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Use of Murine CXCR-4 as a Second Receptor by Some T-Cell-Tropic Human Immunodeficiency Viruses

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The human CXCR-4 molecule serves as a second receptor for primary, T-cell-tropic, and laboratory-adapted human immunodeficiency virus type 1 (HIV-1) isolates. Here we show that murine CXCR-4 can support the entry of some of these HIV-1 isolates. Differences between mouse and human CXCR-4 in the ability to function as an HIV-1 receptor are determined by sequences in the second extracellular loop of the CXCR-4 protein.

Human immunodeficiency virus types 1 and 2 (HIV-1 and 2) and simian immunodeficiency virus cause AIDS in their respective hosts (5, 27). AIDS is characterized by the depletion of $CD4^+$ T lymphocytes, which represent a major target of viral infection in vivo (23). Infection of other $CD4^+$ cell types, such as monocytes, monocytes/macrophages, tissue dendritic cells, and brain microglia, has been suggested to be important for the pathogenesis and transmission of the primate immunodeficiency viruses (20, 28, 29, 34, 45, 47, 55).

The tropism of primate immunodeficiency viruses for $CD4^+$ cells is explained by the utilization of the CD4 glycoprotein as a primary receptor for virus entry into the cell (18, 33, 40). The viral envelope glycoproteins, which mediate virus entry, consist of the gp120 exterior envelope glycoprotein and the gp41 transmembrane glycoprotein (2, 48). The gp120 glycoprotein binds the CD4 molecule (40), following which the concerted action of the gp120 and gp41 glycoproteins results in the fusion of viral and cellular membranes (31, 35, 53). The interaction of the viral envelope glycoproteins expressed on the infected cell surface with adjacent $CD4^+$ cells results in the formation of syncytia by an analogous process (37, 52).

Host cell factors in addition to CD4 have been suggested to determine the efficiency of primate immunodeficiency virus envelope glycoprotein-mediated membrane fusion. Some human and animal cells have been shown to be resistant to HIV-1, HIV-2, or simian immunodeficiency virus infection and syncytium formation even when human CD4 was expressed on the cell surface (3, 15, 39, 41). HIV-1 variants that infect either primary monocytes/macrophages or immortalized $CD4^+$ cell lines, in addition to primary T lymphocytes, have been identified. The macrophage-tropic primary HIV-1 isolates cannot infect T-cell lines, laboratory-adapted viruses cannot infect primary monocytes/macrophages, and T-cell line-tropic primary viruses exhibit dual tropism for these cell types (10, 26, 50). Changes in the viral envelope glycoproteins, in particular in the third variable (V3) region of the gp120 exterior envelope glycoprotein, determine these phenotypes (8–13, 32, 43, 51, 56, 57). HIV-1 tropism has been explained by the requirement for specific members of the chemokine receptor family to be expressed on the target cell membrane, in addition to CD4, for HIV-1 entry and fusion. Most T-cell line-tropic primary viruses and laboratory-adapted viruses utilize a CXC chemokine receptor called CXCR-4 (21, 25) (also called LESTR, HUMSTSR, or fusin) (24, 38), while most macrophage-tropic primary HIV-1 isolates use the C-C chemokine receptor CCR5 (1, 14, 19, 22). The structure of the V3 loop on the HIV-1 gp120 envelope glycoprotein is a major determinant of which chemokine receptor can be used as an entry cofactor (14, 17, 44). The binding of macrophage-tropic HIV-1 gp120 glycoproteins to CD4 creates a high-affinity binding site for CCR5, indicating that HIV-1 entry involves sequential interaction with CD4 and chemokine receptors (54, 59). Evidence for interactions of a laboratory-adapted HIV-1 gp120 with a complex containing CD4 and CXCR-4 has also been reported (36). The natural ligands for the chemokine receptors (SDF-1 for CXCR-4 [7, 42] and RANTES/MIP-1α/MIP-1β for CCR5 [46, 49]) inhibit infection with the particular HIV-1 variants that utilize these molecules for entry (7, 16, 42).

