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Raymond F Schinazi, Emory University
Ivana Massud, Atlanta Veterans Affairs Medical Center
Kimberly L. Rapp, Atlanta Veterans Affairs Medical Center
Meta Cristiano, Emory University
Mervi Acuzar Detorio, Emory University
Richard A. Stanton, Emory University
Matthew A. Bennett, Atlanta Veterans Affairs Medical Center
Monique Kierlin-Duncan, Atlanta Veterans Affairs Medical Center
Johan Lennerstrand, Atlanta Veterans Affairs Medical Center
James H Nettles, Emory University

Journal Title: Antimicrobial Agents and Chemotherapy
Volume: Volume 55, Number 5
Publisher: American Society for Microbiology | 2011-05, Pages 2054-2060
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/AAC.01700-10
Permanent URL: http://pid.emory.edu/ark:/25593/f0hzv

Final published version: http://aac.asm.org/content/55/5/2054

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Accessed March 22, 2020 4:21 AM EDT
Selection and Characterization of HIV-1 with a Novel S68 Deletion in Reverse Transcriptase

Raymond F. Schinazi,* Ivana Massud, Kimberly L. Rapp, Meta Cristiano, Mervi A. Detorio, Richard A. Stanton, Matthew A. Bennett, Monique Kierlin-Duncan, Johan Lennerstrand,† and James H. Nettles

Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Veterans Affairs Medical Center, Decatur, Georgia, 30033

Received 7 December 2010/Returned for modification 3 February 2011/Accepted 16 February 2011

Resistance to human immunodeficiency virus type 1 (HIV-1) represents a significant problem in the design of novel therapeutics and the management of treatment regimens in infected persons. Resistance profiles can be elucidated by defining modifications to the viral genome conferred upon exposure to novel nucleoside reverse transcriptase (RT) inhibitors (NRTI). In vitro testing of HIV-1_LAI-infected primary human lymphocytes treated with \( \beta \)-2',3'-dideoxy-2',3'-dideoxynucleosides (DNC; Dexelvuclitabine; Reverse) produced a novel deletion at codon 68 (S68Δ) alone and in combination with K65R that differentially affects drug response. Dual-approach clone techniques utilizing TOPO cloning and pyrosequencing confirmed the novel S68Δ in the HIV-1 genome. The S68Δ HIV-1 RT was phenotyped against various antiviral agents in a heteropolymeric DNA polymerase assay and in human lymphocytes. Drug susceptibility results indicate that the S68Δ displayed a 10- to 30-fold increase in resistance to DFC, lamivudine, emtricitabine, tenofovir, abacavir, and amdoxovir and modest resistance to stavudine, \( \beta \)-2',3'-oxa-5-fluorocytidine, or 9-(\( \beta \)-2',3'-dideoxynucleosine (ddI), 1-(\( \beta \)-dideoxolan)-thymine (DOT) and lopinavir. Modeling revealed a central role for S68 in affecting conformation of the \( \beta \)-3' finger region and provides a rationale for the selective resistance. These data indicate that the novel S68Δ is a previously unrecognized deletion that may represent an important factor in NRTI multidrug resistance treatment strategies.

There are significant detriments associated with the management and treatment of human immunodeficiency virus type 1 (HIV-1) infection that limit treatment efficacy, including drug resistance, adherence, tolerability, and long-term toxicity. The error-prone HIV-1 reverse transcriptase (RT), along with the high rate of turnover for infected cells, contributes to a rapid mutation rate coupled with diverse viral quasispecies.

