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# Murine CXCR-4 Is a Functional Coreceptor for T-Cell-Tropic and Dual-Tropic Strains of Human Immunodeficiency Virus Type 1

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**The human chemokine receptor hCXCR-4 serves as a coreceptor for T-cell-tropic (T-tropic) and dual-tropic strains of human immunodeficiency virus type 1 (HIV-1). We have isolated a homolog of hCXCR-4 from a murine T-cell cDNA library and have examined its ability to function as an HIV-1 coreceptor. mCXCR-4 was found to be 91% identical to the human receptor at the amino acid level, with sequence differences concentrated in extracellular domains. Surprisingly, coexpression of both hCD4 and mCXCR-4 on either simian or murine cell lines rendered them permissive for HIV-1-induced cell fusion, indicating that mCXCR-4 is a functional HIV-1 coreceptor. As with hCXCR-4, coreceptor function was restricted to T-tropic and dual-tropic HIV-1 strains. Ribonuclease protection analysis indicated that mCXCR-4 mRNA was expressed in only two of six murine cell lines tested. In contrast, Northern blot analysis of human and mouse tissues revealed that CXCR-4 is widely expressed in both species in vivo. Overall, these data suggest that the reported lack of susceptibility of hCD4$^+$ murine cells to HIV-1 infection in vitro is, at least in part, due to a lack of mCXCR-4 expression rather than a lack of coreceptor function.**

It has long been evident that with a few exceptions, expression of human CD4 (hCD4) on the surface of human cell lines is sufficient to render them permissive for T-cell-tropic (T-tropic) human immunodeficiency virus type 1 (HIV-1) infection and cell fusion (reviewed in reference 20). In contrast, the majority of hCD4-expressing nonhuman cell lines remain refractory to infection or cell fusion (3, 8, 14, 17, 18, 26). Recently, a number of human chemokine receptors have been shown to function as coreceptors for HIV-1, with considerable diversity between HIV-1 strains as to which coreceptors are recognized and which domains are required for coreceptor function. Thus, culture-adapted or T-tropic HIV-1 strains utilize exclusively hCXCR-4 as a coreceptor, while macrophage-tropic (M-tropic) strains predominantly utilize hCCR-5. Dual-tropic strains are capable of functional interaction with both hCCR-5 and hCXCR-4. In addition, hCCR-3 and hCCR-2b support membrane fusion and infection by a more restricted subset of M-tropic and dual-tropic HIV-1 strains (1, 7, 10, 12–14, 24, 25, 28).

**Molecular cloning of a murine homolog of hCXCR-4.** We and others have previously shown that the murine homolog of hCCR-5 is nonfunctional as a coreceptor for M-tropic, dual-tropic, and T-tropic HIV-1 strains despite extensive homology at the amino acid level (4, 5). The *mCCR-5* gene has therefore provided a useful reagent for mapping domains of hCCR-5 that are required for functional interaction with M-tropic and dual-tropic HIV-1 envelopes. In order to adopt a similar strategy for characterization of the interactions between T-tropic envelopes and hCXCR-4, a murine T-cell cDNA library was screened with an hCXCR-4 probe to identify the murine homolog mCXCR-4. Since a large body of literature has established the lack of susceptibility of hCD4$^+$ murine cells to HIV-1 infection (3, 8, 14, 17, 18, 26), such a homolog would be predicted to be nonfunctional as an HIV-1 coreceptor. A single clone was identified which hybridized to an hCXCR-4 probe under conditions of high stringency. Sequence analysis revealed a high degree of homology to sequences located towards the 5′ end of both hCXCR-4 and a rat CXCR-4 homolog, termed LCR-1 (16, 27). Oligonucleotide primers based on this sequence and on the known 3′ end of the coding region of the rat LCR-1 sequence were then used in the PCR to obtain an approximately 1-kb DNA fragment that was cloned with the T/A vector kit (Invitrogen). Sequence analysis revealed that the predicted amino acid sequence of this mCXCR-4 specific DNA fragment was 91% homologous to that of hCXCR-4 and identical to the mCXCR-4 sequence subsequently published by two other groups (15, 21).

