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# CD4-Independent Infection by Human Immunodeficiency Virus Type 2 Strain ROD/B: the Role of the N-Terminal Domain of CXCR-4 in Fusion and Entry

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**The human immunodeficiency virus type 2 (HIV-2) strain ROD/B can efficiently use the 7tm chemokine receptor CXCR-4 as a primary receptor to enter CD4-negative cells. We have stably expressed CXCR-4 on mink lung Mv-1-lu and feline kidney CCC cells (normally restrictive to HIV entry) and have shown efficient fusion, entry, and replication of ROD/B. Mutation of the two N-linked glycosylation sites on CXCR-4 ($N_{11}$→I, and $N_{176}$→Q) or pretreatment of CCC or Mv-1-lu cells expressing wild-type CXCR-4 with the glycosylation inhibitor tunicamycin increased fusion and entry by ROD/B. Deletion of portions of the N terminus of CXCR-4 resulted in a 3- to 10-fold decrease in cell-free infection by ROD/B and complete inhibition of cell-cell fusion by both ROD/B and another HIV-2 strain, CBL23. These data suggest that the N-terminal domain of CXCR-4 is involved in but is not essential for the efficient fusion of ROD/B with CD4-negative cells. Deletion of the C-terminal (intracellular) domain of CXCR-4 did not significantly affect entry by ROD/B, indicating that intracellular signalling through this domain does not play a significant role in entry by HIV-2.**

Although CD4 is the primary receptor for the human and simian immunodeficiency viruses (HIV-1, HIV-2, and SIV), it is known that other cofactors are required for entry of these viruses into target cells (5, 11, 24). The restriction to HIV-1 entry into certain CD4-positive cells is due to the absence of a specific cofactor essential for fusion. Recently, two members of the seven transmembrane (7tm) G-protein-coupled chemokine receptor family of proteins, CXCR-4 (18) (also termed Fusin or Lestr) and CCR-5, have been shown to function as coreceptors, together with CD4, for T-cell-line- and macrophage-tropic HIV-1 isolates, respectively (1, 13, 15). Other members of this family including CCR-3 and CCR-2b also act as cofactors for some primary HIV-1 isolates (4, 14). The natural ligands for some of these receptors block entry of HIV-1 (8, 15, 17, 28). Recent reports showing the formation of complexes between gp120, CD4 and CXCR-4 (22) and evidence for direct binding of gp120 to CCR-5 (37, 40) are beginning to give insights into the mechanisms that HIV uses to fuse and enter cells.

Several laboratories have shown that some HIV isolates can infect lymphoid and nonlymphoid cells (6, 19, 25, 35, 36) in the absence of CD4. Although infection in most of these examples is extremely inefficient, the selection of an HIV-2 strain, ROD/B (derived from the prototype HIV-2 strain ROD/A), that can directly induce cell-cell fusion and efficient infection of certain CD4-negative human cell lines has been reported (6). The highly cytopathic nature of this infection indicates that HIV-2 can efficiently utilize one or more alternate receptors independently of CD4. ROD/B is able to use the 7tm chemokine receptor CXCR-4 to efficiently enter CD4-negative cells (16, 31). We also found that ROD/B can use CCR-3 and the orphan receptor V28 to enter CCC cells but only after pretreatment of the virus with soluble CD4 (sCD4) (31).

In this article, we define some of the regions on CXCR-4 essential for the entry into CD4-negative cells by ROD/B. Deletion of both the N terminus and C terminus and mutation of the two N-linked glycosylation sites of CXCR-4 have been studied for their ability to allow the entry of ROD/B into mink (Mv-1-lu) and feline (CCC) cells.

## MATERIALS AND METHODS

**Cells.** The human T-cell line H9 (30) was maintained as a suspension culture in RPMI 1640 medium (Gibco), supplemented with 10% (vol/vol) fetal calf serum (FCS), 60 μg of penicillin per ml, and 100 μg of streptomycin per ml. The nonprimate lines, cat kidney CCC (10) and mink lung Mv-1-lu (20), were maintained as monolayers in Dulbecco's modified Eagle's medium (Gibco) supplemented with 5% (vol/vol) FCS, 60 μg of penicillin per ml, and 100 μg of streptomycin per ml.

