The interaction of human immunodeficiency virus type 1 (HIV-1) with CD4 and one of a cadre of chemokine receptors triggers conformational changes in the HIV-1 envelope (Env) glycoprotein that lead to membrane fusion. The coreceptor activity of the second extracellular loop of CXCR4, which is restricted to dual tropic and T-tropic strains, was insensitive to the removal of charged residues either singly or in combinations by alanine scanning mutagenesis or to the conversion of acidic residues to lysine. Conversion of Asp-187 to a neutral residue exclusively unmasked activity with M-tropic Env in fusion and infection experiments. Insertion of the D187V mutation into chimeras containing extracellular loop 2 of CXCR4 in a CXCRI2 framework also resulted in the acquisition of M-tropic coreceptor activity. The independence of CXCR4 coreceptor activity from charged residues and the extension of its repertoire by removing Asp-187 suggest that this interaction is not electrostatic and that coreceptors have the potential to be utilized by a spectrum of Env, which may be masked by charged amino acids in extracellular domains. These findings indicate that the primary structural determinants of coreceptors that program reactivity with M-, dual, and T-tropic Env are surprisingly subtle and that relatively insignificant changes in CXCR4 can dramatically alter utilization by Env of varying tropism.

Human immunodeficiency virus, type 1 (HIV-1) infection is initiated by the interaction of the envelope (Env) glycoprotein with its primary receptor, CD4, on the plasma membrane of the host cell (1–3). Progression to membrane fusion does not occur unless the target cell also expresses one of a cadre of chemokine receptors (Ref. 4; reviewed in Ref. 5), which function as fusion cofactors (6, 7). These members of the serpentine receptor superfamily transduce the signals of proinflammatory chemokines. The physiologic effects on the directed migration of leukocytes are mediated through linkage to guanine nucleotide binding proteins (G-proteins) (reviewed in Ref. 8), as is characteristic of this receptor type. In contrast, the precise mechanism for their role as coreceptors in HIV-1 Env-mediated fusion has not been fully elucidated, although some insights into structure-function relationships and the consequences of Env-coreceptor interactions have been gleaned.

Whereas virtually all Env glycoproteins can associate with CD4 (9), binding to a discrete region in the first immunoglobulin-like domain (10), there is selective utilization of chemokine receptors by Env at various stages of infection (11), thereby imparting the specificity of viral tropism. M (macrophage)-tropic strains of HIV-1 require CCR5 for entry into target cells (12–16), and individuals carrying a protective allele encoding a nonfunctional protein have a significant degree of resistance to infection (17–19). T-tropic strains spawned late in the evolution of AIDS predominantly use CXCR4 (20, 21). There is emerging evidence that dual tropic viruses, which exhibit a more promiscuous utilization of coreceptors (16), represent intermediates in this evolution (11).

Fusion of the viral and target cell membranes is mediated by the envelope glycoprotein in many viral systems, including HIV (reviewed in Ref. 22). During this process, Env undergoes a dramatic change in conformation that ultimately results in exposure of the fusion peptide of gp41, enabling it to interact directly with the plasma membrane of the target cell. The association of gp120 with CD4 elicits the unmasking of cryptic epitopes in the former (23, 24), but this activated configuration cannot effect fusion. The structure of this complex is, however, permissive for association of Env with a cognate coreceptor, which triggers the former to assume a fusogenic conformation. Thus, it is likely that it is the binding of coreceptors to the activated form of Env that leads to mobilizing the exposure of the fusion peptide of gp41. To this end, a complex of soluble CD4 and a T-tropic Env has been reported to coimmunoprecipitate with CXCR4 (25), and a similar trimolecular complex containing CCR5 has been demonstrated with M-tropic Env in ligand binding experiments (26, 27).

Structure-function relationships of CCR5 and CXCR4 coreceptor activity have been analyzed in order to gain insight into the interactions described above and into the mechanisms underlying the inhibition of viral infection by the cognate chemokines for CCR5 (28) and CXCR4 (20, 21) and by candidate small molecule inhibitors of coreceptor function. These studies indicate that multiple domains of CCR5 and CXCR4 are required for coreceptor activity (29–35). Furthermore, it is clear from experiments with chimeric receptors and point mutants that
there is differential utilization of CCR5 by virus strains, with dual tropic viruses being more sensitive to changes in CCR5 than M-tropic strains (33). Subtle differences in how viruses interact with CXCR4 have been observed as well (32, 34). Such analysis may identify the structural motifs that enable multiple coreceptors to be utilized by Env glycoproteins of dual tropic viruses. The available data suggests that the N terminus of CCR5 (29, 33) and the second extracellular loop (ECL) of CXCR4 (34) are critical to coreceptor activity for Env of 89.6. Since ECL2 of CXCR4 is also crucial for coreceptor function for T-tropic Env, it may play a pivotal role in coreceptor function in the evolving spectrum from M-tropism to T-tropism. This domain has a net positive charge in CCR5 and a net negative charge CXCR4.