**Murine CXCR-4 is a functional coreceptor for T-tropic and dual-tropic HIV-1 strains.** To determine whether mCXCR-4 constitutes a functional HIV-1 coreceptor, the entire coding region was subcloned into the eukaryotic expression vector pBC12/CMV (9), and coreceptor function was assayed as previously described (5). Briefly, COS cells were cotransfected with expression vectors encoding hCD4 and the candidate coreceptor along with a reporter plasmid containing the secreted alkaline phosphatase (SEAP) indicator gene under the transcriptional control of the HIV-1 long terminal repeat. After 48 h, these indicator cells were cocultivated with COS cells which had been transfected with infectious T-tropic, M-tropic, or dual-tropic HIV-1 proviruses. SEAP activity in culture supernatants was determined after 48 h of coculture as previously described (5).

As shown in Fig. 1, cocultivation of COS indicator cells expressing hCD4 and hCXCR-4 with cells producing the T-tropic virus IIIB or the dual-tropic virus 89.6 (12) resulted in marked activation of the indicator construct. SEAP activation was not observed when hCD4/hCXCR-4-expressing indicator cells were cocultivated with cells producing the M-tropic virus BaL, which is unable to use hCXCR-4 as a coreceptor (1, 5, 10, 13), or when indicator cells expressing CD4 alone were cocultivated with any of the HIV-1-producing cells.

Surprisingly, when indicator cells expressing hCD4 and mCXCR-4 were cocultivated with virus-producing cells, acti-

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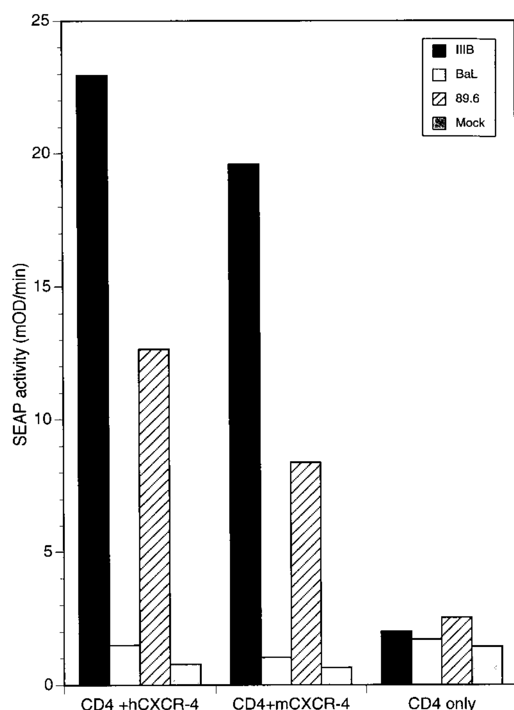


FIG. 1. mCXCR-4 is a functional coreceptor when expressed in COS cells. COS cells were transfected with 400 ng of pBC12/CMV/CD4 and 400 ng of pBC12/HIV/SEAP along with 400 ng of the indicated coreceptor expression plasmid (5). The parental pBC12/CMV plasmid (9) was used as a negative control. SEAP activity in culture supernatants was determined after cocultivation with COS cells producing the IIIB, BaL, or 89.6 strain of HIV-1. Shaded bars indicate SEAP activity observed after cocultivation with mock-transfected COS cells. The results shown are representative of four independent experiments. mOD, milli-optical density units.

vation of SEAP expression was also observed. Like hCXCR-4, this activation was restricted to instances in which indicator cells were cocultivated with IIIB or 89.6 virus producers. Thus, these data demonstrate that mCXCR-4 can indeed function as a specific coreceptor for at least one T-tropic HIV-1 strain and one dual-tropic HIV-1 strain.

