose virus contained sequence variants within KF11 possessed the A163X mutation, which reduces the epitope binding affinity to HLA-B*5703 (35). Fourteen of these 24 subjects also carried the S165X mutation, but none carried the S165X mutation alone (Fig. 1B; \( P = 0.0009, \) Fisher’s exact test). These data suggest that S165X mutations are selected subsequent to A163G, perhaps to compensate for structural changes imposed on the viral capsid by the A163G mutation. In addition, the study by Yu et al. (35) showed that variation at residue 163 (A163X) was consistently associated with an increase of slightly greater than 1 log unit in peptide concentration to achieve 50% maximal recognition (SD50) (comparing median SD50s; 14 subjects studied), and variation at both residues 163 and 165 (A163X S165X) was associated with a \( >10^4 \)-fold increase in peptide concentration required to achieve SD50, showing that the additional mutation at position 165 reduces TCR recognition further.

Viral sequences from the HLA-B*5703-negative individuals provide further insight into the possible role of S165X. The presence of A163X and/or S165X polymorphisms in the HLA-B*5703-negative subjects was strongly linked with the occurrence of characteristic HLA-B*5703-associated mutations, so-called footprints (26) (see Fig. 1C, footnote b) within the ISW9 and TW10 epitopes: 40 of 49 (82%) HLA-B*5703-negative subjects with A163X and/or S165X polymorphisms carried HLA-B*5703 footprints (Fig. 1B) compared to 129 of 382 (34%) of HLA-B*5703-negative controls lacking either A163X or S165X (Fig. 1C; \( P = 1.4 \times 10^{-10} \)). Excluded from this control group were study subjects expressing any of HLA-B*5702/B*5801/B*13/B*1510, which select for escape mutations at the same residues within ISW9 and TW10 (13, 21). The mutation V168I that arises in 10% of the HLA-B*5703-negative subjects is not associated with expression of any other HLA class I allele, and its role is at present unclear. These data suggest that the KF11 mutations A163X and S165X seen in HLA-B*5703-negative individuals originally developed in HLA-B*5703-positive subjects and were transmitted together with mutations within ISW9 and TW10. However, in contrast to HLA-B*5703-positive subjects, S165X is observed without A163X in 13 HLA-B*5703-negative subjects, suggesting that the A163X mutation may undergo reversion in the absence of HLA-B*5703.

In order to test this hypothesis, we sequenced virus from four selected HIV-infected HLA-B*5703-negative individuals possessing mutations in KF11 and from whom longitudinal samples were available. RNA was extracted from plasma and reverse transcribed (Reverse-iT first strand synthesis kit; Abgene), cloned using a TOPO TA cloning kit (Invitrogen), and sequenced as previously described (21). Virus from these four individuals carrying both A163G and S165N variants were sequenced longitudinally; however, none exhibited reversion of G163A over 17 to 26 months of follow-up (Table 1).

An additional HIV-infected HLA-B*5703-negative individual, SK233, whose virus possessed a single A163G mutation in KF11, was also investigated. Characteristic mutations in all three HLA-B*5703 epitopes were present (Table 1), suggesting transmission from a HLA-B*5703-positive donor. As anticipated from previous studies (3, 21), A146P and A248G

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**FIG. 2.** Maximum-likelihood phylogenetic tree of Gag sequences in patient SK233. Branch color corresponds to the time period in months during which each plasma clone was isolated. The circles at the branch tips indicate changes in TW10: 242N (closed), 242S (open), and 242T (no circle). The arrow indicates the position of change in KF11 from 163G to 163A. This tree was inferred using PHYML (11) under the GTR plus G plus I model with the clade C consensus sequence (ConC) as an outgroup (http://www.hiv.lanl.gov/content/hiv-db). Nodal sequences were described by using PAUP* (32) with the model parameters estimated by PHYML and were used to determine where amino acid changes occurred on the phylogeny. All branch lengths are drawn to scale.
were stable over the 30-month period of the study, while T242N started to revert at 6 months after enrollment in the study via the intermediate T242S. However, unlike the HLA-B*5703-negative donors carrying the A163G S165N double mutation, the A163G mutation occurring alone in KF11 also underwent reversion. Furthermore, this reversion to the consensus amino acid Ala occurred within the first 6 months postenrollment, more rapidly than that of T242N to Thr. These Gag reversions, albeit in a single study subject, were coincident with a relatively rapid decline in absolute CD4 absolute count and increase in viremia (547 to 218/mm³ and 10,800 to 47,100 copies/ml, respectively) over 30 months.

Phylogenetic analysis (Fig. 2) showed first that the early gag sequence changes in SK233 are predominantly reversions (that is, the sequences became more closely related to consensus over the first 6 months of sequencing), whereas subsequent to that time, sequence changes started to diverge from the consensus. These data are consistent with those previously published (12, 22). Second, specific to the HLA-B*5703 escape mutations, this tree shows the rapid reversion of A163G in contrast to the much slower reversion of T242N.