**Viruses.** The HIV-2 strain ROD/B (6) was derived from C8166 cells chronically infected with ROD/A (6). The HIV-2 strain CBL-23 has been described previously (6).

**Construction of plasmids.** The construction of CXCR-4 N-terminal deletions and point mutations (Table 1) is described elsewhere (29).

**Transfection of cells.** Six-well trays were seeded at $10^5$ cells/well and incubated overnight at 37°C. The cells were transfected with Lipofectamine (Gibco). Plasmid (1 μg) in 15 μl of phosphate-buffered saline (PBS) was mixed with 5 μl of Lipofectamine diluted with 10 μl of PBS and was incubated for 30 min at room temperature. The cells were washed twice with Optimem (Gibco) before being overlaid with 800 μl of Optimem followed by the DNA-Lipofectamine complexes. The cells were incubated for 5 h at 37°C and then washed with Optimem; to each well was added 2 ml of Dulbecco's modified Eagle's medium (Gibco) supplemented with 5% (vol/vol) FCS, 60 μg of penicillin per ml, and 100 μg of streptomycin per ml. Stably transfected pools of cells were selected with 1 mg (for CCC cells) or 2 mg (for Mv-1-lu cells) of G418 (Gibco) per ml 48 h posttransfection. G418-resistant cells were usually obtained after 1 to 2 weeks of selection.

**Determination of cell surface expression of CXCR-4 constructs.** Cell surface expression of the CXCR-4 constructs was determined by fluorescence-activated cell sorting (FACS). Approximately $10^6$ CCC cells transfected with the different CXCR-4 constructs were removed from the tissue culture dish with trypsin/versene and washed with ice-cold PBS. The cells were resuspended in 100 μl of PBS–1% FCS–0.1% sodium azide and incubated on ice for 20 min before being centrifuged and resuspended in 50 μl of PBS–1% FCS–0.1% sodium azide containing 5 μg of monoclonal antibody (MAb) 12G5 per ml (16). The cells were incubated for 60 min at 20°C, washed twice in PBS–1% FCS–0.1% sodium azide,

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TABLE 1. Sequences of N-terminal deletions of CXCR-4[a]

| Protein | Sequence |
|---|---|
| CXCR-4 | MEGISIYTSD**N**YTEEMGSGDYDSMKEPCFREENANFNKiflptiysiifltg |
| N1 | MSD**N**YTEEMGSGDYDSMKEPCFREENANFNKiflptiysiifltg |
| N2 | MGSGDYDSMKEPCFREENANFNKiflptiysiifltg |
| N3 | MKEPCFREENANFNKiflptiysiifltg |

[a] The extracellular domain is shown in capital letters, and the first putative transmembrane domain is shown in lowercase letters. The putative N-linked glycosylation site in the N-terminal domain is highlighted in boldface type.

and resuspended in 50 μl of PBS–1% FCS–0.1% sodium azide containing fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Sera-labs) at a 1:40 dilution. After incubation for a further 60 min at 20°C, the cells were washed three times in PBS–0.1% sodium azide and finally resuspended in 100 μl of PBS–0.1% sodium azide and added to 300 μl of formol saline (4% formaldehyde in 0.5% NaCl and 1.5% $Na_2SO_4$). They were then analyzed by FACS (Becton Dickinson).

**Cell-free virus infections.** Virus supernatant stocks were serially diluted in fivefold steps, and 250-μl aliquots were added to appropriate cell types in 24-well trays. Unless otherwise stated, the virus was allowed to adsorb for 5 h at 37°C before unbound virus was removed and replaced with appropriate medium. After overnight incubation, 1 μg of azidothymidine (Sigma) per ml was added, and the infected cells were immunostained after a further 3 to 5 days of incubation.

**Cell-cell fusion assays.** Cells transfected the CXCR-4 constructs were seeded in 24-well trays at $2 \times 10^5$ cells per well and incubated overnight. H9 cells ($10^6$ per well) chronically infected with ROD/B were overlaid onto the cultures and incubated for a further 24 h at 37°C. The cells were washed in PBS and fixed and stained for 10 min in methanol containing 1% methylene blue and 0.25% basic fuchsin. The cell layers were then washed with tap water and examined for syncytia by low-power microscopy. An estimate of the percentage of nuclei incorporated into syncytia in adherent cells was recorded (see Table 2).

