Impact of HLA-B*(star)81-Associated Mutations in HIV-1 Gag on Viral Replication Capacity

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**Journal Title:** Journal of Virology  
**Volume:** Volume 86, Number 6  
**Publisher:** American Society for Microbiology | 2012-03-01, Pages 3193-3199  
**Type of Work:** Article | Final Publisher PDF  
**Publisher DOI:** 10.1128/JVI.06682-11  
**Permanent URL:** https://pid.emory.edu/ark:/25593/s5ng1

Final published version: [http://dx.doi.org/10.1128/JVI.06682-11](http://dx.doi.org/10.1128/JVI.06682-11)

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*Accessed January 13, 2019 5:48 PM EST*
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HIV-1 attenuation resulting from immune escape mutations selected in Gag may contribute to slower disease progression in HIV-1-infected individuals expressing certain HLA class I alleles. We previously showed that the protective allele HLA-B*81 and the HLA-B*-81-selected Gag T186 mutation are strongly associated with a lower viral replication capacity of recombinant viruses encoding Gag-protease derived from individuals chronically infected with HIV-1 subtype C. In the present study, we directly tested the effect of this mutation on viral replication capacity. In addition, we investigated potential compensatory effects of various polymorphisms, including other HLA-B*-81-associated mutations that significantly covary with the T186S mutation. Mutations were introduced into a reference subtype B backbone and into patient-derived subtype C sequences in subtype B and C backbones by site-directed mutagenesis. The exponential-phase growth of mutant and wild-type viruses was assayed by flow cytometry of a green fluorescent protein reporter T cell line or by measurement of HIV-1 reverse transcriptase activity in culture supernatants. Engineering of the T186S mutation alone into all patient-derived subtype C sequences failed to yield replication-competent viruses, while in the subtype B sequence, the T186S mutation resulted in impaired replication capacity. Only the T186S mutation in combination with the T190I mutation yielded replication-competent viruses for all virus backbones tested; however, these constructs replicated slower than the wild type, suggesting that only partial compensation is mediated by the T190I mutation. Constructs encoding the T186S mutation in combination with other putative compensatory mutations were attenuated or defective. These results suggest that the T186S mutation is deleterious to HIV-1 subtype C replication and likely requires complex compensatory pathways, which may contribute to the clinical benefit associated with HLA-B*81.

Certain HLA class I alleles are strongly and consistently associated with slower human immunodeficiency virus type 1 (HIV-1) disease progression (7, 23). For example, HIV-1 subtype C-infected African individuals expressing HLA-B*-57, HLA-B*-5801, or HLA-B*-81 have significantly lower viral loads and/or higher CD4 counts (18, 19). Understanding the mechanisms underlying this advantage may have implications for HIV-1 vaccine design.

Previous studies have reported significant viral fitness costs associated with specific immune escape mutations in HIV-1 Gag CD8⁺ T cell epitopes that are restricted by clinically favorable HLA alleles (5, 9, 10, 20, 31). Immune-mediated HIV-1 attenuation may therefore represent one mechanism underlying the protective effects of certain beneficial HLA alleles. However, studies investigating protective mechanisms and immune-driven fitness costs have focused largely on the HLA-B*-57, HLA-B*-5801, or HLA-B*-27 alleles. Recently, we demonstrated that in chronic HIV-1 subtype C infection, the clinically favorable allele HLA-B*81 is strongly associated with a reduced replication capacity of recombinant viruses encoding patient-derived Gag-protease sequences (16, 35). In these sequences, the HLA-B*-81-associated T186S mutation in the HLA-B*-81-restricted TL9 CD8⁺ T cell epitope (HXB2 Gag codons 180 to 188) was strongly correlated with reduced virus replication (16, 35). This suggests that HLA-B*81-mediated selection pressure on Gag can attenuate HIV-1 replication, which may explain in part the clinical benefit of HLA-B*81. However, the direct effect of the T186S mutation on HIV-1 replication capacity has yet to be confirmed using site-directed mutagenesis studies.