Chimerae combining chemokine receptors that function as HIV-1 receptors and those that do not support HIV-1 entry have been created (4, 6, 22a, 48a). The activity of these chimeric molecules as second receptors has been studied in an attempt to define components of the chemokine receptor extracellular regions important for HIV-1 entry. These studies have revealed complex determinants for second receptor function, with contributions from the amino-terminal domain as well as each of the three extracellular loops (4, 6, 48a).

We examined the ability of murine CXCR-4 to support the entry of T-cell line-tropic primary and laboratory-adapted HIV-1 isolates that had previously been shown to utilize human CXCR-4 as a second receptor (14). Two isoforms of murine CXCR-4, which differ by a 2-residue insertion near the amino terminus, have been shown to be translated from alternatively spliced mRNAs (30a, 41a). Both murine CXCR-4 isoforms were tested for HIV-1 coreceptor activity. Recombinant HIV-1 variants encoding chloramphenicol acetyltransferase (CAT) and containing different HIV-1 envelope glycoproteins were produced as previously described (14, 31). These viruses were incubated with Cf2Th canine thymocytes transiently transfected with pcDNA3 plasmids expressing human CD4 and human CXCR-4, or human CD4 and either of the

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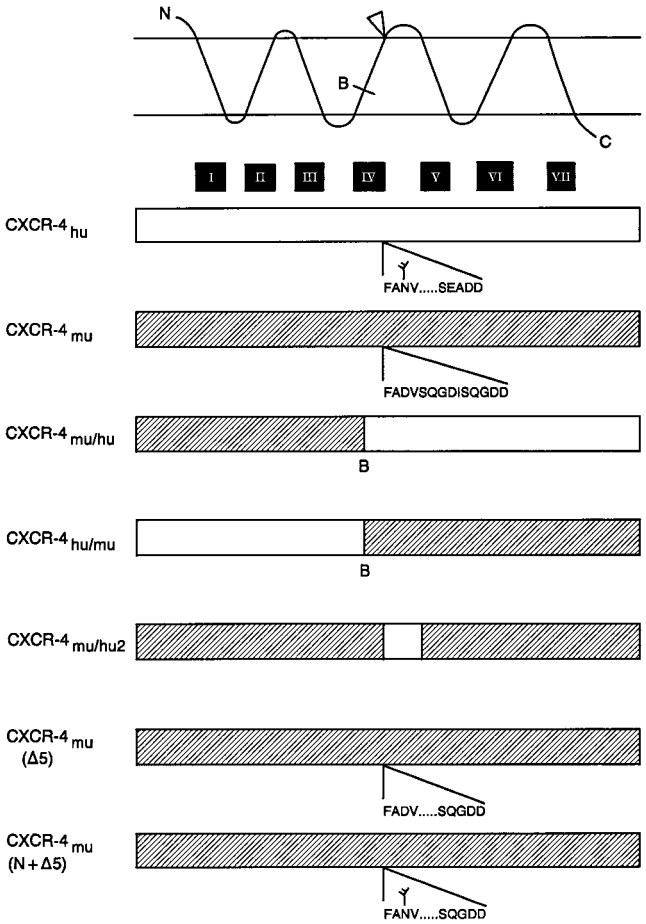

FIG. 1. Schematic representation of the CXCR-4 constructs used in this study. The CXCR-4 derivatives are chimeric molecules containing murine CXCR-4 (CXCR-4_{mu}) sequences (diagonally striped rectangles) as well as sequences (open rectangles) derived from human CXCR-4 (CXCR-4_{hu}). The junction between the CXCR-$4_{mu/hu}$ and CXCR-$4_{hu/mu}$ chimeric segments corresponds to the *Bam*HI site indicated in the parental structure (B). To create the CXCR-$4_{mu/hu2}$ chimera, a *Blp*I site was introduced into the human CXCR-4 gene at a position corresponding to the natural *Blp*I site in the murine CXCR-4 gene. A *Bam*HI-*Blp*I fragment of the murine CXCR-4 gene was then replaced by the corresponding human CXCR-4 fragment. The structures of murine CXCR-4 mutants are also shown. The sequences surrounding the inserts in the human and mouse CXCR-4 second extracellular loop, and the sequences present in the CXCR-4 variants tested, are shown beneath the rectangles. Solid boxes, transmembrane regions; N, N terminus; C, C terminus; open arrowhead, region of CCR5 sequence shown in detail, including site of potential N-linked glycosylation, indicated by ϒ. (Fig. 1).

