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Discordant Movement of CD4-Positive T-Cell Count in HIV-1 Infected Patients with HAART Failure

Aiko Okano, Masakazu Matsuda, Tomoko Chiba, Kenji Moriya, Kaneo Yamada[1] and Wataru Sugiura*

AIDS Research Center, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashimurayama, Tokyo 208-0011 and [1]St. Marianna University School of Medicine, Sugao 2-16-1, Miyamae-ku, Kawasaki, Kanagawa 216-8511

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Highly active antiretroviral treatment (HAART) with at least one protease inhibitor (PI) and two nucleoside reverse transcriptase inhibitors (NRTIs) is now the recommended first-line antiretroviral prescription for HIV-1 infection (1,2). While its introduction has greatly improved the prognoses of many of patients (3-5), HAART has been unsuccessful in a considerable number of cases (6,7). This treatment failure is due mainly to the emergence of drug resistant HIV-1. Once the drug resistant virus appears, it will quickly predominate in the virus population due to selective advantage under continued drug administration. Insufficient suppression of virus replication, gradual decrease of peripheral CD4+ T-cell count, and consequent accelerated disease progression are frequent outcomes (8-10). Recently, however, several groups have reported cases in which the rise and fall of CD4+ T-cell counts and viral copy numbers during HAART did not follow the rule above (11,12).

We examined 236 HIV-1 infected patients who had been treated in 17 hospitals collaborating with our laboratory and followed up for more than 4 years (from November 1996 to September 2000).

CD4+ T-cell count and clinical data were provided by the collaborating hospitals. Viral load (VL) was measured by

*Corresponding author: Fax: +81-42-561-7746, E-mail: wsugiura@nih.go.jp

using AMPLOCOR HIV-1 MONITOR™ Test Version 1.5 (Roche Diagnostics, Branchburg, N.J., USA).

HIV-1 *pol* sequences were analyzed for drug resistance genotypes. RNA was extracted form 200 μl of plasma by using an RNA purification kit (Boehringer Mannheim, Indianapolis, Ind., USA), and the 821 bp reverse transcriptase (RT) and 380 bp protease (PR) regions were reverse transcribed and amplified by nested PCR in a previously reported manner (13). The amplified fragments were directly sequenced using dye-terminator chemistry (14) and the auto-sequencer ABI-377 (Applied Biosystems, Foster City, Calif., USA).

The 236 cases were classified according to their VL response and changes in CD4+ T-cell count (Table 1). VL responses were classified into two groups, either (i) responding: VL achieved below the detectable level (<50 copies/mL) or >2 log copies/mL of reduction from the baseline before treatment, or (ii) non-responding: not applicable to category (i). Each VL response class was categorized into three according to CD4+ T-cell counts: (a) increase: absolute increase from the baseline before treatment, (b) decrease: absolute decrease from the baseline before treatment, (c) no change: no significant increase or decrease during the observation period.

As summarized in Table 1, in the VL responding group (n = 109), the number of cases applicable to categories (a), (b), and (c) were 80, 22, and 7, respectively. In the VL non-responding group (n = 127), the number of cases applicable to (a), (b), and (c) were 41, 64, and 22 cases, respectively. Thus, the responding group had a significantly high number of category (a), i.e., cases with increased CD4+ T-cell count, ($P < 0.001$). In contrast, the non-responding group had significantly high numbers of categories (b) + (c), cases whose CD4+ T-cell did not increase during the therapy ($P < 0.001$). As has been previously reported, and found as well in our samples, some cases have shown discordance between VL response and CD4+ T-cell response. Forty-one VL non-responding cases demonstrated increased CD4+ T-cell count, and seven VL responding cases showed a decrease in CD4+ T-cell count.

Table 1. Classification of 236 cases by treatment response and change in peripheral CD4+ T-cell count

		CD4+ T-cell count		
		Increase	No change	Decrease
VL Response	Responding	80	22	7
	Non-responding	41	64	22

* Underline: Discordant cases

Patient profiles of each category are summarized in Table 2. No significant difference was seen in regard to sex, sexual behaviors and treatment protocols. Only parameter in which a significant difference could be seen was the lower median age, 30.5 years, of the patients with "VL non-responding-CD4+ increase", i.e., (ii) (a) cases.

