JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2005, p. 484–487 Vol. 43, No. 1
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.1.484–487.2005

Polymorphism of the Human Immunodeficiency Virus Type 2 (HIV-2) Protease Gene and Selection of Drug Resistance Mutations in HIV-2-Infected Patients Treated with Protease Inhibitors

F. Damond,[1,2] F. Brun-Vezinet,[1,2] S. Matheron,[1] G. Peytavin,[1] P. Campa,[1] S. Pueyo,[3] F. Mammano,[2] S. Lastere,[1,2] I. Farfara,[1] F. Simon,[4] G. Chene,[3] and D. Descamps[1,2]*

Hôpital Bichat-Claude Bernard[1] and INSERM U552,[3] Paris, and INSERM U330, Bordeaux,[2] France, and Institut Pasteur, Dakar, Senegal[4]

Received 8 November 2003/Returned for modification 12 March 2004/Accepted 12 September 2004

We described the baseline polymorphism of the human immunodeficiency virus type 2 (HIV-2) protease gene from 94 treatment-naive patients and the longitudinal follow-up of 17 protease inhibitor-treated patients. Compared to the HIV-2 consensus sequences, baseline polymorphism involved 47 positions. Substitutions selected under treatment were observed at positions corresponding to HIV-1 resistance mutations as well as at positions of currently unknown impact on HIV-1.

Human immunodeficiency virus type 2 (HIV-2) is endemic in west Africa and has spread in the last decade in Europe (1, 9, 13, 21). Many studies have reported antiretroviral drug resistance profiles for HIV-1-infected patients receiving therapy (5), but less is known for patients infected with HIV-2 (20, 22). In France, a cohort initiated in 1994 and covering almost all the HIV-2-infected patients living in France monitored in hospital centers is the largest one in industrialized countries (14). The objectives of our study were to characterize the polymorphism of the HIV-2 protease (PR) gene and to determine which treatment-associated changes were selected in vivo in the PR gene during a PR inhibitor (PI)-containing regimen in HIV-2-infected patients included in the French cohort.

Patients and methods. Polymorphism of the PR genes from 94 PI-naive HIV-2-infected patients was assessed. Patients were representative of the 341 patients of the French cohort regarding gender (60% female), age (mean, 38.8 years), country of birth (73% originated from west Africa), Centers for Disease Control and Prevention (CDC) stage (80% stage A according to CDC criteria), CD4 cell count (median, 466 cells/mm^3), and viral load (median: 3 log copies/ml). Characterization of treatment-associated changes under the PI regimen was performed for 17 out of the 94 patients by comparing PR sequences before the initiation of treatment and under treatment. These 17 patients received, for a median duration of 12 months (range: 2 to 48 months), indinavir (IDV; $n = 7$), saquinavir plus boosted ritonavir (SQV/RTV; $n = 2$), SQV ($n = 2$), RTV ($n = 2$), or nelfinavir (NFV; $n = 4$). Six out of these 17 patients received a second PI-containing regimen (NFV [$n = 2$] or SQV/RTV [$n = 4$]) for a median duration of 14 months (range, 5 to 41 months). Appropriate informed consent was obtained from all patients.

* Corresponding author. Mailing address: Laboratoire de Virologie, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, 75018 Paris, France. Phone: 33 1 40 25 61 54. Fax: 33 1 40 25 67 69. E-mail: diane.descamps@bch.ap-hop-paris.fr.

PR genotypic analyses. PR gene sequencing was performed retrospectively on available plasma specimens collected between 1994 and 2001 and stored at −80°C. HIV-2 RNA was extracted from 1 ml of plasma, and the PR gene was amplified from 10 μl of RNA and sequenced as previously described (18). Sequence alignments were performed with Sequence Navigator software, and sequences were compared to HIV-2 clade A and B consensus sequences (Los Alamos database: http://hiv-lanl.gov) (15). Changes in amino acids were compared to those associated with resistance in HIV-1 identified from the International AIDS Society-USA (IAS-USA) panel expert list (www.iasusa.org) (8). Phylogenetic analyses, performed with PR gene nucleotide sequences as described elsewhere (10), indicated that 68 patients were infected by a subtype A, 25 were infected by a subtype B, and one was infected by subtype H (7).

