

Antiretroviral Resistance Mutations in Human Immunodeficiency Virus Type 1 Reverse Transcriptase and Protease from Paired Cerebrospinal Fluid and Plasma Samples

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Twenty-four adults infected with human immunodeficiency virus type 1 (HIV-1) with central nervous system symptoms were studied for antiretroviral resistance mutations in HIV-1 RNA obtained from paired cerebrospinal fluid (CSF) and plasma samples. Paired sequences were obtained from 21 and 13 patients for reverse transcriptase (RT) and for protease, respectively. Mutations conferring resistance to the RT inhibitors zidovudine, lamivudine, or nevirapine were detected in 14 patients, including 11 pretreated and 3 drug-naïve subjects. The mutation patterns in the 2 compartments were different in most patients. Genotypic resistance to protease inhibitors was detected in both plasma and CSF from 1 patient treated with multiple protease inhibitors. However, accessory protease inhibitor resistance mutations at polymorphic sites were different in plasma and CSF in several patients. Partially independent evolution of viral quasiespecies occurs in plasma and CSF, raising the possibility that compartmentalization of drug resistance may affect response to antiretroviral treatment.

Establishment of chronic human immunodeficiency virus type 1 (HIV-1) infection in the central nervous system (CNS) occurs early and eventually leads to AIDS dementia complex in up to 20% of untreated individuals [1]. High levels of HIV-1 RNA in the cerebrospinal fluid (CSF) correlate with the presence and degree of cognitive impairment and neuropathological abnormalities [2].

Functional and genetic studies have shown that HIV-1 isolates obtained from the brain or CSF may represent a special group of viruses and evolve separately because of tissue-specific selective forces [3]. Sequencing studies have shown that genome regions encoding for the gp120 envelope protein V3 domain [4] and for reverse transcriptase (RT) [5] derived from the CNS differ from those obtained from blood or lymph nodes for the same patients. Furthermore, a lack of correlation between CSF

and plasma HIV-1 RNA levels has been reported in patients not undergoing antiretroviral therapy [6, 7].

Antiretroviral treatment may differently affect HIV-1 replication in the CNS and in the peripheral blood because of specific virological and pharmacological factors. Indeed, discordant changes in peripheral blood and CSF HIV-1 RNA levels have been reported in response to antiretroviral therapy [7, 8]. Experimental data on CNS penetration by available antiretroviral drugs are still incomplete. Although zidovudine first proved effective in the treatment of HIV-1-associated dementia [9], promising results have been reported recently for CNS penetration by other nucleoside analogues such as stavudine and lamivudine, the nonnucleoside analogue nevirapine, and the protease inhibitor indinavir [10]. It is crucial to achieve and maintain therapeutic drug levels in the CNS, because suboptimal activity may fail to suppress HIV-1 replication, thereby favoring selection of drug-resistant virus even in the presence of complete virus clearance in the systemic circulation. The same set of RT mutations previously documented in zidovudine-resistant isolates from the blood compartment have been anecdotally described in CNS-derived HIV-1 sequences, with some evidence for a different pattern between the 2 compartments [11, 12]. In addition, discordant cases in the distribution of lamivudine-resistant HIV-1 genotypes in plasma and CSF have recently been reported [13]. The present report describes HIV-1 RT and protease sequences obtained from paired CSF and plasma samples for patients under different antiretroviral regimens and patients without anti-HIV-1 therapy.

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Materials and Methods

Patients and samples. Paired CSF and plasma samples were obtained from 24 adult patients attending the Division of Infectious Diseases of the General Hospital of Careggi (Florence, Italy). Of these, 16 were under treatment with combined antiretroviral compounds, whereas 8 had never been treated (drug-naive). All of the patients had CNS symptoms at the time of sampling (table 1). Quantitation of HIV-1 RNA in plasma was done with the Amplicor HIV-1 Monitor test (Roche Molecular Systems, Branchburg, NJ) according to the manufacturer's instructions. CSF samples were shown to be free of red blood cell contamination.

