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Characterization of a dual-tropic Human immunodeficiency virus (HIV-1) strain derived from the prototypical X4 isolate HXBc2

Shi-hua Xiang [a,b,*], Beatriz Pacheco [a], Dane Bowder [b], Wen Yuan [a,1], Joseph Sodroski [a,c,d]

[a] Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute and Department of Microbiology and Immunobiology, Division of AIDS, Harvard Medical School, Boston, MA 02215, United States
[b] Nebraska Center for Virology, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583, United States
[c] Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115, United States
[d] Ragon Institute of MGH, MIT and Harvard, United States

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) coreceptor usage and tropism can be modulated by the V3 loop sequence of the gp120 exterior envelope glycoprotein. For coreceptors, R5 viruses use CCR5, X4 viruses use CXCR4, and dual-tropic (R5X4) viruses use either CCR5 or CXCR4. To understand the requirements for dual tropism, we derived and analyzed a dual-tropic variant of an X4 virus. Changes in the V3 base, which allow gp120 to interact with the tyrosine-sulfated CCR5 N-terminus, and deletion of residues 310/311 in the V3 tip were necessary for efficient CCR5 binding and utilization. Thus, both sets of V3 changes allowed CCR5 utilization with retention of the ability to use CXCR4. We also found that the stable association of gp120 with the trimeric envelope glycoprotein complex in R5X4 viruses, as in X4 viruses, is less sensitive to V3 loop changes than gp120-trimer association in R5 viruses.

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Introduction

The entry of human immunodeficiency virus type 1 (HIV-1) into the host cell is mediated by the viral envelope glycoproteins (Choe et al., 1998; Wyatt and Sodroski, 1998). The envelope glycoproteins, gp120 (SU) and gp41 (TM), constitute a trimeric complex that is anchored on the virion surface by the membrane-spanning segments of gp41 (Chan et al., 1997; Farzan et al., 1998; Weissenhorn et al., 1997; Zhu et al., 2003). The mature envelope glycoproteins form a trimer in which three gp120 subunits are noncovalently bound to three membrane-anchored gp41 subunits (Helseth et al., 1991). The initial binding of gp120 to the cellular receptor CD4 triggers conformational changes in gp120 that allow the subsequent interaction with one of the chemokine coreceptors, usually CCR5 or CXCR4 (Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Dragic et al., 1996; Feng et al., 1996; Wu et al., 1996). Coreceptor binding is thought to induce additional conformational changes in the envelope glycoproteins that lead to the fusion of the viral and target cell membrane (Hoffman and Doms, 1999).

HIV-1 can be classified into three phenotypes based on the virus' ability to use the CCR5 and/or CXCR4 coreceptor (Berger et al., 1998). R5 viruses use CCR5 as the coreceptor, X4 viruses use CXCR4 as the coreceptor and R5X4 (dual-tropic) viruses can use both coreceptors. HIV-1 primarily infects human CD4-positive T cells and macrophages. Cellular tropism can be determined by coreceptor usage (Rana et al., 1997). R5 viruses infect primary macrophages and T lymphocytes, whereas X4 viruses infect primary T lymphocytes and T-cell lines (Rana et al., 1997). The coreceptor usage, and thus, cellular tropism, is mainly determined by the third variable loop (V3 loop) of the gp120 exterior envelope glycoprotein (Chavda et al., 1994; Chesebro et al., 1996; Hwang et al., 1991).

The V3 loop of HIV-1 gp120 is about 34–37 residues in length but exhibits significant variability among different isolates (Hartley et al., 2005). Structurally, the V3 loop can be divided into three regions: the base, the stem and the tip (crown) (Huang

* Corresponding author at: Nebraska Center for Virology, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Morrison Center 337, 4240 Fair Street, Lincoln, NE 68583-0900, United States.
Fax: +1 402 473 3323.
E-mail addresses: sxiang2@unl.edu, shxiang2@gmail.com (S.-h. Xiang).
[1] Current address: Department of Medicine, Division of Infectious Diseases, School of Medicine, University of Virginia, Charlottesville, VA 22908.