**Treatment of cells with tunicamycin.** The cells were seeded at $10^5$ cells/ml on 24-well trays and grown for 24 h at 37°C before medium containing 0.1 μg of tunicamycin (Sigma) per ml was added. The cultures were allowed to grow for a further 24 h before the medium was removed and fresh medium containing serially diluted virus was added. The virus was allowed to adsorb for 5 h at 37°C, when it was replaced with more fresh medium. After overnight incubation, 1 μg of azidothymidine per ml was added, and the infected cells were immunostained after a further 3-day incubation.

**Immunostaining HIV-2-infected cells.** Infection assays were typically performed in 24-well tissue culture trays. At 3 days after infection, the medium was removed and the cells were washed twice in PBS and fixed with a 50:50 mixture of methanol-acetone at −40°C for 2 min. After removal of the methanol-acetone, the cells were washed once with PBS followed by PBS containing 1% (vol/vol) FCS. The primary antibody (pooled rat MAbs against ROD/B envelope [26]), 250 μl/well diluted 1:50 in PBS–1% FCS) was added to the fixed cells and allowed to bind for 1 h at room temperature, and then the monolayer was washed twice in PBS–1% FCS. Goat anti-rat immunoglobulin G–β-galactosidase conjugate (Sera-labs) at a dilution of 1:300 (in PBS–1% FCS) was added for 1 h at room temperature, and then the monolayer was washed twice in serum-free PBS. Approximately 500 μl of 5-bromo-4-chloro-3-β-D-galactopyranoside (X-Gal) substrate (0.5 mg/ml) in PBS containing 3 mM potassium ferricyanide, 3 mM potassium ferrocyanide, and 1 mM magnesium chloride was added to each well. Infected cells stained blue within 1 to 2 h of the addition of substrate.

## RESULTS

**HIV-2 (ROD/B) uses the 7tm receptor CXCR-4 for efficient entry into CD4-negative cells.** Mink Mv-1-lu cells and feline CCC cells were chosen to study the role of CXCR-4 as the main CD4-independent receptor for ROD/B because they are restrictive to entry but are permissive for replication of the virus (25). The cells were stably transfected with a cDNA clone of human CXCR-4 in the expression plasmid pCDNA3 (29) and infected with ROD/B. Efficient entry into and infection of both Mv-1-lu and CCC cells was observed, although the titer obtained in Mv-1-lu cells expressing CXCR-4 was fivefold reduced compared with that in CCC cells expressing CXCR-4 (Fig. 1). A low level of infection (≤10 focus-forming units per ml) of untransfected Mv-1-lu or CCC cells by ROD/B or CBL23 was consistently observed (Fig. 1). This infectivity was neutralized with an HIV-2-neutralizing MAb (MAb 8e [reference 26 and data not shown]), suggesting that these HIV-2 strains are able to use (albeit inefficiently) a nonhuman homolog of CXCR-4 present on Mv-1-lu or CCC cells.

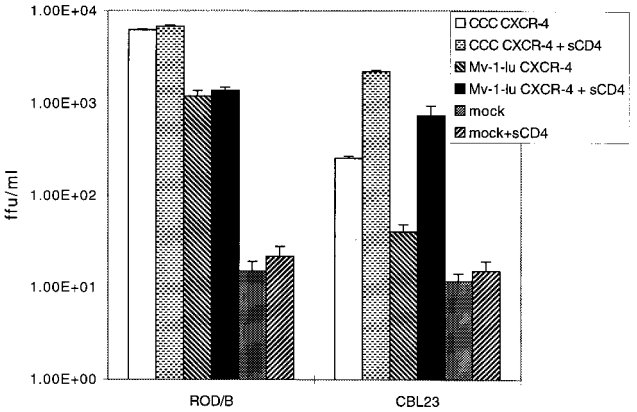


FIG. 1. Cell-free infection titers of ROD/B and CBL23 on CCC or Mv-1-lu cells expressing human CXCR-4, in the presence or absence of sCD4 (5 μg/ml). The titers are expressed as focus-forming units per milliliter.

