A Monoclonal Antibody (12G5) Directed against CXCR-4 Inhibits Infection with the Dual-Tropic Human Immunodeficiency Virus Type 1 Isolate HIV-1_{89.6} but Not the T-Tropic Isolate HIV-1_{HXB}

JULIE M. STRIZKI,¹ JULIE DAVIS TURNER,² RONALD G. COLLMAN,³ JAMES HOXIE,²
AND FRANCISCO GONZÁLEZ-SCARANO^{1*}

Departments of Neurology and Microbiology,¹ Department of Medicine (Hematology and Oncology Division),² and Department of Medicine (Pulmonary Division),³ University of Pennsylvania Medical Center, Philadelphia, Pennsylvania 19104-6146

Received 10 February 1997/Accepted 26 March 1997

We used a monoclonal antibody (12G5) directed against an extracellular domain of CXCR-4 to investigate the role of this receptor in infection of immortalized lymphoid cell lines, peripheral blood mononuclear cells (PBMCs), and primary brain microglia with a dual-tropic strain of human immunodeficiency virus (HIV-1_{89.6}) and a T-tropic strain (HIV-1_{HXB}). Addition of antibody 12G5 to cells prior to and during infection with HIV-1_{89.6} inhibited p24 production 100- to 10,000-fold in CEMx174 and 174-CD4 cells and about 10-fold in PBMC cultures but had no activity against infection of either monocyte-derived macrophages or brain microglia. In contrast, 12G5 had little or no effect on infection of CEMx174 cells with HIV-1_{HXB} or HIV-1_{HXB}. To identify the region of the HIV-1_{89.6} envelope that confers sensitivity to 12G5, we used chimeric molecular clones. Chimeras containing the V3 loop region of HIV-1_{89.6} were inhibited by 12G5 to the same degree as wild-type HIV-1_{89.6}, whereas replication of those viruses containing the V3 loop of HIV-1_{HXB} was not inhibited by the antibody. A similar pattern was seen in infections of a U87 glioblastoma line that coexpresses CD4 and CXCR-4. Antibody 12G5 was also able to block fusion between HeLa-CD4 cells and CEMx174 cells chronically infected with HIV-1_{89.6} but had no effect on fusion mediated by cells chronically infected with HIV-1_{HXB}. Taken together, these results suggest that different strains of HIV-1 may interact with different sites on CXCR-4 or may have different binding affinities for the coreceptor.

The chemokine receptor CXCR-4, a member of the serpentine family of G-protein-associated receptors, has recently been shown to function in conjunction with CD4 as a cofactor for human immunodeficiency virus type 1 (HIV-1) infection and for virus-mediated cell-to-cell fusion (2, 9). This cofactor or coreceptor is used principally by T-cell-tropic or syncytium-inducing strains of HIV-1 (2, 6, 9), and it is expressed in a wide variety of cells and tissues, including lymphocytes, monocytes, endothelial cells, colon, liver, kidney, and some cells of the central nervous system (CNS), including microglia and neurons (8, 13, 14). Functional studies have shown that coexpression of CXCR-4 and CD4 in nonprimate cells renders them susceptible to infection and fusion with T-tropic isolates of HIV-1. Although the exact mechanism through which CXCR-4 mediates viral entry and fusion is unknown, Lapham et al. (12) have shown by immunoprecipitation that in the presence of CD4, gp120 forms a complex with CXCR-4, suggesting that both CXCR-4 and CD4 interact directly with the viral envelope. Further support for a direct interaction between gp120 and CXCR-4 is the ability of some HIV-2 isolates to infect CXCR-4-positive cells in the absence of CD4 (7, 15).

The monoclonal antibody (MAb) 12G5, previously described by Endres et al. (7), specifically binds CXCR-4 and can be used to detect its expression on cell surfaces. Furthermore, this antibody can inhibit infection of a variant HIV-2 strain

that can infect CD4-negative, CXCR-4-positive cells. Although both dual-tropic and T-tropic viruses appear to use CXCR-4 equivalently in either single-step infections or fusion assays, we report that MAb 12G5 inhibits infection of some cells with HIV-1_{89.6} but not HIV-1_{HXB} or its molecular clones. To gain insights into the interaction between these viruses and the T-tropic coreceptor, we also performed a series of studies with chimeric viruses incorporating critical regions of the HIV-1_{89.6} envelope in the background of HIV-1_{HXB}.

