Virus Research 144 (2009) 306–314

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

HIV-1 clade C *env* clones obtained from an Indian patient exhibiting expanded coreceptor tropism are presented with naturally occurring unusual amino acid substitutions in V3 loop

Lavina Gharu, Rajesh Ringe, Sudhanshu Pandey, Ramesh Paranjape, Jayanta Bhattacharya*

Department of Molecular Virology, National AIDS Research Institute, Indian Council of Medical Research, G-73, MIDC, Bhosari, Pune 411026, Maharashtra, India

ARTICLE INFO

Article history:
Received 6 April 2009
Accepted 17 April 2009
Available online 3 May 2009

Keywords:
HIV-1
Envelope
Coreceptors
Expanded tropism
V3 loop
Clade C

ABSTRACT

HIV-1 subtype C is predominantly circulating in India and has been reported to be strictly CCR5 tropic irrespective of disease stages. In the present study, we examined *env* clones obtained from a late stage Indian patient with a history of multiple sexual partners and opportunistic infections for coreceptor usage and V3 loop sequence. The *env* clones were found to exploit several coreceptors in addition to CCR5 in a cell-associated and cell-free manner. Analysis of V3 loop sequence revealed that the NARI-VB105 *env* clones were presented with unique amino acid substitutions with GPGR motif, atypical of clade C envelope. Further genetic analysis showed the V3 sequences albeit belonging to subtype C; however clustered distinctly to that of other clade C envelopes originated in different geographical regions. Modelling data revealed that NARI-VB105 V3 loop contained several basic residues giving rise a high net positive charge of +8 to these envelopes.

© 2009 Elsevier B.V. All rights reserved.

HIV-1 clade C is the major subtype circulating in India that almost exclusively transmits via CCR5 throughout the course of infection (Cecilia et al., 2000). Most of the published data that were primarily focused on the viral subtypes circulating in India showed that subtype C accounts for the majority of the HIV infections in India (Mandal et al., 2002; Siddappa et al., 2004), though other subtypes and recombinant forms have also been reported (Lakhashe et al., 2008; Lole et al., 1999; Tripathy et al., 2005). Binding of HIV-1 envelope to CD4 results in conformational rearrangements in gp120 and facilitates formation of heterotrimeric complex between gp120, CD4 and coreceptor (Trkola et al., 1996) thus facilitating virus–host membrane fusion for entry. Apart from principle coreceptors, CCR5 and CXCR4, several members of β-chemokine family such as CCR2b, CCR3, CCR8 and several orphan 7 transmembrane (TM) having close homology with chemokine receptor family e.g., GPR15 (BOB), STRL-33 (BONZO), GPR-1, etc. support HIV-1 entry. The *env* gene of HIV-1 undergoes substantial divergence right from the *env* sequence acquired from the transmitted virus throughout the prolonged course of infection and leading to switch (Coetzer et al., 2008) and/or expansion of alternate coreceptor usages (Dash et al., 2008; Peters et al., 2004; Willey et al., 2003). Very recently, Shimizu et al. (2009) demonstrated that G protein-coupled receptors such as FPRL1 and GPR1 play a potential role in the context of HIV-1 infection as much as CCR5 and CXCR4 *in vivo*. Although subtype C viruses are predominantly CCR5 tropic, CXCR4 variants have also been reported (Coetzer et al., 2007; Lahuerta et al., 2008; Morris et al., 2001; Peeters et al., 1999); however it is not clear why HIV-1 clade C circulating in India predominantly uses CCR5 throughout the course of infection irrespective of any disease stage.

In the present study, we have investigated V3 loop sequence and coreceptor binding properties of HIV-1 subtype C envelope obtained from an ART naïve patient (NARI-VB105) in year 2000 with a history of multiple sexual partners, blood transfusion, genital ulcer disease and Herpes simplex and Herpes zoster infections associated with ophthalmic branch of trigeminal nerve. The HIV-1$^+$ patient peripheral blood mononuclear cells (PBMCs) were obtained from Virus Repository of National AIDS Research Institute, Pune, India. However, unlike most primary viruses isolated from Indian patients that have been reported to be strictly CCR5 tropic (Cecilia et al., 2000) NARI-VB105 primary isolate was found to infect several coreceptors besides CCR5, which led us investigate further the properties of *env* genes obtained from this patient. The primary virus of NARI-VB105 was obtained by co-cultivating HIV-1$^+$ PBMC with donor PBMC essentially as described by Rodriguez et al. (2006) and subsequently tested in CD4$^+$ GHOST indicator cells to evaluate coreceptor usage along with eight primary CCR5-using primary viruses obtained from AIDS patients with CD4 count of less than 250 cu/mm. GHOST indicator cells were infected with different dilutions of primary isolates and incubated for 72 h in a humidified CO_2 incubator at 37 °C before infectivity measured by

* Corresponding author. Tel.: +91 20 2712 1343; fax: +91 20 2712 1071.
E-mail address: jbhattacharya@nariindia.org (J. Bhattacharya).

0168-1702/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2009.04.020

Table 1
Infectivity titers of primary viruses in GHOST indicator cells.