For many CD8⁺ T cell escape mutations, secondary mutations that fully or partially compensate for replicative costs of escape at the primary site have been described (5, 10, 31, 33). In patient-derived sequences, we previously identified several polymorphisms that positively covaried with the T186S mutation and/or negatively covaried with the consensus 186T residue (35). Among HIV-1 sequences harboring the T186S mutation, there was no statistically significant relationship between the number of covarying residues and HIV-1 replication capacity (35). However, further exploration revealed that viruses harboring the HLA-B*-81-associated Q182S and/or T190X polymorphisms replicated significantly better than those with the T186S mutation alone, highlighting these polymorphisms as the most likely candidates for compensatory mutations. The extent to which these polymorphisms may potentially compensate for the T186S mutation has yet to be confirmed and quantified directly using site-directed mutagenesis studies.

In the present study, we directly tested the effect of the HLA-B*-81-associated T186S mutation on HIV-1 replication capacity through introduction of this mutation into the laboratory-adapted HIV-1 NL4-3 reference strain, as well as patient-derived
viral subtype C Gag-protease sequences, by site-directed mutagenesis. The replication capacities of the mutant viruses were assayed in a green fluorescent protein (GFP) reporter T cell line, as previously described (6, 22, 35), or by the measurement of reverse transcriptase (RT) activity in the culture supernatants. In addition, various polymorphisms covarying with the T186S mutation, in particular the HLA-B*81-associated Q182S and T190A/I mutations (35), were introduced into subtype B and C viral backbones, and the replication capacities of these constructs were assessed. The results confirmed a profound fitness cost conferred by the T186S mutation and suggest that compensation of this defect is complex and may be suboptimal in general.

**MATERIALS AND METHODS**

Site-directed mutagenesis of HIV-1 NL4-3 sequence and patient-derived HIV-1 gag-protease sequence (SK-254). Gag mutations T186S, Q182S, T190A, T190I, T186S/Q182S, and T186S/T190I were engineered into the HIV-1 subtype B NL4-3 plasmid (pNL4-3) by use of a QuickChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, CA) and custom-designed mutagenesis primers (Table 1). In addition, since these mutations are relatively rare in subtype B sequences, they were similarly introduced using custom-designed mutagenesis primers (Table 1) into a patient-derived subtype C HIV-1 Gag-protease sequence (SK-254; GenBank accession number HM593258) chosen for its high amino acid similarity (96.4%) to the consensus subtype C Gag-protease sequence (35). Prior to site-directed mutagenesis of the SK-254 Gag-protease, the SK-254 gag-protease amplicon was purified (PCR purification kit; Qiagen, Valencia, CA) and cloned into pCR2.1-TOPO (Invitrogen, Carlsbad, CA) per the manufacturer’s instructions, and the resulting clone was purified using a plasmid DNA maxiprep kit (Qiagen). As an additional experiment, the T186S mutation was introduced into 6 different HIV-1 subtype C gag sequences (GenBank accession numbers FJ606176, FJ606180, FJ606254, FJ606288, FJ606318, and JQ219842) derived from recently infected treatment-naive individuals from the Zambia Emory HIV Research Project (12) and cloned into the MJ4 plasmid, an infectious HIV-1 subtype C molecular clone derived from a primary isolate (24). For all constructs, DNA sequencing was performed to confirm the presence of introduced mutations.

**Virus generation and assay of mutant, patient-derived (SK-254) Gag-protease sequences in an HIV-1 subtype C MJ4 backbone.** To measure the effect of the various mutations on replication capacity in a purely subtype C background, the mutated SK-254 gag sequences (as well as the 6 patient-derived subtype C gag sequences) were cloned into the HIV-1 subtype C MJ4 plasmid (24). PCR was performed to amplify the mutated SK-254 gag gene from TOPO vector/SK-254 gag-protease by use of a Phusion hot start polymerase kit (Fisher, Pittsburgh, PA) and the following primers designed to introduce a BclI restriction site (in bold) at the 3’ end of gag: 5’ AGG CTA GAA GGA GAG AGA TG 3’ and 5’ TCT ATA GGT ATT TGA TCA TAC TGT CCT 3’ (BclI Rev; HXB2 nucleotides 775 to 792) and 5’ TCT ATA AGT ATT TGA TCA TAC TGT CCT 3’ (BclI Rev; HXB2 nucleotides 2420 to 2446). The MJ4 5’ long terminal repeat (LTR), containing an NcoMIV restriction site at the 5’ end, was amplified using the following primers: 5’ CCA AAT CGG CAA AAT CCC 3’ (MJ4for1b; MJ4 vector sequence) and 5’ CCC ATC TTC TCT CTA GCC 3’ (HXB2 nucleotides 775 to 794). The 5’ end of the purified SK-254 gag PCR product was then joined to the 3’ end of the MJ4 LTR fragment by splice overlap extension (SOE) PCR using primers MJ4for1b and BclI Rev. Following purification of the MJ4 LTR-gag product, this product and the MJ4 vector were both digested with NcoMIV (Roche, Indianapolis, IN) and BclI (Roche). The MJ4 LTR-gag insert was then ligated into the vector at a 3:1 ratio, using T4 ligase (Roche). JM109 competent bacteria were transformed with the ligation products, and the MJ4/SK-254 gag plasmid was purified using a Qiagen miniprep kit.