Table 1 summarizes CXCR-4 expression on a group of T- and B-cell lines and primary cells used in this study as determined by either fluorescence-activated cell sorting (FACS) analysis or immunohistochemistry with MAb 12G5 (7, 15). Many T-lymphoblast lines are reactive with 12G5, and Table 1 shows that CEM.3 cells, the B-cell line 721.174 (represented by a clone engineered to express CD4, 174-CD4), and CEMx174 cells all express CXCR-4. The glioblastoma cell lines U87 and U87-CD4 are negative for 12G5 staining (7) and do not express CXCR-4 mRNA (9); however, following transduction with a CXCR-4 retroviral vector, these cells become immunoreactive with 12G5 and are susceptible to infection with T-tropic HIV-1 strains (7). MAb 12G5 is also reactive with primary peripheral blood mononuclear cells (PBMCs) but minimally or not reactive with monocyte-derived macrophages (15). However, brain microglia are strongly positive for 12G5 immunostaining and have been shown to express CXCR-4 mRNA (13).

Inhibition of HIV-1_{89.6} by MAb 12G5. To determine the effect of 12G5 binding on HIV-1 infection, CEMx174 cells were preincubated with either 5, 10, 20, or 40 µg of the anti-

* Corresponding author. Mailing address: Department of Neurology, University of Pennsylvania Medical Center, Clinical Research Building, 415 Curie Blvd., Philadelphia, PA 19104-6146. Fax: (215) 573-2029.

TABLE 1. CXCR-4 expression on cells used in this study determined by MAb 12G5 immunoreactivity

Cell type	Description	12G5 reactivity ^a
Lines		
CEM.3	T lymphoblastoid	+
721.174	B lymphoblastoid	+
CEMx174	T-B lymphoblast hybrid	+
174-CD4	CD4 ⁺ B lymphoblast	+
U87	Glioblastoma	-
U87-CD4	CD4 ⁺ glioblastoma	-
U87-CD4-Les	CD4 ⁺ glioblastoma	+
HeLa-CD4	CD4 ⁺ carcinoma	+
Primary cells		
PBMC	Normal human blood	+
Macrophage	Normal human blood	±
Microglia	Human brain	+

^a Cells were analyzed for 12G5 immunoreactivity by FACS analysis as described by Endres et al. (7), with the exception of the macrophage and PBMC cultures, which were analyzed as described by McKnight and colleagues (15), and the microglial cultures, which were evaluated by immunohistochemical analysis as described by Lavi et al. (13). +, strongly positive; +/-, weakly positive; -, no reactivity above background.

body or 20 μ g of an isotype-matched control antibody per ml for 1 h at 37°C. The cells were subsequently infected at a multiplicity of 0.005 to 0.01 with HIV-1_{89.6} for 1 to 2 h at 37°C, washed, and cultured in the presence of antibody. Viral replication was determined by p24 production in the culture supernatants on day 6 after infection. Figure 1 demonstrates that addition of the anti-CXCR-4 antibody to the cells prior to infection had a dose-dependent inhibitory effect on HIV-1_{89.6} replication. Maximal inhibition of infection was noted at a concentration (10 to 20 μ g/ml) that correlates with the saturating concentrations of 12G5 binding to cell surfaces as determined by FACS staining (7).

To extend this observation, we examined the effect of the antibody on replication over the course of infection with either HIV-1_{89.6} or the T-tropic laboratory isolate HIV-1_{IIIB} in both CEMx174 cells and a CD4-positive B-cell line, 174-CD4 (17). Figure 2 shows growth curves of HIV-1_{89.6} and HIV-1_{IIIB} in

FIG. 1. Dose-dependent inhibition of HIV-1_{89.6} infection by anti-CXCR-4 MAb 12G5. CEMx174 cells (1.5×10^5) were preincubated with various concentrations of MAb 12G5 1 h prior to infection with HIV-1_{89.6} (5 ng of p24). The cells were washed extensively and incubated in the presence of the indicated concentration of antibody. Six days after infection, the supernatant p24 concentration was determined by antigen capture enzyme-linked immunosorbent assay. The plotted values represent one of three experiments with similar results.

