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Macrophage Tropism of Human Immunodeficiency Virus Type 1 and Utilization of the CC-CKR5 Coreceptor

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The recent identification of the CC-CKR5 β chemokine receptor as a major cofactor for entry of macrophage-tropic isolates of human immunodeficiency virus type 1 (HIV-1) raises the question of whether macrophage tropism is determined by utilization of this chemokine receptor. We observe that in addition to macrophage-tropic isolates of clades A, B, and E, macrophage-tropic isolates of clade F also utilize the CC-CKR5 molecule for entry. However, using single-round replication-competent reporter viruses carrying the envelope genes of T-cell line-tropic or macrophage-tropic phenotypic recombinant and mutant HIV-1 strains in infection of stable cell lines that coexpress the CD4 and chemokine receptors, we were unable to establish a strict correlation between macrophage tropism and utilization of the CC-CKR5 chemokine receptor. This latter finding suggests that a cofactor other than CC-CKR5 serves to determine entry into primary macrophages.

Variation in the ability of human immunodeficiency virus type 1 (HIV-1) isolates to infect different cell types and to induce syncytium formation is well recognized (34). Although all HIV-1 isolates can infect primary $CD4^+$ T lymphocytes, they differ in their ability to infect immortalized T-cell lines and primary macrophages (12, 26). In general, HIV-1 strains can be classified as T-cell line tropic, macrophage tropic, or dual tropic. T-cell line-tropic viruses replicate well in immortalized $CD4^+$ T-cell lines and are syncytium inducing (SI) in infected cells but fail to infect primary macrophages. Macrophage-tropic isolates do not productively infect T-cell lines and are non-syncytium inducing (NSI). Dual-tropic viruses replicate well in both immortalized T-cell lines and primary macrophages and exhibit the SI phenotype. Changes in these biological properties of viral isolates obtained from infected individuals over the course of infection have been shown to correlate with disease progression (4, 11, 43, 51). Most viruses isolated at the time of seroconversion and during the asymptomatic phase of infection are macrophage tropic and NSI (43, 53, 54). With time, T-cell line-tropic, SI variants with increased replicative capacity appear that are often associated with the development of AIDS (20, 50). Dual-tropic viruses may represent an important transitional phenotype (18).

Genetic analyses of the HIV-1 genome have demonstrated that the third hypervariable region (V3 loop) of the viral envelope glycoprotein gp120 contains major determinants for the differences in cell tropism and SI ability of HIV-1 (6, 10, 14, 15, 27, 29, 31, 39, 40, 44, 49, 52). However, other domains within both envelope gp120 and gp41 can also influence these specific envelope functions. For example, the V1/V2 domain and specific mutations in the V2 region of envelope gp120 have been shown to modulate these biological phenotypes of HIV-1 (2, 32, 33, 48). With respect to cell tropism, we previously reported that three and two amino acid changes within the V3 loop of envelope gp120 of the T-cell line-tropic SF2 and the macrophage-tropic SF162 strains, respectively, can alter their cell tropism (29, 45). The SF2 mutant virus Mu3 infects both T-cell lines and primary macrophages (and hence is dual tropic), whereas the SF162 mutant virus Dbl loses its ability to infect macrophages but replicates efficiently in a number of immortalized $CD4^+$ T-cell lines (and hence is T-cell line tropic). Furthermore, three amino acid substitutions in the V2 domain of the T-cell line-tropic SF2 gp120 generated a mutant virus, MuG2, that now replicates efficiently in primary macrophages but not in T-cell lines (33). Immunochemical analyses of virion-associated gp120 of SF2 and SF162 with monoclonal antibodies indicated that the type and extent of conformational changes that the V3 loop of virion-associated gp120 undergoes upon virion-CD4 binding are different for viruses displaying different cell tropisms (46). We suggested that these differences in postbinding V3 loop conformational changes in turn influence subsequent events that mediate viral entry, among which could be the interaction of the V3 loop with other cellular receptors (46, 47).