MJ4/SK-254-Gag mutant virus stocks were generated by transfection of 293T cells with mutated MJ4/SK-254 gag plasmids according to published protocols (2). Briefly, 293T cells at approximately 70% confluence were in-

**TABLE 1 Forward primers used to introduce specific Gag mutations into SK-254 HIV-1 Gag-protease and pNL4-3**

<table>
<thead>
<tr>
<th>Template</th>
<th>Mutation(s)</th>
<th>Primer (5’–3’)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK-254</td>
<td>T186S</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>Q182S</td>
<td>CACAAAGATTTAACCCACCATGTTAATATAGTTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>T190A</td>
<td>CACAAAGATTTAACACCATGTTAATATAGTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>T190I</td>
<td>CACAAAGATTTAACACCATGTTAATATAGTGGGGGAC</td>
</tr>
<tr>
<td>SK-254 T186S</td>
<td>T186S/Q182S</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>T186S/T190A</td>
<td>CACAAAGATTTAACCTCATTTAAATATAGTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>T186S/T190I</td>
<td>CACAAAGATTTAACCTCATTTAAATATAGTGGGGGAC</td>
</tr>
<tr>
<td>NL4-3</td>
<td>T186S</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>Q182S</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>T190A</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>T190I</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
</tr>
<tr>
<td>NL4-3 T186S</td>
<td>T186S/Q182S</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
</tr>
<tr>
<td></td>
<td>T186S/T190A</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
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<tr>
<td></td>
<td>T186S/T190I</td>
<td>CACAAAGATTTAACCTCATTTAAATACGATTAAAACACCAGTGGGGGAC</td>
</tr>
</tbody>
</table>

“Mutated Gag codons (HXB2 numbering) are shown in bold. The reverse mutagenesis primers comprised the reverse complement of the forward primers shown.”
Recombinant virus stocks were generated successfully for all mutation combinations engineered into the laboratory-adapted NL4-3 subtype B strain. All NL4-3 mutant viruses displayed lower replication capacities (68 to 84% of wild-type levels), with the exception of the Q182S mutant, which replicated to 95% of wild-type levels (Fig. 2). The NL4-3 T186S/Q182S mutant replicated to the same levels as the T186S mutant (68 to 70% of wild-type levels), while the T186S/T190A and T186S/T190I mutants replicated 8 to 16% faster than the T186S and T186S/Q182S mutants but 16 to 22% slower than the wild type (Fig. 2).

Due to the fact that these HLA-B*81-associated mutations were initially identified in patient-derived HIV-1 subtype C sequences, they were next introduced into a patient-derived subtype C Gag-protease sequence (SK-254) closely resembling the consensus subtype C sequence. In order to generate replicating virus stocks, the SK-254 Gag-protease was inserted into an HIV-1 NL4-3 subtype B backbone by homologous recombination approach (22, 35). Notably, the T186S, T186S/Q182S, and T186S/T190A mutations in the NL4-3/SK-254 backbone failed to yield

FIG 1 Structure of HIV-1 Gag p24 capsid. Residues 182, 186, and 190 are shown as yellow, pink, and blue spheres, respectively. Helices are represented by gray ribbons, strands are represented by black arrows, and coils are shown in green. This diagram was modified from structure 1GWP (32), viewed in PyMOL.

FIG 2 Replication capacities of NL4-3 viruses encoding HLA-B*81-associated mutations. Replication capacities of wild-type NL4-3 virus and NL4-3 viruses encoding Gag mutations T186S, Q182S, T190A, T190I, T186S/Q182S, T186S/T190A, and T186S/T190I are shown. Replication capacities of the mutant viruses are expressed relative to the replication capacity of wild-type NL4-3 virus. Bars represent the means for at least 3 independent experiments, and error bars represent standard deviations from the means.