Last, we undertook in vitro fitness assays in order to test the replicative capacity of viruses with single and double mutations within KF11. These individual mutants were constructed in an NL4-3 background using the GeneTailor site-directed mutagenesis system (Invitrogen, United Kingdom) as previously described (23). The plasmid was PCR amplified in a mutagenesis reaction with two overlapping primers, one of which contained the target mutation. The primers for the 163G mutant were p241260-1287 (5'-AGT AGT AGA GAA GGG TTT CAG CCC A-3') and p241276-1248 (5'-CCT TCT CTT CTA CTT TTA CCC ATG CA-3'), and the primers for the 163G/165N mutant were p241263-1293 (5'-AGT AGT AGA GAA GGG TTT GAA CAA CCC A-3') and p241282-1251 (5'-TGA AAC CCT TCT CTT CTA CTT TTA CCC AT-3') (mutagenesis sites are underlined). Viral stocks were produced by cotransfection of 5 µg of p83-2-derived mutants (5' half of HIV-1NL4-3) and 5 µg p83-10 (3' half of HIV-1NL4-3 strain), containing a green fluorescent protein (GFP) reporter gene (24, 34). Viral replication kinetic experiments were performed in triplicate in MT4 cells to a multiplicity of infection of 0.001 and measured over time by the percentage of GFP-positive cells by fluorescence-activated cell sorting analysis.

FIG. 3. Fitness consequences of A163G and A163G S165N mutations within KF11. (A) Levels of p24 expression in Jurkat cells after transfection with HIV-1NL4-3 and A163G and A163G/S165N mutants as detected in the supernatant by an enzyme-linked immunosorbent assay. (B) Infectivity of HIV-1NL4-3 and A163G and A163G/S165N mutants expressed as the percentage of GFP-positive (GFP+) cells over time following transfection, measured by fluorescence-activated cell sorting analysis. (C) Replication kinetics experiments were undertaken using identical starting titers of virus to control for potential differences in transfection efficiency of the variant and wild-type plasmids. MT4 cells were infected in triplicate with a multiplicity of infection of 0.001, the percentage of GFP-positive cells was plotted on a log scale and fitted to linear regression. (D) Infectivity levels at day 3 (with error bars showing standard deviations). (E) HIV-1 loads (showing median values [short horizontal lines]) of HLA-B*5703-positive individuals from South Africa. WT, wild type.
Replication kinetics showed a delay in the infectivity rate of the A163G mutant compared to the Ala-163-expressing NL4-3 virus. This fitness cost of A163G was partially restored by the additional S165N mutation (Fig. 3A to D). These data may explain the persistence of the A163G S165N mutant in HLA-B^*5703-negative persons and the rapid reversion of A163G when it occurs alone. Consistent with these data, HLA-B^*5703-positive individuals possessing the A163G S165X double mutation tend to have somewhat higher viral loads compared to those with the single A163X mutation, although there were few comparisons and the differences were not statistically significant (Fig. 3E).

This study of sequence changes in KAFSPEVIPMF, the dominant HLA-B^*5703-restricted epitope in chronic HIV infection, shows that mutations within the epitope are strongly associated with HLA-B^*5703, typically at positions 2 and 4 in the epitope (A163X and S165X). S165X (most commonly S165N) is selected subsequent to A163X (most commonly A163G) in HLA-B^*5703-positive subjects. In vivo reversion of A163G when it occurs alone is more rapid than that of T242N in TW10 in one HLA-B^*5703-negative subject studied. In vitro fitness assays here demonstrate a fitness cost of the A163G escape mutation that is partially compensated for by S165N. In vivo reversion of A163G is slow when it occurs in combination with S165N (longer than 17 to 26 months in 4 HLA-B^*5703-negative subjects studied), but the presence of S165N mutations occurring in the absence of A163G in 13 HLA-B^*5703-negative subjects (and in no HLA-B^*5703-positive subjects) supports in vitro fitness data showing that compensation for A163G by S165N is incomplete. However, the timing of infection of the five study subjects whose viral sequences were followed longitudinally was unknown, limiting the conclusions that can be drawn regarding the speed of reversion of A163X and the durability of persistence of the A163X S165X double mutation.

These data are consistent with other studies where control of HIV or SIV is associated with certain CD8 T-cell responses, escape from which incurs a significant fitness cost to the virus. The fact that HLA-B^*5703 presents two epitopes within p24 Gag, TW10 and KF11, in which the viral escape mutations T242N and A163G, respectively, inflict a fitness cost on the virus suggests that this may represent at least part of the mechanism for the association between HLA-B^*5703 and control of HIV. Other major histocompatibility complex class I alleles associated with control of HIV/SIV include HLA-B^*2705, Mamu-A^*01, and Mane-A^*11, all target capsid protein epitopes, escape from which results in a fitness cost to the virus (5, 7, 14). As a result of the reversion that occurs post transmission, these epitopes may remain available throughout the epidemic. However, the selection of compensatory mutations would tend to eliminate key epitopes, perhaps ultimately rendering alleles such as HLA-B^*5703 ineffective (6).

The presence of A163X and/or S165X mutations in as many as 49 out of 485 HLA-B^*5703-negative subjects in Durban, South Africa, demonstrates that approximately 10% of HLA-B^*5703-negative persons carry virus likely to have originated in HLA-B^*5703-negative subjects, even in a population such as that in South Africa, where the phenotypic frequency of this allele is only 4% and the epidemic is relatively young. Although HLA-B^*5703-positive subjects have low viral loads in chronic infection, reducing the risk of transmission, a high proportion of transmissions are believed to occur during acute infection (2). Also, HLA-B^*5703 footprints that were originally selected in HLA-B^*5703-positive individuals can evidently persist in HLA-B^*5703-negative subjects, who do not necessarily have low viral loads in chronic infection, allowing for transmission of this virus between individuals where neither the donor nor recipient carries HLA-B^*5703. Indeed, an example of transmission of HLA-B^*57-positive variants from the father to child, via the HLA-B^*57-negative mother, has been reported (29). Further studies are needed to determine the extent to which particular CD8 T-cell epitopes and the alleles restricting them will remain associated with immune control as the HIV epidemic progresses.

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21. Reference deleted.