FIG. 2. Differential inhibition of infection by anti-CXCR-4 MAb 12G5. 174-CD4 and CEMx-174 cells (1.5×10^5 of each) (17) were preincubated with 20 and 40 μ g, respectively, of MAb 12G5 or an isotype-matched control antibody per ml or with 10 μ g of a mixture of anti-CD4 antibodies (OKT3a, clone 19 and clone 21) per ml and then infected with either HIV-1_{IIIB} or HIV-1_{89.6} (multiplicity of infection of 0.01). One half volume of the medium was collected at regular intervals and replaced with medium containing the appropriate antibody, and the p24 concentration of those supernatants was measured by an antigen-capture enzyme-linked immunosorbent assay. □, no antibody; ◇, anti-CD4; △, MAb 12G5; ○, control MAb.

both cell types treated with medium alone, a control antibody (20 μ g/ml), an anti-CD4 cocktail (10 μ g/ml), or 12G5 (20 or 40 μ g/ml). As previously observed, 12G5 inhibited p24 production by HIV-1_{89.6} in both the CEMx174 and 174-CD4 cells 50- to 100-fold in comparison with the control antibody. As expected, the anti-CD4 cocktail completely inhibited infection of both viruses; however, the anti-CXCR-4 antibody had no effect on replication of the T-tropic isolate. This result was unexpected since most of the T-tropic virus envelopes examined to date have been shown to utilize CXCR-4 for fusion and entry (1, 2, 4, 9).

To map the region of the HIV-1_{89.6} gp120 responsible for its sensitivity to 12G5 inhibition, we used a panel of chimeric envelope viruses constructed from the proviral clones of HIV-1_{89.6} and HIV-1_{HXB} (10). The genetic maps of the envelope regions of the parental and chimeric viruses are depicted in Fig. 3. The first set of chimeric viruses, designated Hx(89.6BB) and 89.6(HxBB), use conserved *Bgl*I sites to swap a fragment of gp120 containing the V3 loop and CD4 binding domains of HIV-1_{89.6} and HIV-1_{HXB}, respectively, into the background of the other virus. The second set of chimeric viruses, 89.6(HxBM) and 89.6(HxMB), was prepared by cloning the *Bgl*I-*Mst*I fragment containing the HxB V3 loop and the *Mst*I-*Bgl*I fragment, containing the HxB CD4 binding region, respectively, into the HIV-1_{89.6} proviral genome. All of the chimeric clones were propagated in CEMx174 cells to generate viral stocks.

CEMx174 cells or phytohemagglutinin- and interleukin-2-stimulated PBMCs were pretreated with 10 μ g of 12G5 or a control antibody and infected, and half of the volume of the culture supernatants was collected every 3 to 4 days and re-

FIG. 3. HIV recombinants used in these experiments. Chimeric viruses incorporating regions of the HIV-1_{HxB} (HxB) *env* on the background of HIV-1_{89.6} have been described elsewhere (10). Restriction sites used to construct the chimeras: B, *Bgl*II; M, *Mst*II.

placed with an equal volume of medium with or without antibody. Figure 4 shows growth curves for each of the viruses in CEMx174 cells treated with medium alone, 12G5, or a control antibody. As previously seen with HIV-1_{IIIB}, one of its molecular clones, HIV-1_{HxB}, was not substantially inhibited by the anti-CXCR-4 antibody. Additionally, the chimeric virus 89.6

FIG. 4. MAb 12G5 inhibition of infection by chimeric viruses. CEMx174 cells (10^5) were preincubated with 10 μ g of anti-CXCR-4 antibody 12G5 ($\diamond$) or isotype-matched control antibody ($\circ$) per ml or with medium alone ($\square$) and then infected for 1 h with each of the parental or chimeric viruses (5 ng of p24). The cells were washed, the medium replaced with the appropriate concentration of antibody, and the supernatants were collected as indicated in the legend to Fig. 2. Viruses that express the HxB V3 loop are indicated on the left; those that express the 89.6 V3 loop are indicated on the right.