Several early studies have indicated that in addition to the CD4 receptor, other cell surface coreceptors are required for mediating HIV entry (3, 9, 13, 17, 23, 36). The fact that HIV-1 isolates differ in cell tropism suggests that multiple cell type-specific cofactors may be required. Such cofactors have recently been identified. A C-X-C (α) chemokine receptor for stromal cell-derived factor 1 (SDF-1), previously named LESTR/fusin and now designated CXCR4 (8, 38), was first shown to act as a coreceptor for T-cell line-tropic strains (25). Subsequent studies revealed that a member of the family of chemokine receptors for C-C (β) chemokines, CC-CKR5, served as a major coreceptor for macrophage-tropic isolates from clades A, B, C, and E (1, 16, 21, 22, 24). These chemokine receptors belong to the family of G protein-coupled seven-transmembrane-domain proteins. On binding to their natural ligand, chemokine receptors transduce signals through associated heterotrimeric G proteins, resulting in rapid calcium influx and inflammatory responses (5, 37, 41).

These findings of differential chemokine coreceptor usage of T-cell line-tropic versus macrophage-tropic viruses raise the question of whether cell tropism of HIV-1 is determined by utilization of a specific chemokine receptor. To address this question, a previously described envelope *trans*-complementation assay was used to generate luciferase reporter viruses

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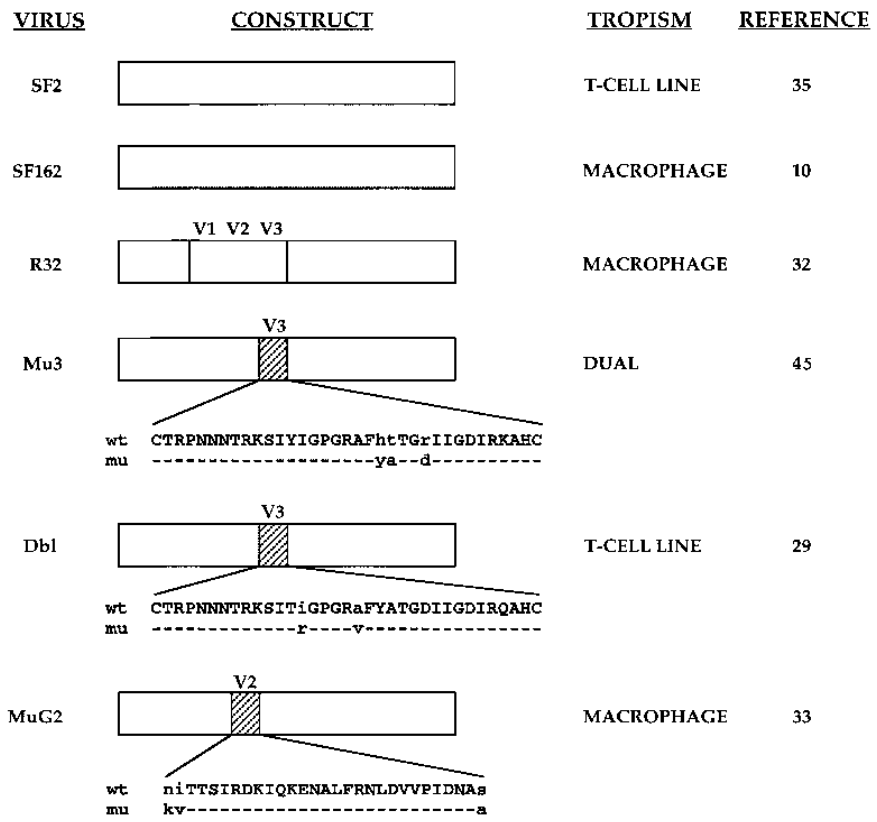