Infection of CCC cells expressing CXCR-4 was blocked by 4 μg of 12G5 (MAb against human CXCR-4 [16, 27]) per ml but was not blocked by up to 20 μg of an anti-CD4 MAb (Q4120) per ml, further indicating that ROD/B is specifically using the transfected human CXCR-4 to enter the CCC cells (Fig. 2).

**Cell-cell fusion of H9 cells chronically infected with ROD/B or CBL23 is mediated by CXCR-4.** H9 cells chronically infected with ROD/B efficiently fused with CCC cells transfected with CXCR-4 but not with nontransfected CCC cells. Addition of sCD4 (5 μg/ml) had little effect on the number or size of syncytia observed. However, fusion of H9 cells chronically infected with HIV-2 strain CBL23 with CXCR-4-transfected CCC cells could be enhanced significantly by the addition of sCD4 (Table 2).

**Infection of Mv-1-lu or CCC cells expressing CXCR-4 is enhanced by pretreatment of the cells with tunicamycin.** Treatment of mink Mv-1-lu or feline CCC cells expressing human CXCR-4 with the glycosylation inhibitor tunicamycin increased the apparent titer of ROD/B by 10- and 2-fold, respectively (Fig. 3a). Tunicamycin inhibits the first reaction in the lipid-saccharide pathway, blocking N-linked glycosylation completely (34). We have shown previously that treatment of a

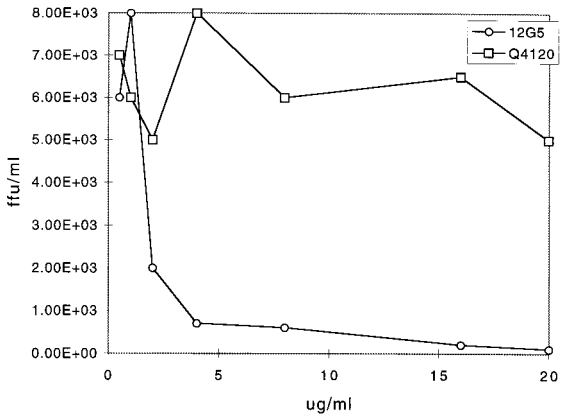


FIG. 2. Infection of CCC cells expressing CXCR-4 by ROD/B in the presence of increasing concentrations of the anti-CXCR-4 MAb 12G5 (16) or the anti-CD4 MAb Q4120 (MRC AIDS reagent program no. ARP318).

TABLE 2. Cell-cell fusion of chronically infected H9 cells with CCC cells expressing various CXCR-4 deletions and mutants

| Protein | Syncytium formation by[a]: | | | | % Surface expression[b] |
|---|---|---|---|---|---|
| | ROD/B | | CBL23 | | |
| | −sCD4 | +sCD4 | −sCD4 | +sCD4 | |
| CXCR-4 | +++ | ++++ | + | +++ | 100 |
| N1 | ± | ± | − | − | 100 |
| N2 | − | − | − | − | 13 |
| N3 | − | − | − | − | 7 |
| G1 | ++++ | ++++ | + | ++ | 37 |
| G2 | ++++ | ++++ | + | ++ | 48 |
| G1G2 | ++++ | ++++ | + | ++ | 52 |
| C1 | ++ | +++ | + | ++ | 57 |

[a] An estimate of the percentage of nuclei incorporated into syncytia in adherent cells was recorded as follows: −, <2%; +, 2 to 5%; ++, 5 to 20%; +++, 20 to 50%; ++++, >50%.

[b] Surface expression of the different CXCR-4 constructs was determined by FACS with the CXCR-4-specific MAb 12G5 (16). Surface expression levels of the CXCR-4 mutants were calculated from the median immunofluorescence values and are shown as a percentage of the level for wt CXCR-4.

variety of CD4-negative human cells with tunicamycin removes the block to infection by ROD/B and postulated that differences in the complexity of glycosylation on these different cells were at least partially responsible for the cell tropism pattern of ROD/B on CD4-negative cells (35). The observation that infection of Mv-1-lu and CCC cells expressing CXCR-4 can also be enhanced after inhibition of glycosylation in these nonprimate cells suggests that the presence of complex glycans interferes either with the binding of ROD/B to CXCR-4 or with a postbinding fusion step. To investigate this further, we mutated the two potential N-linked glycosylation sites on CXCR-4.

