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Comparison of Human Immunodeficiency Virus Type 1 (HIV-1) Protease Mutations in HIV-1 Genomes Detected in Plasma and in Peripheral Blood Mononuclear Cells from Patients Receiving Combination Drug Therapy

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Detections of mutations in the protease gene of human immunodeficiency virus type 1 in plasma and peripheral blood mononuclear cells (PBMC) were sought in two matched populations of 23 individuals receiving combination drug therapy with or without protease inhibitors. In the control group (23 patients not receiving protease inhibitors), no primary resistance mutations were found. In contrast, primary resistance mutations (especially at codons M46, V82, and L90) were found in 16 of 23 patients (70%) treated with protease inhibitors. In 30% of the cases, these mutations were detected in plasma but not in PBMC.

Replication of drug-resistant human immunodeficiency virus type 1 (HIV-1) during multidrug therapy may be a major cause of treatment failure (1). In this respect, detection of resistance-associated mutations in HIV-1 genomes from treated patients is receiving increasing attention. As pointed out by D'Aquila (3), if all mutations that confer resistance to antiretroviral drugs were identified and all possible interactive effects of the different mutations were catalogued, characterization of viral genotypes at all relevant positions would be sufficient for determining the nature and magnitude of resistance phenotypes. Routine identification of these mutations should ideally be based on analysis of reverse transcriptase (RT) and protease gene sequences, i.e., without culture and/or cloning assays (10). The material to be sequenced can thus be amplified from peripheral blood mononuclear cells (PBMC) or, alternatively, from plasma after amplification of HIV-1 RNA by RT-PCR. According to recent reports (6, 7, 9, 13), mutations conferring resistance to zidovudine (e.g., codon 215) can be detected in plasma several months before their occurrence in PBMC. Similarly, envelope genotypic variants may be present in plasma HIV-1 RNA and not in PBMC HIV-1 DNA (14). The underlying idea is that the HIV-1 DNA sequences found in circulating PBMC from infected patients are those that were present earlier in plasma virus populations (3). However, it should be noted that these studies were performed with patients receiving monotherapy. Thus, the influence of other antiretroviral drugs on the emergence (and possibly reversion) of specific resistance mutations has not been determined in detail. Moreover, most of these studies were based on quantitative point mutation assays (6) and not on direct sequencing of the *pol* gene. Finally, comparison of the resistance mutation patterns in plasma and PBMC has not been performed for the protease gene.

We therefore studied the detection of mutations in the protease gene of HIV-1 in plasma and PBMC of 23 individuals receiving combination drug therapy including at least one protease inhibitor (group 1). The data were compared with a matched population of 23 subjects not receiving protease inhibitors (group 2). The mean HIV-1 plasma viral loads were 5.08 $\log_{10}$ (range, 2.59 to 6.42 $\log_{10}$) and 5.55 $\log_{10}$ (range, 2.90 to 7.88 $\log_{10}$) for groups 1 and 2, respectively. Genomic DNA was extracted from PBMC with the QIAamp tissue kit (Qiagen, Courtaboeuf, France). Plasma HIV-1 RNA was purified with the QIAamp viral RNA kit (Qiagen) and amplified with the RT SuperScript RNase H (Gibco) by using primer 3′e-prB. HIV-1 DNA and cDNA were amplified by two rounds of nested PCR with *Taq* DNA polymerase and supplied buffer (Boehringer Mannheim). The first round was performed with primers 3′e-prB and 5′e-prB. The second round was performed with the product of the first PCR round with primers 3′prB and 5′prB. The material was amplified with a model 9600 thermocycler (Perkin-Elmer). The sequences of the primers used are as follows: 3′e-prB, TTTTGGGCCATCCATTC CTGGCTT; 3′prB, ACTGGTACAGTTTCAATAGG; 5′e-prB, AGAGCTTCAGGTCTGGGG; 5′prB, GAAGCAGGA GCCGATAGACA (4). The purified PCR products were sequenced with primers 3′pb and 5′pb with the ABI PRISM dye terminator cycle sequencing kit with Ampli*Taq* DNA polymerase FS (Applied Biosystems) and were analyzed with the Applied Biosystems 377 automatic sequencing system. The sequences were aligned on the HXB2 protease gene with Sequence Navigator software (Applied Biosystems). The entire HIV-1 protease gene was directly sequenced from the PCR products to minimize the introduction of artifacts resulting from culture and/or cloning (2). The use of fluorescent dye terminator cycle sequencing (12) allowed the detection of single mutations and was also effective in the detection of mixed viral populations representing at least 10 to 20% of the total genomes, as previously reported for different sequencing strategies (8). Only mutations resulting in an amino acid change were considered.