FIG. 1. Genomic organization and tropism of SF2 and SF162 recombinant, mutant viruses. Dotted bars indicate DNA sequences of SF162, and open bars indicate those of SF2. The corresponding amino acid substitutions in the V2 and V3 regions (hatched) of the mutant viruses are designated. Tropisms of the viruses have been determined and are reported in the indicated references.

which are single-round replication competent and which carry envelope genes of divergent clade A (SF170), E (93Th966.8), and F (Br029.2) HIV-1 isolates and phenotypic variants of clade B isolates (19). The biological properties of the clade B viruses whose envelope genes were used to produce the reporter viruses are summarized in Fig. 1. The functionality and tropism of the non-clade B Env-pseudotyped viruses generated were determined by infecting peripheral blood mononuclear cells (PBMC) and macrophage cultures with 30 ng of the p24 equivalent from the various reporter viruses. The results are summarized in Table 1.

We observe that all the non-clade B isolates examined infected primary PBMC and macrophages. There is a difference in the efficiency of entry into PBMC mediated by the envelopes of these viruses. The level of luciferase activity was highest in cells infected with viruses pseudotyped by the clade A SF170 Env-pseudotyped virus and lowest for the clade F Br029.2 Env-pseudotyped virus. The low infectivity of the Br029.2 Env clone has been reported previously (28) and may be due to differences in the level of envelope glycoproteins packaged within the variously pseudotyped virions as indicated by the gp120/p24 ratios of these viruses (data not shown). The coreceptor usage of these reporter viruses was then investigated by infection of stable $CD4^+$ human osteosarcoma cells (HOS.CD4 cells) that coexpress either the CXCR4, CC-CKR1, CC-CKR2b, CC-CKR3, CC-CKR4, or CC-CKR5 receptor. The establishment of these cell lines has been described previously (21). Infection of HOS.CD4 cells transfected with the pBabepuro expression vector (HOS.CD4.Babe) served to control for background entry of viruses into HOS.CD4 cells independent of the presence of coreceptors. The results are summarized in Fig. 2 and show that all the non-clade B isolates examined preferentially utilized the CC-CKR5 coreceptor for entry. These observations confirm and extend previous findings on coreceptor usage by divergent HIV-1 isolates (16). The efficiency of entry into these cells is again highest for SF170 Env and lowest for Br029.2 Env.

TABLE 1. Infection of PBMC and primary macrophages by divergent HIV-1 isolates[a]

Virus	Clade	Luciferase activity (cps) in:	
		PBMC	Macrophages
SF162	B	225	3,955
SF170	A	2,390	7,100
93Th966.8	E	385	955
Br029.2	F	90	80

[a] Single-round replication-competent viruses pseudotyped with Env glycoproteins from clade B (SF162), clade A (SF170), clade E (93Th966.8), and clade F (Br029.2) isolates were used in infection of PBMC and primary macrophages. Luciferase activity in the infected cells was assayed at 7 days postinfection. Background luciferase activities, as determined by infection of PBMC and macrophages with virions containing no envelopes (15 and 30 cps, respectively), have been substracted from the values presented. The results of a single representative experiment are shown.

The preferential utilization of the CC-CKR5 coreceptor by macrophage-tropic isolates of subtypes A, B, E, and F is in support of a role of this receptor in entry into primary macrophages. To examine whether macrophage tropism is determined exclusively by usage of this coreceptor, viruses