Determination of HIV-1 RT and protease sequences. RNA was extracted from plasma and from CSF by use of the QIAmp Viral RNA kit (Qiagen, Hilden, Germany). Two separate regions of the HIV-1 *pol* gene, one encoding RT amino acids 30–230 and the other encoding the whole protease, were amplified by reverse transcription-nested polymerase chain reaction (PCR), and crude PCR products were then directly sequenced with infrared-labeled primers, as described elsewhere [14]. Reconstruction experiments with mixtures of titrated cloned templates showed that mixed populations are unequivocally detected, provided that they represent at least 10%–20% of the total population, depending on the bases involved in the polymorphism and the composition of the neighbor region [14]. Mutations involved in specific drug resistance were retrieved from the HIV-1 resistance database [15]. RT and protease sequences have been deposited to GenBank under accession numbers AF158749 through AF158790 and AF158791 through AF158816, respectively.

Phylogenetic analysis. Pairwise distances were computed by use of the DNADIST program contained in the Phylip software package, version 3.57 (<http://evolution.genetics.washington.edu/phylip.html>), and were calculated according to the Kimura 2-parameter model. The mean of inpatient pairwise distances between plasma and CSF sequences was compared with the mean interpatient plasma and CSF distances by use of one-way analysis of variance. Neighbor-joining phylogenetic trees were constructed by use of the DRAWTREE program contained in the same Phylip package.

Results

HIV-1 RT sequences were obtained from paired plasma and CSF samples from 21 individuals: 14 patients undergoing antiretroviral treatment and 7 drug-naive subjects. Paired HIV-1 protease sequences were obtained from 13 patients, including 3 drug-naive subjects and 2 individuals who had been pretreated with protease inhibitors. Table 1 shows CNS diagnosis, CSF white blood cell count, and HIV-1 RT and protease mutations involved in drug resistance that were detected in the 2 compartments, as well as CD4 cell counts, HIV-1 RNA load in plasma, treatment history, and duration of infection.

Zidovudine resistance mutations (M41L, D67N, K70R, L210W, T215Y, and K219Q) were detected in paired plasma and CSF samples in 11 patients. However, the pattern of mutations in the 2 compartments differed in 5 of these individuals.

In addition, there were 3 patients (nos. 3, 5, and 7) harboring a single zidovudine resistance mutation (M41L) in the CSF but not in the plasma sample analyzed. The presence of a different zidovudine resistance profile in the 2 compartments was independent of the duration of zidovudine treatment. The lamivudine resistance mutation (M184V/I) was detected in paired plasma and CSF samples from 4 patients but was also present only in plasma in patient no. 5 and only in CSF in patient no. 1. Thus, zidovudine and/or lamivudine resistance mutations were found in 3 (nos. 3, 5, and 23) of the 7 drug-naive subjects examined. Moreover, the patterns of the mutations in plasma and CSF were not identical in any of these 3 individuals. Mutations conferring resistance to nevirapine and possibly to other nonnucleoside RT inhibitors (A98G, V108I, and Y181C) were detected in paired plasma and CSF samples from 2 subjects (nos. 7 and 8), only 1 of whom had been treated with nevirapine. Finally, the H208Y mutation conferring resistance to foscarnet was found in both plasma and CSF from patient no. 8 but only in plasma from patient no. 17.

The key mutation V82S, known to confer resistance to protease inhibitors indinavir and ritonavir, was detected in 1 (patient 9) of the 2 subjects under treatment with ritonavir for whom the protease sequences were obtained. The pattern of protease and RT inhibitor resistance mutations was identical in plasma and CSF from this patient. In addition, 11 of the 13 patients analyzed harbored HIV-1 genomes with accessory mutations at protease polymorphic sites involved in restoration of viral fitness in protease inhibitor-resistant viruses. A discordance in the distribution of these mutations between plasma and CSF sequences was found in 4 patients.

The mean inpatient distance between sequences obtained from plasma and CSF was significantly lower than the mean interpatient distance among sequences derived from the same compartment ($P < .001$ for both RT and protease), as shown by the inferred phylogenetic tree (figure 1). The distance between pairwise plasma and CSF sequences did not significantly correlate with the duration of HIV-1 infection ($R^2 = 0.10$ and $R^2 = 0.02$ for RT and protease, respectively; P , NS).