Polymorphism of the HIV-2 PR gene. Comparison to the Los Alamos database consensus sequences indicated that 47 positions were polymorphic (Table 1). Most of the differences (>20%) were located at positions 14, 17, 40, 41, and 70 for subtype A and at positions 12, 14, 41, 62, 71, 75, and 92 for subtype B. Variability at residue 14 was the most frequently observed for both subtypes: 71% for subtype A and 52% for subtype B. HIV-2 PR polymorphisms were observed at positions 10, 36, 46, 54, 71, 73, 77, and 90; mutations at these residues are known to be associated with major or minor changes in HIV-1 drug resistance. Differences at codons 46 and 90 were observed only in HIV-2 subtype A. Additionally, several differences at positions of unknown impact on HIV-1 resistance were found in more than 5% of cases.

Selection of mutations in 17 PI-treated patients. Before treatment, the median CD4 cell count and plasma viral load were 237 cells/mm^3 (range: 8 to 359 cells/mm^3) and 491 copies/ml (range: <250 to 1,485,000 copies/ml), respectively (Table 2). Compared to viruses with their respective baseline sequences, viruses with treatment-associated changes described as PI major or minor resistance mutations for HIV-1 according to the IAS-USA resistance panel emerged in 12 of 17 patients

TABLE 1. Frequency of amino acid polymorphism in the PR in naive HIV-2-infected patients compared to consensus sequences for HIV-1 clade B and HIV-2 subtypes A and B

Position	HIV-1 clade B consensus	HIV-2 clade A consensus	Substitution(s) (position and amino acid)[a]	HIV-2 clade B consensus	Substitution(s) (position and amino acid)[a]	%
7	Q	K	7N (1)		#	1
8	R			R	8K (1)	1
9	P			P	9T (1)	1
10	L	V	10I (4)	V	10I (2), 10R (1)	7
11	V			V	11S (1)	1
12	T	T	12I (2) 12R (1)	K	12Q (3), 12R (1), 12K/Q (1)	8
14	K	Y	14H (38), L (1), P (2)	C	14H (3), 14N (6), 14S (3), 14S/N (1)	54
15	I	I	15V (7), 15F (1)	I	15V (2)	10
17	G	G	17D (20), 17K/D/E (1), 17E (2)	G	17D (1)	24
18	Q			Q	18K (1)	1
20	K	V	20I (1)			1
21	E	E	21D (1)	E	21D (1)	2
22	A			V	22I (1)	1
35	E	G	35E (1)			1
36	M	I	36V (4)			4
37	N	E	37D (1)			1
40	G	S	40D (15), 40N (10), 40G/D (1), 40E/D (2), 40E (1)	S	40N (1)	30
41	R	N	41D (14)	N	41D (7)	21
43	K	S	43V/I (1), 43N (2), 43I (1), 43R (1), 43V (1)	T	43I (1)	7
44	P	P	44S (1)			1
45	K			K	45R (2)	2
46	M	I	46V (7), 46M (1)			8
54	I	I	54M (2)	I	54M (1)	3
56	V			T	56A (1), 56V (1)	2
57	R	K	57R (2)			2
60	D	K	60R (4), 60E (1)	K	60R (2)	5
61	Q			D	61V/I (1)	1
62	I	V	62I (2), 62A (1)	V	62I (9)	12
65	E	K	65R (7), 65I (2), 65T (1)			10
66	I			V	66A (1)	1
68	G	N	68S (1)			1
70	K	R	70K (19), 70T (3)	R	70I (1), 70K (2), 70V (1)	26
71	A	V	71I (2), 71I/L (1)	V	71I (7), 71L (1)	11
72	I	R	72K (5)			5
73	G	A	73G (1)			1
74	T	T	74N (1)			1
75	V	I	75R (1), 75L (1)	I	75V (3), 75L (2)	7
77	V	T	77M/I (1)			1
79	P	D	79E (2)			2
85	I	F	85L (1)			1
86	G	G	86A (1)			1
87	R	R	87E (1)			1
89	L	I	89V (4), 89D/E (1)			5
90	L	L	90S/L (2), 90L/M (1)			3
91	T	T	91A (9)	N	91S (1)	10
92	Q	A	92S (4), 92V (2)	T	92I (1), 92S (7)	14
95	C			F	95I (1)	1

[a] Values in parentheses are numbers of patients.

during treatment (8). Of the seven patients receiving IDV, selection of the I82F substitution was observed alone ($n = 3$) and associated with the L90M substitution ($n = 1$) and with V10I ($n = 2$). Substitutions I82M ($n = 1$) and I36V and R70K ($n = 1$) were detected in two patients. Among the four patients receiving SQV, one patient harbored virus with the I54L and L90M treatment-associated changes and one had a mixture at residue 48 (G48G/R) associated with substitutions I36V and I46V. In the two patients that received RTV, I82F alone and the L90M change associated with I54M and V71A were selected. Among the four patients treated with NFV, two had the L90M change associated with I54M and/or V71I. Many other treatment-associated changes ($n = 25$) were selected in 13 patients. Valine or leucine at amino acid 84 was found in four of the six patients that received a second PI-containing regimen.