In vitro testing of HIV-1_LAI-infected primary human lymphocytes treated with \( \beta \)-2',3'-dideoxy-2',3'-dideoxynucleosides (DNC; Dexelvuclitabine; Reverse) produced a novel deletion at codon 68 (S68Δ) alone and in combination with K65R that differentially affects drug response. Dual-approach clone techniques utilizing TOPO cloning and pyrosequencing confirmed the novel S68Δ in the HIV-1 genome. The S68Δ HIV-1 RT was phenotyped against various antiviral agents in a heteropolymeric DNA polymerase assay and in human lymphocytes. Drug susceptibility results indicate that the S68Δ displayed a 10- to 30-fold increase in resistance to DFC, lamivudine, emtricitabine, tenofovir, abacavir, and amdoxovir and modest resistance to stavudine, \( \beta \)-2',3'-oxa-5-fluorocytidine, or 9-(\( \beta \)-2',3'-dideoxynucleosine (ddI), 1-(\( \beta \)-dideoxolan)-thymine (DOT) and lopinavir. Modeling revealed a central role for S68 in affecting conformation of the \( \beta \)-3' finger region and provides a rationale for the selective resistance. These data indicate that the novel S68Δ is a previously unrecognized deletion that may represent an important factor in NRTI multidrug resistance treatment strategies.

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Cod, Inc., East Falmouth, MA) in 500 ml of RPMI 1640 (Mediatech, Inc., Herndon, VA) containing 20% heat-inactivated fetal bovine serum (HyClone, Logan, UT), 83.3 IU of penicillin/ml, 83.3 µg of streptomycin/ml, 1.6 mM l-glutamine (Mediatech), for 2 to 3 days prior to use. HIV-1 LAI obtained from the Center for Disease Control and Prevention (Atlanta, GA) was used as the virus for selecting a resistant pool. A multiplicity of infection of 0.1 was selected to begin the infected pool, as determined by a limiting dilution method in human PBM cells.

Selection of resistant virus. To select resistant virus, naïve activated human PBM cells were treated with DFC at 0.1 µM for 1 h prior to inoculation with HIV-1 LAI (WT) as previously described (35). Briefly, the vehicle (control) and DFC-treated human PBM cell groups were infected for 1 h. Supplemented RPMI 1640 containing interleukin-2 (2 IU/ml) was then added for a final concentration of 10⁶ cells/ml. Virus was passaged every 6 days with a fresh treatment of DFC, ranging from 0.0 to 6.0 µM over 52 weeks. The RT activity in supernatant was measured weekly and used to determine the percent inhibition by DFC (35).

HIV-1 genome isolation and amplification. Total RNA was isolated from culture supernatants by using a commercial QIAamp Viral RNA kit (Qiagen, Inc., Valencia, CA). RT-PCR was performed using SuperScript III RT (Invitrogen, Carlsbad, CA) and DCA primer II primers (Ambion, Austin, TX) to generate cDNA from purified RNA. PCR was performed using Platinum Taq polymerase and M13 universal primer sequences (Ambion) as described previously (12). The ligation was transformed into Alpha-Select competent cells (New England Biolabs). The digested products were gel extracted by using a QIAEX II kit (Qiagen). The ligation was transformed into Alpha-Select competent cells (New England Biolabs). The digested products were gel extracted by using a QIAEX II kit (Qiagen).

Site-directed mutagenesis for protein expression. Site-directed mutagenesis was performed on a hybrid plasmid (designated MC002) created by digesting self-ligated pCR2.1 (Invitrogen) and pNL4-3 (AF3244930) with restriction enzymes EcoRI and SpeI. The 4.2-kb band from pNL4-3 containing RT was ligated into pCR2.1 using the cutting sites EcoRI/SpeI. The primers used had the S68 codon AGT removed, thus introducing the deletion into pNL4-3 RT. The ligation was confirmed by sequencing in both directions. To ligate the RT of pNL4-3 with the deletion at codon 68 into protein expression vector pE60 (Invitrogen), MC002 was amplified and cloned into the NcoI/BglII site by using T4 DNA ligase (New England Biolabs). The digested products were gel extracted by using QIAEX II (Qiagen). The ligation was transformed into Alpha-Select competent bacteria (BioLine, Taunton, MA). Plasmid DNA was extracted by using a HiSpeed plasmid maxi kit (Qiagen) and sequenced in multiple directions to confirm the mutagenesis.

Heteropolymeric DNA polymerase assay. The principle and performance of this nonradioactive RT assay has previously been described by Lennerstrand et al. (25). The following modifications were introduced to obtain ATP primer unblocking reaction in the assay, the ATP (Amersham/GE Healthcare) was set to physiological concentration (3.2 mM). In addition, ATP was only used in assay with virus pellets as a sample, not only to obtain primer unblocking but also to protect degradation of substrate in the crude sample. Furthermore, the deoxy-nucleoside triphosphate (dNTP) level (including Brd-UTP) was increased from 1 to 4 µM for assay with the virus pellet samples. Subsequently, in an assay with recombinant purified RT enzyme, only 1 µM dNTP (no ATP) was used.

