CORRESPONDENCE

Highly Active Antiretroviral Therapy Failure and Protease and Reverse Transcriptase Human Immunodeficiency Virus Type 1 Gene Mutations

To the Editor—To assess the role that genotypic mutations within the protease (PR) domain play in determining treatment failure, Young et al. [1] determined the sequence of PR and reverse transcriptase (RT) genes of human immunodeficiency virus type 1 (HIV-1) isolates from 35 patients in whom highly active antiretroviral therapy (HAART) was unsuccessful. Even though the authors found an unexpectedly low number of PR mutations (range, 0–6) and observed that failure may occur with only a few mutations, they emphasized the importance of patient drug exposure. Unquestionably, compliance and pharmacologic factors are important in determining the efficacy of any antiretroviral regimen, but they should not be considered the sole cause of treatment failure.

For this study, we selected 8 severely immunodeficient (median CD4+ cell count, 107) patients whose treatment had failed (as defined by a rise in plasma virus load or a decrease of ≤1 log10). The patients had received a combination regimen including a PR inhibitor, and their therapy was directly supervised by a responsible family member, leaving no doubt of treatment adherence. Most patients had received multiple nucleoside RT inhibitors for >24 months, either as monotherapy or as combination therapy, and at least two PR inhibitors (median duration, 11.5 months; range, 6–18). The sequences of plasma-derived PR- and RT-coding regions were determined with a 373 sequencer (Perkin-Elmer Cetus, Norwalk, CT) and compared by use of Factura software (BCM Search Launcher; Baylor College of Medicine, Houston) with the reference HXB2±HIV-1.

At the time of plasma collection, all but 2 patients were receiving combination therapy with stavudine, lamivudine, and indinavir (or nelfinavir [1 patient]); the mutations detected in the PR and RT genes are listed in table 1. Mutations were categorized as either “primary” (with discernible effect on the drug susceptibility of the virus) or “secondary” [2, 3]. The primary mutations associated with PR inhibitor resistance were V82A (63% of patients), L90M (50% of patients), M46I/L (25% of patients), and G48V (12.5% of patients). Secondary mutations mainly included L63P (100% of patients) and L10I and I54V (63% each of patients). Except for 2 patients in whom L63P was detected as a single mutation, the remaining 6 patients presented a variable pattern with up to 7 mutations; however, in agreement with published reports [4, 5], we estimated that only a modest (0- to 30-fold) susceptibility decrease resulted in most patients and that only 2 patients (nos. 7 and 8) had a higher reduction of PR inhibitor susceptibility. Multiple RT mutations associated with lamivudine resistance were detected in all patients, only 1 of whom was receiving zidovudine; additional RT mutations compatible with previous RT inhibitor therapy were T69D (25% of patients) and L74V (12.5% of patients). With the current lamivudine-based regimen, 86% of patients also had mutation M184V; and mutations K101E, K103N, and V108I were detected in a patient who was receiving efavirenz.

Overall, in our experience, analysis of PR and RT genes gave results closely resembling those of Young et al. [1]. However, we do not feel that a large number of mutations are essential for establishment of PR inhibitor resistance, and because we are certain of our patients’ adherence, some comments might be appropriate. First, it appears that it is the association or interaction of mutations, not the single mutation in itself, that determines the decrease in drug resistance. Moreover, particular mutations, such as L90M, are more efficient than others, such as V82A, in determining drug resistance, and most data regarding drug resistance derive from monotherapy studies, so little is known concerning the development of phenotypic resistance to multidrug regimens. In other words, it is the quality and the association rather than the quantity of site substitutions that are crucial for treatment failures. In addition, as stated by Young et al. [1], mutations at the PR cleavage sites, which are currently not under investigation, would also augment the possibility of treatment failure.

Second, the importance that RT inhibitor resistance has for HAART failure must be stressed. In our experience, most patients currently on PR inhibitor therapy have been previously administered multiple RT inhibitors, and the effect of these previous treatments on successive options remains uncertain. Furthermore, as observed in our series, after prolonged zidovudine administration, a large number of patients will become candidates for a stavudine-based regimen, but stavudine phosphorylation is normally reduced in these circumstances [6], thus leading to development of some cellular resistance, which differs according to the individual patient. Last, the extensive use of earlier RT inhibitors, such as zidovudine and didanosine, obliges clinicians to adopt newer drugs, such as lamivudine, which selects for M184V. Although the role of this mutation is not certain in the context of a multidrug regimen, it is known that M184V as a single mutation confers high-level phenotypic resistance to lamivudine [7].

Therefore, the reasons for treatment failure are extremely complex, and each patient has an accumulation of individual factors that distinguishes him or her from other patients. The effect that single mutations have on phenotypic resistance in the context of a combination regimen should be carefully investigated.