In our study, we analyzed 123 HIV-2 PR sequences from 94 PI-naive patients and from 17 treated patients before and after introduction of therapy. Only two other studies have reported natural polymorphism of the HIV-2 PR gene (4, 19). We found that 47 of the 99 amino acid positions of HIV-2 PR were polymorphic, which is more than the number reported by Colson et al. (4), probably due to the higher number of sequences analyzed in our study. We found that positions 14, 17, 40, 41, and 70 were highly polymorphic, in contrast to what was found for HIV-1 PR, with the exception of position 41, which has

TABLE 2. Treatment-associated changes selected in the PR genes of HIV-2-infected patients receiving a PI-containing regimen

First-line PI regimen	At baseline				At time of protease gene genotyping					
									Amino acid changes in the PR gene during therapy	
	Patient	Subtype	Viral load (cells/ml)	CD4 cells/mm^3	Viral load (cells/ml)	CD4 cells/ mm^3	PI regimen	Mo from start of therapy	Amino acid changes known to be involved in HIV-1 resistance	Amino acid changes of unknown impact in HIV-1 resistance[a]
IDV	1	A	<250	231	ND[b]	445	IDV	8	—[c]	T80Y/T
	2	A	19800	64	836	44	IDV	26	I36I/V	R70K
	3	A	250	252	3,855	173	IDV	6	—	—
	4	A	16980	8	14,545	152	IDV	21	V10I, I82F, L90L/M	K7N, F85L, I89V
					188,500	93	SQV/R	26	V10I, I82F, I84V, L90M	S43I, R70K, I84L, F85L, I89V
	5	A	360	360	84,000	246	IDV	7	I82F	—
					4,220	209	IDV	9	V10I, I82F	I89I/V
					355	238	SQV/R	46	V10I, T77T/I, I82F, I84L, L90M	A34S, K65K/R, F85F/L, I89V, A92A/V
	6	B	<250	31	<250	83	IDV	14	—	L23M, S40N, T43I, K45R, I64V, I82M
					<250	92	SQV/R	19	V10V/I, I54I/L, I84V	L23M, S40N, T43I, K45R, 146I/T, E63E/A, I64V, I95V, I82M
	7	C	10,530	315	1,470	450	IDV	9	—	N41Y
					3,935	293	IDV	24	I82F	I15I/V, A34E, N41Y
					<250	501	IDV	36	I82F	A34E, N41Y
					2,580	492	IDV	48	I82F	A34E, N41Y/H, R72K, M95M/I
SQV	8	A	<250	262	<250	251	SQV	2	—	—
	9	A	15,000	42	3,240	111	SQV/R	12	I46I/V	I36V, G48G/R
	10	A	490	249	531	350	SQV	14	I54L, L90M	—
					1,583	123	NFV	55	V10I, I84V, L90M	E63Q, V71I
	11	B	<250	86	<250	146	SQV/R	31	—	—
RTV	12	A	16,450	24	20,780	38	RTV	9	I82F	G55R/G
					58,080	21	SQV/R	23	—	S40D, K60R
	13	A	4,780	42	5,177	106	RTV	6	I54I/M, V71V/A, L90L/M	L38L/F, K45K/R, I64I/V
					12,840	77	NFV	16	I54M, L90M	I64V, V71V/I
NFV	14	A	1,485,000	48	39,840	80	NFV	12	I54M	V71V/I, T74N
					9,316	95	NFV	14	I54M, L90M	V71V/I, T74N
	15	A	97,980	257	1,058	154	NFV	7	L90M	V71I, A92A/V
	16	B	<250	359	<250	331	NFV	11	V33J	I15V, T56A, V71I, R87K
					<250	237	NFV	25	F53F/V33J	I15V, I32L, T56A, V62A, V71I, G78G/E
	17	B	<260	296	<250	398	NFV	20	—	V22I, K45R, T56A, I64V

[a] *, amino acid changes associated with polymorphism of the PR gene in HIV-2 are underlined.
[b] ND, not determined.
[c] —, None.

been previously described as highly polymorphic in HIV-1 (2). Residue 14, moderately polymorphic in HIV-1, is the most frequently polymorphic residue in HIV-2. In contrast to position 63 in HIV-1, where polymorphism is around 60%, this position (63E) is largely conserved in HIV-2 (2, 11). We found also sequence variability at 8 residues corresponding to those associated with PI resistance in HIV-1. Recently two other studies reported similar results although some of the residues implicated were different (4, 19); in particular, Pieniazek et al. found a 30N substitution in 1 out of 75 infected patients.