The level of resistance to NRTI-triphosphate (NRTI-TP) by the S68 deletion mutants compared to WT RT was determined as the 50% inhibitory concentration (IC₅₀) based on RT activity. Fold increases in resistance values were determined by dividing the IC₅₀ for the mutant by the IC₅₀ for the respective WT strain. The RT activity was linear during the assay time within the substrate range used, and thus steady-state kinetics were assumed. The NRTI-TPs used were ZDV-TP, DFC-TP, and (−) FTC-TP, all prepared in our laboratory with purity >96% as determined by liquid chromatography-tandem mass spectrometry.

Molecular modeling. All models of drug interaction were extrapolated from X-ray crystallographic atomic coordinates, available to the public at the RCSB Protein Data Bank (PDB; www.pdb.org) (1).

Ligand model selection. Four experimental data sets were obtained from the PDB for reference modeling of the S68c mutation. All selected coordinate sets contained HIV-RT with terminated and cross-linked DNA (template-primer [T/P]) and small molecule nucleotide ligands (4, 18, 43). 1RTD and 1T05 contain the WT RT/DNA complex with the ligands thymidine-5'-triphosphate (dTTP) and tenofovir-diphosphate (TDFP), respectively (18, 43). Files 3Y1T and 35SM contain the K65R mutant form of HIV-RT with T/P and 2'-deoxycytidinose-5'-triphosphate (dATP) and TDFP solved by the same lab as 1T05 (4).

Bioinformatics: data assembly and sorting. Custom scripting was used to download and process more than 150 available structures of HIV-RT. The 66-kDa A-chain of the ternary complex with the DNA ligand was chosen as a structural reference (18). Every other structure was fit to the reference coordinate space through a command scripted Chimera interface to the Needleman-Wunsch algorithm (29). All were fit to the single reference coordinate space by using a BLOSUM-62 matrix, a gap extension penalty of 1, and a secondary structure score of 20% (13). Pairwise RMSD of C-alpha carbons were calculated for chains. The data were plotted and used to select relevant clusters of structure and ligand models for detailed protein and ligand modeling as described above.

Fitting and analysis of protein-ligand interactions. All structures were fit to the 1RTD coordinate space as described in Bioinformatics using UCSF Chimera for visualization and protein preparation (30). Ramachandran plots were generated by using the SwissPDB viewer (31). Ligands were prepared and modeled in the Molecular Operating Environment (MOE; Chemical Computer Group, Montreal, Quebec, Canada) using the Protonate 3D and ligand interaction diagramming algorithms (3, 24).

Modeling WT and single and double mutations. To reduce bias from manual loop model building, random-number seeds were applied through the UCSF Modeler application to generate five different HIV-1, p66 chains, three-dimensional models given identical primary sequence (7). Sequences input for WT, S68Δ, K65R, and S68Δ/K65R double mutants were fit against 1RTD, 1T05, and 35SM as templates, yielding 60 theoretical models that were back-fit against the experimental structures for calibration and statistical analysis. Rotamers were mapped and scored using the Dunbrack rotamer library accessed through Chimera and MOE rotamer explorer as described previously (29).

Nucleotide sequence accession number. The sequences obtained in the present study have been deposited in GenBank under accession number GQ245682.

RESULTS

In vitro selection of S68A. In the presence of DFC, virus containing the S68 deletion was first detected in supernatant of HIV-1 LAI-infected human PBM cells at week 14 as a mixture of S68Δ and WT viruses (Table 1). Population sequencing, from amino acids 1 to 500 in the HIV-1 RT region, revealed S68Δ as the dominant mutation by week 19. It then became a mixture containing K65R and WT sequences by week 25, and the K65R mutation became dominant by week 28. No other mutations in the RT region were detected. Adjustment of the DFC concentration was at times necessary in order to increase virus replication.