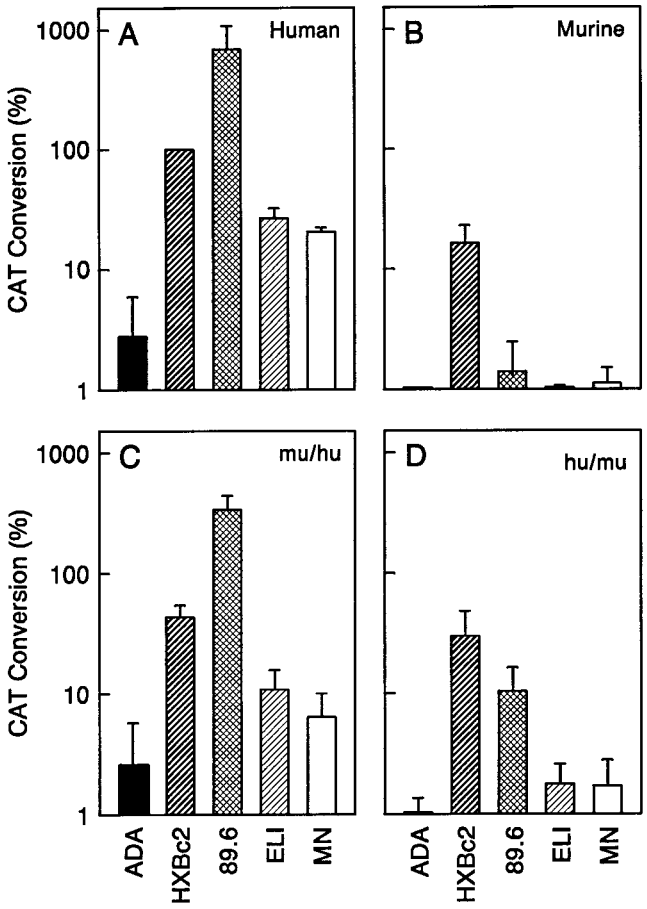

FIG. 2. CAT activity in transfected Cf2Th cells infected with recombinant HIV-1 variants. Cf2Th cells expressing human CD4 and human CXCR-4 (A), human CD4 and murine CXCR-4 (long isoform) (B), or human CD4 and a human-murine chimeric CXCR-4 protein (CXCR-$4_{mu/hu}$ [mu/hu] or CXCR-$4_{hu/mu}$ [hu/mu]) (C and D, respectively) were exposed to recombinant viruses containing envelope glycoproteins of the ADA, HXBc2, 89.6, ELI or MN isolate. The results of the CAT assay performed on the Cf2Th cell lysates, normalized for protein content, are shown. In some cases, dilutions of the cell lysates were used to keep the CAT assay within the linear range, and, thus, some CAT conversions greater than 100% are reported. The values reported are means of duplicate infections derived from three separate experiments. The CAT conversions observed for target Cf2Th cells transfected with the human CD4-expressing plasmid only were as follows (sources of envelope glycoproteins are given in parentheses): 0.8% (ADA), 0.4% (HXBc2), 0.4% (89.6), 0.6% (ELI), and 0.7% (MN).

two mouse CXCR-4 isoforms (Fig. 1). Measurement of CAT activity in the Cf2Th cells provided an assessment of the abilities of the different chemokine receptors to support the entry of HIV-1 variants containing different envelope glycoproteins. None of the recombinant viruses containing HIV-1 envelope glycoproteins entered Cf2Th cells expressing only human CD4 (see the legend for Fig. 2). When human CXCR-4 was coexpressed with CD4 in these cells, viruses with envelope glycoproteins from laboratory-adapted (HXBc2 and MN) and T-cell line-tropic primary (89.6 and ELI) strains infected the Cf2Th cells efficiently (Fig. 2A). As expected (14), a virus with macrophage-tropic primary isolate (ADA) envelope glycoproteins infected these cells much less efficiently.