**CXCR-4 expression in murine tissues and immortalized cell lines.** The large majority of human cell lines can be rendered susceptible to infection by T-tropic strains of HIV-1 by expression of hCD4 and are therefore presumed to express the hCXCR-4 coreceptor (3, 8, 20, 21). Thus, the observation that mCXCR-4 is a functional HIV-1 coreceptor when expressed in African green monkey (COS) cells was unexpected, given that many murine cell lines have been shown to be refractory to infection by HIV-1 even when expressing hCD4 (3, 8, 14, 17, 18). We therefore determined whether CXCR-4 is expressed in a range of immortalized murine cell lines. For this purpose, a 210-bp fragment of mCXCR-4 was PCR amplified and subcloned into pCRII (Invitrogen) to generate a probe for ribonuclease protection analysis of mCXCR-4 mRNA expression levels. As a positive control, a directly analogous fragment of hCXCR-4 was used to probe HeLa cell-derived RNA. Cytoplasmic RNA extraction and ribonuclease protection analysis were performed as previously described (6). mCXCR-4 mRNA was not detected in the murine fibroblast cell lines 3T3 and L, the T-cell line CTLL-2, or the pre-B-cell line 319 (Fig. 2). In contrast, a protected fragment of the predicted size, which comigrated with a protected fragment observed upon analysis of HeLa cells, was observed in the murine T-cell line EL4 and the B-cell line A20. Therefore, mCXCR-4 RNA is expressed in some murine cell lines, but its expression appears to be significantly more restricted than in immortalized human cells (14, 16).

The limited expression of mCXCR-4 in murine cell lines could suggest that mCXCR-4 expression is more restricted in murine tissues in vivo than in human tissues. To test this hypothesis, mouse and human multiple-tissue Northern blots (Clontech) were analyzed with probes synthesized from the full-length coding sequences of mCXCR-4 and hCXCR-4, respectively (Fig. 3). These experiments revealed that the approximately 2-kb CXCR-4 mRNA is present in multiple tissues, but it is particularly abundant in heart, lung, spleen (mouse), and placenta (human). A minor band of ~4.3 kb is likely to represent unspliced CXCR-4 RNA, since the genomic *mCXCR-4* gene has been shown to contain a single intron of ~2.3 kb (15). Importantly, we obtained no evidence for differential expression of CXCR-4 in the two species in vivo.

**Murine CXCR-4 is a functional coreceptor when expressed in a murine cell line.** Although the lack of expression of CXCR-4 in at least some immortalized murine cell lines could account for their lack of susceptibility to HIV-1 infection or fusion even when expressing hCD4, it is nevertheless also possible that murine cells perform a posttranslational modification that renders mCXCR-4 nonfunctional as an HIV-1 coreceptor. To address this question, we examined the ability of mCXCR-4 to mediate HIV-1 fusion in murine cells by performing coreceptor function assays with the CXCR-4-negative murine fibroblastic cell line L as indicator cells, in place of the simian

