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Clinical and Virological Characterization of CRF07_BC Infection

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ABSTRACT

Background: The circulating recombinant form (CRF) 07_BC is the most prevalent HIV-1 strain among injection drug users (IDUs) in Taiwan. It contains a 7 amino-acid deletion in its p6gag. We conducted a cohort study to compare viral loads and CD4 cell count changes between patients infected with subtype B and CRF07_BC and to elucidate its mechanism.

Methods: Twenty-one patients infected with CRF07_BC and 59 patients with subtype B were selected from a cohort of 667 HIV-1/AIDS patients whom have been followed up for 3 years. Generalized estimated equation was used for statistical analysis. The replicative kinetics, tropism and cytopathic effects were determined. HIV-1 NL4-3 which containing a 7 amino-acid deletion in p6gag (7d virus) was generated and its virological properties were compared with the wild-type.

Results: The results showed that patients infected with CRF07_BC had significantly lower viral loads (about 58,000 copies per ml less) than patients with subtype B infection (p = 0.002) using generalized estimated equation. The replicative capacity of nine CRF07_BC and four subtype B isolates were compared and the results showed that the former had significantly lower replicative capacity than the latter although all of them were CCR5- tropic and non-syncytium inducing viruses. An HIV-1-NL4-3 mutant virus which contains a 7 amino-acid deletion in p6gag (designated as 7d virus) was generated and its live cycle was investigated. The results showed that 7d virus had significantly lower replication capacity, poorer protease-mediated processing and viral proteins production. Electron microscopic examination of cells infected with wild-type or 7d virus demonstrated that the 7d virus had poorer and slower viral maturation processes: more viruses attached to the cell membrane and higher proportion of immature virions outside the cells. The interaction between p6gag and Alix protein was less efficient in cells infected with 7d virus.

Conclusions: In conclusion, patients infected with CRF07_BC had significantly lower viral loads than patients infected with subtype B and it may due to the deletion of 7 amino acids which overlaps with Alix protein-binding domain of the p6gag.

RESULTS

Table 1. Demographic data, risk factors and clinical characteristics of HIV-1-infected patients recruited in the study.

	HIV-1 infected population					
	Male (%)		Female (%)		Total (%)	
	(N = 627)		(N = 40)		(N = 667)	
Age (yrs)	n = 612		n = 37		n = 649	
15–29	287	(46.9)	12	(32.4)	299	(49.2)
30–50	283	(46.2)	19	(51.4)	302	(42.5)
≥50	42	(6.9)	6	(16.2)	48	(8.3)
Mode of infection	n = 561		n = 39		n = 600	
Homosexual contact	388	(69.2)	0	(0)	388	(64.7)
Heterosexual contact	51	(9.1)	20	(51.3)	71	(11.8)
Injecting drug use	116	(20.7)	19	(48.7)	135	(22.5)
Receipt of blood products	6	(1.1)	0	(0)	6	(1.0)
HBV coinfection	n = 280		n = 21		n = 301	
HBsAg, positive	68	(24.3)	3	(14.3)	71	(23.6)
HCV coinfection	n = 361		n = 27		n = 388	
HCVAb, positive	105	(29.1)	16	(59.3)	121	(31.2)
Antiretroviral treatment	n = 627		n = 40		n = 667	
HAART	444	(70.8)	22	(55.0)	466	(69.9)
HIV-1 subtype	n = 262		n = 10		n = 272	
B	215	(82.1)	4	(40.0)	219	(80.5)
C	3	(1.1)	0	(0)	3	(1.1)
CRF01_AE	14	(5.3)	3	(30.0)	17	(6.3)
CRF07_BC	29	(11.1)	3	(30.0)	32	(11.8)
CRF08_BC	1	(0.4)	0	(0)	1	(0.4)

Table 2. Distribution of HIV-1 subtypes and circulating recombinant forms (CRFs) in different groups of patients recruited in this cohort study.

	Male					
Subtype/CRF	Heterosexual	MSM	IDUs	Others	Female[a]	Total
B	16 (69.6)	154 (93.9)	9 (25.7)	36 (90.0)	4 (40)	219 (80.5)
C	0 (0)	3 (1.8)	0 (0)	0 (0)	0 (0)	3 (1.1)
CRF01_AE	7 (30.4)	4 (2.4)	0 (0)	3 (7.5)	3 (30)	17 (6.3)
CRF07_BC	0 (0)	2 (1.2)	26 (74.3)	1 (2.5)	3 (30)	32 (11.8)
CRF08_BC	0 (0)	1 (0.6)	0 (0)	0 (0)	0 (0)	1 (0.4)
Total	23 (100)	164 (100)	35 (100)	40 (100)	10 (100)	272 (100)

[a]Includes 4 heterosexual female infected with subtype B, 3 heterosexual female infected with CRF01_AE, and 3 IDUs infected with CRF07_BC.