When human CD4 and the longer of the murine CXCR-4 isoforms were coexpressed in the Cf2Th cells, the HIV-1 variant with the HXBc2 envelope glycoproteins was able to infect the cells (Fig. 2B). The efficiency of this infection was lower than that seen in Cf2Th cells expressing CD4 and human CXCR-4. A very low but detectable level of infection of the Cf2Th cells expressing CD4 and the longer of the mouse CXCR-4 isoforms was seen for the 89.6, ELI, and MN viruses, whereas the ADA virus did not infect these cells. A cDNA that could express only the shorter murine CXCR-4 isoform yielded results identical to those obtained with the longer mouse CXCR-4 isoform (data not shown).

To examine the role of species-specific sequence differences in the abilities of human and murine CXCR-4 to support HIV-1 entry, chimeric CXCR-4 proteins were produced (Fig. 1). Reciprocal chimerae containing the amino-terminal half of CXCR-4 derived from either human or mouse CXCR-4, with the carboxy-terminal half derived from the CXCR-4 protein of the other species, were expressed in Cf2Th cells with human CD4. Figure 2C shows that the chimera with the carboxy-

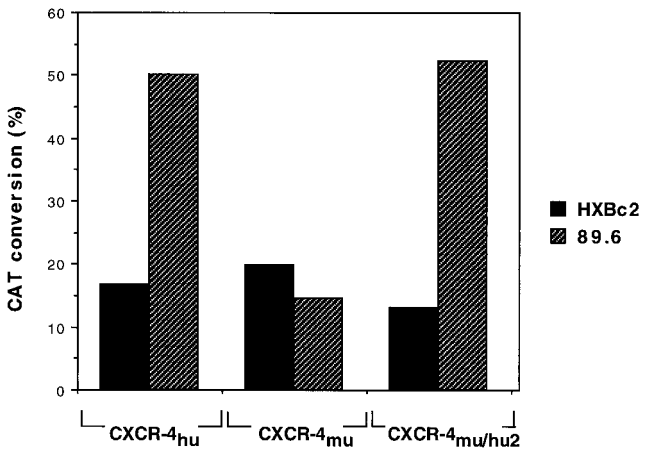

FIG. 3. Effect of expression of the CXCR-$4_{mu/hu2}$ chimera on HIV-1 infection of Cf2Th canine thymocytes. Cf2Th cells expressing human CD4 and either wild-type human CXCR-4, wild-type murine CXCR-4, or the CXCR-$4_{mu/hu2}$ chimera were incubated with recombinant viruses containing the envelope glycoproteins of the HXBc2 or 89.6 HIV-1 isolate, and CAT activity was measured. The values shown are from a representative experiment, which was repeated with comparable results.

terminal human CXCR-4 sequences supported HIV-1 entry with a pattern and efficiency similar to those exhibited by human CXCR-4. The chimera with the carboxy-terminal murine CXCR-4 sequences behaved comparably to the murine CXCR-4 protein, although entry of the 89.6 virus was slightly better on cells expressing the chimera, compared with the wild-type murine CXCR-4 (Fig. 2D). These results indicate that CXCR-4 sequences in the carboxy-terminal half of the protein are the major determinant of the observed functional differences between human and mouse CXCR-4 with respect to HIV-1 entry.

In the carboxy-terminal extracellular domains, most of the sequence differences between human and murine CXCR-4 are located in the second extracellular loop. To examine whether these differences could explain the different activities of human and murine CXCR-4 proteins as HIV-1 coreceptors, a chimera containing the second extracellular loop of human CXCR-4 in a murine CXCR-4 background (CXCR-$4_{mu/hu2}$) was created (Fig. 1). The activity of the CXCR-$4_{mu/hu2}$ chimera in supporting the entry of viruses with the HXBc2 and 89.6 envelope glycoproteins was indistinguishable from that of human CXCR-4 (Fig. 3). Similar results were obtained even when a lower multiplicity of infection was used (data not shown). These results indicate that differences between human and mouse CXCR-4 in the ability to function as an HIV-1 receptor are largely determined by sequences in the second extracellular loop of the CXCR-4 protein.

