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Adaptation of HIV-1 to cells with low expression of the CCR5 coreceptor

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ABSTRACT

The binding of the human immunodeficiency virus (HIV-1) envelope glycoprotein (Env) trimer $((gp120/gp41)_3)$ to the receptors CD4 and CCR5 triggers virus entry into host cells. To identify Env regions that respond to CCR5 binding, HIV-1 was serially passaged on a CD4-positive canine cell line expressing progressively lower levels of CCR5. HIV-1 replication was observed in cells expressing ~1300 CCR5 molecules/cell. Env changes that conferred this low-CCR5 replication phenotype were located outside of the known CCR5-binding region of the gp120 Env subunit and did not apparently increase CCR5 binding affinity. The adaptation-associated changes, located in the gp120 α1 helix and in the gp41 HR1 heptad repeat and membrane-proximal external region (MPER), enhanced HIV-1 replication in cells at all levels of CCR5 expression. The adapted Envs exhibited a greater propensity to undergo conformational changes, as evidenced by increased exposure of conserved regions near the CD4- and CCR5-binding sites.

1. Introduction

The metastable human immunodeficiency virus (HIV-1) envelope glycoprotein (Env) trimer $((gp120/gp41)_3)$ mediates entry into target cells (Wyatt and Sodroski, 1998). Virus entry is triggered by Env binding sequentially to CD4 and a coreceptor, most often CCR5 but also CXCR4 (Klatzmann et al., 1984; Dalgleish et al., 1984; Cocchi et al., 1995; Deng et al., 1996; Feng et al., 1996; Alkhatib et al., 1996; Choe et al., 1996; Doranz et al., 1996; Dragic et al., 1996). CD4 binding drives Env from its unliganded conformation (State 1) through an intermediate (State 2) to the full CD4-bound conformation (State 3) (Munro et al., 2014; Herschhorn et al., 2016). In State 3, Env assumes a pre-hairpin intermediate conformation in which the CCR5-binding site on gp120 and the heptad repeat (HR1) coiled coil on gp41 are formed and exposed (Wu et al., 1996; Trkola et al., 1996; Furuta et al., 1998; Si et al., 2004; He et al., 2003; Koshiba and Chan, 2003). The hydrophobic fusion peptide at the gp41 N-terminus is thought to interact with the target cell membrane during this process (Freed et al., 1990; Kowalski et al., 1987). CCR5 binding (for CCR5-tropic (R5) HIV-1) or CXCR4 binding (for CXCR4-tropic (X4) HIV-1) triggers the pre-hairpin intermediate (State 3) to form the gp41 six-helix bundle (Lu et al., 1995; Chan et al., 1997; Tan et al., 1997; Weissenhorn et al., 1997). The formation of this very stable, post-fusion six-helix bundle is thought to promote the fusion of the viral and target cell membranes (Melikyan et al., 2000).

HIV-1 variants with lower requirements for CD4, including viruses that are completely CD4-independent, have been generated in the laboratory (Kolchinsky et al., 1999; Edwards et al., 2001; Dumonceaux et al., 1998; Zhang et al., 2002). CD4-independent viruses exhibit the ability to sample downstream Env conformations (State 2 and/or State 3) spontaneously, a property dictated by determinants in both gp120 and gp41 subunits (Haim et al., 2011; Kolchinsky et al., 2001a; Kolchinsky et al., 2001b; Edwards et al., 2002; LaBranche et al., 1999; Hoffman et al., 1999; Dumonceaux et al., 2001; Musich et al., 2011). Natural HIV-1 variants derived from the central nervous system often exhibit a reduced dependence on CD4 and efficiently infect macrophages and microglia, which express low levels of CD4 (Martin et al., 2001; Thomas et al., 2007; Peters et al., 2004; Gorry et al., 2002; O'Connell et al., 2013).

The vast majority of transmitted/founder HIV-1 and most HIV-1 strains in individuals with established infections are CCR5-tropic (Peters et al., 2004; Lin et al., 2012; Keele et al., 2008; Melby et al., 2006). Clinical observations suggest that blocking Env-CCR5 binding will suppress HIV-1 infection. For example, in 2009, an HIV-1-infected patient with acute myeloid leukemia received a stem cell transplant from a donor homozygous for *CCR5Δ32*, which encodes an N-terminally deleted CCR5 protein that does not support HIV-1 infection (Liu et al., 1996). Since then, this individual has had an undetectable viral load and a sustained reconstitution of his immune system in the absence of antiretroviral therapy (Hutter et al., 2009; Allers et al., 2011).

