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Letters to the Editor

P-Glycoprotein Expression by Peripheral Blood Mononuclear Cells from Human Immunodeficiency Virus-Infected Patients Is Independent from Response to Highly Active Antiretroviral Therapy

During the last few years, highly active antiretroviral therapy has considerably reduced human immunodeficiency virus (HIV) disease progression (11). However, the treatment outcome is not always satisfactory (7). This can depend on different virological, immunological, behavioral, or pharmacological factors (5). In the framework of aspects, the interaction between P-glycoprotein (P-gp) and antiretroviral drugs has been evidenced (8, 13, 15). P-gp is a transmembrane phosphoglycoprotein belonging to the ATP-binding cassette superfamily that is able to transport several substrates through the cell membrane acting as a cationic pump (2, 3, 4, 6, 9, 14). Specifically, studies on protease inhibitors (PI) have evidenced that the transporter activity of P-gp may contribute to the reduced bioavailability of these drugs, which can act as substrates for P-gp (4, 15, 16). However, the influence of HIV infection on P-gp expression is still a matter of debate (1, 9), and as yet, no data are available on the effects of highly active antiretroviral therapy on its expression.

The intensity of P-gp expression on peripheral blood mononuclear cells (PBMC) from healthy donors is 1.8 ± 0.5, as indicated by an intensity index described below (see footnote to Table 1). Recently, it has been reported that P-gp expression on PBMC was reduced in HIV-positive (HIV^+) persons when compared with that found in healthy donors (10). Using the same approach, we analyzed the influence of antiretroviral treatment and that of viral and immunological parameters on P-gp expression on PBMC from 18 HIV^+ persons. We defined three groups (Table 1): (i) HIV^+ patients naive for antiretroviral therapy ($n = 5$); (ii) HIV^+ patients with a successful response to the therapy ($n = 8$); and (iii) HIV^+ patients with failing virological response to the therapy ($n = 5$). Treatment protocols of all patients are reported in Table 1.

In this pilot study, the expression of P-gp in total PBMC, $CD4^+$ T lymphocytes, and $CD14^+$ monocytes from naive, responder, and nonresponder patients was analyzed by flow cytometry. The intensity of P-gp expression on total PBMC of HIV^+ patients was very low (0.99 ± 0.12), confirming previous observations (10). Further analysis among $CD4^+$ T cells and $CD14^+$ monocytes showed a higher intensity of P-gp expression on $CD14^+$ monocytes than in $CD4^+$ T lymphocytes (2.2 ± 1.3 versus 1.04 ± 0.16 [$P < 0.05$]). To asses whether the lack of virological response to the therapy treatment may be correlated with a different intensity of P-gp expression on the cell surface, we compared P-gp expression in the three groups of HIV^+ patients described above (naive, responder, and nonresponder). No differences among these groups were observed in P-gp expression on total PBMC (naive, 1.04 ± 0.02; responder, 1.00 ± 0.02; and nonresponder, 0.92 ± 0.10), $CD4^+$ T lym-

TABLE 1. Analysis of clinical parameters and P-gp expression in total PBMC, $CD4^+$ T lymphocytes, and $CD14^+$ monocytes from HIV^+ patients[a]

No. of group	Patient code	Therapy	Viremia (copies/ml)	No. of CD4 cells/mm^3	Treatment protocol	P-gp expression		
						PBMC	$CD4^+$	$CD14^+$
1	CM0612	Naive	2,900	322		1.05	1.23	2.00
	GR1411	Naive	2,400	629		1.10	1.21	6.21
	AS1512	Naive	2,300	433		0.99	1.06	1.75
	VC1512	Naive	190,000	267		1.01	1.22	1.74
	BG2211	Naive	25,000	630		1.03	1.00	1.65
2	DF0612	Responder	<80	657	2 NRTI + 1 NNRTI	0.99	0.96	1.45
	D10612	Responder	<80	719	2 NRTI + PI	1.05	1.13	1.22
	MC0612	Responder	<80	808	2 NRTI + PI	1.08	1.00	1.95
	ER1411	Responder	<80	356	2 NRTI + 1 NNRTI	0.88	0.88	3.55
	AG1512	Responder	<80	420	2 NRTI + PI	1.03	1.27	1.34
	GW1512	Responder	<80	867	2 NRTI + PI	1.00	0.99	1.15
	BA2211	Responder	<80	805	2 NRTI + 1 NNRTI	1.03	0.96	2.74
	OA2211	Responder	<80	309	2 NRTI + PI	0.97	0.96	2.74
3	AG0612	Nonresponder	12,000	363	2 NRTI + PI	1.07	1.07	2.02
	FL1512	Nonresponder	84,000	20	2 NRTI + PI	0.53	0.57	1.38
	PE1512	Nonresponder	13,000	373	2 NRTI + PI	1.01	0.98	1.55
	DT2211	Nonresponder	46,000	299	2 NRTI + PI	0.98	1.17	3.31
	CF0612	Nonresponder	98,000	64	2 NRTI + 1 NNRTI	1.00	1.11	1.49

[a] Cytometric analysis of surface antigen expression was performed as previously described (12) by using the following monoclonal antibodies: anti-CD14-phycoerythrin (clone M5E2; Becton Dickinson, Mountain View, Calif.), anti-CD4-phycoerythrin-Cy5 (clone RPA-T4; Becton Dickinson), and anti-P-gp–fluorescein isothiocyanate (clone 12.10), which was kindly provided by Dr. M. Cianfriglia (Rome, Italy). Briefly, 5×10^5 PBMC were washed in 1% phosphato-buffered saline, 1% bovine serum albumin, and 0.1% sodium azide and were incubated for 15 min at 4°C with monoclonal antibodies. Samples were fixed in 1% paraformaldehyde, immediately acquired by a FACScalibur flow cytometer (Becton Dickinson), and analyzed with CellQuest software (Becton Dickinson). P-gp expression was analyzed in different cellular subsets: total PBMC, $CD4^+$ T lymphocytes, and $CD14^+$ monocytes. Data are indicated as an intensity index, which is obtained by dividing the median fluorescence intensity seen with the P-gp-specific monoclonal antibody by that seen with the immunoglobulin G2a isotype control (p170/immunoglobulin G), as previously described (10). NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, nonnucleoside reverse transcriptase inhibitor.

phocytes (naive, 1.14 ± 0.05; responder, 1.02 ± 0.04; and nonresponder, 0.98 ± 0.11), and $CD14^+$ monocytes (naive, 2.67 ± 0.90; responder, 2.16 ± 0.39; and nonresponder, 1.95 ± 0.36). The frequency of P-gp-positive cells was independent of both the viremia levels and the T-cell count, and no difference between patients treated with PI and patients naive for PI treatment was observed. Altogether, these observation indicate that differences in P-gp levels did not appear to determine virological responses to antiretroviral therapy.

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