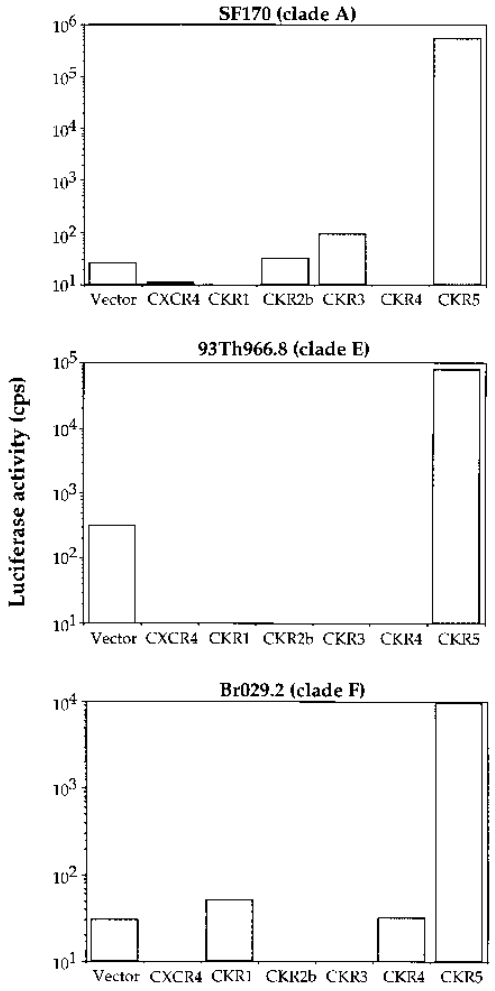

FIG. 2. Preferential utilization of the CC-CKR5 coreceptor by non-clade B HIV-1 isolates. Stable HOS.CD4 cells coexpressing the indicated chemokine receptors were inoculated with 15 ng of the p24 equivalent from single-round replication-competent luciferase reporter viruses expressing the envelope from clade A (SF170), clade E (93Th966.8), and clade F (Br029.2) isolates. 72 hours later, the cells were lysed, and the luciferase activity (in counts per second) in the cell lysates was determined according to the protocol provided by the manufacturer (Promega). HOS.CD4 cells cotransfected with pBabepuro vector alone (vector) served as the negative control for these studies.

pseudotyped with Env glycoproteins from the T-cell line-tropic SF2, the macrophage-tropic SF162, and phenotypic recombinant (R32) and mutant (Mu3, MuG2, and Dbl) viruses generated on the genomic background of these isolates were used in infection of the stable cell lines. The differences in target cell preference of the isolates whose envelopes were used to generate the luciferase reporter viruses has previously been characterized and is summarized in Fig. 1. Infection with reporter viruses that contain envelope glycoproteins from the prototypic T-cell line-adapted HXB2 strain serves as a positive control in these infections. The coreceptor usage of HXB2 had previously been described (7, 21, 22, 24). The results are presented in Table 2.

We observe that the HXB2, SF2, and MuG2 Env glycoproteins were able to mediate some degree of entry into HOS.CD4 cells in the absence of cloned coreceptor expression. This is consistent with previous reports of infection of $CD4^+$ nonlymphoid cells by some strains of HIV-1 (14, 30, 36). In agreement with previously published reports (7, 21, 22, 24), HXB2 Env preferentially interacts with the CXCR4 receptor expressed by HOS.CD4. In contrast, viruses pseudotyped with Env glycoproteins from SF162 and R32, both macrophage-tropic viruses, preferentially utilize the CC-CKR5 coreceptor for entry, whereas the dual-tropic Mu3 virus uses both CXCR4 and CC-CKR5. However, we find that T-cell line-tropic SF2 can utilize both CXCR4 and CC-CKR5. SF2 can therefore use CC-CKR5 but does not enter macrophages. Furthermore, the other macrophage-tropic virus, the V2 mutant MuG2, retains the parental T-cell line-tropic SF2 strain's ability to utilize both the CXCR4 and CC-CKR5 coreceptors. The T-cell line-tropic mutant of SF162, Dbl, also utilizes both coreceptors. The same coreceptor usage was seen when using U87 glioma cells coexpressing CD4 and either CXCR4 or CC-CKR5 as targets (data not shown). These results suggest that neither T-cell line tropism nor macrophage tropism correlates with qualitative utilization of these chemokine receptors and that other cell type-specific factors (i.e., osteosarcoma versus glioma) do not appear to play a major role in influencing coreceptor usage by these viruses. Infection of HOS.CD4 cells that coexpress other characterized chemokine receptors (e.g., CC-CKR1, CC-CKR2b, CC-CKR3, or CC-CKR4) with these pseudotyped viruses also failed to reveal a correlation of specific chemokine receptor usage and HIV-1 tropism (data not shown).