**Mutation of the N-linked glycosylation sites on CXCR-4 increases the efficiency of entry of ROD/B.** The two potential N-linked glycosylation sites on CXCR-4 (one in the N-terminal domain and the other in the second extracellular loop) were mutated by site-directed mutagenesis (two single mutants, G1 [$N_{11}$→I] and G2 [$N_{176}$→Q], and a double mutant, G1G2 [$N_{11}$→I, $N_{176}$→Q]). At least one of these sites has been shown to be glycosylated (2). Upon transfection of either the single mutants (G1 or G2) or the double mutant (G1G2), ROD/B infection of CCC cells was enhanced twofold in comparison with wild-type (wt) CXCR-4 (Fig. 3b). These data are compatible with the previous results obtained by treating cells with tunicamycin (35). Cell surface expression of the glycosylation mutants was calculated at between 37 and 52% of wt CXCR-4 (as determined by FACS with the CXCR-4-specific MAb 12G5 [16] [Table 2]), suggesting that the increase in ROD/B infection is specific to the lack of carbohydrate rather than being caused by an increase in cell surface expression. In addition to enhanced cell-free infection, an increase in cell-cell fusion by ROD/B was also observed with CCC cells expressing these glycosylation mutants (Table 2). However, no enhancement of cell fusion by CBL23 was observed with the glycosylation mutants (Table 2).

**N-terminal deletions of CXCR-4 interfere with cell fusion but not cell-free infection by ROD/B.** The enhancement of infection by ROD/B upon mutation of an N-linked glycosylation site in the N-terminal domain suggested either that part of the envelope binding determinant is defined by this region of CXCR-4 or that this domain is involved in the fusion process. We therefore tested a set of deletions covering the N-terminal (extracellular) domain of CXCR-4 (29) to determine the role

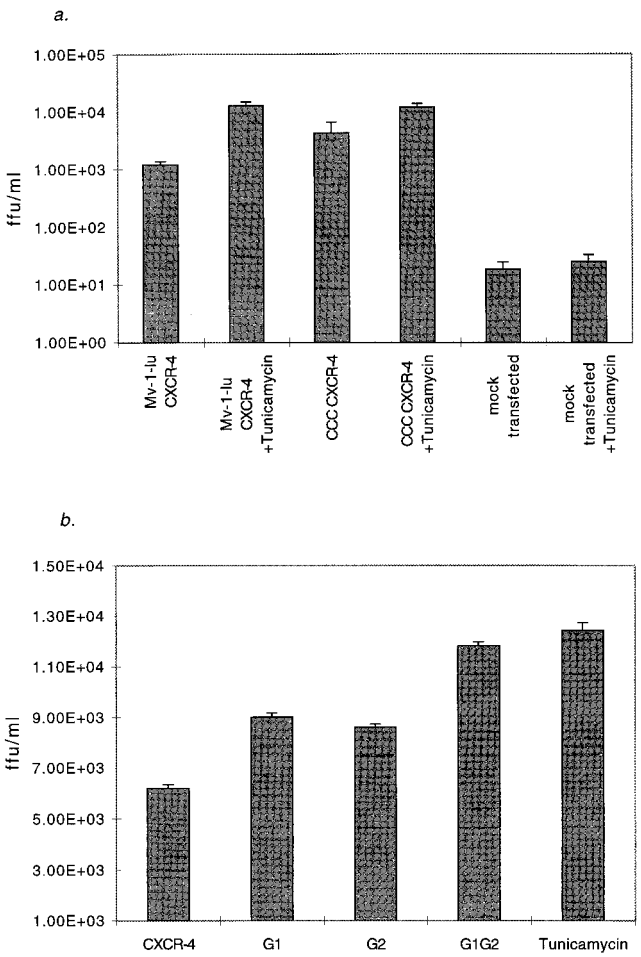


FIG. 3. (a) ROD/B cell-free infection of CCC or Mv-1-lu cells expressing human CXCR-4 is enhanced by pretreatment of the cells with the glycosylation inhibitor tunicamycin. (b) ROD/B cell-free infection of CCC cells expressing human CXCR-4 or N-linked glycosylation mutants of CXCR-4. Two single mutants, G1 ($N_{11}$→I) and G2 ($N_{176}$→Q), and a double mutant, G1G2 ($N_{11}$→I; $N_{176}$→Q), were used.