Discussion

Differential evolution of drug resistance in plasma and CNS may affect long-term efficacy of therapeutic regimens. In this study population, sequencing was used to compare the pattern of drug resistance mutations in HIV RNA from paired CSF and plasma samples. Amplification of RNA sequences was unsuccessful in several cases, particularly for protease. Possible reasons for amplification failures include low HIV-1 RNA titer in CSF and greater polymorphism in the protease than in the RT region.

The mutations most frequently detected in plasma and in CSF were those conferring resistance to zidovudine, reflecting

Table 1. Characterization of the patients studied and antiretroviral resistance mutations found in human immunodeficiency virus type 1 (HIV-1) RNA obtained from paired plasma and cerebrospinal fluid (CSF) samples.

Patient, sample	Reverse transcriptase inhibitor resistance mutations ^a	Protease inhibitor resistance mutations ^a	WBC count ^b	Central nervous system diagnosis	CD4 ^c RNA ^d infection ^e	HIV-1 RNA ^d infection ^e	Month of infection ^e	Treatment history (months of treatment)
1 Plasma CSF	67N 70R 219Q 70R 184Vw	63Pw None	0	Toxoplasma encephalitis	223	4.58	77	ZDV (66), ZDV+ddI (5)
2 Plasma CSF	67N 210W 215Y 210W 215Y 219Q	63P 63P 77Iw	2	Toxoplasma encephalitis	3	5.36	107	ZDV (29), ddI (36), ZDV+3TC (7), ZDV+3TC+RTV (3)
3 Plasma CSF	None 41Lw	ND ND	19	HIV-encephalopathy	182	4.85	27	Naive
4 Plasma CSF	ND ND	None None	2	Systemic tuberculosis	34	3.88	122	ZDV (36), ZDV+ddC (12)
5 Plasma CSF	184V 41L	ND ND	20	Toxoplasma encephalitis	192	5.54	2	Naive
6 Plasma CSF	41L 210W 215Y 41L 210W 215Y	60E 36I 60E	2	Systemic lymphoma	30	5.50	47	ZDV (36) ZDV+ddI (8)
7 Plasma CSF	98G 41L 98G	63P 77Iw 63P	2	Systemic lymphoma	26	5.40	107	ZDV (48), ddI (4)
8 Plasma CSF	41L 67N 108I 181C 184I 208Y 210W 215Y 41L 67N 108I 181C 184I 208Y 210W 215Y	ND ND	7	HIV encephalopathy	40	5.68	103	ZDV (6), ddI (10), ZDV+ddC (5), ZDV+3TC (8), ZDV+3TC+SQV (12), D4T+3TC+IDV (4), SQV+RTV+D4T+NVP (2)
9 Plasma CSF	41L 215Y 41L 215Y	33F 60E 63P 71V 77I 82S 33F 60E 63P 71V 77I 82S	0	Cryptococcal meningitis	95	5.81	121	ZDV (36), ZDV+ddI (19), ZDV+3TC+RTV (4)
10 Plasma CSF	None None	ND ND	80	Cryptococcal meningitis	10	5.00	75	Naive
11 Plasma CSF	ND ND	36I 36I	2	Cryptococcal meningitis	7	5.12	10	Naive
12 Plasma CSF	None None	ND ND	4	Tuberculous meningitis	206	5.90	43	Naive