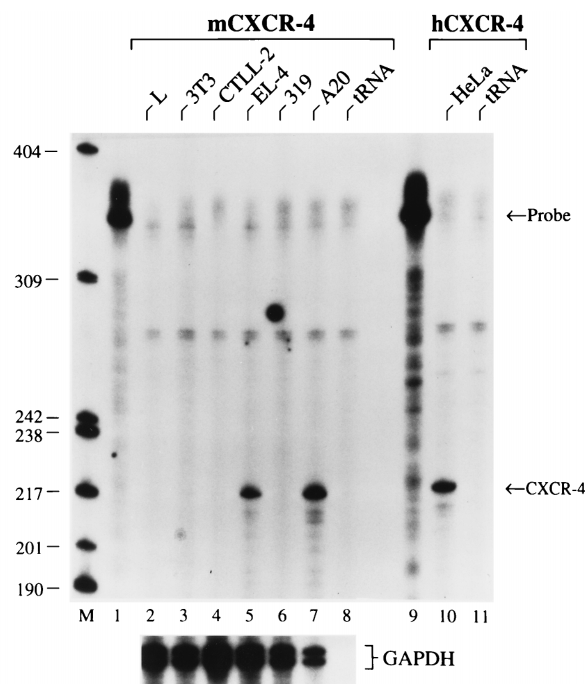


FIG. 2. Expression of CXCR-4 in immortalized murine cell lines. Cytoplasmic RNA from the indicated murine (lanes 2 to 7) and human (lane 10) cell lines was hybridized with a species-specific CXCR-4 riboprobe, digested with RNase $T_1$ as described previously (6), and protected fragments were analyzed on denaturing acrylamide gels. $^{32}$P-labeled *Msp*I-digested pBR322 DNA was used as a marker to approximate the size of the protected fragments, which is predicted to be 210 nucleotides for both mCXCR-4 and hCXCR-4. A second, identical aliquot of RNA was simultaneously probed with a murine glyceraldehyde phosphate dehydrogenase (GAPDH) riboprobe to verify RNA integrity. Molecular sizes are indicated in nucleotides.

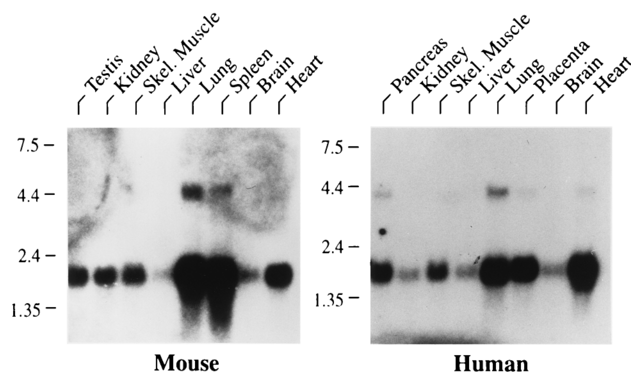


FIG. 3. Expression of CXCR-4 in vivo. Mouse and human multiple-tissue Northern blots were probed with similar, species-specific CXCR-4 probes. The position of molecular size markers is indicated in kilobases.

COS cells used previously (Fig. 1). Although the absolute levels of SEAP activity observed were reduced compared to those in similar experiments with COS cells (since L cells do not express simian virus 40 T antigen, none of these simian virus 40 *ori*-containing expression plasmids are amplified after transfection), SEAP expression was clearly activated after cocultivation with IIIB-producing but not BaL-producing COS cells (Fig. 4). Activation required the presence of a coreceptor, but was observed whether hCXCR-4 or mCXCR-4 was expressed in these murine indicator cells. Therefore, mCXCR-4 is an active HIV-1 coreceptor irrespective of whether it is expressed in the context of a primate or a murine cell line.

In this article, we demonstrate that mCXCR-4 can serve as a functional coreceptor for two strains of HIV-1 that can also utilize hCXCR-4 (Fig. 1 and 4). This was an unexpected result, given that it has been repeatedly reported that a wide range of murine cell lines remain refractory to HIV-1 infection or fusion even when they express hCD4 (3, 8, 14, 17, 18). In at least some cases (for example, in studies with the murine cell lines 3T3 and L [18]), this can be readily explained by a lack of mCXCR-4 expression (Fig. 2). In fact, the apparent absence of mCXCR-4 mRNA in the majority of murine cell lines is in itself somewhat surprising, in that the majority of human cell lines are presumed to express hCXCR-4, because hCD4 expression is sufficient to render them susceptible to T-tropic HIV-1 infection (3, 8, 20, 21). In addition, CXCR-4 expression patterns are not obviously different in the two species in vivo (Fig. 3).