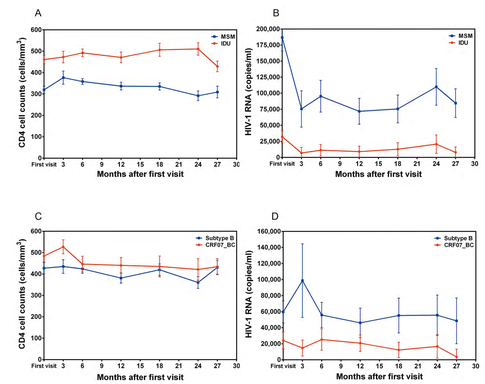


Figure 1. Comparisons of the changes of CD4 cell count and HIV-1 viral loads after the first clinical visit among the following four groups of treatment naïve patients. Men who have sex with men (MSM, 357 patients for CD4 cell count and 371 patients for viral loads analysis) vs. injection drug users (IDUs, 129 patients for CD4 cell count and 128 patients for viral loads analysis) (Figure 1A and 1B); patients infected with CRF07_BC vs. infected with subtype B (Figure 1C and 1D). A generalized estimating equation model was used for the analyses.

Table 3. Univariate and multivariate generalized estimating equations models of factors associated with CD4 cell counts.

		Univariate		Multivariate model I		Multivariate model II[a]	
	No.	difference	p	difference	p	difference	p
Gender							
Male	578						
Female	37	20.8	0.541	43.0	0.187	52.2	0.082
Age (yrs)							
15–29	271						
30–50	281	−31.1	0.084	−43.6	0.012	−36.4	0.040
≥50	46	−153.2	<0.001	−140.6	<0.001	−170.7	<0.001
Mode of transmission							
Homosexual contact	357						
Heterosexual contact	70	−59.8	0.032	−33.9	0.228	-	-
Injecting drug use	129	40.7	0.066	103.3	0.001	-	-
Receipt of blood products	6	−164.4	0.115	−118.5	0.222	-	-
HIV-1 subtype							
B	201						
C	3	85.6	0.317	68.2	0.384	70.5	0.367
CRF01_AE	15	−147.7	0.004	−103.3	0.041	−118.0	0.020
CRF07_BC	31	9.9	0.746	−24.4	0.510	53.1	0.084
CRF08_BC	1	−225.6	<0.001	−132.3	<0.001	−135.5	<0.001
HIV viral loads (copies/ml)							
<40	225						
40–400	34	−42.8	0.008	−44.1	0.007	−41.4	0.010
401–10,000	93	−107.1	<0.001	−99.0	<0.001	−97.7	<0.001
>10,000	109	−169.9	<0.001	−151.2	<0.001	−153.6	<0.001
Received ART							
No	261						
Yes	354	118.3	<0.001	50.0	0.005	41.6	0.020

[a]The mode of transmission was removed from multivariate model II.

Table 4. Univariate and multivariate generalized estimating equation model of factors associated with HIV-1 viral loads.

		Univariate		Multivariate model I		Multivariate model II[a]	
	No.	difference	p	difference	p	difference	p
Gender							
Male	603						
Female	38	−26767.8	<0.001	−20826.2	0.013	−26658.9	<0.001
Age (yrs)							
<30	286						
30–50	290	−4936.2	0.449	1520.8	0.823	−3313.5	0.633
≥50	48	−4747.7	0.627	2665.1	0.802	8246.7	0.404
Mode of transmission							
Homosexual contact	371						
Heterosexual contact	70	−3605.2	0.706	10465.9	0.387	-	-
Injecting drug use	128	−22329.5	<0.001	−73432.1	<0.001	-	-
Receipt of blood products	6	−7205.5	0.752	−35456.4	0.227	-	-
HIV-1 subtype							
B	217						
C	3	1607.7	0.940	23058.5	0.269	19596.9	0.325
CRF01_AE	16	−10620.5	0.291	−43785.9	0.004	−37612.3	0.004
CRF07_BC	30	−21153.8	0.005	−9074.4	0.472	−58045.6	<0.001
CRF08_BC	1	−7883.3	0.086	−113409.7	<0.001	−106777.7	<0.001
CD4 cell count (cells/mm³)							
≥50	22						
51–250	82	−263241.1	<0.001	−240696.2	<0.001	−246030.4	<0.001
251–500	209	−313911.1	<0.001	−290801.3	<0.001	−296881.6	<0.001
>500	160	−334294.9	<0.001	−309993.4	<0.001	−309618.0	<0.001
Received ART							
No	270						
Yes	371	−97661.2	<0.001	−102574.2	<0.001	−88088.5	<0.001

[a]The mode of transmission was removed from multivariate model II.