The primary structural basis for the requirement of ECL2 of CXCR4 for coreceptor activity was investigated to gain insight into the mechanism of Env-mediated fusion. Since the V3-loop, which has been implicated in determining coreceptor specificity (39–44), is generally more basic in T-tropic Env proteins (43–45), the contribution of charged residues in the second ECL of CXCR4 to coreceptor function was determined. While no individual charged residue or cluster was critical for coreceptor activity with the IIIB or 89.6 Env, removal of a single, specific acidic residue from this domain conferred coreceptor activity with an array of Env from M-tropic strains without significantly altering this function for T-tropic or dual tropic Env. These findings provide further insight into the complexity of the interaction between gp120 and coreceptors during the progression of infection and furnish a model coreceptor system that could be widely applicable to the study of HIV-1 entry into target cells and to virally mediated gene therapy of AIDS.

**EXPERIMENTAL PROCEDURES**

**Preparation of Mutant Receptors—Constructs encoding wild type CXCR4 and CCR5 in the pcDNA3 vector described previously were used as templates for site-directed mutagenesis using the Chameleon double-stranded site-directed mutagenesis kit (Stratagene, San Diego, CA). Clones containing the programmed mutation(s) were identified by nucleotide sequence analysis of the targeted region, and the nucleotide sequence of the entire open reading frame was confirmed prior to analysis in fusion assays to exclude the possible introduction of extraneous mutations. A chimeric receptor containing the N-terminal extracellular domain of CCR5 and the complementary region of CXCR4, designated 5444, and a battery of chimeras composed of CXCR4 and CCR5, both of which were prepared by polymerase chain reaction-ligation-polymerase chain reaction as described previously, were also used as templates for site-directed mutagenesis (34).**

**Env-mediated Fusion Assay**—The coreceptor function of wild type chemokine receptors, variants containing point mutations, and chimeras was determined using a modified fusion assay (36) employing a luciferase reporter gene as described previously (33, 34). Briefly, constructs encoding the candidate coreceptor, CD4, and luciferase under the transcriptional control of a T7 promoter were cotransfected into the QT6 cell line using calcium phosphate precipitation. 16–18 h posttransfection, the transfectants were mixed with either HeLa or QT6 effector cells in which the expression of HIV-1 Env and T7 polymerase was directed using a vaccinia virus system (37, 38). Following incubation for 24 h to permit transient expression, the cells were infected with viral stocks in the presence of 4 μg of polybrene/ml in a total volume of 500 μl. Three days postinfection, an additional 0.5 ml of medium was added. Four days postinfection, the cells were harvested by resuspension in 150 μl of 0.5% Triton X-100 in phosphate-buffered saline, and 50–75-μl aliquots were assayed for luciferase activity using commercial reagents (Promega, Madison, WI) in a Wallac 1450 Microbeta luminometer.

**Analysis of Cell Surface Expression**—The expression of chemokine receptor chimeras and point mutants on the surface of transfected cells was measured by flow cytometry using monoclonal antibodies to CXCR4 (1D5G5), CCR5 (12D1 and R&D 45529; R&D Systems, Minneapolis, MN), and CXCR2 (10H2). Cells were stained at room temperature with the appropriate monoclonal antibody, washed, incubated with a secondary antibody labeled with phycoerythrin, and analyzed using an Elite flow cytometer (Coulter Electronics, Inc., Miami, FL).