Here, we show that treatment-associated changes occurred in the HIV-2 PR gene at sites corresponding to some of those conferring HIV-1 PI resistance. These changes could not be directly associated with the use of a specific PI, and their emergence did not appear to follow an ordered accumulation. Only one study described the selection of I82F, I54M, and V71I in one HIV-2-infected patient who received IDV for 12 months (20). Interestingly, although we studied a limited number of patients, I82F appeared only in patients receiving IDV or RTV, as reported for HIV-1 (5). Arginine at amino acid 48 and methionine at amino acid 82, observed in two of our patients treated with SQV and IDV, respectively, have not been reported to confer PI resistance in HIV-1 (Stanford HIV RT and Protease Sequence Database [http://hivdb.stanford.edu]). Under NFV selective pressure, two of three of the viruses from HIV-1-infected patients selected for D30N and one of three of the viruses displayed L90M (12, 17). Here, in patients receiving NFV there was selection for L90M associated with V71I and but not for D30N. This predominant selection of the L90M substitution was also observed in HIV-1 subtypes C and G (3). This suggests that a change at residue 90 is more advantageous and/or better tolerated than a change at residue 30. Substitution at residue 84, known to be associated in HIV-1 with cross-resistance to RTV, SQV, and IDV (16), was observed only in HIV-2 patients receiving a second-line PI regimen.

Since currently available PIs were designed to fit into the active site of HIV-1 PR, the differences observed in the sequence of HIV-2 may have an impact on the efficacy of HIV-2 PR inhibition. In addition, the effect of the polymorphism described here on the phenotypic susceptibility of HIV-2 strains is still unknown and needs to be evaluated by in vitro 50% inhibitory concentration measurement and mutagenesis experiments. The availability of HIV-2 plasma viral load (6) will allow studies of the correlation between the emergence of resistance mutations in vivo and virologic response.

This study was supported by a grant from the Agence Nationale de Recherche sur le SIDA and European Union grant QL K2-CT-2001-02360.

