RESEARCH NOTE

THE EFFECTS OF ANTIRETROVIRAL DOSE MODIFICATION ON THE RE-EMERGENCE OF HIV-1 WILD-TYPE STRAINS

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Abstract. Four human immunodeficiency virus type 1 (HIV-1) treatment-naïve Thai patients began antiretrovival therapy with a triple drug regimen -zidovudine plus lamivudine plus indinavir; this regimen was modified at week 20 of therapy because of drug toxicity. The virus in all patients was suppressed to lower than 400 copies/ml while they were taking the triple antiretroviral drug regimen. However, suppression was lost after changing the antiretroviral regimen. A comparison of HIV-1 DNA sequences taken from the baseline (day 0) and week 24 showed no significant overgrowth in HIV-1 drug-resistant strains. There was no difference in the protease and reverse transcriptase (RT) mutation profiles. Resistant variants did not emerge, even after sub-therapeutic levels of antiretroviral drugs had been introduced to these patients for 4 weeks. These findings may have clinical implications for long-term treatment strategies.

Virus replication is a highly dynamic process in human immunodeficiency virus (HIV) infections (Ho, 1995; Wei *et al*, 1995). Errors of reverse transcriptase (RT) (Mansky and Temin, 1995), recombination events during viral replication, and the high rate of virus production (up to 10^{10} virions per day) (Coffin, 1995) result in multiple variants within an infected individual. There is rapid selection of adapted viruses via co-receptor selection, immune pressure, and/or effective antiviral drugs (Wei *et al*, 1995). Potent inhibitors of viral replication may reduce the amount of plasma HIV-1 RNA to levels that are undetectable even by sensitive assays. Increases in plasma RNA levels, despite continuing therapy, are often due to the replication of drug-resistant mutant viruses that are selected from the virus population by antiretroviral treatment (Ho, 1995; Loveday *et al*, 1995; Schuurman *et al*, 1995).

Little is known about the effect on viral fitness of the mutations' conferring resistance to the antiretroviral drugs. *In vitro* studies have shown that drug-resistant mutants have a lower replication capacity than the wild-type virus in the absence of drug selection pressure (Boucher *et al*, 1993; Chow *et al*, 1993). The lower replication capacity of drug-resistant mutants may also account for their low frequency in the virus populations of untreated individuals (Najera *et al*, 1994). Interruptions (total withdrawal) in the selection pressure imposed by antiretroviral treatment may delay the emergence of resistant mutations *in vivo* because the mutant viruses have a lower replication capacity than the wild-type in the absence of drugs.

This study analyzed the evolution of wild type and mutant HIV-1 in patients treated with a combination of zidovudine (ZDV), lamivudine (3TC) and indinavir (IDV) and considered viral evolution in the same patients when they had a sub-therapeutic drug levels.

Four HIV-1 infected treatment naïve Thai patients (patients 092, 103, 107 and 123) were selected from Ramathibodi Hospital, Bangkok, Thailand. The antiretroviral drug regimen was

a combination of 150 mg of ZDV and 300 mg of 3TC twice a day (bid), and 800 mg of IDV three times a day (tid). All patients adhered to this regimen for 19 to 20 weeks. Doses were then reduced because of drug toxicity: the modified regimen was 100 mg of ZDV and 150 mg of 3TC bid, and 800 mg of IDV tid for patients 092 and 103; IDV was withdrawn from the regimen for patients 107 and 123. Plasma samples were collected at baseline (day 0 before starting antiretroviral drug treatment) and at weeks 2, 4, 8, 12, 16, 24 and 36. Total HIV RNA was quantitated with the Amplicor Monitor assay; plasma samples were processed according to the manufacturer's specifications. When plasma RNA samples fell below 400 copies per ml, separate aliquots of plasma were assayed by using the Roche Ultrasensitive Assay (a detection limit of 20 copies per ml).

Reverse transcription-polymerase chain reaction (RT-PCR) of the reverse transcriptase (RT) and protease gene was performed on plasma viral RNA using the SuperScript One-step RT-PCR System (Life Technologies). Direct sequencing of the PCR product was performed using the dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA, USA). Sequencing reaction products was analyzed on an ABI 310 Genetic Analyzer (PE Biosystems). Amino acid substitutions were identified by comparison of the plasma RNA sequences with a pNL4-3 reference sequence. The sample sequences were submitted to GenBank and assigned the accession numbers AF187038-9, AF191187-8 and AF191192-5.

The patients in this study had a suppressed viral load of below log 2.6 copies/ml while taking triple-drug therapy (Table 1). Suppression was lost after changing the antiretroviral regimen. At week 24, the major population of rebound-virus was ZDV-, 3TC- and IDV-sensitive (genotypically) in all patients. Comparison of the amino acid sequence of the protease and RT genes in successive samples showed that no amino acid substitution that was with significant resistance to any antiretroviral drugs was found (Table 2). There was no significant overgrowth in HIV-1 drug-resistant strains at 4 weeks after reducing the antiretroviral dose; in addition, there was no significant difference in the protease and RT mutation profiles of samples tested before starting therapy (day 0) and at week 24. Viral fitness and antiviral potency may explain the outgrowth of antiretroviral drug-sensitive viruses with continued therapeutic pressure. Drug-resistant HIV-1 variants, in theory, should replicate faster than HIV-1 with a lesser degree of resistance than the wild-type in the presence of drugs. But in this study, the major population of the rebound-virus, with a marked increase in replication, was wild-type. This suggests that at week 24 (4 weeks after dose reduction) the wild-type was the fitter. A mutation that conferred high level resistance, but resulted in an unfit virus, emerged slowly or not at all. The wild-type virus remained capable of rapidly outmatching the protease or RT mutant strains, even in the presence of sub-therapeutic drugs. However, resistant viruses might exist in these patients but were not detected by the population-sequencing method, which has approxi-

Table 1
Plasma HIV-1 viral load at each visit.

Patient number	Viral load ($\log_{10}$)							
	Baseline	Week2	Week4	Week8	Week12	Week16	Week24	Week36
092	5.07	3.00	2.78	2.60	2.60	3.00	5.53	4.99
103	4.48	3.00	2.60	2.37	2.42	2.74	4.52	4.39
107	4.42	3.00	2.78	2.42	2.31	2.42	4.66	4.14
123	4.52	2.60	2.60	2.78	2.60	2.60	4.44	4.40

mately 20% sensitivity for a given variant. It could be expected that the resistant viruses would probably be selected sooner or later by this sub-therapeutic pressure. Further investigations are required to confirm the evolution of wild-type HIV-1 after dose reductions, as to well as to determine the characteristics of both wild-type and resistant strains in a larger population of HIV-1 infected patients.

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Table 2

Treatment history, viral load and sequencing results from successive plasma samples of four patients who reduced antiretroviral medication.

Patient	Therapy start	Interrupting day	Sampling day	Viral load (Log_{10})	Codon Wild type AA	Reverse transcriptase									Protease										
						41	67	69	70	179	184	210	215	219	10	20	36	46	48	54	63	71	82	84	90
						M	D	T	K	V	M	L	T	K	L	K	M	M	G	I	L	A	V	I	L
092	day 0	-	day 0	5.07		-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	I	-	-
		week19	week 24	5.53		-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	I	-	-
103	day 0	-	day 0	4.48		-	-	-	-	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		week 20	week 24	4.52		-	-	-	-	D	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-
107	day 0	-	day 0	4.42		-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-
		week 20	week 24	4.66		-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-
123	day 0	-	day 0	4.52		-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-
		week 20	week 24	4.44		-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-

AA = Amino acid.