of this domain in the fusion of HIV-2. Although expression of these deletions in CCC cells led to a decrease (3- to 10-fold) in the cell-free infection by ROD/B (Fig. 4), infection significantly above the control transfection was still observed. However, fusion of CCC cells expressing these N-terminal deletions

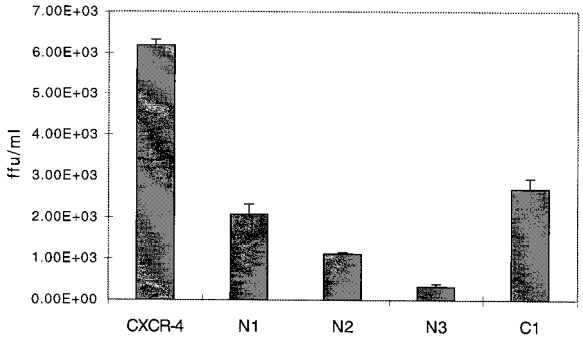


FIG. 4. ROD/B cell-free infection of CCC cells expressing human CXCR-4 or N-terminal deletions (Table 1) or a C-terminal deletion ($\Delta K_{310}$→$S_{352}$) of CXCR-4.

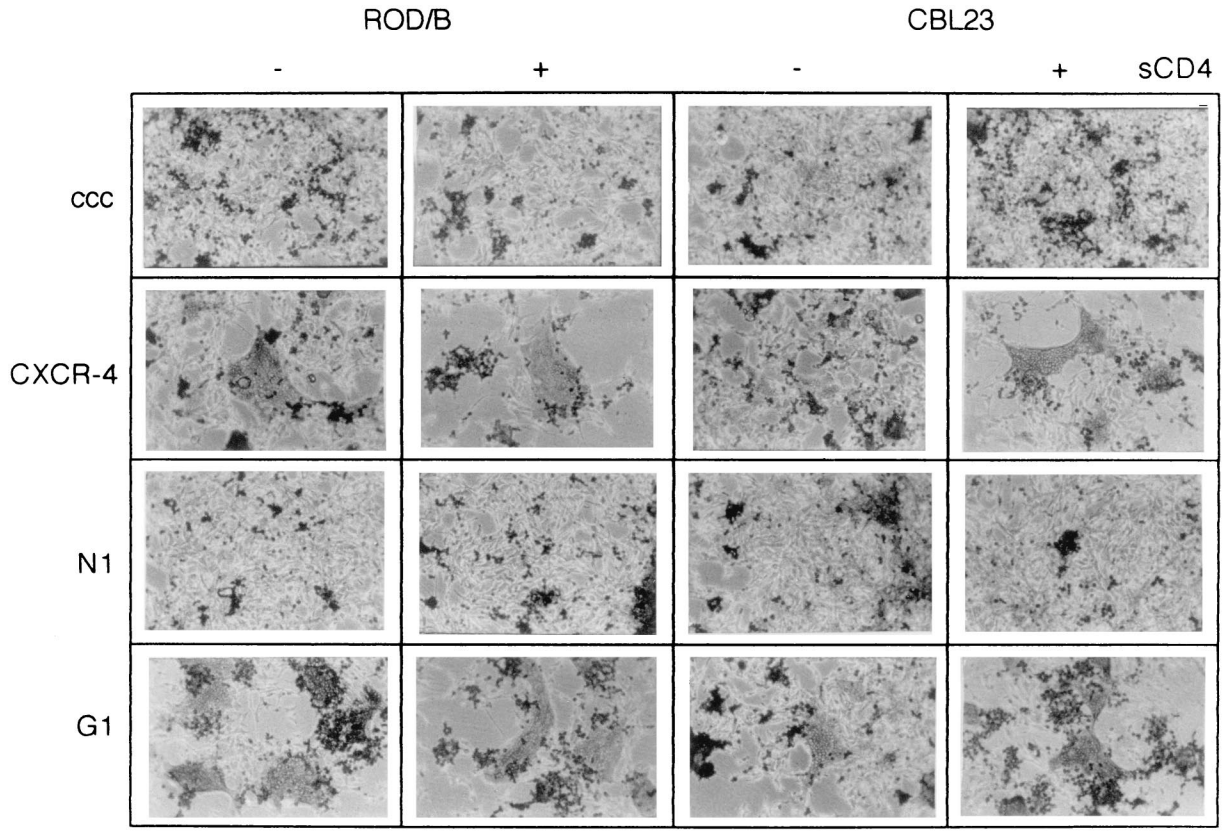


FIG. 5. Cell-cell fusion of CCC cells expressing CXCR-4, N-terminal deletion N1, or glycosylation mutant G1 with H9 cells (black spheres in the photographs) chronically infected with ROD/B or CBL23.

with H9 cells chronically infected with either ROD/B or CBL23 was almost completely blocked (Fig. 5; Table 2). The block to fusion imposed by deletion of parts of the N terminus could not be relieved by the addition of sCD4 to the medium.

Cell surface expression of the N-terminal deletions of CXCR-4 (Table 2), determined by FACS with the CXCR-4-specific MAb 12G5 (16), revealed that N1 was expressed at identical levels to that of CXCR-4 but N2 and N3 were poorly expressed on the cell surface (13 and 7% of CXCR-4 levels, respectively). The lower level of cell surface expression of the two longest N-terminal deletions (N2 and N3) compared with CXCR-4 could explain the lack of cell-cell fusion, which may rely on high levels of expressed receptor to proceed efficiently. However, the cell surface expression of N1 was identical to that of CXCR-4 but cell-cell fusion was still greatly impaired, suggesting that fusion requires an intact N terminus to proceed efficiently.

The fact that cell-cell but not cell-free fusion is blocked suggests that there are distinct requirements for these two processes and that the N terminus of CXCR-4 is involved in the fusion reaction.