FIG. 5. Inhibition of infection of U87-CD4-CXCR4 cells U87-CD4-CXCR4 cells (2×10^4) (5, 7) seeded in 96-well plates were infected with chimeric viruses (≈ 1 ng of p24) after preincubation for 1 h with MAb 12G5 (10 μ g/ml), and the supernatant p24 was measured on day 6 after infection.

(HxBB), which has the HxB V3 loop and CD4 binding domain, and 89.6(HxBB) virus, which expresses only the V3 loop of HxB, were not sensitive to inhibition by MAb 12G5. In contrast, both of the chimeric viruses that contained the V3 loop region of 89.6, HxB(89.6BB) and 89.6(HxMB), were sensitive to inhibition by 12G5 to the same or even greater degree as the parental virus HIV-1_{89.6}. These results implicate the V3 loop of HIV-1_{89.6} in its use of CXCR-4, much like the V3 loops of other M-tropic viruses define the use of CCR5 and other chemokine receptors (4). When the chimeric viruses were used to infect phytohemagglutinin-stimulated PBMCs from several different donors, we saw a similar pattern of inhibition with 12G5 (data not shown). However, the parental strain HIV-1_{89.6} was inhibited only about 10-fold, probably because HIV-1_{89.6} can also use CCR5, which is also expressed in PBMCs, as an entry cofactor (4, 6, 16).

U87-CD4-CXCR-4. To rule out the possibility that the results with the HxB viruses and V3 chimeras in CEMx174 cells were due to their use of a different coreceptor, we tested MAb 12G5 for inhibition of infection of an astrogloma cell line (U87-CD4-CXCR-4) that stably expresses CD4 and CXCR-4 (5). Unlike the parental U87 and U87-CD4 cells, which do not support infection by HIV-1, the U87-CD4-CXCR-4 cells are susceptible to infection by T-tropic HIV-1 isolates (7). U87-CD4-CXCR-4 cells were plated in 96-well plates and incubated with medium alone or medium containing 20 μ g of MAb 12G5 per ml 1 h prior to infection and were infected overnight. The concentration of p24 in the supernatant was measured 6 days later and is plotted in the graph shown in Fig. 5. As previously observed in the CEMx174 cells, neither HIV-1_{HxB} nor the chimeric virus expressing the V3 loop and CD4 binding domain of HIV-1_{HxB} was substantially inhibited by 12G5. In contrast, the viruses expressing the V3 loop of HIV-1_{89.6} were quite sensitive to the MAb. This result suggests while HIV-1_{89.6} and HIV-1_{HxB} both utilize CXCR-4 for entry and infection, differences exist in the binding properties of these envelopes for the coreceptor.

Cell-to-cell fusion. We also examined the ability of 12G5 to inhibit gp120-mediated cell fusion by HIV-1_{89.6} and HIV-1_{IIIB}, using a cell-cell fusion assay. HeLa CD4 cells, which normally express CXCR-4 (9), were preincubated with 20 μ g of MAb

FIG. 6. Cell-to-cell fusion of CEMx174 and HeLa CD4 cells. CEMx174 cells were chronically infected with either HIV-1_{IIB} or HIV-1_{89.6} and then mixed with HeLa CD4 cells that had been preincubated with 20 μ g of either MAb 12G5 or a control, isotype-matched MAb per ml. After an overnight incubation, the plates were stained with DiffQuick and inspected for syncytium formation, and representative photographs were obtained. CEMx174 cells infected with either virus induced syncytia in HeLa CD4 cells pretreated with a control antibody (top panels), whereas cells preincubated with 12G5 did not fuse after HIV-1_{89.6}-infected cells were added to the culture (bottom right) but fused extensively after HIV-1_{IIB}-infected cells were added (bottom left).

12G5 per ml for 1 h, then chronically infected CEMx174 cells were added, and the mixture was incubated overnight. The monolayer containing fused cells was washed and stained with the DiffQuick Solution II stain, and the monolayers were photographed. As expected (Fig. 6), there was extensive syncytium formation between CEMx174 cells infected with either HIV-1_{IIB} or HIV-1_{89.6}; however, MAb 12G5 was able to inhibit only fusion mediated by the HIV-1_{89.6} envelope and had no apparent effect on the fusion between CEMx174 cells infected with HIV-1_{IIB} and the uninfected HeLa-CD4 cells.