A search in the Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu) did not reveal matching
sequences for the S68Δ indicating that it is a novel mutation (Table 2) (2, 10, 20, 21, 42, 44, 22). To confirm the mutant population, the virus obtained from supernatants at weeks 11, 12, 14, 16, 17, 18, 19, 20, 23, 26, 27, 29, 30, 37, and 52 was TOPO cloned and sequence analysis was conducted on at least 10 clones per week (Fig. 1). Similar results were found using population sequencing (Table 1) and TOPO cloning (Fig. 1). The mutation K65R appeared at week 14 in 10% of the total population and became 100% at week 29. On the other hand, S68Δ was apparent at week 14 as 70% of the total population, and it continued to be detected until week 52, where it appeared as a mixture in the same genome with K65R (63%). Interestingly, two clones of 10 at week 23 contained the mutations S68Δ in combination with T69A or T69S on the same genome. These results demonstrate that S68Δ virus obtained from the DFC selection process can occur independently or as a mixture with WT, K65R, T69A, or T69S viruses.

Pyrosequencing analysis (FLX system) of samples from weeks 14, 20, 23, and 52, detected S68Δ, K65R, and other 64 polymorphisms. Of the detected polymorphisms, an average of 26 occurred at a frequency higher than 3%, and all are non-synonymous mutations (Table 3). The populations occurring at a frequency greater than 3% or higher are unlikely to represent sequence artifact making this a conservative cutoff for identification of authentic variants (19, 28, 45). Direct comparison of the FLX system versus TOPO cloning revealed a 2- to 10-fold difference in the frequency of virus polymorphisms in samples obtained at weeks 14 and 20 (Table 4). The two sequencing methods showed less difference in the results from the samples obtained at weeks 23 and 52. A higher number of emerging polymorphisms during early time points could be responsible for these finding. The polymorphisms were scored using the Stanford University HIV Drug Resistance Database, revealing that none were associated with known NRTI and NNRTI drug resistance. The FLX system results support the existence of the S68Δ with or without the K65R mutation on the same genome. In addition, these results provide information related to polymorphisms that evolved under DFC treatment and were not detected by conventional DNA sequencing.

**Susceptibility to RT inhibitors.** To determine the susceptibility of the S68Δ virus against various NRTI and NNRTI, HIV_S68Δ-23 was expanded and tested. HIV_S68Δ-23 was sequenced to ensure the dominant population was the deletion at codon 68.

Drug susceptibility assays indicated that virus with the S68 deletion produced a >30-fold increased resistance to the NRTIs DFC, TDF, 3TC, and (−) FTC, a 5- to 20-fold increased resistance to DVG, DAPD, and ABC, and a modest resistance (<5-fold) to ddc, d4T, D-FDOC, or DOT but remained susceptible to ZDV (Fig. 2). The correlation coefficient for the dose-response curve was higher than 0.9 for all of the assays (data not shown). As expected, the HIV_S68Δ-23 susceptibility to an NNRTI (Sustiva) and protease inhibitor (PI; lopinavir) was not markedly changed.

**Enzymatic characterization of the recombinant S68 deletion RT.** Enzymatic studies with the HIV-1 RT obtained from the recombinant and particle-derived S68Δ virus in the presence or absence of 3.2 mM ATP resulted in a 2.5-fold, 6- to 8-fold, and 8- to 10-fold increases in resistance to ZDV-TP, DFC-TP, and (−)-FTC-TP (Fig. 3). As expected, the control M184V enzyme had a significant >10-fold increase in resistance to (−)-FTC-TP but was not resistant to DFC-TP. Neither the S68Δ nor the M184V recombinant RTs showed significant resistance to ZDV-TP, confirming the results of the cell-based susceptibility assays.