13	Plasma	ND	101 361 63P	15	Disseminated <i>M. avium-intracellulare</i> infection	59	5.64	58	ZDV (42), ZDV+ddI (12)
	CSF	ND	101w 361 63P						
14	Plasma	67N 70R 103R 184V 219Q	ND	9	Neurosyphilis	490	4.66	49	ZDV (15), ZDV+ddI (13), D4T+3TC+RTV (2), IDV+D4T+3TC (3), D4T+3TC+SQV (5), RTV+SQV+D4T+3TC (3)
	CSF	67N 70R 103R 184V 219Q	ND						
15	Plasma	None	63P	13	HIV-encephalopathy	112	5.48	2	Naive
	CSF	None	63Pw						
16	Plasma	None	63P 77I	2	Cryptococcal meningitis	282	5.18	22	ZDV (12), ddI (9)
	CSF	None	63P 77I						
17	Plasma	208Yw 210W 215Y	ND	2	HIV encephalopathy	48	4.78	86	ZDV (48), ddI (24)
	CSF	70R 210W 215Y	ND						
18	Plasma	None	ND	2	Progressive multifocal leukoencephalopathy	54	4.22	113	ZDV (45), ZDV+ddI (4)
	CSF	None	ND						
19	Plasma	None	77I	9	HIV encephalopathy	60	5.70	1	Naive
	CSF	None	77I						
20	Plasma	41L 184V 210W 215Y	ND	2	HIV encephalopathy	92	4.61	112	ZDV (36), ddC+3TC+SQV (12), 3TC+d4T+SQV (3)
	CSF	41L 184Vw 210W 215Y	ND						
21	Plasma	None	63P	12	Systemic lymphoma	81	5.78	124	ZDV+ddI (3)
	CSF	None	63P						
22	Plasma	41L	60E 63P	29	Progressive multifocal leukoencephalopathy	367	5.51	129	ZDV (72), ddI (12)
	CSF	41L	60E 63P						
23	Plasma	184V 215Y	ND	13	HIV encephalopathy	670	4.72	13	Naive
	CSF	41L 184Vw 210W	ND						
24	Plasma	67N 219Q	ND	2	Cryptococcal meningitis	77	6.57	57	ZDV (30), ddI (10), ZDV+ddI (1)
	CSF	67N 70R 219Q	ND						

NOTE. 3TC, lamivudine; ddC, zalcitabine; ddI, didanosine; d4T, stavudine; IDV, indinavir; ND, not determined; NVP, nevirapine; RTV, ritonavir; SQV, saquinavir; ZDV, zidovudine.

^a Number and uppercase letter indicate the mutant amino acid in 1-letter code at that codon number. The lowercase w indicates simultaneous presence of the mutant and wild-type amino acid. Only amino acid changes that have been shown to be involved in drug resistance are indicated.

^b White blood cells/mL.

^c CD4⁺ cells/mm³.

^d log₁₀ HIV-1 RNA copies/mL of plasma.

^e Months elapsed since documented seroconversion.