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et al., 2005). The V3 stem is more variable in sequence, whereas the base and tip are relatively conserved. Because the V3 loop is the main determinant of coreceptor usage (Chesebro et al., 1996; Hoffman et al., 2002; Shioda, Levy, and Cheng-Mayer, 1992; Willey, Theodore, and Martin, 1994), it has been intensively studied for the purposes of understanding interactions with the coreceptors and predicting coreceptor usage of HIV-1 isolates. In general, the V3 loops of X4 viruses have a greater number of positive charges than those of R5 viruses (Jensen et al., 2003; Low et al., 2007); however, distinct sequence characteristics have not been defined for the V3 loops of dual-tropic viruses. Some studies have shown that residues 306, 321 and 322, the N-linked glycan at residue 301, and the total number of positive charges in the V3 loop are important for determining coreceptor preference (Cardozo et al., 2007; de Jong et al., 1992; Fouchier et al., 1995; Ogert et al., 2001; Polzer et al., 2002). In addition, some bioinformatics tools have been developed to predict coreceptor usage (Chueca et al., 2009; Jensen et al., 2003). However, the prediction of coreceptor usage for a given V3 loop based only on the V3 amino acid sequence is still imperfect.

In this report, we explore in more detail the interactions between the V3 loop sequences and coreceptors CCR5 and CXCR4. We identify an interesting derivative of the prototypic X4 strain, HXBc2, that has acquired the ability to use CCR5 but still retains CXCR4 usage. Two residues in the base of the V3 loop were found to be critical for this dual-tropic phenotype. Modeling based on available x-ray crystal and NMR structures and mutagenesis data suggest that these residues contact the tyrosine-sulfated N-terminus of the chemokine receptor. Another pair of HXBc2 amino acid residues at the tip of the V3 loop was found to be detrimental to CCR5 binding and had to be deleted to allow CCR5 tropism. In addition to analyzing the effects of these changes on HIV-1 tropism, we examined the impact of the V3 changes on envelope glycoprotein trimer stability and discovered that R5X4 and X4 HIV-1 exhibit similar phenotypes that are distinct from those of R5 HIV-1.

Results

Generation of a dual-tropic HXBc2 variant.

We wished to study the specific role of certain residues in the gp120 V3 loop that are important for coreceptor interaction, with the aim of understanding the mechanism by which some HIV-1 viruses acquire dual tropism. Fig. 1 shows an alignment of the V3 loops of some common X4-, R5-, and dual-tropic viruses. Most natural HIV-1 isolates lack residues 310–311 (QR in some laboratory-adapted X4-tropic viruses). Residues 325 and 326 (NM in the laboratory-adapted X4-tropic viruses) are more commonly DI in primary R5 or R5X4 HIV-1 strains, suggesting that these residues could be important for CCR5 interaction. Previous studies have shown the importance of some of these motifs in virus tropism (Hung et al., 1999); however, the exact mechanism by which these changes affect viral phenotypes has not been defined. To investigate the contribution of these residues to coreceptor choice, we generated HXBc2 mutants containing a deletion in residues 310–311 (ΔQR) and/or the changes N325D/M326I (DI) (Fig. 2A). The infectivity of these V3 loop mutants was analyzed in Cf2Th cells expressing CD4 and either CXCR4 or CCR5. Corroborating the hypothesized role of these residues in coreceptor usage, we observed that, like the R5-tropic ADA virus and the dual-tropic 89.6 virus, the DIΔQR mutant was able to infect Cf2Th cells expressing CD4 and CCR5 efficiently (Fig. 2B). Surprisingly, the DIΔQR mutant retained the ability to infect CD4/CXCR4 cells efficiently and thus exhibited dual tropism.

To characterize further the contribution of the two V3 elements to tropism, we generated HXBc2 viruses containing only the DI substitutions (DI) or the QR deletion (ΔQR). When these changes were individually introduced, the resulting mutant viruses were unable to mediate entry of HIV-1 into CD4/CCR5 cells. In addition, their infectivity in CD4/CXCR4 cells was reduced compared to that of the wild-type HXBc2 (27% and 68% for the DI and ΔQR mutants, respectively) (Fig. 2B). The substitution of an alanine residue for Arg 311 in the context of the DI mutant (DI/R311A) abolished the infectivity in CD4/CCR5 and in CD4/CXCR4 cells (Fig. 2B). By contrast, changing Arg 306 to Ser did not alter coreceptor usage in the context of either HXBc2 or the DI mutant (Fig. 2B). Together, these data suggest that both the ΔQR and DI changes in the V3 region are necessary for the dual-tropic phenotype.