### Table 1. Population sequencing from amino acids 1 to 300 in the HIV-1 RT region demonstrates selection of the S68Δ virus in supernatant from DFC-treated human PBM cells

<table>
<thead>
<tr>
<th>DFC selection pressure (concn [μM])&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Culture wk</th>
<th>Mutation by population sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>4</td>
<td>WT</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>WT</td>
</tr>
<tr>
<td>1.0</td>
<td>11</td>
<td>WT</td>
</tr>
<tr>
<td>1.0</td>
<td>12</td>
<td>WT</td>
</tr>
<tr>
<td>0.1</td>
<td>14</td>
<td>S68Δ/WT</td>
</tr>
<tr>
<td>0.1</td>
<td>15</td>
<td>WT</td>
</tr>
<tr>
<td>0.1</td>
<td>16</td>
<td>WT</td>
</tr>
<tr>
<td>1.0</td>
<td>17</td>
<td>S68Δ/WT</td>
</tr>
<tr>
<td>1.0</td>
<td>18</td>
<td>S68Δ/WT</td>
</tr>
<tr>
<td>1.0</td>
<td>19</td>
<td>S68Δ</td>
</tr>
<tr>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>S68Δ</td>
</tr>
<tr>
<td>1.0</td>
<td>22</td>
<td>S68Δ</td>
</tr>
<tr>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23</td>
<td>S68Δ</td>
</tr>
<tr>
<td>2.0</td>
<td>24</td>
<td>S68Δ</td>
</tr>
<tr>
<td>3.0</td>
<td>25</td>
<td>S68Δ/K65R/WT</td>
</tr>
<tr>
<td>3.0</td>
<td>26</td>
<td>S68Δ/K65R/WT</td>
</tr>
<tr>
<td>3.0</td>
<td>27</td>
<td>S68Δ/K65R/WT</td>
</tr>
<tr>
<td>6.0</td>
<td>28</td>
<td>K65R</td>
</tr>
<tr>
<td>6.0</td>
<td>29</td>
<td>K65R</td>
</tr>
<tr>
<td>6.0</td>
<td>30</td>
<td>K65R</td>
</tr>
<tr>
<td>1.5</td>
<td>36</td>
<td>K65R</td>
</tr>
<tr>
<td>6.0</td>
<td>49</td>
<td>K65R</td>
</tr>
<tr>
<td>6.0</td>
<td>52</td>
<td>S68Δ/K65R</td>
</tr>
</tbody>
</table>

<sup>a</sup> *, Adjustment of the DFC concentration was necessary in order to increase virus replication, thus providing material for sequence analysis.

### Table 2. Alignment of S68Δ with previously published deletions and/or mutations in codons 63 to 73 of the HIV-1 β3-β4 loop

<table>
<thead>
<tr>
<th>Virus (reference[s])</th>
<th>Deletion and/or mutation(s)</th>
<th>Amino acid at position:</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>64</td>
<td>65</td>
</tr>
<tr>
<td>S68Δ</td>
<td>S68Δ</td>
<td>I</td>
</tr>
<tr>
<td>AF271766 (2, 10, 21)</td>
<td>D67Δ, T69G, K70R</td>
<td>I</td>
</tr>
<tr>
<td>AF311203 (42)</td>
<td>K70Δ, S68N</td>
<td>I</td>
</tr>
<tr>
<td>DQ394304 (16)</td>
<td>K70Δ, S68G</td>
<td>I</td>
</tr>
<tr>
<td>AF311157 (42)</td>
<td>T69Δ, D67S, S68G</td>
<td>I</td>
</tr>
<tr>
<td>EF154395 (44)</td>
<td>T69Δ, S68G, K70G</td>
<td>I</td>
</tr>
<tr>
<td>pNL4-3</td>
<td>I</td>
<td>K</td>
</tr>
</tbody>
</table>
bility assays described above. Both enzymatic assay and recombinant RT assay demonstrated similar fold increases in resistance with or without ATP. Thus, these data demonstrated that S68Δ resulted in resistance to several clinically important nucleoside analogs but remained susceptible to ZDV, thus supporting the previously published cell-based studies (6, 9, 15, 40).