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replicating virus stocks. Replication-competent virus stocks were generated successfully for the remaining NL4-3/SK-254 mutants, i.e., the Q182S, T190A, T190I, and T186S/T190I mutants; however, their replication capacities were 11 to 30% lower than that of the wild-type virus (Fig. 3). The NL4-3/SK-254 Q182S mutant replicated to 70% of wild-type levels (Fig. 3), while in contrast, the NL4-3 Q182S mutant had a similar replication capacity to that of wild-type NL4-3 (Fig. 2).

The failure to generate NL4-3/SK-254 mutant viruses containing the T186S mutation, with the exception of the T186S/T190I mutant, indicated a profound deleterious effect of the T186S mutation, with partial compensation mediated by the T190I mutation. This was largely consistent with data generated in the NL4-3 background, which showed that mutations at codon 190 rather than the Q182S mutation may partially, although weakly, compensate for the fitness cost of the T186S mutation. It should be noted, however, that the observed fitness cost of the T186S mutation and the compensatory effect of the T190I mutation in NL4-3 were modest compared with the same substitutions introduced into an NL4-3/SK-254 background. Another notable difference between the virus backbones was the slightly improved replication of the T186S/T190A mutant compared with the T186S/T190I mutant in NL4-3 (Fig. 2), while in the NL4-3/SK-254 background, replicating stocks of the T186S/T190A mutant were not generated successfully (Fig. 3).

Replication capacities of mutant, patient-derived (SK-254) Gag-protease sequences in an HIV-1 subtype C MJ4 backbone. The introduction of HLA-B*81-associated Gag mutations into a subtype B laboratory strain and then into a subtype B/C Gag-protease recombinant virus may not be sufficient to fully reveal the implications of these polymorphisms in a subtype C context. Variants at Gag residues 182, 186, and 190 were rare in HIV-1 subtype B viruses from a North American cohort (each present at <1.5%) (4). Moreover, discordant results obtained for the Q182S mutation and the T186S/T190A combination in subtype B versus subtype B/C Gag-protease backgrounds motivated further confirmation in a subtype C backbone. Therefore, subtype C SK-254 Gag was introduced into MJ4, a subtype C infectious molecular clone derived from a primary isolate (24). For these experiments, virus was recovered following proviral transfection of 293T cells, and RT assays were used to assess the replication capacity of each MJ4/SK-254 mutant. Although measurement of RT activity does not directly assess cell-to-cell spread of virus (1), replication capacity results obtained using the RT assay were comparable to those obtained by flow cytometry detection of GFP-positive HIV-1-infected cells (data not shown).

Consistent with data generated in the NL4-3/SK-254 background, only virus stocks harboring the Q182S, T190A, T190I, and T186S/T190I mutations in the MJ4/SK-254 background were generated successfully. All of these mutants displayed low replication capacities (29 to 71% of wild-type levels) (Fig. 4). The generation of virus stocks from 293T cells failed for the T186S/T190A mutant, and although low-titer virus stocks (assayed on JCR5BL cells) were obtained from 293T cells for the T186S and T186S/Q182S mutants, these stocks did not replicate in the GXR cell line used to assay replication capacity. These results further confirm the deleterious effect of the T186S mutation and suggest partial rescue of this defect by the T190I mutation but little or no rescue by the other covarying mutations tested.

Additional mutagenesis experiments in the subtype C backbone. Gag Q65H, E177D, and L343I polymorphisms were also found previously to covary with the T186S mutation (35) and could therefore potentially influence replication capacity in the presence of the T186S mutation. Therefore, these polymorphisms were also introduced into MJ4/SK-254-Gag by site-directed mutagenesis, in combination with the T186S mutation. However, the
T186S/E177D and T186S/L343I mutants did not yield infectious virus stocks, and the T186S/Q65H mutant yielded low-titer virus stocks that did not replicate in the GXR cell line, indicating that these mutations do not compensate for the fitness cost of the T186S mutation (data not shown). The Gag I256V polymorphism was previously negatively correlated with the T186S mutation in our chronically infected cohort (35). Since this polymorphism is already present in SK-254 Gag, we introduced the consensus residue 256I in combination with the T186S mutation to investigate whether this could rescue the T186S mutation-mediated defect; however, this mutant also did not replicate in the GXR cell line (data not shown).