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Small-molecule CCR5 antagonists have been used to treat HIV-1 infection (Anastassopoulou et al., 2011; Lalezari et al., 2005; Roche et al., 2011; Tilton et al., 2010a). Maraviroc (MVC) is an FDA-approved HIV-1 entry inhibitor that binds in the hydrophobic pocket formed by the CCR5 transmembrane helices and stabilizes a CCR5 conformation that resists efficient gp120 binding (Garcia-Perez et al., 2011; Tan et al., 2013). HIV-1-infected individuals treated with MVC exhibited reduced viral loads, followed by selection for previously undetected CXCR4-using viruses or the evolution of CCR5-tropic viruses capable of using MVC-bound CCR5 as a coreceptor (Lalezari et al., 2005; Tilton et al., 2010a; Tilton et al., 2010b; Westby et al., 2007; Jiang et al., 2015; Berro et al., 2011; Westby et al., 2007). These latter MVC-resistant viruses have altered residues in the gp120 V3 stem that enhance binding affinity to the CCR5 N-terminus and to the drug-bound extracellular loops, despite overall decreases in entry and replication capacity (Roche et al., 2011; Tilton et al., 2010a; Tilton et al., 2010b; Berro et al., 2012). HIV-1 adapted to vicriviroc, an investigational CCR5 antagonist, displayed altered residues in gp41 that have been proposed to lead to increased triggering of the fusion peptide as well as gp120 changes that enhanced affinity for the CCR5 N-terminus (Lalezari et al., 2005; Berro et al., 2012). These studies indicate that HIV-1 can evolve to use conformationally altered CCR5 for viral entry.

Strategies to interfere with CCR5 binding would benefit from additional knowledge about the structures of the CCR5-bound Env trimer and downstream intermediates, the Env determinants of these conformational transitions, and the molecular dynamics required for viral entry. Here, we generate HIV-1 isolates that infect cells with low levels of CCR5, hypothesizing that the adaptation-associated changes in Env will identify regions that are critical for CCR5 binding or CCR5-triggered membrane fusion. We tested the extent to which HIV-1 can evolve to adapt to limiting amounts of CCR5 by progressively reducing the levels of CCR5 expressed on target cells and monitoring the compensatory changes in the adapted HIV-1 Env. The starting virus for these studies was a chimeric HIV-1 with the envelope glycoproteins from HIV-$1_{JR\text{-}FL}$, a macrophage-tropic brain-derived virus that was already able to infect cells with moderately low levels of CCR5 (O'Brien et al., 1990). After extensive passage, viruses that can infect cells with ~1300 CCR5 molecules per cell were generated. The adapted viruses demonstrated enhanced infectivity compared to the starting virus during cell-cell transmission, but cell-free infectivity was poor. The Env changes required for the adaptation to low CCR5 usage did not result in an increase in CCR5 binding. However, the adapted viruses were more sensitive to CD4 triggering and to neutralization by particular antibodies, indicating that the Env from these viruses is predisposed to make transitions from a State 1 conformation.

2. Results

2.1. Adaptation of HIV-$1_{NL4.3(JR\text{-}FL)}$ to replicate in cells expressing low levels of CCR5

We adapted an HIV-1 with the HIV-$1_{JR\text{-}FL}$ Env to infect cells with low levels of CCR5 by serially passaging the virus in cells in which the level of CCR5 expression was gradually decreased. Cf2Th canine cells constitutively expressing human CD4 and expressing human CCR5 in a Tet-regulated fashion were used as target cells for the adaptation (Fig. 1). Two Cf2Th clones that express high levels of CD4 and either low or high ranges of cell-surface CCR5 upon doxycycline treatment, herein called R5-Low and R5-High cells, were used for HIV-1 adaptation. An initial stock of HIV-$1_{NL4.3(JR\text{-}FL)}$ was prepared by transfecting 293T cells with the proviral plasmid and harvesting the cell supernatant three days later. Cf2Th-CD4/CCR5 cells, which constitutively express human CD4 and human CCR5, were incubated with the 293T cell supernatants and cultured. Reverse transcriptase activity was detected in the supernatants by 30 days of culture (data not shown). Cell supernatants with reverse transcriptase activity were used to reinfect Cf2Th-CD4/CCR5 in the presence of 2 μg/mL polybrene to enhance infection. Viruses in the supernatants of these cells were used for adaptation of HIV-$1_{NL4.3(JR\text{-}FL)}$ to CD4-expressing cells with low levels of CCR5.