Computational analysis of S68 and K65 structural relations. Comparative analyses of WT and K65R RT experimental complexes suggest a potential mechanism of drug interaction and resistance that is structurally mediated through residues 68 and 69. Ramachandran plotting of peptide backbone angles for the WT/TTP and WT/TDP enzyme/ligand complexes (1RTD and 1T05) highlight S68 as the most strained residue in phi/psi space, followed by M184 and T69 in both (data not shown). The same type of plot comparing WT/TDP (1T05) to the K65R/TDP complex, published recently by the same lab (3JSM) (4), reveals a marked movement of S68 to the Ramachandran favored region accompanying the K65R mutation (Fig. 4a and c). Molecular representations of the experimental structures illustrate the S68 residue in an extended solvent exposed geometry in the WT to one less strained in the K65 mutant (Fig. 4b and d).

While theoretical modeling of side-chain mutations is often extended from existing protein structural backbones and discussed in terms of ligand interactions, deletion or insertion modeling requires independent analysis of flexible protein space. The UCSF Modeler software was used to test possible loop conformations available to an S68Δ mutation with or without an accompanying K65R mutation (7). The models for S68Δ and S68Δ/K65R gave lowest overall RMSD from the experimental references based upon the K65R geometry.

**DISCUSSION**

Deletions occur <0.2% within the β3-β4 hairpin loop-coding region of HIV-1-infected subjects treated with NRTI, and they appear most likely to emerge in viruses from subjects with extensive histories of antiretroviral therapy (27, 33, 42). These deletions in the HIV-1 RT have been associated with resistance to NRTI when they appear in combination with other mutations in the RT-coding region. For instance, deletions that occur in the RT region at codons 67 and 70 have been known to occur in combination with mutation T69G or Q151M (16, 21).

In the present study, S68Δ was found to be independent of any other reported mutations and/or deletions in the HIV-1 β3-β4 loop (Table 2). Sequences used for comparison to S68Δ were generated from clinical samples that have undergone multiple drug treatments for HIV-1 (2, 10, 20, 21, 42, 44). The presence of a novel deletion in position 68 of the HIV-1 RT genome was selected when the infected cells were exposed to DFC. The presence of the S68Δ was confirmed in vitro using TOPO cloning and pyrosequencing.

These data establish, for the first time, that S68Δ can be detected in vitro under single drug pressure. In contrast to previous reports where an S68G mutation appeared in subjects

**TABLE 3. Frequency of polymorphisms detected in supernatant from DFC-treated human PBM cells using ultradeep pyrosequencing**

<table>
<thead>
<tr>
<th>Wk</th>
<th>Total no. of polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;0.5%</td>
</tr>
<tr>
<td>14</td>
<td>72</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>23</td>
<td>64</td>
</tr>
<tr>
<td>52</td>
<td>62</td>
</tr>
</tbody>
</table>

**TABLE 4. Comparison of the population frequencies of S68Δ and K65R obtained with TOPO cloning and pyrosequencing**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Population frequency (%) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 14</td>
</tr>
<tr>
<td>S68Δ TOPO cloning</td>
<td>70.0</td>
</tr>
<tr>
<td>S68Δ pyrosequencing</td>
<td>6.0</td>
</tr>
<tr>
<td>K65R TOPO cloning</td>
<td>5.0</td>
</tr>
<tr>
<td>K65R pyrosequencing</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Direct comparison revealed 2- to 10-fold differences in the population frequencies for virus obtained on weeks 14 and 20 and slight difference by weeks 23 and 52. These differences may be due to an increase in the percentage of new polymorphisms in the early weeks and more error-prone (5 to 10 errors/kb) than those obtained from Sanger sequencing (0.01 errors/kb). The K65R and S68Δ haplotypes were as previously reported (19, 46).
that already had K65R (32), the present study demonstrates that the S68Δ can appear independent of and before K65R (Table 1). The mechanism by which the S68Δ virus confers a decrease in DFC efficiency is largely unknown. According to the enzymatic studies, S68Δ RT showed similar resistance to NRTI-TP (ZDV-TP, FTC-TP, and DFC-TP) with or without ATP. These data suggest that the mechanism of S68Δ resistance is ATP independent and most likely occurs by enhancing substrate discrimination.

The serine deletion in position 68 is located in the fingers subdomain of the HIV-1 RT, between residues that make important contacts with the incoming dNTP. This region helps position, bind, and activate the incoming dNTP for catalytic incorporation into the growing DNA chain during polymerization (18). Our models suggest S68Δ alone or in combination with K65R could impair the dNTP incorporation through repositioning of charge groups above the plane of catalysis defined by the bound triphosphate. As a consequence, virus replication rate could decrease overall, with selective resistance to certain chemically related nucleoside analogs.