REFERENCES

1. **Bock, P. J., and D. M. Markovitz.** 2001. Infection with HIV-2. AIDS **15:** S35–S45.
2. **Bossi, P., M. Mouroux, A. Yvon, F. Bricaire, H. Agut, J.-M. Huraux, C. Katlama, and V. Calvez.** 1999. Polymorphism of the human immunodeficiency virus type 1 (HIV-1) protease gene and response of HIV-1-infected patients to a protease inhibitor. J. Clin. Microbiol. **37:**2910–2912.
3. **Cane, P. A., A. de Ruiter, P. Rice, M. Wiselka, R. Fox, and D. Pillay.** 2001. Resistance associated mutations in subtype C HIV-1 protease inhibitor treated and untreated patients, Antivir. Ther. **6**(Supp. 1)**:**114.
4. **Colson, P., M. Henry, C. Tourres, D. Lozachmeur, H. Gallais, J. A. Gastaut, J. Moreau, and C. Tamalet.** 2004. Polymorphism and drug-selected mutations in the protease gene of human immunodeficiency virus type 2 from patients living in southern France. J. Clin. Microbiol. **42:**570–577.
5. **Condra, J. H., W. A. Schleif, O. M. Blahy, L. J. Gabryelski, D. J. Graham, J. C. Quintero, A. Rhodes, H. L. Robbins, E. Roth, M. Shivaprakash, et al.** 1995. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature **374:**569–571.
6. **Damond, F., M. Gueudin, S. Pueyo, I. Farfara, D. L. Robertson, D. Descamps, G. Chène, S. Matheron, P. Campa, F. Brun-Vézinet, and F. Simon.** 2002. Plasma RNA viral load in human immunodeficiency virus type 2 subtype A and subtype B infections. J. Clin. Microbiol. **40:**3654–3659.
7. **Damond, F., M. Worobey, P. Campa, I. Farfara, G. Collin, S. Matheron, F. Brun-Vezinet, D. L. Robertson, and F. Simon.** 2004. The identification of a highly divergent HIV-2 in France and a proposal for a new HIV-2 classification. AIDS Res. Hum. Retrovir. **20:**666–672.
8. **D'Aquila, R. T., J. M. Schapiro, F. Brun-Vézinet, B. Clotet, B. Conway, L. M. Demeter, C. Loveday, R. M. Grant, V. A. Johnson, D. R. Kuritzkes, R. W. Shafer, and D. D. Richman.** 2002. Drug resistance mutations in HIV-1. Top. HIV Med. **10:**21–25.
9. **Kanki, P. J., and K. M. De Cock.** 1994. Epidemiology and natural history of HIV-2. AIDS **8:**S85–S93.
10. **Kimura, M.** 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16:**111–120.
11. **Kozal, M. J., N. Shah, N. Shen, R. Yang, R. Fucini, T. C. Merigan, D. D. Richman, D. Morris, E. Hubbell, M. Chee, and T. R. Gingeras.** 1996. Extensive polymorphisms observed in HIV-1 clade B protease gene using high-density oligonucleotide arrays. Nat. Med. **2:**753–759.
12. **Masquelier, B., G. Peytavin, C. Leport, C. Droz, S. Duran, R. Verdon, J. M. Besnier, G. Chene, F. Raffi, and F. Brun-Vezinet.** 2002. Mechanisms of early virologic failure in antiretroviral-naive patients starting protease inhibitor-containing regimens: the APROVIR Study. J. Infect. Dis. **186:**1503–1507.
13. **Matheron, S., G. Mendoza-Sassi, F. Simon, R. Olivares, J. P. Coulaud, and F. Brun-Vezinet.** 1997. HIV-1 and HIV-2 AIDS in African patients living in Paris. AIDS **11:**934–936.
14. **Matheron, S., S. Pueyo, F. Damond, F. Simon, A. Lepretre, P. Campa, R. Salamon, G. Chene, and F. Brun-Vezinet.** 2003. Factors associated with clinical progression in HIV-2 infected-patients: the French ANRS cohort. AIDS **17:**2593–2601.
15. **Myers, G., B. Foley, J. W. Mellors, B. T. Korber, K. T. Jeang, and S. Wain-Hobson.** 1996. Human retroviruses and AIDS 1996: a compilation and analysis of nucleic acid and amino acid sequences. Theoretical Biology and Biophysic Group, Los Alamos National Laboratory, Los Alamos, N.M.
16. **Palmer, S., R. W. Shafer, and T. C. Merigan.** 1999. Highly drug-resistant HIV-1 clinical isolates are cross-resistant to many antiretroviral compounds in current clinical development. AIDS **13:**661–667.
17. **Patick, A. K., M. Duran, Y. Cao, et al.** 1996. Personal communication.
18. **Pieniazek, D., D. Ellenberger, L. M. Janini, A. C. Ramos, J. Nkengasong, M. Sassan-Morokro, D. J. Hu, I. M. Coulibally, E. Ekpini, C. Bandea, A. Tanuri, A. E. Greenberg, S. Z. Wiktor, and M. A. Rayfield.** 1999. Predominance of human immunodeficiency virus type 2 subtype B in Abidjan, Ivory Coast. AIDS Res. Hum. Retrovir. **15:**603–608.
19. **Pieniazek, D., M. Rayfield, D. Hu, J. Nkengasong, V. Soriano, W. Heneine, C. Zeh, S. Agwale, C. Wambebe, L. Odama, S. Wiktor, and M. Kalish.** 2004. HIV-2 protease sequences of subtypes A and B harbor multiple mutations associated with protease inhibitor resistance in HIV-1. AIDS **18:**495–502.
20. **Rodés, B., A. Holguín, V. Soriano, M. Dourana, K. Mansinho, F. Antunes, and J. González-Lahoz.** 2000. Emergence of drug resistance mutations in human immunodeficiency virus type 2-infected subjects undergoing antiretroviral therapy. J. Clin. Microbiol. **38:**1370–1374.
21. **Soriano, V., P. Gomes, W. Heneine, A. Holguin, M. Doruana, R. Antunes, K. Mansinho, W. M. Switzer, C. Araujo, V. Shanmugam, H. Lourenco, J. Gonzalez-Lahoz, and F. Antunes.** 2000. Human immunodeficiency virus type 2 (HIV-2) in Portugal: clinical spectrum, circulating subtypes, virus isolation, and plasma viral load. J. Med. Virol. **61:**111–116.
22. **van der Ende, M. E., C. Guillon, P. H. Boers, T. D. Ly, R. A. Gruters, A. D. Osterhaus, and M. Schutten.** 2000. Antiviral resistance of biologic HIV-2 clones obtained from individuals on nucleoside reverse transcriptase inhibitor therapy. J. Acquir. Immune Defic. Syndr. **25:**11–18.