**RESULTS**

**Charged Residues in ECL2 of CXCR4 Do Not Contribute to Coreceptor Activity with Dual Tropic and T-tropic Env**—Previous studies using CXCR4/CXCR2 chimeras have demonstrated the importance of ECL2 of CXCR4 in coreceptor activity with Env glycoproteins encoded by IIIB (T-tropic) and 89.6 (dual tropic) (34). In order to dissect the motifs that are involved in this function, an aggressive mutagenesis strategy was initially focused on charged amino acid residues in ECL2 and adjacent transmembrane-spanning helices. Alanine (Ala) scanning mutagenesis was performed to identify charged residues in this domain of CXCR4 that may be involved in the interaction with dual tropic and T-tropic envelope glycoproteins. This loop contains 5 acidic and 2 basic amino acid residues, and the fourth transmembrane-spanning domain (tm4) contains 1 acidic residue, as depicted in Fig. 1.

Variants were prepared in which the charged residues were individually converted to Ala by site-directed mutagenesis. Fusion coreceptor activity of the Ala-scanning mutants with IIIB and 89.6 Env glycoproteins did not differ significantly from that of the wild type receptor (data not shown), indicating that no single residue was critical to coreceptor activity. There was a suggestion that removal Glu-179 and Asp-182 may result in a subtle but consistent enhancement of coreceptor activity. Since 6 of 13 residues in the proximal region of ECL2 of CXCR4 are charged, it was reasoned that several may contribute to a structure that interacts with Env. To test this possibility, variants in which multiple charged residues were converted to neutral ones were prepared and analyzed for coreceptor func-

![Diagram of the predicted topology of the second extracellular domain and adjacent transmembrane-spanning helices of CXCR4.](image-url)
tion (data not shown). Glu-179 was replaced with Gln in order to maintain potential hydrophilic boundaries of tm4 while removing the net charge. CXCR4-E179Q/D181A/D182N showed a moderate decrease in utilization by IIIB and 89.6. The conversion of Asp-187 and Lys-188 to Ala, with or without D193A, did not have a dramatic effect on coreceptor activity. Surprisingly, a variant in which all acidic and one of the basic (Arg-189) residues were converted to neutral amino acids (E179Q/D181A/D182N/R188A/D193A) retained significant coreceptor activity with IIIB (~50%) and 89.6 (~25%).

The absence of a significant impact of remodeling the conformation of ECL2 by the removal of 6 of 7 charged amino acids on the fusion cofactor activity for dual tropic and T-tropic Env suggests that they are not directly involved in the structure that associates with Env. To test the possibility that noncharged residues participate in this structure, Phe-189 and Tyr-190 were replaced with Ala, and a hydrophobic stretch in the distal portion of ECL2 was interrupted by converting Val-197 to Asn. Neither of these mutations significantly altered fusion coreceptor function with IIIB and 89.6 (data not shown).

**Mutagenesis of ECL2 of CXCR4 Unmasks Cryptic M-tropic Activity**—The excess of acidic residues in ECL2 of CXCR4 is contrasted by an excess of basic amino acids in the corresponding domain of CCR5, which does not support fusion with T-tropic Env (33). To determine whether the net charge of ECL2 is critical to determining the coreceptor activity of CXCR4 to include T-tropic and dual tropic but not M-tropic Env, each acidic residue in this domain was converted individually to Lys. Analysis of these charge conversion mutants failed to reveal a significant alteration in coreceptor activity with IIIB and 89.6 (data not shown). Surprisingly, one of these variants, CXCR4-D193K, seemed to have minimal enhancement of utilization by 89.6.

Conversion of Asp-171, which is located in tm4, to Lys resulted in a marked loss of coreceptor function with the 89.6 (6% of wild type CXCR4) and IIIB (16%) Env glycoproteins. However, this variant was utilized as a fusion coreceptor by the BK132 and DH12 Env glycoproteins at levels approximately 25% of wild type CXCR4 (data not shown). A variant in which multiple acidic amino acid residues were switched to Lys, CXCR4-E179K/D181K/D182K, was produced to mimic the charge of the proximal region in ECL2 of CCR5. This mutant demonstrated a dramatic loss of function. Neither of these variants were detected on the cell surface by immunofluorescent staining (data not shown), suggesting that the mutations interfered with intracellular trafficking.