Mutations at position 65 in HIV RT are known to influence the nucleotide biding specificity of the enzyme (4, 8, 47). Furthermore, a new resistance profile, which includes K65R with S68G, was detected in persons failing ABC, ddI, and d4T treatment. The authors of those studies suggested that the presence of the S68G mutation might increase the replicative capacity of the mutant virus K65R (32, 41). Thus, these findings underscore the potential relevance of the S68Δ on virus replication, which developed resistance not only against DFC but also against other important NRTIs involved in the HIV-1 therapy.

According to previous reports, mutations that occur in the HIV-1 RT region between amino acids 62 and 78 significantly increase NRTI resistance and restore the replication defect associated with the K65R mutation (16, 41). After evaluation of HIV_S68Δ-23 against 13 NRTIs, it was found that this virus developed >5-fold increased resistance (FI50) in the presence of DFC, DAPD, 3TC, (−)-FTC, TDF, DXG, and ABC, indicating that the S68Δ in the HIV-1 genome has potential clinical relevance and should be monitored in HIV-infected persons.

Early structural studies of resistance mutations effecting nucleoside analogs relied upon atomic coordinates of HIV-RT derived from one of two relevant experimental structures, 1R7D and 1T05 (18, 43). These contain the ternary WT protein complexed with template-primer and phosphorylated 2′-deoxynucleotide dNTP and TDP, respectively (18, 43). Our study benefits from two recent crystallographic sets of the resistant mutant K65R-RT ternary complex with TDP and dATP as ligands (4). Evaluation of protein geometries across these four experimental structures reveals a structural anomaly at S68/D69 (Fig. 4). The S68/D69 protein backbone is highly strained in the WT structures, which appears pinned in that conformation by the tight pairing of charge-localized K65 to beta/gamma phosphate oxygens (18, 43).

To minimize bias from manual model building, the UCSF Modeller algorithm was used to build more than 60 models of WT, S68Δ, K65R, and the S68Δ/K65R double-mutant models for further analysis (see Materials and Methods for details).

According to our models, resistance to S68Δ/K65R may be explained through a mechanism repositioning catalytic residues through loop dynamics. The least-strained loop conformation formed by deletion of S68 was found to be closer to K65R than WT structures. These findings suggest that S68Δ and K65R may exist compatibly on the same genome, but we expected that the double mutation may be even less fit than single mutations. Since both S68Δ and K65R effect binding “above” the catalytic plane defined by the incoming triphosphate, substrates with substitutions below the plane, such as natural 3′-OH, azido would be preferred for incorporation over ddNTP, nonsubstituted 1-nucleoside, or acyclic analogs. The spring-like strain placed in loop β3-β4 by residue S68 in relation to placement of K65 may increase processivity and promiscuity of substrates in WT HIV-1RT versus either mutant. Conversely, the rearranged backbone conformation needed to accommodate either S68Δ or K65R substitutions diminish loop dynamics and promote side chain placements.
that increase substrate selectivity while reducing overall enzyme efficiency.

In conclusion, the present study reveals the significance of a novel deletion (S68Δ/H9004) in the hairpin loop of HIV-1 RT that may have potential clinical relevance when it occurs alone or in combination with the K65R mutation.

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service grants SR37-AI-0419801 and SP30-AI-50409 (CFAR) and by the Department of Veterans Affairs. Computational modeling was performed with assistance of the Emory School of Medicine Biomolecular Computing Resource (BIMCORE). Hardware and software was supported in part through an Academic Excellence Grant from Sun Microsystems and an Academic Research Award from Accelrys Corp.

R.F.S. is the inventor of DFC, (−)-FTC, and 3TC. The royalties for (−)-FTC were sold to Gilead Sciences and Royalty Pharma in 2005. However, R.F.S. continues to receive royalties from the sales of 3TC until 2011. R.F.S. is also a major shareholder in RFS Pharma, LLC, a company that currently develops Amdoxovir for HIV-1 infections.

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