To verify that our observations were representative of subtype C isolates in general, we introduced the T186S mutation into six additional patient-derived subtype C Gag sequences cloned into the MJ4 background. Consistent with the observations in MJ4/SK-254, all patient-derived recombinant viruses, which were chosen to have various replication capacities (unpublished data), were rendered defective upon introduction of the T186S mutation (Fig. 5). We also previously identified a nonconsensus residue, 256V, that negatively covaried with 186S (35). Interestingly, three of the six additional patient-derived sequences tested encoded the consensus residue 256I, while three encoded the I256V polymorphism, confirming that the T186S mutation has a deleterious effect regardless of variation at codon 256.

**Discussion**

We previously reported that the T186S mutation, associated with the protective allele HLA-B*81, is strongly linked to a lower viral replication capacity of recombinant viruses encoding Gag-protease from individuals chronically infected with HIV-1 subtype C, an observation which may have clinical relevance (35). A previous study also identified codon 186 as a site where HLA-driven mutations revert upon transmission to an HLA-mismatched host, suggesting a fitness cost for these polymorphisms (21). However, these studies were not able to directly assess the impact of the T186S mutation on viral replication. To address this, we introduced the T186S mutation, by site-directed mutagenesis, into a laboratory-adapted strain of HIV-1 as well as into recombinant viruses expressing patient-derived subtype C Gag sequences in both subtype B and C backbones. A significant deleterious effect of the T186S mutation on HIV-1 replication was observed consistently for all sequences tested. The introduction of the T186S mutation into a range of viruses carrying patient-derived subtype C sequences, with various replication capacities, resulted in replication-defective viruses, while the introduction of the T186S mutation into a laboratory-adapted subtype B strain significantly reduced replication capacity. Consistent with these data, a viral fitness cost associated with residue 186M following site-directed mutagenesis of HIV-1 subtype B center-of-tree Gag p24 in the NL4-3 backbone was recently described (29).

The mechanisms responsible for decreased replication capacity of the T186S mutant have yet to be investigated fully. Mutations in the capsid N-terminal domain (residues 133 to 277), in which helix 3 of Gag p24 (Fig. 1) is found, generally impair virus maturation and core morphology and, consequently, virus infectivity (11, 13). Previous studies have examined the effect of the capsid T54A mutation, equivalent to the Gag T186A mutation, on virus replication (34, 37). Alanine scanning mutagenic analysis of the Gag capsid revealed that the Gag T186A mutation reduced the efficiency of capsid formation (34), and it is possible that similar mechanisms could apply to the T186S mutation. Further work suggested that defective virus replication of the T186A mutant was mediated at least in part by an altered cyclophilin A (CypA)-capsid interaction, reduced stability of capsid formation, and reduced reverse transcription (37). Interestingly, the T186A mutation was rare in our chronic HIV-1 subtype C-infected cohort, occurring in only 1 of 406 sequences (35). With the exception of this sequence, the nonconsensus residue at codon 186 was always S.

Using patient-derived subtype C Gag-protease sequences, we previously observed that Gag-protease recombinant viruses harboring the T186S mutation in the presence of other covarying HLA-B*81-associated mutations, i.e., Q182S and T190X, replicated better than those with the T186S mutation alone, suggesting that the Q182S and T190X mutations may compensate for the fitness cost of the T186S mutation (35). In the present study, site-directed mutagenesis experiments with all virus backbones tested indicated that the Q182S mutation did not compensate for the T186S mutation-mediated defect. However, polymorphisms at Gag codon 190, most consistently T190I, partially restored the fitness of the T186S mutant, to different degrees, in the various backbones tested. When the T186S mutation was engineered alongside either the T190I or T190A mutation into the subtype B NL4-3 strain, the double mutant replicated slightly better than the T186S single mutant, and this was more pronounced for the T186S/T190A double mutant. For the patient-derived subtype C SK-254 Gag sequence in both subtype B and C backbones, the T186S single mutant failed to replicate but was rendered replication competent (yet not to wild-type levels) in the presence of the T190I mutation only, indicating partial compensation by the T190I mutation, not the T190A mutation, in subtype C. In support of a modest compensatory role for codon 190 variants but not for the Q182S mutation, in our HIV-1 subtype C-infected cohort (35), mutations at codon 190 occurred more frequently in conjunction with the T186S mutation (in 13 of 18 cases) than without the T186S mutation, while the Q182S mutation occurred less frequently in conjunction with the T186S mutation (in 7 of 21 cases) than without the T186S mutation (Fisher’s exact test; P = 0.02). Furthermore, in HLA-B*81-expressing individuals lacking the T186S mutation, polymorphisms at codon 190 were rarely present (2 of 25 cases), while polymorphisms at residue 182 were common.
(13 of 25 cases) (Fisher’s exact test; P = 0.002). Forty-six percent of the patient-derived sequences harboring the T186S mutation also harbored a mutation at position 190.