The difference in charge between ECL2 of CCR5 and CXCR4 and the frequent acquisition of positively charged residues in the V3 loop of Env upon conversion from M- to T-tropism (43–45) raised the possibility that a negatively charged ECL2 is required to limit the coreceptor function of CXCR4 to T-tropic Env and that changing the charge of this domain could be associated with a gain of coreceptor utilization by M-tropic Env. To test this possibility, the Ala scanning and charge conversion point mutants described above were tested in cell-cell fusion assays with JRFL. None of the CXCR4 variants containing charge conversion mutations were found to acquire coreceptor activity with this M-tropic Env (Fig. 2A). Parallel analysis of the Ala-scanning mutants, also shown in Fig. 2A, revealed that a single point mutant, CXCR4-D187A, functioned as a coreceptor for JRFL, demonstrating approximately 25% of the activity of CCR5. This point mutant was also found to be utilized as a fusion coreceptor by other M-tropic Env, including ADA, Bal, and SF162 (data not shown).

Analysis of CXCR4 variants with multiple mutations for M-tropic coreceptor activity revealed that variants containing the D187A mutation exhibited coreceptor activity with JRFL (data not shown). This activity was not altered significantly by the addition of R188A, D193A, F189A/Y190A, or V197N, but it was diminished by E179Q/D181A/D182A, E179Q/D181A/D182A/R188A, and E179Q/D181A/D182A/R188A/D193A. The acquisition of M-tropic coreceptor activity was not observed in variants lacking D187A.

To determine the requirements at amino acid residue 187 of CXCR4 for the maximum acquisition of M-tropic coreceptor activity, saturation mutagenesis was performed. As shown in Fig. 2B, the conversion of CXCR4-Asp-187 to Val, Phe, and Ser was associated with the acquisition of coreceptor activity with JRFL, whereas replacement with Asn resulted in limited M-tropic coreceptor activity, which was absent when Lys was substituted. CXCR4-D187V consistently demonstrated a significant level of fusion coreceptor activity with M-tropic Env, with a mean value that was greater than 50% of wild type CCR5. However, these mutations had minimal effects on the coreceptor activity of CXCR4 with IIIB and 89.6 (data not shown).

The M-tropic coreceptor activity of CXCR4-D187V could be inhibited by the addition of SDF-1, the ligand for CXCR4, and ALX40–4C, a pharmacologic inhibitor of CXCR4, to the fusion reaction, but not by AOP-RANTES, a CCR5-specific antagonist (Fig. 2C).

**Structural Determinants of CXCR4-D187V M-tropic Coreceptor Activity**—We have previously reported findings that implicated the involvement of the N terminus, ECL2, and ECL3 of CCR5 (33) and ECL1 and ECL2 of CXCR4 (34) in fusion coreceptor activity. Chimeras composed of CXCR4 and CXCRI containing CXCR4 Asp-187 point mutations were analyzed in fusion assays with M-tropic Env in order to gain insight into domains required for this activity. Since CXCR4-D187V was found to have the highest M-tropic coreceptor activity of the variants examined, this point mutation was introduced into a panel of chimeras containing ECL2 of CXCR4, including hybrids 4442, 2442, 2242, 2444, and 2244. As shown in Fig. 3A, the chimeras 2444-D187V and 4442-D187V showed significant coreceptor activity with JRFL. Fusion assays with wild type Env inserted into 2442, 2242, and 2244 were higher than negative controls and wild type CXCR4 but were less than the other chimeras. The ability of the D187V mutation to confer some M-tropic coreceptor activity in each CXCR4/2 chimera containing ECL2 of CXCR4 suggests that the segment of this receptor present in the 2242 chimera, which extends from tm4 to tm6, contains the motif(s) responsible for its fusogenic activity but does not exclude the contribution of other (extracellular) domains. The acquisition of coreceptor activity with M-tropic Env was not associated with a loss of utilization by IIIB and 89.6, as shown in Fig. 3B. The T-tropic coreceptor activity of the chimeras containing D187V was similar to that previously described for the chimeras with the wild type sequences (34).

Previous experiments have demonstrated that a chimera containing the N-terminal extracellular domain of CCR5 and the remainder of CXCR4 is a fusion coreceptor for M-, T-, and dual tropic Env (34). The D187V mutation was introduced into this multispecific coreceptor to determine whether it would enhance its utilization by M-tropic Env. The coreceptor activity of the 5444-D187V variant was not significantly different from that of the 5444 hybrid composed of wild type sequences (Fig. 3A).

