Evolution of Human Immunodeficiency Virus Type 1 Populations after Resumption of Therapy following Treatment Interruption and Shift in Resistance Genotype

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Conventional genotyping of human immunodeficiency virus type 1 often reveals a shift from a drug-resistant genotype to a wild-type genotype after treatment interruption. A real-time polymerase chain reaction–based technique was used to detect minority resistant populations in 13 patients who showed genotype reversion after interruption of treatment for 3 months. Sixty-two percent of patients in whom the V82A and L90M protease mutations were no longer detectable by conventional genotyping still harbored minority resistant variants, in proportions ranging from 0.1% to 21%. None of the patients with these minority resistant variants who received a protease-inhibitor regimen on resumption of therapy had a response to treatment. However, population sequencing and clonal analysis of plasma samples obtained 1–2 months after resumption of treatment revealed the presence of wild-type virus during the initial decline in plasma virus load, which indicates that minority resistant variants were not rapidly selected.

Strategies that involve the interruption of treatment in patients with human immunodeficiency virus (HIV) type 1 infection who have virologic failure of antiretroviral therapy have been suggested as a means of improving outcomes when treatment options are limited. Because drug-resistant variants have a lower replication capacity in vivo in the absence of drug selection pressure than do wild-type viruses [1–4], it has been postulated that changes in the genotypic drug-resistance pattern that occur after a short interruption in antiretroviral therapy may lead to improvements in control of HIV-1 replication and, therefore, result in durable benefits. Several groups have observed a shift from a drug-resistant to a wild-type circulating virus population after interruption of treatment in patients who previously had received many failing therapies [5–9]. However, some concern exists that the pre-interruption, drug-resistant virus strain will reemerge when therapy is resumed, as a result of drug selection pressure exerted on persistent minority species in plasma and lymphocytes.

We used an ultrasensitive real-time polymerase chain reaction (PCR) to detect minority resistant populations in the plasma of patients who had genotype reversion, as determined by population-based sequencing, after treatment interruption and to assess the influence of these strains on the virus response after treatment was resumed.

Patients, Materials, and Methods

Patients. Twenty-one HIV-1-infected patients who had genotype reversion after 3 months of treatment interruption following receipt of multiple failing regimens were selected for study [7]. None of the primary mutations associated with resistance to reverse-transcriptase (RT) and protease (PR) inhibitors were detected after treatment had been interrupted. Patients received a new salvage regimen that consisted of 3–5 drugs selected according to each patient’s treatment history.

CD4+ T lymphocyte counts. CD4+ T lymphocytes in peripheral blood were counted by flow cytometry (Epics Profile; Coulter), using commercially available monoclonal antibodies (Becton Dickinson).

Plasma HIV-1 RNA. The level of HIV-1 RNA in plasma was measured using the Amplicor HIV-1 Monitor version 1.5 PCR assay (Roche Diagnostics) according to the manufacturer’s instructions.

Selective real-time PCR. Virus populations that included the V82A and L90M mutations, which confer resistance to PR inhibitors, were quantified by real-time PCR on an ABI 7700 Sequence Detection System (Applied Biosystems), as described elsewhere [10]. The limits of detection for the V82A and L90M mutations in the PR gene were 1% and 0.05%.

Sequencing of the HIV-1 pol gene. Nucleic acid isolation and PCR amplification of the HIV-1 RT and PR regions were performed as described elsewhere [7]. PCR products from several samples were cloned with a TA cloning kit (Invitrogen BV). Recombinant
plasmids were used to transform competent *Escherichia coli* cells, according to the manufacturer’s protocol, and transformants were grown on ampicillin plates. A total of 18–22 clones from the PCR products of each region were selected. Plasmid DNA containing RT or PR inserts was prepared and sequenced on both strands. Nucleotide sequences are available from GenBank (accession numbers AF264307–AF264345, AF264383–AF264421, AF444877–AF445024, and AF449896–AF449903).

**Statistical analysis.** Patients who had plasma HIV-1 RNA levels <200 copies/mL at months 3 and 6 after treatment resumption were considered to be “responders.” Patients who had plasma HIV-1 RNA levels <200 copies/mL at month 3 but experienced a virus rebound (i.e., return to a plasma HIV-1 RNA level ≥200 copies/mL) between month 3 and month 6 were considered to be “transient responders.” Patients who had plasma HIV-1 RNA levels ≥200 HIV-1 RNA copies/mL at months 3 and 6 were considered to be “nonresponders.”