Cf2Th cells expressing human CD4 constitutively and human CCR5 in a Tet-regulated manner (Fig. 1) were incubated with the HIV-$1_{NL4.3(JR\text{-}FL)}$ virus and then passaged. By three rounds of passage, viruses emerged that replicated on a mixture of 70% R5-High cells and 30% R5-Low cells in 1 μg/mL doxycycline (Fig. 2A). By 9 rounds of passage, viruses that replicated on R5-Low cells in 75 ng/mL doxycycline were obtained.

When additional rounds of virus passage were conducted at decreasing levels of target cell CCR5 expression, we observed decreasing reverse transcriptase activity in the cell supernatants (Fig. 2A). Viral replication was not detected beyond round 12, in which we detected reverse transcriptase activity in the supernatants of R5-Low cells in 12.5 ng/mL doxycycline. Cell-free viruses in the supernatant of the round 12 cultures did not detectably replicate in R5-Low cells maintained in the same levels of doxycycline (data not shown). QuantibritePE was used to estimate the number of CCR5 molecules/cell by quantifying the epitopes for the 2D7 anti-CCR5 antibody per cell (BD Biosciences). Replication of HIV-$1_{NL4.3(JR\text{-}FL)}$ was not detected in the CD4-expressing cells with less than ~1300 CCR5 molecules per cell (Fig. 1).

To confirm that the viruses generated by this adaptation procedure could replicate in cells expressing low levels of CCR5, we infected R5-Low cells at 50 and 100 ng/mL doxycycline, as well as Cf2Th-CD4/CCR5 cells, with cell supernatants from round 3 (J3), round 9 (J9) and round 11 (J11) that were normalized for the level of the p24 Gag protein. Fig. 2B compares the replication capacity of the J3 and J9 viruses with that of the parental HIV-$1_{NL4.3(JR\text{-}FL)}$ virus for each cell type and doxycycline concentration. In Cf2Th-CD4/CCR5 cells, J3 and J9 viruses infected 2-fold and 4-fold more efficiently than the parental HIV-$1_{NL4.3(JR\text{-}FL)}$, respectively. Both J3 and J9 viruses replicated better than HIV-$1_{NL4.3(JR\text{-}FL)}$ in R5-Low(100) and R5-Low(50) cells. The production of the J3 virus in R5-Low(100) cells was comparable to that of the parental HIV-$1_{NL4.3(JR\text{-}FL)}$ in Cf2Th-CD4/CCR5 cells; by contrast, HIV-$1_{NL4.3(JR\text{-}FL)}$ replicated only marginally in the R5-Low(100) cells. Both J3 and J9 viruses replicated in R5-Low(50) cells, whereas no HIV-$1_{NL4.3(JR\text{-}FL)}$ reverse transcriptase activity was detected in these cells. These observations indicate that the J3 and J9 viruses have adapted to replicate better than the parental HIV-$1_{NL4.3(JR\text{-}FL)}$ virus on cells expressing low levels of CCR5.

The J11 viral pool did not detectably replicate in any cell type, suggesting that some adaptive changes occurring after round 9 were deleterious to cell-free infection in this context.

2.2. Adaptive changes in the HIV-$1_{JR\text{-}FL}$ Env

We isolated the genomic DNA of the cells collected at the time point of peak reverse transcriptase activity in the supernatant at each round of adaptation, PCR amplified the integrated HIV-1 provirus, and sequenced the *env* gene. The wild-type HIV-$1_{JR\text{-}FL}$ *env* sequence was maintained throughout multiple rounds of replication in Cf2Th-CD4/CCR5 cells (data not shown). In the viruses adapted to replicate in R5-Low cells, multiple changes were observed. Changes that were retained through multiple rounds of adaptation are shown in Fig. 3A. Three changes, S115N, R564H, and E662K, were found in all three adapted viruses, J3, J9, and J12. Both J9 and J12 had, in addition, an S164N change, and J9 had an E831D change.

None of the above passage-associated Env changes were observed in previous studies in which HIV-1 was adapted to replicate on Cf2Th cells lacking CD4 (Kolchinsky et al., 1999) or expressing New World monkey receptors (Pacheco et al., 2008). Thus, the observed Env changes apparently arose as an adaptation to the specific requirements imposed by the low-CCR5 cells. None of the observed Env changes has been previously implicated in the interaction of gp120 with CCR5.