**CXCR4 D187V Also Confers Sensitivity to Infection with Pseudotyped Viruses Containing M-tropic Env**—The coreceptor function of the CXCR4-D187V variants was tested in infection experiments to provide independent evidence of the acquisition of activity with the extended repertoire of Env glycoproteins.
FIG. 2. Residues in ECL2 involved in determining coreceptor activity of CXCR4 with M-tropic Env. CXCR4 variants were tested for coreceptor activity in fusion assays using effector cells expressing the indicated Env glycoproteins and T7 polymerase and target cells programmed to express human CD4, candidate coreceptor variants, and luciferase under the transcriptional control of a T7 promoter. Luciferase activity in detergent lysates was determined 8 h after mixing. The coreceptor activity of CCR5 was arbitrarily set at 100% in fusion experiments with M-tropic Env. The coreceptor activity of CXCR4 variants containing Ala-scanning mutations or Asp/Glu to Lys conversions with M-tropic Env was determined in experiments using the JRFL Env (A). The results are the mean values of duplicate analysis in at least four independent experiments. The coreceptor activity of CXCR4 variants in which various residues were substituted for Asp-187 was determined with JRFL (B). The ability of CXCR4 and CCR5 ligands (SDF-1 and AOP-RANTES, respectively), a pharmacologic inhibitor of CXCR4 (ALX40–4C), and a monoclonal antibody to CD4 (number 19) to inhibit the utilization of CXCR4-D187V by T-tropic (IIIB) and M-tropic (JRFL) Env is shown in C. Values are expressed as percentages of inhibition.

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As shown in Fig. 4, target cells transfected with CXCR4 variants in which Asp-187 was substituted with Ala, Val, Phe, and Ser could be infected with pseudotyped viruses containing the ADA and Bal Env glycoproteins at levels greater than wild type CXCR4. Conversion to Asn and Lys did not confer utilization by these M-tropic viruses, although the levels of infection by viruses containing the IIIB Env were similar among the Asp-187 mutants.

Independence of Coreceptor Activity from Level of Cell Surface Expression—The expression of the receptor variants was determined by flow cytometric analysis of QT6 cells in transient expression assays. With the exception of CXCR4-D182K and CXCR4-E179K/D181K/D182K, the expression of all of the point mutants and chimeras on the cell surface could be detected, albeit in varying levels. Typically, the level of expression was less than that of wild type CXCR4. To determine whether the differences in coreceptor activity in the current cell-cell fusion assay could be dependent upon levels of cell surface expression, parallel fusion and flow cytometry experiments were performed. Following optimization of the transfection efficiency, transfectants expressing varying levels of CXCR4, CCR5, CXCR4-D187V, and the 5444 chimera were prepared by using serial dilutions of the construct plasmid with compensatory amounts of vector plasmid as carrier. Transfec-
DISCUSSION

The present study demonstrates that substitution of one specific acidic amino acid in ECL2 of CXCR4 with a noncharged residue results in the acquisition of significant M-tropic coreceptor activity in cell-cell fusion and infection assays. This effect is exclusive to the Asp-187 residue and is conferred in significant levels when it is replaced with a hydrophobic amino acid. The ability of such a minor alteration in CXCR4 to exert a dramatic extension of its coreceptor repertoire is surprising, particularly since it is not associated with a commensurate loss of T-tropic coreceptor activity. Although it is likely that this mutation alters the conformation of ECL2, it did not influence the binding of a monoclonal antibody that recognizes an epitope involving ECL2 or the capability of SDF-1, the cognate ligand, to inhibit fusion. Analysis of chimeric receptors revealed that ECL2 of CXCR4 containing D187V was sufficient for inducing fusion with M-tropic Env glycoproteins. The ability to confer M-tropic coreceptor activity to CXCR4 by a point mutation raises the possibility that an array of chemokine receptors have structural determinants that are sufficient for coreceptor activity with dual tropic Env are determined by the primary structure of the contact point(s) of each. This has also been assumed to be the mechanism for the basis of the selective utilization of coreceptors by HIV-1 Env of varying tropisms. Previous studies have revealed that subtle amino acid changes in Env (39–42), notably the acquisition of basic residues in the V3 loop (43–45), have a significant effect on the evolution from M- to T-tropism. However, limited insight into the structural correlates in chemokine receptors responsible for this switch in specificity is available. Preliminary evidence suggests that the structures in CCR5 and CXCR4 that program coreceptor activities are topologically complex and represent extremes in the spectrum of this function. This is further emphasized by the finding that the domains required for coreceptor activity with dual tropic Env are different for CCR5 and CXCR4, providing additional evidence for the divergence in function of these two coreceptors. However, the current data suggest that both CCR5 and CXCR4 have structural determinants that are sufficient for coreceptor utilization by M-tropic Env but that this activity is silent in wild type CXCR4.

