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Analysis of the near full length genomes of HIV-1 subtypes B, F and BF recombinant from a cohort of 14 patients in São Paulo, Brazil

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Abstract

The human immune deficiency virus (HIV) exhibits strikingly tremendous amount of genetic variability. Such feature is critically important for the virus to adapt to environmental changes by escaping the host immune system and by escaping candidate vaccine. Therefore, understanding of such diversity is fundamental for the design of successful drugs or vaccine, which is urgently needed to bring the HIV/AIDS epidemic under control. In this study, we investigated the magnitude of diversity of the HIV-1 near full-length genomes from patients previously assigned as infected with non-recombinant HIV-1 subtypes B and F1 variants based on small portion of viral genome. HIV-1 proviral DNA was extracted from 14 samples previously classified in our laboratory as six subtypes B and eight subtypes F on the basis of small amplicon sequencing. Reamplifications of DNA from these samples were carried out by an overlapping PCR followed by direct sequencing. The data were phylogenetically inferred. Sequence analysis revealed that two out of six partially identified subtype B and six out of eight partially identified subtype F were in fact BF recombinants throughout their full genomes. Two pairs BF recombinants had identical genomic recombination structure and distinct from the Argentinean CRF 12_BF strains, probably represents a novel circulating recombinant forms in Brazil. Our data provided new genetic material of some of the HIV-1 subtypes currently circulating in the country and points to the widespread of BF recombinants which are expected to change the epidemic nature by approaching the level of subtype B in Brazil.

Keywords: Full length genome; HIV-1 subtypes; HIV-1 recombination

1. Introduction

Human immunodeficiency virus-1 (HIV-1) is the causative agent of AIDS and can persist in individuals for years before causing disease. The HIV-1 genome is approximately 9.5 kb and displays significant sequence variations as a result of constant mutation and evolutionary pressure. Based on these genetic variations and pattern observed in phylogenetic reconstruction, researchers have classified the virus into groups, subtypes and sub-subtypes (Robertson et al., 2000). Currently, three groups (M, main; O, outlier; N, neither) have so far been recognized. HIV-1 group M viruses are responsible for the current global epidemic and are further classified into nine (A–D, F–H, J and K) subtypes, approximately equidistant, although subtypes B and D seem to be more closely related. Moreover, early sequencing studies have provided evidence of interstrand crossovers between genomes of different HIV subtypes (Sabino et al., 1994; Robertson et al., 1995). Such interclade recombinant strains are consistently reported from regions where two or more clades are predominant. Recombinant strains from

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unlinked epidemiological sources that exhibit identical patterns of mosaicism have been classified separately as circulating recombinant forms (CRFs) (Carr et al., 1998). There are currently 16 defined CRFs, namely CRF01–CRF16 and are epidemiologically important as subtypes. HIV-1 subtypes and CRFs show considerably different patterns of distribution in different geographical regions.

In South America, HIV epidemic is concentrated among people at increased risk of infection like injecting drug users, and men who have sex with men. The most prevalent HIV genetic subtypes are subtypes B, BF recombinants and F (Hierholzer et al., 2002). CRF12_BF and its related recombinant forms are widely circulating in Argentina (Carr et al., 2001; Thomson et al., 2002).

Brazil is the Latin American country that has been hardest hit by the HIV epidemic and has the second highest number of HIV-1 cases in the Americas, after the USA with an estimated number of 610,000 HIV-1/AIDS cases at the end of 2001 (Global AIDS program, 2004). HIV-1 subtype B is a major genetic form circulating in the country, however, existence of small proportion of other subtypes such as F, C, B/C and B/F have been consistently reported (Sabino et al., 1994; Cornelissen et al., 1996; Bongertz et al., 2000; Caride et al., 2000; Brindeiro et al., 1999). Typing strategies of these strains are based on sequencing of one or multiple short fragments of the viral genome, which may not accurately define the true HIV-1 genetic structure. Efforts to search for CRFs in Brazil in two recent studies did not find a common ancestry of Brazilian recombinants or their relationship to CRF12_BF (Thomson et al., 2004; Sa Filho et al., 2005). The current study was undertaken to characterize HIV-1 near full-length genomes from patients previously assigned as infected with non-recombinant HIV-1 subtypes B and F1 variants based on small portion of viral genome.

2. Materials and methods

2.1. Samples

A total of 14 HIV-1 strains isolated from adult Brazilian patients attending HIV/AIDS treatment centre were selected as representative of pure subtype B or F1 variants based on small portion of their genome. These isolates were genotyped in our laboratory by sequencing part of *pol* region to determine their genotypic resistance to antiretroviral therapy. Sequence informations from the V3–V5 *env* genome regions were available from some samples. Partial genome sequencing revealed eight isolates as subtype F1 and six as subtype B variants. Based on these results, the isolates were submitted for complete genome characterizations. All patients were from São Paulo (located at the south-east region of Brazil) and reportedly to had acquired their infection through sexual contact. Other characteristics of the subjects are given in Table 1.

2.2. Methods

DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the QIAamp blood kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. Proviral DNA was amplified from the purified genomic DNA by PCR using primers and methods described by Thomson et al. (2002) with little modifications. Briefly, five overlapping subgenomic fragments were amplified in 50-μl volume reaction mixture containing 0.5–1 μg of template DNA, 10 nmol of each dNTP, 20 pmol each of primers, 2.0 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.3) and 0.50 U of Taq DNA polymerase (Invitrogen). Thermal cycling was essentially hot-started (Mastercycler Gradient thermocycler; Eppendorf Scientific) at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 58.5 °C for 30 s,

Table 1
Characteristics of patient samples included in this study

Sample	Year of collection	Sex	Age	Mode of transmission	Subtype identification		
					Partial genome region	subtype	Complete genome
01BR042	2001	M	38	Heterosexual	F1	*rt*[a]	BF1
01BR047	2001	M	38	Heterosexual	F1	*Prot*[b], *rt*	BF1
01BR087	2001	M	49	Bisexual	F1	*prot*, *rt* and *env*[c]	F1
01BR125	2001	F	39	Heterosexual	F1	*prot*, *rt* and *env*	F1
01BR226	2001	F	43	Heterosexual	F1	*prot*, *rt*	BF1
01BR323	2001	F	44	Heterosexual	F1	*prot*, *rt* and *env*	BF1
02BR002	2002	F	29	Heterosexual	B	*prot*, *rt* and *env*	B
02BR005	2002	F	29	Heterosexual	B	*prot*, *rt*	BF1
02BR006	2002	F	30	Heterosexual	B	*prot*, *rt*	BF1
02BR008	2002	M	37	Homosexual	B	*prot*, *rt*	B
02BR011	2002	M	27	Bisexual	B	*prot*, *rt*	B
02BR013	2002	F	35	Heterosexual	B	*prot*, *rt*	B
02BR033	2002	M	36	Bisexual	F1	*prot*, *rt*	BF1
02BR034	2002	M	31	Bisexual	F1	*prot*, *rt*	BF1

[a] Reverse transcriptase.
[b] Protease.
[c] Envelope (V3–V5).