Quantitative variables were analyzed with the Mann-Whitney U test for group comparisons and the Wilcoxon test for paired values. Qualitative variables were analyzed with the χ² test or Fisher’s exact probability test. *P < .05* was considered to be statistically significant.

**Results**

**Patient characteristics.** According to the CDC adult AIDS classification system [11], 10 patients were at stage A, 4 patients were at stage B, and 7 patients were at stage C of infection. The median CD4⁺ cell count before the treatment interruption was 269 × 10⁶ cells/L, and the median plasma HIV-1 RNA load was 4.4 log copies/mL. The median number of antiretroviral drugs that had been prescribed before the treatment interruption was 7. Ten (48%) of 21 patients had received nonnucleoside RT inhibitors. The median duration of the most recent failing treatment regimen was 10 months. No opportunistic complications were seen during the treatment interruption. The median change in plasma HIV-1 RNA load was +0.6 log copies/mL, and the median change in CD4⁺ cell count was −81 × 10⁶ cells/L.

When treatment was resumed, 3–5 drugs were selected by a physician who was blind to the results of genotypic resistance testing. Eight patients were given a regimen that included a PR inhibitor, and 13 patients were given a new salvage regimen that did not include a PR inhibitor. Seven (33%) of 21 patients were responders, 4 patients (19%) were transient responders, and 10 patients (48%) were nonresponders. The median change in CD4⁺ cell count after resumption of therapy, measured from the end of the treatment interruption, was +51 × 10⁶ cells/L at month 6. In 2 responders, 1 transient responder, and 7 nonresponders, CD4⁺ cell counts did not return to preinterruption levels within 6 months after resumption of therapy.

**Minority resistant populations.** Because only virus species representing >20% of the total population can be detected using conventional genotyping, an ultrasensitive real-time PCR procedure was developed for measuring the proportion in plasma of viruses harboring the key mutations V82A and L90M, which confer resistance to PR inhibitors. Population-based sequencing analysis detected at least 1 of these mutations in 13 patients before the interruption of therapy: 4 patients harbored strains with both mutations, 5 patients harbored strains with the V82A mutation, and 4 patients harbored strains with the L90M mutation. Real-time PCR analysis after treatment interruption revealed minority resistant populations in the plasma of 8 (62%) of those 13 patients. The proportions of residual V82A mutation (<1%–20.7%) did not differ significantly from the proportions of residual L90M mutation (<0.05%–13%).

Five patients with strains that harbored the V82A and/or the L90M PR mutation before treatment interruption were given a PR inhibitor–based regimen. Four patients had minority resistant virus populations harboring these mutations before treatment resumption; 3 were nonresponders, and 1 was a transient responder. The remaining patient, who had no minority resistant population harboring V82A or L90M PR mutations before treatment resumption, was a responder. Four patients with minority resistant variants were given non–PR inhibitor regimens; 3 of these patients were nonresponders, and 1 was a responder.

Among the total study population, responders were more likely to be in stage A of HIV infection than were nonresponders and transient responders (6 of 7 vs. 4 of 14 patients, respectively; *P = .04*) and had higher CD4⁺ cell counts at the time of treatment resumption (378 × 10⁶ vs. 142 × 10⁶ cells/L, respectively; *P = .04*).

**Kinetics of the reemergence of HIV-1–resistant populations.** The HIV-1 resistance genotype after resumption of treatment was determined by population sequencing of plasma RNA from the 10 nonresponders and the 4 transient responders. Samples were obtained at month 3 after treatment resumption for the nonresponders and at the time of virus rebound for the transient responders. The HIV-1 resistance genotype after treatment resumption was compared with the preinterruption HIV-1 resistance genotype for each patient (table 1). In all nonresponders, a particular genotype was identified as a drug-resistance genotype after therapy had failed for 3 months. Plasma RNA sequences from samples obtained before treatment interruption and 3 months after resumption of therapy were very similar at critical codons that confer resistance to RT and PR inhibitors in the majority of patients. Interestingly, major mutations that confer resistance to PR inhibitors were detected in patients 1, 2, 5, and 14 while these patients were receiving a combination of nucleoside and nonnucleoside RT inhibitors. A virus with the wild-type genotype in the RT and PR regions was identified in 2 transient responders, both of them patients for whom therapy was interrupted because of drug toxicity (patients 9 and 16).