In the drug resistance genotype comparison (Table 3), the incidence of D67N, K103N, and M184V/I RT-related resistance mutations was significantly higher in the discordant

Table 2. Patients profiles of 236 study participants classified into 6 groups

VL Response CD4+ cell counts	Responding			Non-responding			Total (n=236)
	Increase* (n=80)	No change (n=22)	Decrease** (n=7)	Increase** (n=41)	No change (n=64)	Decrease* (n=22)	
Sex							
Male	73	22	7	38	61	20	221
Female	7	0	0	3	3	2	15
Median age	36.0	33.5	36.0	30.5	34.0	36.0	35.0
(Range)	(20, 63)	(21, 53)	(19, 62)	(15, 71)	(17, 56)	(18, 70)	(15, 71)
Sexual behavior							
Hemophiliacs	44	15	6	23	37	12	137
Homosexual	12	1	0	9	14	14	50
Heterosexual	18	5	1	9	9	5	47
Homo/Hetero	3	1	0	1	3	0	8
Transfusion	2	0	0	1	0	0	3
Unknown	1	0	0	0	0	0	1
Other	0	0	0	0	1	0	1
Treatment							
NRTI							
AZT	74	17	3	39	56	20	209
d4T	50	10	3	30	39	17	149
ddC	29	6	2	26	23	11	97
ddI	46	9	3	37	38	17	150
3TC	70	18	3	19	53	20	183
PI							
Indinavir	40	5	3	22	26	10	106
Ritonavir	28	2	2	23	17	10	82
Saquinavir	68	5	2	30	22	12	139
Nelfinavir	51	13	4	17	34	14	133

* Concordant group
** Discordant group

Table 3. Summary of drug resistance mutations observed in 6 groups of patients

VL Response CD4+ cell counts	Responding			Non-responding			Total (n=236)
	Increase (n=80)	No change (n=22)	Decrease (n=7)	Increase (n=41)	No change (n=64)	Decrease (n=22)	
Mutations to PI							P value**
K10I/R/V	12	3	3	22	22	7	0.097
K20M/R	8	0	1	11	9	6	0.970
L24I	1	1	0	4	0	1	0.466
D30N*	4	0	1	6	5	0	0.059
V32I	1	0	0	1	2	0	0.466
L33F	2	0	1	4	3	3	0.640
M36I	15	6	3	11	16	9	0.252
M46I/L*	6	1	0	15	12	6	0.455
G48V*	0	1	0	2	3	1	0.953
I54V/L	1	1	1	12	6	5	0.577
L63P	46	9	4	36	46	17	0.472
A71V/T	15	4	0	16	22	4	0.090
G73S	0	0	0	3	0	0	0.194
V77I	31	5	1	23	31	9	0.250
V82A/F/T*	8	1	1	11	11	5	0.721
I84V	0	0	1	5	3	1	0.324
N88D	5	0	1	11	6	3	0.230
L90M*	9	2	2	14	9	6	0.576
Mutations to RT							
M41L	26	1	2	17	10	8	0.693
K65R	2	0	0	0	0	2	0.050
D67N	18	2	1	22	9	6	0.045
T69D	7	1	1	11	3	5	0.721
K70R*	13	1	2	14	5	5	0.346
L74V*	0	1	1	3	6	0	0.194
V75T*	2	0	1	3	0	1	0.667
K103N*	2	0	0	7	0	0	0.040
V106A*	1	0	0	0	2	0	–
V108I*	0	0	0	0	0	0	–
Y181C/I*	0	0	0	1	1	0	0.460
M184V/I*	28	6	4	30	10	8	0.004
Y188L/C/H*	0	0	0	0	1	0	–
G190A/S*	0	0	0	0	0	0	–
T215Y/F*	28	2	3	25	10	11	0.401
K219Q	7	1	1	11	4	5	0.721

* Primary mutations

** P value was calculated with data of the concordant "VL non-responding-CD4+ decrease" group (n=22) and discordant "VL non-responding-CD4+ increase" group (n=41)

"VL non-responding-CD4+ increase" group (n = 41) than in the concordant "VL non-responding-CD4+ decrease" group (n = 22) (P = 0.045, 0.040, and 0.004, respectively). Regarding protease inhibitor-resistant mutations, no significant association could be seen between mutations and the discordance pattern.

Our results confirmed that virologic failure did not always result in acute immunologic failure. The cause of this discordance remains unclear, necessitating further research. The further study might help answer fundamental questions regarding HIV-1 pathogenesis and may provide clues to the proper timing for changing antiretroviral regimens after virologic failure.

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