The inability of HIV-1 to infect hCD4$^+$ nonhuman cell lines is not restricted to murine cells. Instead, it is observed with many, albeit not all, cell lines derived from nonhuman species, including, for example, the simian cell line COS used here as an indicator cell (3, 8, 14, 17, 18). While we cannot exclude the possibility that some species express CXCR-4 homologs that are nonfunctional as HIV-1 coreceptors, this is unlikely to be the case for all nonhuman species. Indeed, the rat homolog of CXCR-4, which is ~95% identical to the murine CXCR-4 protein examined here, was recently shown to function as an effective HIV-1 coreceptor for T-tropic HIV-1 strains (22). Instead, it appears possible that CXCR-4 expression, while common in immortalized human cells, is more unusual in permanent cell lines derived from other mammalian species. This report and the recent work of others have shown that CXCR-4 is expressed in some murine cell lines of lymphoid origin, but is absent in the majority of cell lines derived from other tissues (15, 23). The loss of expression of specific chemokine receptors upon immortalization is not without precedent, in that hCCR-5, the major coreceptor for M-tropic HIV-1 strains, is expressed in primary human T cells but not in the majority of immortalized human T-cell lines (1, 10, 20).

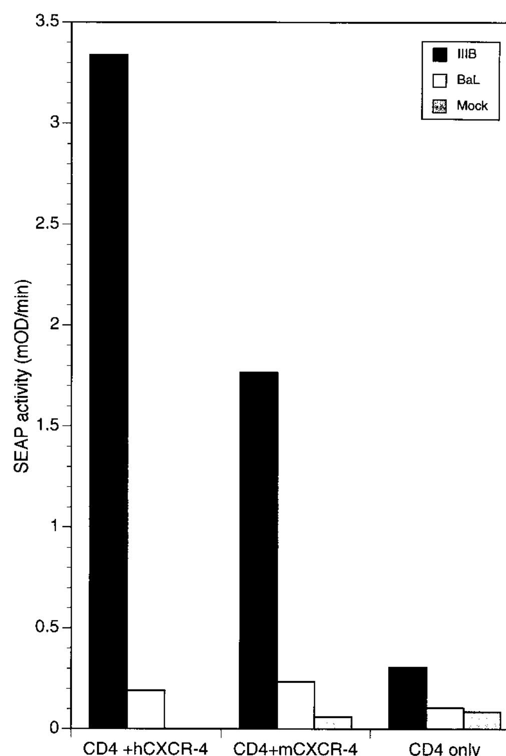


FIG. 4. mCXCR-4 is a functional coreceptor when expressed in murine cells. L cells were transfected with expression plasmids and the indicator construct as described in the legend to Fig. 1. SEAP activities were determined after cocultivation with COS cells producing the IIIB or BaL strain of HIV-1. Shaded bars indicate SEAP expression after cocultivation of the same indicator cells with mock-transfected COS cells. The results shown are representative of three independent experiments. mOD, milli-optical density units.

While a lack of expression of mCXCR-4 can account for the inability of T-tropic HIV-1 isolates to infect a number of hCD4$^+$ murine cell lines, this cannot explain the previously reported inability of HIV-1 to infect primary hCD4$^+$ murine lymphocytes or an hCD4$^+$ form of the murine B-cell line A20, both of which appear to express significant levels of mCXCR-4 (3, 15, 17, 23) (Fig. 2 and 3). In the case of the primary cells, this negative result could, at least in part, reflect other intracellular blocks to viral replication, including a lack of HIV-1 Tat function in rodent cells (2, 19). However, the finding that hCD4$^+$ A20 cells are unable to form syncytia when cocultured with cells expressing the IIIB envelope protein (3) is more difficult to explain. Similarly, although CXCR-4 expression appears to be sufficient to render the large majority of human CD4$^+$ cells susceptible to T-tropic infection, this is not invariably the case in that primary human macrophages, which are reported to express significant levels of hCXCR-4, are not infectable by T-tropic HIV-1 isolates (11). This apparent anomaly, which may relate to the level of CD4 and/or CXCR-4 coreceptor expression required for efficient infection, will need to be addressed in future studies.

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