Our data do not exclude a role for the CC-CKR5 receptor in infection by macrophage-tropic viruses. It is conceivable that CC-CKR5 is the principal coreceptor for macrophage-tropic viruses on $CD4^+$ lymphocytes and that T-cell line-tropic and dual-tropic viruses acquire the ability to utilize additional chemokine coreceptors. Our findings with SF2 and Dbl, however, suggest that a cofactor other than CC-CKR5 serves to determine entry into primary macrophages. The studies in which RANTES, MIP-1α, and MIP-1β, potent agonists of CC-CKR5, did not block infection of macrophages (24) and in some cases enhanced macrophage infection (42) support this conclusion. Alternatively, the expression of the chemokine receptors in the

TABLE 2. Luciferase activity in transfected HOS cells infected with T-cell line-tropic and macrophage-tropic Env-pseudotyped HIV-1 strains[a]

Molecule(s) expressed in target cells	Luciferase activity (cps) in HOS cells infected with viruses pseudotyped with envelope glycoprotein from:						
	HXB2 (T)	SF2 (T)	SF162 (M)	R32 (M)	Mu3 (D)	Dbl (T)	MuG2 (M)
CD4	2,600	2,623	23	0	0	145	258
CD4 and CXCR4	11,500	8,875	1,140	0	1,635	164,620	997
CD4 and CC-CKR5	3,500	29,559	240,888	14,535	22,448	160,763	2,736

[a] HOS cells expressing CD4 and chemokine receptors were inoculated with 25 ng of the p24 equivalent from single-round replication-competent luciferase reporter viruses pseudotyped with envelope glycoproteins from the viruses indicated. Values presented show luciferase activity (cps) in the infected cell lysates collected 3 days postinoculation from one representative experiment. Background luciferase activities, as determined by inoculation with virions containing no envelope glycoproteins, were subtracted from the values presented. T, T-cell line-tropic; M, macrophage tropic; D, dual tropic.

stably transfected cell lines used in our study may be qualitatively (perhaps influenced by the molecular constitution of the membrane) or quantitatively different from that of primary lymphocytes and macrophages in vivo, and hence the pattern observed here may be different from that in vivo. Possible overexpression of CC-CKR5 in stably transfected cells may compensate for a low-affinity interaction between T-cell line-tropic Env glycoproteins and CC-CKR5, leading to viral entry via this coreceptor in these cell lines. However, the maintenance in these HOS.CD4 cells of the preferential utilization of the CXCR4 coreceptor by HXB2-pseudotype virions argues against this possibility. Nevertheless, it will be important to evaluate the effect, if any, of differential quantitative expression of the chemokine receptors on their use by viruses of various pseudotypes, to assess the binding affinities of T-cell line-tropic and macrophage-tropic Env glycoproteins to CC-CKR5, and to determine the level of cell surface expression of the CC-CKR5 receptor on primary macrophages in order to establish its role in macrophage infection.

The majority of HIV-1 strains that initiate infection in vivo and that persist throughout the course of the disease are macrophage tropic and NSI (43, 53, 54). As the disease progresses, viruses that replicate to higher levels in PBMC and that can infect and induce syncytium formation in an immortalized T-cell line appear (4, 11, 20, 43, 50, 51). Thus, the molecular identification of the cofactor that facilitates HIV-1 entry into macrophages is critical for understanding HIV-1 pathogenesis and transmission and may also be useful for designing therapeutic strategies to control HIV infection. Our data suggest that cofactors other than those currently identified may exist that determine tissue-specific HIV entry.

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