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Table 1. Analysis of protease inhibitor (PI)- and reverse transcriptase inhibitor (RTI)-associated mutations in protease and reverse transcriptase genes in 8 patients with highly active antiretroviral therapy failure.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Antiretroviral drugs</th>
<th>Codons (protease region)</th>
<th>Codons (reverse transcriptase region)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RTIs</td>
<td>L</td>
<td>K</td>
</tr>
<tr>
<td>2</td>
<td>AZT/ddI/3TC/d4T</td>
<td>L/I</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>AZT/ddC/ddI/3TC</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>4</td>
<td>AZT/ddI/ddC/ddI/ddI</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>AZT/ddC/ddC/ddC/ddC</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>6</td>
<td>AZT/ddC/ddC/ddC/ddC</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>7</td>
<td>AZT/ddC/ddC/ddC/ddC</td>
<td>L</td>
<td>R</td>
</tr>
<tr>
<td>8</td>
<td>AZT/ddC/ddC/ddC/ddC</td>
<td>L</td>
<td>R</td>
</tr>
</tbody>
</table>

NOTE. Reference strain, HXB2. AZT, zidovudine; ddC, zalcitabine; d4T, stavudine; 3TC, lamivudine; ddI, didanosine; EFV, efavirenz; SQV, saquinavir; IDV, indinavir; NFV, nelfinavir; RTV, ritonavir. At time of sample collection, combination regimens were d4T + 3TC + IDV (patients 1–3, 5, 6), d4T + 3TC + NFV (patient 4), AZT + 3TC + RTV (patient 7), and EFV + SQV + NFV (patient 8). Patient 8 also had mutations K101E, K103N, and V108I; and mutations at codons 211 and 214 in RT region were detected in 63% and 75% of patients, respectively.
To the Editor

Monno et al. suggest that prior treatment with zidovudine might have led to reduced phosphorylation of stavudine, which most of their patients received. The affect of prior zidovudine treatment on stavudine phosphorylation remains controversial. Recent data strongly suggest that prior zidovudine experience does not result in any significant difference in the levels of stavudine triphosphate and hence may not be a significant factor in determining the virologic response to stavudine [8].

The basis for therapy failure is clearly multifactorial. We agree that it is the specific pattern of resistance mutations rather than their number that determines the efficacy of drug therapy. The role of drug-resistance mutations in determining failure of antiretroviral therapy should be considered in the context of the entire sequence of the PR and RT genes and the sequences of the substrate gag-pol polyprotein cleavage sites. The relative contribution of individual mutations in determining viral phenotype remains to be fully elucidated. Analysis of the growing number of databases that combine genotypic and phenotypic data will no doubt increase our understanding of this complex subject.

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References


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Reply

To the Editor—Monno and colleagues [1] report the genetic sequences of human immunodeficiency virus type 1 (HIV-1) protease (PR) and reverse transcriptase (RT) from 8 patients for whom combination antiretroviral therapy failed [1]. Their results are qualitatively similar to ours [2]. We found that mutations in both the PR and RT genes of HIV-1 were common in a cohort of 35 patients for whom combination antiretroviral therapy that included a PR inhibitor had failed.

We attempted to emphasize in our report that, even though multiple mutations may be required for high-level phenotypic resistance to PR inhibitors in vitro [3, 4], failure of combination therapy was frequently associated with a small number of mutations in the PR gene. Thus, we are in agreement with Monno et al. [1], who state that a large number of mutations may not be necessary for PR inhibitor resistance. We agree also that resistance to RT inhibitors, particularly lamivudine and the nonnucleoside RT inhibitors, is an important factor in the failure of combination regimens, as demonstrated recently by several groups [5–7]. Although we suggested that problems with treatment adherence and pharmacologic factors might play a role in determining treatment outcome (particularly in patients for whom therapy failed without evidence of PR inhibitor resistance), we did not mean to imply that these factors were the sole causes of treatment failure.

Monno et al. suggest that prior treatment with zidovudine might have led to reduced phosphorylation of stavudine, which most of their patients received. The affect of prior zidovudine treatment on stavudine phosphorylation remains controversial. Recent data strongly suggest that prior zidovudine experience does not result in any significant difference in the levels of stavudine triphosphate and hence may not be a significant factor in determining the virologic response to stavudine [8].

The basis for therapy failure is clearly multifactorial. We agree that it is the specific pattern of resistance mutations rather than their number that determines the efficacy of drug therapy. The role of drug-resistance mutations in determining failure of antiretroviral therapy should be considered in the context of the entire sequence of the PR and RT genes and the sequences of the substrate gag-pol polyprotein cleavage sites. The relative contribution of individual mutations in determining viral phenotype remains to be fully elucidated. Analysis of the growing number of databases that combine genotypic and phenotypic data will no doubt increase our understanding of this complex subject.

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References

6. Holder DJ, Condra JH, Schleif WA, et al. Virologic failure during combination antiretroviral therapy should be considered in the context of the entire sequence of the PR and RT genes and the sequences of the substrate gag-pol polyprotein cleavage sites. The relative contribution of individual mutations in determining viral phenotype remains to be fully elucidated. Analysis of the growing number of databases that combine genotypic and phenotypic data will no doubt increase our understanding of this complex subject.