**Deletion of the C terminus of CXCR-4 has no effect on infection by ROD/B.** The C-terminal domain of CXCR-4 is potentially involved in the transmission of intracellular signals in response to binding of its chemokine ligand (SDF-1) at the cell surface (3, 28). Deletion of this domain (C1; $\Delta K_{310} \rightarrow S_{352}$) had little effect on the ability of ROD/B to use this construct as a receptor for fusion and entry (Fig. 4), suggesting that intracellular signalling through this domain is not involved in the process of viral entry. However, we cannot rule out the importance of signalling through the other intracellular loops in the entry and fusion process of HIV-2.

## DISCUSSION

The HIV-2 strain ROD/B is able to efficiently enter a variety of CD4-negative human cells (6, 35). We have investigated several features of the 7tm chemokine receptor CXCR-4 which are important for its role as a primary receptor for HIV-2 strain ROD/B in the absence of CD4. Expression of a cDNA clone of human CXCR-4 in feline CCC or mink Mv-1-lu cells enabled the efficient CD4-independent fusion and entry of ROD/B into these cells, which are normally refractory to HIV entry. The mutations in the ROD/B envelope (SU and TM) which are responsible for its CD4-independent phenotype (32) have recently been mapped. One amino acid change that significantly enhances a minimal CD4-independent phenotype is in the V3 loop. The V3 loop is the primary tropism determinant in the HIV envelope and is a common target of neutralizing antibodies although it is not involved in the interaction of the envelope with CD4. Choe et al. (4) have recently mapped a determinant for coreceptor utilization to the V3 loop of the HIV-1 gp120 surface envelope glycoprotein, and others (37, 40) have shown that antibodies to the V3 loop can block the interaction of gp120 with CCR-5. It has been proposed that CD4-dependent strains of HIV need an initial interaction with CD4 to induce conformational changes within the envelope which are essential for the subsequent fusion event involving 7tm membrane proteins (13–15, 18). This is apparently the case with HIV-2 strains such as CBL23 which are able to fuse

with CD4-negative cells (such as CCC cells expressing CXCR-4) only after activation of the envelope with sCD4. The mutations in the ROD/B envelope could remove the necessity for CD4 by lowering the activation threshold required for triggering conformational changes in the envelope glycoproteins that lead to fusion or by increasing the affinity of the interaction of the envelope SU with the 7tm receptor.