Besides the HLA-B*81-associated Q182S and T190A/I mutations, other residues that covaried with the T186S mutation in patient-derived subtype C Gag sequences—65H, 177D, 343I, and 256I—were tested in the subtype C backbone for potential compensatory effects, but each failed to rescue the T186S mutation-mediated replication defect. Interestingly, Rolland et al. found that the E177D mutation further decreased the replication capacity of a subtype B virus backbone encoding the T186M mutation (29). The observation that all Gag residues significantly covarying with the T186S mutation in natural subtype C Gag sequences either failed to rescue the T186S mutation-mediated defect in the subtype C backbone or, in the case of the T190I mutation, allowed replication but not to wild-type levels, suggests that the T186S mutation is complex to compensate and that compensation may be suboptimal in general. This is in contrast to the HLA-B*57/5801-associated T242N mutation, which when engineered alone into the HIV-1 subtype C consensus capsid cloned into the subtype C MJ4 strain modestly reduced replication in primary cells and was rescued to wild-type levels by the single compensatory H219Q mutation (3). Consistent with the significant deleterious effect of the T186S mutation and potential suboptimal compensation for this defect, Huang et al. demonstrated evidence for in vivo reversion of the T186S mutation during advanced immunodeficiency (16), while the T242N mutation tended to be preserved during advanced disease, likely due to the accumulation of compensatory mutations throughout the infection course (10).

In our previous study of Gag-protease recombinant viruses, some patient-derived sequences harboring the T186S mutation demonstrated average or high viral replication capacities (35), suggesting that the T186S mutation was effectively compensated in at least some cases. In fact, one patient-derived subtype C sequence harboring the T186S mutation lacked all of the specific residues covarying with the T186S mutation tested here, even though the corresponding recombinant virus displayed an average replication phenotype (35). While we cannot exclude the possibility that complex and/or unique patterns of covarying mutations may be able to effectively compensate for the T186S mutation, no other reproducible patterns of covariation with the T186S mutation have been observed in the natural sequences examined (35). Since the T186S mutation was particularly deleterious in several subtype C Gag sequences, it is likely that context-appropriate sequence changes may need to arise prior to its selection, potentially delaying its emergence in vivo.

Interestingly, although HLA-B*81 expression and carriage of the Gag T186S mutation were strongly associated with a reduced replication capacity in our chronically infected cohort overall, the replication capacities of Gag-protease recombinant viruses derived from HLA-B*81-expressing individuals with or without the T186S mutation were comparable (and both below average) (35). This suggests that other mutations selected by HLA-B*81 may also contribute to replication defects associated with this allele. In fact, mutation away from the consensus at the HLA-B*81-associated codon 182 was linked significantly to a lower replication capacity of viruses encoding patient-derived sequences (35), although this was not as pronounced as for codon 186. We also found that the Q182S mutation in SK-254 Gag significantly decreased viral replication capacity in the present study. Therefore, codon 182 polymorphisms may contribute to a lower fitness of viruses in HLA-B*81-expressing individuals, although not as markedly as for the T186S mutation. Note that in the present study, polymorphisms at codon 190 alone also decreased replication capacity, but these were rarely observed in the absence of the T186S mutation in natural subtype C sequences from HLA-B*81-expressing chronically infected individuals. Deleterious effects of compensatory mutations upon in vitro experimental reversion of escape mutations have been observed by others in bacteriophages (27) and HIV-1 (25).