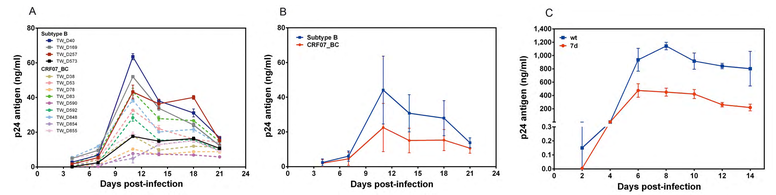
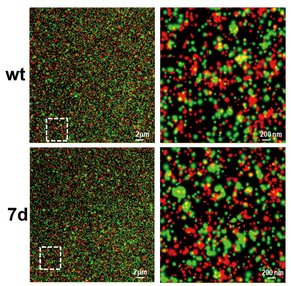


Figure 2. Comparison of the replication kinetics of different HIV-1 isolates from patients infected with CRF07_BC or subtype B (A and B) as well as recombinant HIV-1 virus with or without a 7 amino-acid deletion in the p6gag protein. (A) PBMCs were infected with fixed amounts of different HIV-1 subtype B (square) or CRF07_BC (circle) isolates and cultural supernatants were collected at days 4, 7, 11, 14, 18 and 21 post-infection. (B) The representative replicative curves of CRF07_BC and subtype B isolates deduced from Figure 2A. (C) MT2 cells were infected with wild type (wt) or recombinant mutant virus with a 7 amino-acid deletion at the p6gag (7d). Supernatants were collected at days 2, 4, 6, 8, 10, 12, and 14 post-infection. Viral replication was monitored through p24 antigen production. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to estimate the differences between subtypes, or between the recombinant viruses.



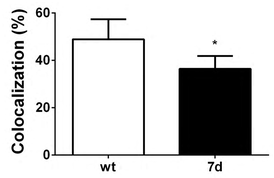


Figure 4. The interaction between Alix protein and wild type/mutant Gag. MAGIC-5 cells were infected with wt or 7d recombinant virus for 48 hours. The Alix and Gag proteins were analyze by TIRF-SR with rabbit anti-Alix polyclonal antibody and mouse anti-p24 monoclonal antibody. Red spots indicate Alix protein. Green spots indicate either wild type or 7d Gag protein. The proportion of co-localization of Alix and Gag protein was quantified using Volocity 3D Image Analysis Software.

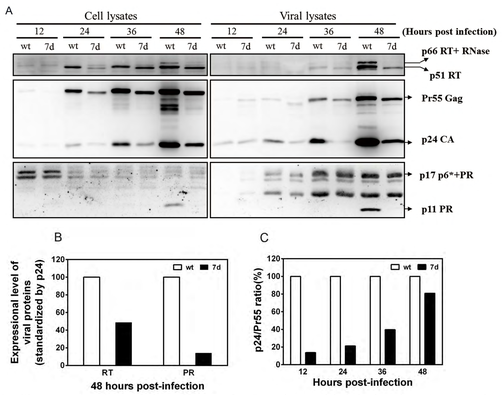
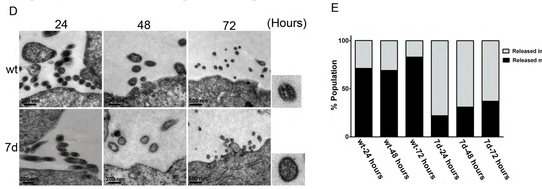


Figure 3. Characterization of the effects of a 7 amino-acid deletion in p6gag to the HIV-1 proteins expression, release and maturation. MT2 cells were infected with wild type (wt) or deleted-type (7d) recombinant viruses. After 12, 24, 36 and 48 hours, supernatant was collected and pelleted by ultracentrifugation. (A) Western blot analysis of the cell lysates (left panel) and viral lysates (right panel) from cells infected with wt or 7d viruses. (B) The relative expression levels of PR and RT in the viral lysates of cells infected with wt or 7d virus. The total arbitrary densitometer units of PR and RT were standardized by p24 and normalized to those of wt in parallel experiments. The images were analyzed with Image J software. (C) The ratios of p24 vs. Pr55 (maturation index) in the viral lysates at different time points after infection were calculated. The total arbitrary densitometer units of each hours post infection were normalized to those of wt in parallel experiments. All results were representative of two independent experiments.



(D) Electron microscopic (EM) examination of the viral particles of cells infected with wt or 7d recombinant viruses. MAGIC-5 cells were fixed and processed for transmission EM at different time points after they were infected with wt or 7d viruses. Scale bar indicates 200nm. (E) Quantification of relative proportions of mature vs. immature virions released at different time points in the cells infected with wt or 7d viruses using EM.

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