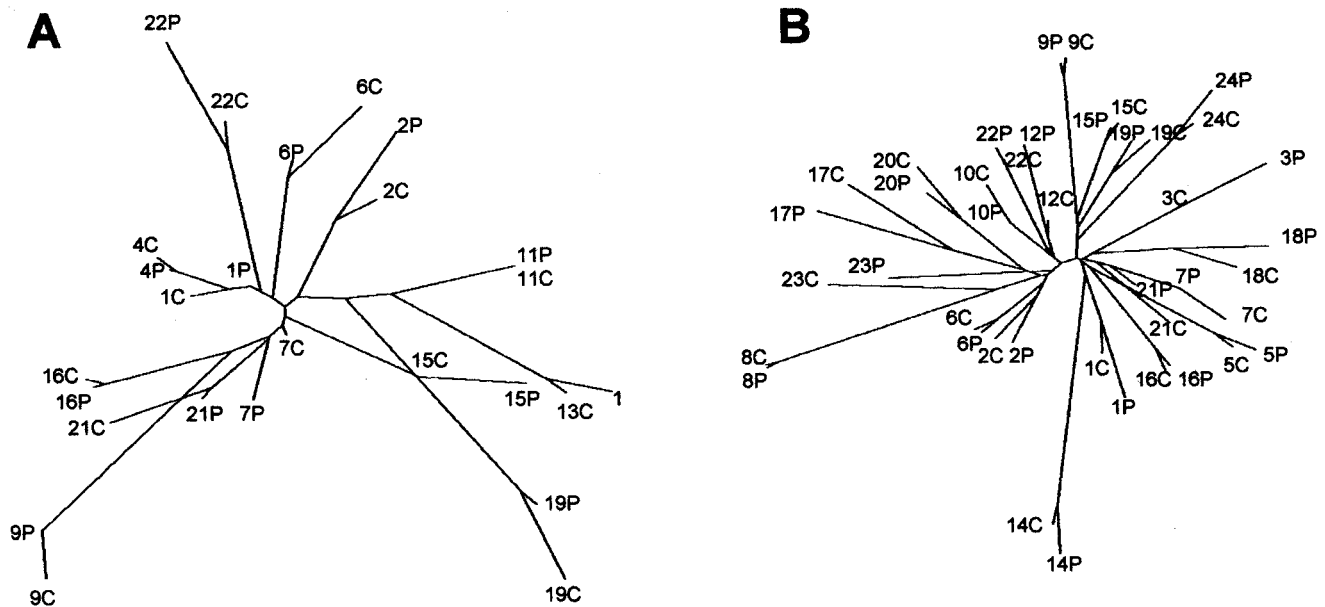


Figure 1. Phylogenetic relationship of genomic human immunodeficiency virus type 1 protease (*A*) and reverse transcriptase (*B*) nucleotide sequences obtained from paired cerebrospinal fluid and plasma samples (C and P, respectively). Each subject is represented by a pair of identical numbers. The phylogenetic tree was constructed by the neighbor-joining method (see Materials and Methods).

its extensive use and its good penetration through the blood-brain barrier (BBB). As suggested by previous works [11, 12], the pattern of resistance mutations in the 2 compartments was different in several patients, indicating independent evolution of viral quasispecies. Interestingly, zidovudine resistance mutations were detected in 3 subjects who had never been treated with zidovudine. Likewise, the M184V/I substitution conferring resistance to lamivudine was detected in 3 lamivudine-pretreated but also in 3 lamivudine-naïve patients. However, 1 of these subjects was under treatment with didanosine, which also may occasionally select for the 184V codon [15]. Resistance to lamivudine was found in only 1 compartment in this subject and in a drug-naïve individual. Although these data strengthen the notion that drug-resistant virus may be transmitted and enter the CNS, it cannot be ruled out that some of the drug-naïve patients made surreptitious use of antiretroviral compounds. The data on the distribution of the M184V RT mutation are similar to those recently reported by Chien et al. [13]. Mutations specifically conferring resistance to didanosine, zalcitabine, and stavudine were not detected in the population studied, confirming a low tendency to select for viruses resistant to these compounds in combination therapy [15]. By contrast, frank genotypic resistance to nevirapine (V108I, Y181C) was documented in both plasma and CSF from the 1 individual under nevirapine treatment, whereas another patient without nevirapine pretreatment harbored the mutation A98G, a naturally occurring polymorphism conferring decreased susceptibility to nevirapine.

Because of the limited number of subjects under treatment

and the unavailability of several protease sequences, the population analyzed did not allow conclusions about a differential evolution of resistance to protease inhibitors in plasma and CSF. Divergence of accessory mutations at polymorphic sites was demonstrated in several subjects. Only 1 patient, who was given a diagnosis of cryptococcal meningitis, was infected with a virus harboring key resistance mutations to ritonavir and indinavir. The complete RT and protease amino acid identity of the virus populations in the 2 compartments suggests that plasma and CSF viral quasispecies have significantly intermixed, making a possible differential evolution hardly observable. Although increased permeability of the BBB may explain HIV-1 population mixing, it must also be noted that brain mass lesions and meningitis may recruit T cells into the CSF, raising the possibility that CSF viral particles are, in part, released by blood-derived lymphocytes productively infected with HIV-1. Because reference parameters for adequate evaluation of BBB functionality were not available, it was not possible to assess the impact of the BBB status on the divergence of CSF and plasma sequences.

Overall, maintenance of independently evolved quasispecies was frequently observed, although some of the differences in resistance mutations between CSF and plasma may have resulted from failure to detect minority populations by the method used. However, phylogenetic analysis of the CSF and plasma population sequences indicated that inpatient evolution leads to closely related *pol* genotypes in the 2 compartments. Phylogenetic analysis of multiple clones derived from plasma and CSF for each subject should be done to detect

inpatient compartmentalization, as previously reported for HIV-1 *pol* sequences obtained from brain, spleen, and lymph nodes of infected patients [5].

In conclusion, our results indicate that plasma and CSF may harbor virus populations with different drug resistance mutation patterns. Virologic and clinical follow-up of patients with detailed characterization of drug resistance profiles in different body compartments is advisable to define a role for compartmentalization of drug resistance in the course of HIV-1 infection.

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