The fitness defect associated with the development of the T186S mutation may partly explain the lower viral loads observed in HIV-1-infected HLA-B*81-expressing individuals. Previous studies have suggested that a balance exists between immune-driven fitness costs and effective CD8+ T cell responses in influencing clinical outcomes (9, 17, 36). It was recently reported that mutation of the consensus residue 186T was associated with lower viral loads in a large sample of HIV-1 subtype C-infected individuals (which included a subset [n = 129] of our chronically infected cohort [35]), not taking HLA expression into account, which is consistent with the fitness cost of residue 186S demonstrated here (29). However, although the average viral load corresponding to sequences with the T186S mutation was lower in our previous study of chronic HIV-1 infection, this was not statistically significant (35); in fact, there was a trend toward higher viral loads in HLA-B*81-expressing individuals harboring the T186S mutation. It is also worth noting that increasing numbers of public T cell clonotypes for simian immunodeficiency virus (SIV) CM9 (residues 181 to 189), which is in close proximity to TL9 in HIV, were associated with lower viral set points in rhesus macaques (28); however, the development of an escape mutation in CM9 with severe viral fitness costs resulted in clinical disease progression in one animal (26). These studies imply that the development of the T186S mutation decreases the effectiveness of CD8+ T cell responses to TL9 that would otherwise control viremia in HLA-B*81-expressing individuals. Indeed, Geldmacher et al. showed reduced gamma interferon (IFN-γ) production in response to the TL9 T186S variant in HLA-B*81-expressing subjects harboring viruses with this mutation (14). It has also been shown that other escape mutations with significant fitness costs, namely, the A163G (9) and R264K (15, 31) mutations, may precede an increase in viral load in individuals who express the associated HLA allele due to decreased effectiveness of CD8+ T cell responses to the mutated epitopes. Therefore, we hypothesize that the clinical benefit in HLA-B*81-expressing individuals could derive partly from a potential delay in TL9 escape, and therefore preservation of an effective CD8+ T cell response, due to the combination of the T186S mutation-mediated fitness defect and the complexity in compensating for this defect, as suggested by our data. Consequently, TL9 may be a good candidate epitope to include together with other conserved epitopes in an HIV-1 vaccine aiming to constrain immune escape and/or reduce viral fitness in the event of partial escape (8, 30). Another possible contributing factor to the lower viral loads in HLA-B*81-expressing individuals could be a viral replication disadvantage incurred by HLA-B*81-driven mutations other than the T186S mutation.

In conclusion, the data presented here clearly and consistently demonstrate that the HLA-B*81-associated T186S mutation has a significant deleterious effect on HIV-1 replication capacity, particularly in HIV-1 subtype C sequences. No compensatory mutations capable of fully rescuing this defect were identified, although muta-
tions at codon 190 (the T190I mutation in particular) may compensate partially for this fitness cost. The impact of the T186S mutation, combined with suboptimal or complex pathways of compensation, may contribute to the clinical benefit of HLA-B*81.

ACKNOWLEDGMENTS

This research was funded by the South African Department of Science and Technology through the National Research Foundation, the Ragon Institute Fund for Innovation and New International Initiatives, and the Mark and Lisa Schwartz Foundation. Additional funding came from the NIH (ROI-AI 64060) (E.H.), J.K.W. was funded by the National Research Foundation and the Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University, V.L.N., was supported by the Columbia University-Southern African Fogarty AIDS International Training and Research Program (AITRP) through the Fogarty International Center, National Institutes of Health (grant 3 D43 TW000231 16S2). Z.L.B. was supported by a New Investigator Award from the NIH AIDS International Training and Research Program (AIDT) through the Fogarty International Center, National Institutes of Health. T.N. held the South African Department of Science and Technology/National Research Foundation Research Chair in Systems Biology of HIV/AIDS.

We acknowledge Taryn Green for technical assistance, Toshiyuki Miura for providing the Gag-protease-deleted NL4-3 plasmid, Johannes Viljoen and the Africa Centre laboratory for providing access to tissue culture and sequencing facilities, and the Emory Center for AIDS Research Immunology Core for flow cytometry (P30 AI050409).

REFERENCES


4. Brockman MA, et al. 2010. Early selection in Gag by protective HLA alleles contributed to decreased HIV-1 replication capacity that may be largely compensated for in chronic infection. J. Virol. 84:11937–11949.