We have shown previously that a block to ROD/B entry into certain human CD4-negative cells could be removed by pretreating cells with the glycosylation inhibitor tunicamycin (35). Tunicamycin inhibits the first reaction in the lipid-saccharide pathway, blocking N-linked glycosylation completely (34). We suggested that complex glycans that are present on the ROD/B CD4-independent receptor and that probably vary among cell types could sterically block the interaction with the ROD/B envelope. Removal of these complex glycans would therefore permit the necessary close interaction between the ROD/B envelope and its primary receptor (CXCR-4). Pretreatment of CCC or Mv-1-lu cells expressing human CXCR-4 (which has been shown to be glycosylated [2]) with tunicamycin enhanced infection by ROD/B. A greater enhancement was observed with Mv-1-lu cells, presumably due to different glycosylation patterns compared with CCC cells. To confirm that the observed enhancement is due to a specific effect on CXCR-4 and not simply to a general reduction in the amount of carbohydrate on the cell surface, we specifically mutated the two N-linked glycosylation sites on CXCR-4 and expressed these mutants in CCC cells. A similar enhancement of both fusion and infection of these cells by ROD/B was observed in comparison with wt CXCR-4, confirming that expression of complex glycans on CXCR-4 can interfere with the interaction of the ROD/B envelope. However, enhancement of fusion with another HIV-2 strain (CBL23) or several HIV-1 strains (29) was not observed when these glycosylation mutants were expressed on CCC cells (or CCC-CD4 cells for HIV-1). These data suggest that different HIV envelopes are able to interact differently with or recognize distinct domains of CXCR-4 during the fusion process. Alternatively, the carbohydrates on CXCR-4 may interfere with regions of the ROD/B envelope distal to the receptor binding domain.

The fact that the N-terminal domains of both CXC and CC chemokine receptors contain the primary high-affinity binding site for chemokine ligands (38) and that these ligands can block HIV-1 infection (3, 8, 15, 28) suggest that this domain may be important for binding or entry of the virus. Deletion of portions of the N-terminal domain of CXCR-4 had a negative effect on cell-free infection by ROD/B, although infection significantly above background levels was still observed even with the longest deletion. Although N-terminal deletions of CXCR-4 can still function as a receptor for ROD/B, cell-cell fusion and syncytium formation were blocked. Fusion of these CXCR-4 deletions with ROD/B- or CBL23-chronically infected H9 cells could not be rescued by addition of sCD4 to the medium. These data suggest that although the N-terminal domain of CXCR-4 is not essential for infection, it may play a role in enhancing or modulating the fusion process with these viruses, perhaps by affecting the triggering of gp41 exposure. We have shown that N-terminal deletions of CXCR-4 can block infection by some HIV-1 strains but have little or no effect on the entry of others (29) and that inhibition of HIV fusion by a MAb to CXCR-4 (12G5 [16]) is both cell type and virus strain dependent (27). These data imply that the site of interaction on CXCR-4 varies among different strains of HIV. In contrast to the data showing that the N-terminal domain of CXCR-4 is not essential for HIV entry, Rucker et al. (33) have shown that the N-terminal domain of CCR-5 is important for determining coreceptor specificity for HIV-1.

It is becoming increasingly clear that different strains of HIV-1 and HIV-2 can use divergent members of the 7tm receptor family (1, 4, 13–15, 18, 31). It has also been suggested that there are differences in how these 7tm receptors are used by different isolates and that these interactions are conformationally complex (27, 33). It appears that strains of HIV-2, in particular, are able to use a larger repertoire of 7tm receptors both as coreceptors (with CD4 [24a]) and as primary receptors (31). The analysis of the fusion mechanism mediated by 7tm molecules may benefit from the use of HIV strains such as ROD/B, which are capable of using 7tm molecules to enter cells efficiently in the absence of CD4 (31). It still remains to be shown whether the use of 7tm receptors as primary receptors in the absence of CD4 has relevance for the pathogenesis of HIV in vivo. However, there is evidence for the productive infection of cells that are apparently CD4 negative in tissues from HIV-infected humans (9, 12, 21, 23, 39). The ability of HIV-2 to utilize divergent members of the family of 7tm receptors (with CXCR-4 being used efficiently in the absence of CD4) may have important implications for infection of the immune, heamatopoietic, and/or nervous systems in the natural history of infection by HIV-2.

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