CXCR4 and CCR5 share limited identity at the level of
FIG. 5. Coreceptor activity is independent of the level of cell surface expression. QT6 cells transiently expressing CXCR4 were analyzed for cell surface expression by flow cytometry with the 12G5 monoclonal antibody (panel A) and for coreceptor activity in cell-cell fusion assays (panel B). Monolayers were transfected with the pcDNA3 vector (a) or with varying amounts of constructs encoding CXCR4-D187V (b, 1.5 µg; c, 0.30 µg; d, 0.15 µg; e, 0.03 µg; f, 0.015 µg; and g, 0.003 µg). The amount of DNA in the transfection reaction was normalized to the concentration shown to give the optimal transfection efficiency by the addition of compensatory amounts of the control vector. Immunofluorescent staining of cells transfected with the control plasmid gave mean peak fluorescence values that were identical to that obtained when transfectants were stained with a subtype-matched monoclonal mouse immunoglobulin. Fusion coreceptor assays were performed to test the coreceptor activity of CXCR4-D187V with IIIB and JRFL as described under “Experimental Procedures.” The amount of the coreceptor construct in the transfection reactions is indicated.
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primary structure, which overall is approximately 30%. The second ECL has been implicated in the coreceptor activity of both (33, 34), but the net charge of these two loops differs dramatically; that of CCR5 is basic, and that of CXCR4 is acidic. The simplest interpretation, that the disparity in the net charge in ECL2 of these receptors is directly responsible for differences in the repertoire of coreceptor activity, was excluded by the findings that the removal of acidic residues and the introduction of basic ones neither significantly altered T-tropic coreceptor function nor conferred M-tropic activity. However, the finding that substitution of a neutral amino acid for one specific acidic residue results in an expanded specificity of coreceptor function raises the possibility that this mutation favors the formation of a conformation permissive for utilization by M-tropic Env, either in ECL2 or in contiguous regions of the coreceptor. The interaction between Env and ECL2 of CXCR4 could involve nonexposed hydrophobic regions of the coreceptor and, presumably, of the Env glycoprotein, which would be likely for a cryptic epitope unmasked by the conformational shift in gp120 induced by interaction with CD4. Several aromatic residues are adjacent to the 187 position of CXCR4 and present in the corresponding region of CCR5 as well. It is unlikely that they participate in the interaction with Env because conversion to Ala did not abolish the coreceptor activity of wild type CXCR4 or the expanded spectrum of coreceptor activity of variants containing mutations at Asp-187.

It is possible that levels of cell surface expression of chemokine receptors and variants may have an impact on coreceptor activity. Experiments were performed to directly address this issue for the cell-cell fusion assay employed in the characterization of the CXCR4 mutants. Parallel flow cytometric analysis of cell surface expression of candidate coreceptors and analysis in fusion assays revealed that the latter activity remains surprisingly constant over a broad range of cell surface expression. This held true when less than 5% of cells had fluorescence values that were greater than that of the negative controls and autofluorescence for the fluorochrome employed (phycoerythrin). It was observed, however, that variants that do not undergo proper intracellular trafficking to the cell surface demonstrate minimal coreceptor activity.

Based on the ability of 89.6 to use ECL2 of CXCR4 and also to interact with this domain of CXCR4, we conclude that the introduction of basic residues as substitutes for acidic amino acids does not result in a conformation that mimics the coreceptor function of CCR5. However, the negative charge of Asp-187 may play a role in determining tropism by preventing the utilization of this coreceptor by M-tropic Env. This raises the possibility that allelic variants of coreceptors could alter the susceptibility to and pathogenesis of HIV-1 infection.

Whereas this type of broadly reactive coreceptor should prove valuable for dissecting mechanisms of Env-mediated fusion that are common to all Env species, it could also prove to be a powerful tool in the gene therapy of AIDS for the targeting of recombinant viruses (55–57) to cells that are infected by HIV quasispecies. In this context, target cells may express Env glycoproteins with varying repertoires of coreceptor specificity; thus, programming diversified tropisms. The effect of this moving target could be minimized by a coreceptor with an extended specificity for Env glycoproteins that includes both M- and T-tropic, as well as dual tropic types.

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