Comparison of the sequences of the human and murine CXCR-4 second extracellular loop reveals a 5-residue insertion in the latter sequence. There are additional amino acid differences in this loop, one of which results in a site of predicted N-glycosylation in human CXCR-4. We wished to examine whether the 5-residue insertion in the mouse CXCR-4 second extracellular loop, compared with that of human CXCR-4, contributed to the different activities of these proteins as HIV-1 second receptors. Thus, two additional murine CXCR-4 mutants were created (Fig. 1). In one of the mutants [CXCR-$4_{mu}(\Delta 5)$], the insertion was removed from the murine CXCR-4. In the other mutant [CXCR-$4_{mu}(N + \Delta 5)$], the potential N-linked glycosylation site present in human CXCR-4 was created in a mouse CXCR-4 mutant lacking the insertion in the second extracellular loop. These two CXCR-4 mutants were coexpressed with human CD4 in Cf2Th cells, which were incubated with the recombinant HIV-1 variants containing the envelope glycoproteins from the ADA, HXBc2, 89.6, ELI, and MN HIV-1 strains. The results of this assay indicate that both mutant mouse CXCR-4 molecules behaved similarly to the wild-type mouse CXCR-4 protein (data not shown). Thus, functionally important sequence differences between the human and mouse CXCR-4 second extracellular loops are not limited to the region of the insertion.

Our results indicate that some T-cell line-tropic HIV-1 strains can utilize the murine CXCR-4 protein as a second receptor. Previous studies finding that some murine cell lines were not infectible by HIV-1 despite the expression of human CD4 (3, 39) may not have employed the most efficient HIV-1 strains or may have used murine cells in which CXCR-4 expression was lower than that achieved in our transfected Cf2Th cells. Alternatively, since the efficiency of mouse CXCR-4 as a second HIV-1 receptor is lower than that of human CXCR-4, certain assays may have been too insensitive to detect this activity. Indeed, some murine cells have been shown to be susceptible to fusion with the envelope glycoproteins of the LAI strain of HIV-1 when human CD4 is expressed in them (3). Also, murine peripheral-blood mononuclear cells have been reported to be infectible by HIV-1 when a mouse CD4 altered to create a high-affinity binding site for gp120 was expressed in these cells (58).

Murine CXCR-4 can bind human SDF-1 and signal calcium transients in response to this interaction (30, 41a). The low efficiency of mouse CXCR-4 as a second receptor for most HIV-1 strains indicates that differences exist between the CXCR-4 sequences critical for SDF-1 binding and those required by the majority of HIV-1 strains. The observation that heterogeneity exists in the efficiency with which different T-cell line-tropic HIV-1 strains use mouse CXCR-4 as an entry cofactor suggests that either qualitative or quantitative differences exist in the interaction of HIV-1 variants with CXCR-4. Similar observations have been made in studies of CCR5 chimerae (6, 48a). This variation may need to be considered in attempts to inhibit HIV-1 infection by targeting the chemokine receptors with small molecules.

Structural differences in the second extracellular loop of human and mouse CXCR-4 determined functional efficiency as a second receptor for T-cell line-tropic HIV-1 strains. While the 5-amino-acid insertion in the second extracellular loop of murine CXCR-4 is the most dramatic of the sequence differences in this segment, our results indicate that this difference is not sufficient to account for the observed differences in second-receptor activity. Recently, it has been reported that rat CXCR-4, which lacks the insertion in the second extracellular loop, can function as a second receptor for some T-cell line-tropic HIV-1 strains (44a). The efficiency of rat CXCR-4 in supporting the entry of viruses with the envelope glycoproteins of the LAI strain of HIV-1 was almost comparable to that of human CXCR-4 (44a). Virus entry mediated by the HXBc2 LAI envelope glycoproteins used in our study was typically less efficient on cells expressing mouse CXCR-4 than on cells expressing human CXCR-4. While experimental variables (e.g., level of receptor expression) might account for these differences, a subset of the 4-amino-acid differences in the second and third extracellular loops of the rat and mouse CXCR-4 proteins might influence the efficiency of HIV-1 coreceptor activity.

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