As recently recommended by the International AIDS Society—USA panel, "primary" mutations conferring drug resistance by themselves were distinguished from "secondary"

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TABLE 1. Primary and secondary resistance mutations in the protease gene based on differences in the nucleotide sequences obtained from plasma and PBMC

Patient	Treatment[a]	Sample	Mutation(s) associated with resistance to protease inhibitors[b]															
			Primary				Secondary											
			M46	G48	V82	L90	L10	K20	L24	L33	I47	I54	L63	A71	V77	I84	N88	I93
P1	ddC + d4t + IDV	PBMC				L/M				I			P	V				L
		Plasma								I			P	V		V		L
P2	ZDV + 3TC + SQV	PBMC																
		Plasma												A/I				
P3	3TC + d4t + SQV	PBMC						K/R					P					
		Plasma						K/R					P	T				
P4	ZDV + 3TC + RTV	PBMC					I						P					L
		Plasma			A		I						P			I/V		L
P5	ZDV + 3TC + NELFI	PBMC											P	V	I			L
		Plasma	I										P	V	I		N/D	L
P6	3TC + d4t + IDV	PBMC											P	A/V				L
		Plasma	I				L/R						P	V				L
P7	ddI + d4t + IDV	PBMC						K/M					V/A		I			
		Plasma	I			L/M		I	I/L				V		I			
P8	ddI + 3TC + SQV + RTV	PBMC											P					
		Plasma	I	V/G	A								P			V		
P9	ddI + d4t + IDV	PBMC																L
		Plasma	L		A		I		I									L
P10	d4t + 3TC + IDV	PBMC											P	V/A	I			
		Plasma			A		I					V	P	V	I			
P11	ZDV + 3TC + RTV	PBMC	M/I		A/V	M/L						I/V	P	V/A	I			L
		Plasma	I		A	M					I/L	V	P	V	I			L
P12	ZDV + 3TC + IDV	PBMC	I/M				I/L	I/T					P	T/A			S/G	
		Plasma	I				I/L	I					P	T			S	

[a] Abbreviations: ddC, dideoxycytosine; d4t, stavudine; IDV, indinavir; ZDV, zidovudine; 3TC, lamivudine; SQV, saquinavir; NELFI, nelfinavir; ddI, dideoxyinosine; RTV, ritonavir.

[b] Mutations are indicated as amino acid changes. When mixed genomes are detected, the amino acids found are indicated and separated with a slash (e.g., L/M for patient P1 at position L90).

mutations that could improve the fitness of virus containing primary mutations (5). For the protease gene, the main primary mutations conferring resistance to antiprotease drugs are M46I/L and/or V82A/F/T for indinavir, V82A for ritonavir, G48V and/or L90M for saquinavir, and D30N for nelfinavir (5). For group 1, i.e., individuals receiving combination therapy with at least one protease inhibitor, primary resistance mutations were detected in 16 cases (70%). In this group, the sequence of the protease gene was the same in PBMC and in plasma for 11 patients (48%). In the 12 remaining patients of this group (52%), differences were detected between the nucleotide sequences from PBMC and those from plasma samples (Table 1). In three cases (patients P2, P3, and P11), the differences consisted of only secondary mutations that were detected in plasma but not in PBMC. In the case of patient P12, the sequence in PBMC revealed a mixture of wild-type and mutated codons, whereas most of these codons were found mutated in the plasma sample. Most important, in a subgroup of seven patients, primary mutations were detected in plasma genomes exclusively (patients P4 to P10). For instance, a mutation at codon 46, which is associated with resistance to indinavir (5), was detected in the plasma samples of five patients (Table 1). A mutation at codon 82, associated with resistance to ritonavir and/or indinavir (5), was detected in the plasma samples of four patients. Interestingly, the mutation D30N, associated with resistance to nelfinavir, was not detected in our study, probably because only two patients were treated with this inhibitor. Finally, in only one case (codon 90 of patient P1) was a mutation detected in PBMC (L90L/M) but not in plasma (L90).

Primary mutations were not detected in the control group of 23 patients (group 2) that did not receive protease inhibitors. The number of secondary mutations varied from 0 (two patients) to 4 (three patients), in agreement with the high polymorphism rate of the protease gene (11). In all cases but one, the same polymorphism mutations were detected in PBMC and plasma (data not shown). Finally, in one patient, a minor mutation was detected in PBMC (V77V/I) and not in plasma. These data show that HIV-1 genomes with primary mutations are selected during treatment with protease inhibitors, consistent with their effects on virus drug susceptibility.

Overall, the information obtained through the sequencing of

plasma HIV-1 RNA allowed, for 7 of 23 patients (30%) receiving protease inhibitors, identification of primary mutations that were not detected in HIV-1 DNA from PBMC and could reflect selection of viruses resistant to specific antiprotease drugs (5). For the other patients in group 1 of this study, i.e., 16 of 23 (70%), HIV-1 plasma RNA sequences did not provide additional information that would have been missed by sequencing of HIV-1 DNA alone.

These data show that primary resistance mutations not found in PBMC were detected in plasma viruses in about one-third of the cases in this study. The significance of these mutations being found exclusively in plasma genomes remains to be established. Nevertheless, sequencing of HIV-1 plasma RNA may be useful in cases of therapeutic escape and/or failure, especially when resistance mutations in the protease gene cannot be detected in PBMC.

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