Binding of DIΔQR mutants to the 412d antibody and CCR5.

The 412d monoclonal antibody mimics the tyrosine sulfate-containing N-terminus of the CCR5 coreceptor (Choe et al., 2003; Huang et al., 2007; Xiang et al., 2005). To assess the binding of the V3 loop mutants to the 412d antibody, cell supernatants containing radiolabeled gp120 glycoproteins were immunoprecipitated with 412d and the bound gp120 was analyzed on an SDS-PAGE gel. As expected, the wild-type HXBc2 gp120 and the X4 ΔQR and R306S mutants did not efficiently bind the 412d antibody (Fig. 2C). However, 412d was able to bind and immunoprecipitate the R5X4 DIΔQR mutant. Surprisingly, 412d also recognized the DI and DI/R306S mutants (Fig. 2C), even though these two mutants were not able to infect CD4/CCR5 cells. Similar results were obtained when the DI change was introduced into the gp120 glycoprotein of another X4 virus, NL4–3 (Fig. 2C). These results indicate that the DI change, but not the ΔQR deletion, is required for 412d antibody binding.

The binding of the V3 loop mutants to CCR5 was also studied. Supernatants from cells expressing radiolabeled gp120 were incubated, in the presence of soluble CD4, with cells expressing CCR5. The bound gp120 glycoprotein was analyzed by SDS-PAGE. In contrast with the 412d binding results, when we tested the DI

		BASE					STEM							TIP									STEM								BASE						
tropism	strain	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322		323	324	325	326	327	328	329	330	331
X4	HXBc2	C	T	R	P	N	N	N	T	R	K	R	I	R	I	Q	R	G	P	G	R	A	F	V	T	I	G	K	-	I	G	N	M	R	Q	A	H	C
X4	NL4-3	C	T	R	P	N	N	N	T	R	K	S	I	R	I	Q	R	G	P	G	R	A	F	V	T	I	G	K	-	I	G	N	M	R	Q	A	H	C
X4	MN	C	T	R	P	N	Y	N	K	R	K	R	I	H	I	-	-	G	P	G	R	-	F	Y	T	T	K	N	I	I	G	T	I	R	Q	A	H	C
X4	CMU08	C	T	R	P	S	N	K	K	R	Q	R	V	T	I	-	-	G	P	G	R	V	Y	Y	S	T	G	D	I	M	G	D	I	R	K	A	H	C
R5/X4	89.6	C	T	R	P	N	N	N	T	R	R	R	L	S	I	-	-	G	P	G	R	A	A	Y	A	R	R	N	I	I	G	D	I	R	Q	A	H	C
R5/X4	SF2	C	T	R	P	N	N	N	T	R	K	S	I	Y	I	-	-	G	P	G	R	A	F	H	T	T	G	R	I	I	G	D	I	R	K	A	H	C
R5	ADA	C	T	R	P	N	N	N	T	R	K	S	I	H	I	-	-	G	P	G	R	A	F	Y	T	T	G	E	I	I	G	D	I	R	Q	A	H	C
R5	YU2	C	T	R	P	N	N	N	T	R	K	S	I	N	I	-	-	G	P	G	R	A	L	Y	T	T	G	E	I	I	G	D	I	R	Q	A	H	C
R5	SF162	C	T	R	P	N	N	N	T	R	K	S	I	T	I	-	-	G	P	G	R	A	F	Y	A	T	G	D	I	I	G	D	I	R	Q	A	H	C

Fig. 1. Sequence alignment of the V3 loops of several common X4-, R5-, and dual-tropic HIV-1 strains. Amino acid position numbering is based on that of the prototypic HXBc2 strain, as per current recommendations (Korber et al., 1998). Key V3 residues that have been previously implicated in coreceptor choice are highlighted with stars. Residues conserved among the V3 loops shown are boxed. A potential N-linked glycosylation site at asparagine 301 is indicated by a black oval.