	CD4+ GHOST (FFU/ml)						
	CCR5	CXCR4	CCR2b	CCR3	CXCR6	GPR15	CX3CR1
NARI-VB105	304,000	293,500	161,000	118,990	149,000	166,490	188,740
NARI-VB27	375,600	–	–	–	–	–	–
NARI-VB51	293,600	–	–	–	–	–	–
NARI-VB95	323,300	–	–	–	–	–	–
NARI-VB96	440,900	–	–	–	–	–	–
NARI-VB97	387,500	–	–	–	–	–	–
NARI-VB98	388,100	–	–	–	–	–	–
NARI-VB100	359,200	–	–	–	–	–	–
NARI-VB106	401,200	–	–	–	–	–	–

Infectivity titers were expressed as focus forming units (FFU) per millilitre of virus stock as a function of intracellular p24 antigen.

p24 antigen immunostaining. Infected cells were washed once in PBS and then fixed in chilled 1:1 vol/vol (−20 °C) of methanol: acetone for 10 min on ice. The cells were then washed once with PBS and incubated at room temperature with PBS containing 1% FCS for 30 min. Anti-p24 Hybridoma supernatant 183-H12-5C (Chesebro et al., 1992) at a dilution of 1:50 in 0.5% PBS/FCS were next added to the cells and incubated further for 1 h at room temperature. Cells were washed again and incubated further with 0.5%

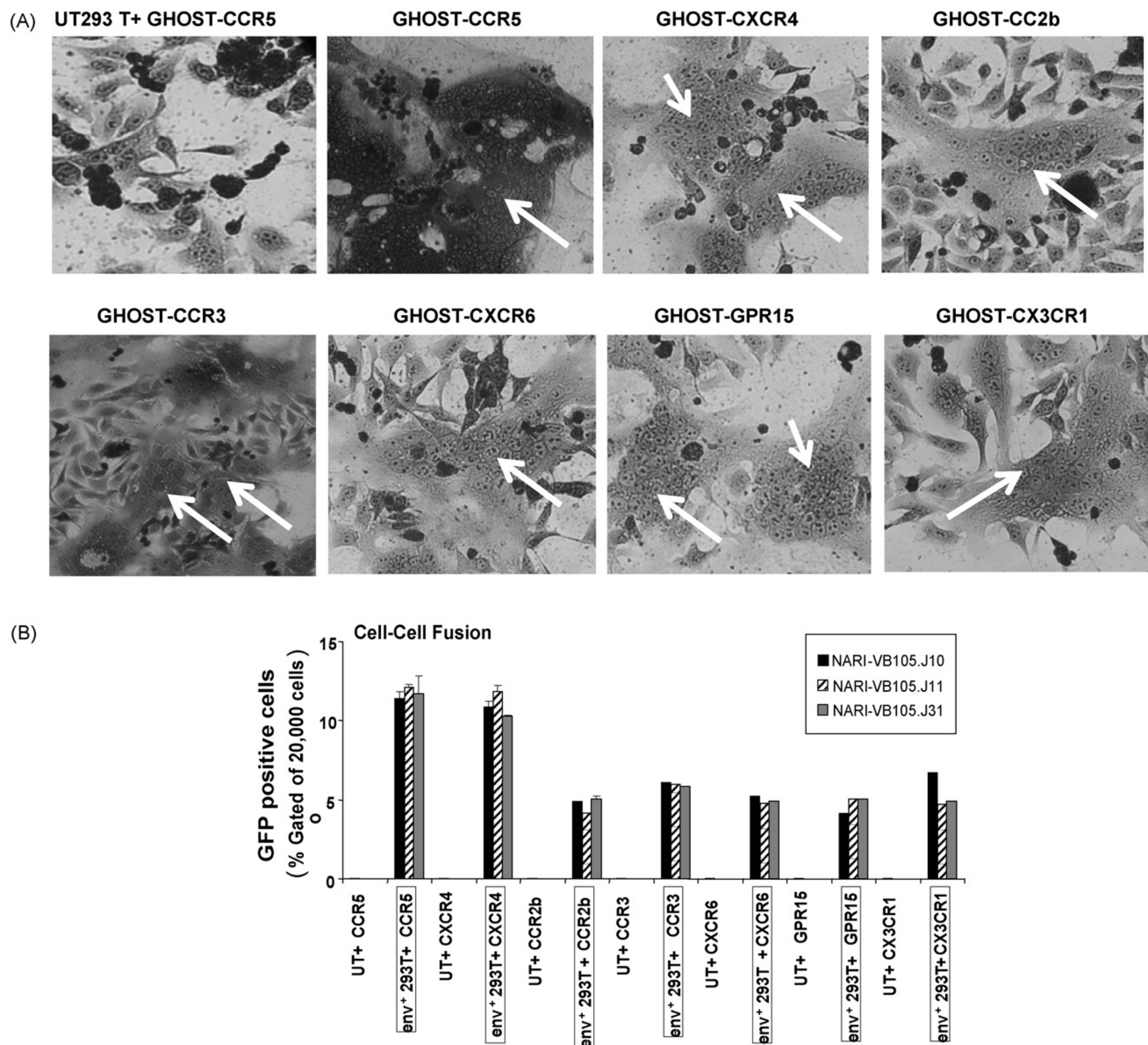

Fig. 1. Coreceptor usages by NARI-VB105 envelope clones. (A) GHOST cells expressing CCR5, CXCR4, CCR2b, APJ, CCR3, CXCR6, GPR-15, and CX3CR1 (V28) were co-cultivated with env+ 293T cells. Post 8–12 h of incubation, multinucleated giant cells (syncytia; indicated by arrows) were stained with methylene blue and basic fuchsin. In mock, un-transfected 293T cells were mixed with GHOST-CCR5 cells. (B) Quantitative fusion assay was done by mixing 293T cells transfected with env clones and GHOST cells expressing different coreceptors as described above and fusion activity measured in a Flow Cytometer (BD FACSCalibur).

Download English Version:

https://daneshyari.com/en/article/3429692

Download Persian Version:

https://daneshyari.com/article/3429692

Daneshyari.com