We analyzed plasma samples obtained shortly after resumption of treatment during the initial phases of plasma HIV-1 RNA decay. Population-based sequencing analyses identified wild-type virus in 2 nonresponders (patients 3 and 15) for whom plasma samples were available 1 month after resumption of treat-
ment (data not shown). We also did clonal analysis in sequential plasma samples from 2 patients who had HIV-1 RNA levels of <200 copies/mL after 3 months: one (patient 19) had a sustained response at month 6, and the other (patient 8) had virus rebound at month 5 (figure 1). No variants harboring major mutations were associated with drug resistance 1–2 months after treatment resumption in either patient. By contrast, the resistant variants identified at the time of virus rebound in patient 8 were very similar to the resistant variants found in that patient before treatment interruption.

Table 1. Changes in amino acid sequence of the drug-resistance genotype after resumption of treatment following treatment interruption in patients who did not have a sustained response to a new antiretroviral regimen.

<table>
<thead>
<tr>
<th>Source, sample</th>
<th>Regimen</th>
<th>Reverse-transcriptase codons</th>
<th>Protease codons</th>
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<td>Referencea</td>
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<td>Nonrespondersb</td>
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Discussion

We used a technique based on real-time PCR to determine the extent to which minority resistant viruses persist in the plasma of patients who have genotype reversion, as determined by population-based sequencing analysis, and whether these minority resistant populations influence the virus response after treatment resumption.

The search for minority resistant populations by real-time PCR focused on the PR mutations V82A and L90M, which are key mutations that confer resistance to PR inhibitors [12]. We found that 62% of patients in whom the V82A and L90M mutations were no longer detectable by population-based sequencing analysis after treatment interruption of 3 months still harbored minority resistant variants in proportions ranging from 0.1% to 21%. This could be the result of the continuous residual replication of sufficiently fit resistant variants that have persisted despite wild-type virus production or of discrete cell proximal activation in patients who harbor many resistant variants in infected cells. Our data show that there were no responders among patients with minority resistant populations who harbored the V82A or L90M mutations and who received PR inhibitor–based regimens on resumption of treatment. However, analysis of the influence of minority resistant populations on the virus response is complicated by the fact that only a small number of patients were reexposed to PR inhibitors and that minority resistant populations harboring critical mutations in the PR or RT regions distinct from the V82A and L90M mutations were not detected.

An important finding of this study is the emergence, after treatment resumption, of strains harboring resistance mutations that were not specific to the new salvage regimen. Indeed, we found major mutations conferring resistance to PR inhibitors in 4 nonresponders after 3 months of treatment resumption, in the absence of PR inhibitor pressure. Laboratory contamination was excluded by genetic analyses and duplicate experiments. There was no evidence of selection of resistant viruses 1–2 months after treatment resumption. It was also reported, in another study, that initiation of a salvage regimen lowered the rate of detection of the majority of HIV-1 mutations associated with drug resistance [13]. Resistant variants could reemerge, once therapy is reintroduced, by activation of cell reservoirs of HIV-1 that bear resistance mutations, when the virus replication is not sufficiently suppressed by the new drugs. This view is supported by the finding of similar patterns of mutations in RNA sequences before treatment interruption and after treatment resumption in most patients.

The shift of HIV-1 populations from drug-resistant viruses to predominantly wild-type viruses after a short interruption in treatment may improve the efficacy of a newly introduced salvage
regimen. Although minority resistant populations were detected by a sensitive PCR technique in the plasma of our patients after 3 months of treatment interruption, the overall response rates were 11 of 21 patients after 3 months and 7 of 21 patients after 6 months. Several investigators have shown that continuation of antiretroviral therapy in patients with persistently elevated plasma HIV-1 RNA may be associated with persistent improvements in CD4+ cell counts [14, 15]. However, a recent study also suggested that full suppression of plasma HIV-1 RNA is needed to achieve an optimal CD4+ cell count response to potent antiretroviral therapy [16]. Randomized trials are needed to assess the clinical benefit of treatment interruption for patients who have failed multiple antiretroviral regimens and for whom treatment options are limited.

In summary, we have shown that minority resistant populations persisted in patients in whom the virus population shifted from a drug-resistant genotype to a wild-type genotype, as determined by population-based sequencing analysis, after treatment interruption. These minority resistant variants were not rapidly selected after a new regimen was instituted. Overall, one-third of patients who had a wild-type genotype after treatment interruption, as determined by population-based sequencing, had a sustained response to the newly introduced treatment regimen.

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References