Microglia. The role of CXCR-4 in HIV-1 infection of the CNS is still unknown, but Lavi and coworkers have described its expression in microglia and other cells of the CNS (13). Microglia, like other members of the monocyte/macrophage lineage, also express mRNA for the M-tropic coreceptor CCR5 (9a). To determine if 12G5 could inhibit infection of HIV-1 in these primary cells, antibody was added to cultures of purified brain microglia 1 h prior to infection with HIV-1_{89.6} or a primary M-tropic isolate, HIV-1_{FASH}. As shown in Fig. 7, MAb 12G5 did not inhibit infection by either HIV-1_{89.6} or HIV-1_{FASH} in these primary cultures. Similarly, in primary monocyte-derived macrophage cultures, which reportedly express only very low levels of CXCR-4 (15), MAb 12G5 had no effect on infection by HIV-1_{89.6} or other M-tropic viruses (data not shown). These results suggest that in the microglial cells, which express CXCR-4, CCR5, and possibly other chemokine

FIG. 7. Microglial infection. Microglial cultures were prepared as previously described (18) and plated at a density of 2×10^5 cells/well in a 24-well plate. The cells were then preincubated for 1 h with 10 μ g of MAb 12G5 per ml or with medium alone and infected overnight with 10 ng of HIV-1_{89.6} or HIV-1_{FASH}. The supernatants were collected at regular intervals and replaced with medium with or without MAb 12G5, and viral replication was assayed by measurement of the concentration of p24 by antigen capture assay.

receptors, HIV-1_{89,6} likely uses a coreceptor other than CXCR-4 for infection.

In this study, we have demonstrated differential inhibition of a T-tropic and a dual-tropic HIV-1 strain by an anti-CXCR-4 MAb. Our results indicate that the antibody had no effect on infection by the prototype T-tropic virus HIV-1_{IIIB}, which is known to use CXCR-4 for fusion and entry (6, 9). In contrast, replication of the dual-tropic virus HIV-1_{89,6}, which can use a number of coreceptors (6), was quite sensitive to moderate concentrations of the antibody. A similar finding was recently reported by McKnight and coworkers, who found differential inhibition of some T-tropic HIV-1 and HIV-2 strains by MAb 12G5 in some T-lymphoblast lines and HeLa-CD4 cells (15). However, they also reported that the T-tropic virus HIV_{LA1} was sensitive to inhibition by 12G5 in RD/CD4 cells, suggesting that CXCR-4 might be expressed differently or be more sensitive to antibody-induced down regulation in this cell type than in lymphoid cell lines. We did not observe any differences in the inhibition patterns of HIV-1_{89,6} and HIV-1_{IIIB} among the different CXCR-4-expressing cell lines used in this study.

The most parsimonious interpretation of our data is that HIV-1_{89,6} and HIV-1_{IIIB}, while capable of using the same coreceptor, bind to different areas of CXCR-4. In this regard, Rucker et al. (16) have recently demonstrated that envelopes from HIV-1_{89,6} or HIV-1_{JRFL} require different regions of the CCR-5 chemokine coreceptor. In those experiments, a 12- to 16-amino-acid deletion at the N terminus of CCR-5 abolished fusion mediated by the HIV-1_{89,6} envelope but had no effect on fusion mediated by HIV-1_{JRFL}. Therefore, it is reasonable to speculate that different T- and dual-tropic viruses have adapted to utilize separate sites on the CXCR-4 coreceptor. Interestingly, the chemokine SDF-1, the natural ligand for CXCR-4, inhibits infection by both of these isolates (3). This finding may indicate that while both viruses use sites involved in SDF-1 binding, only HIV-1_{89,6} uses a region that overlaps with the MAb 12G5 epitope. Alternatively, SDF-1 could mediate inhibition by causing down regulation of the coreceptor at the cell surface rather than by steric hindrance of the gp120–CXCR-4 interaction.

Using a panel of recombinant viruses with chimeric envelopes, we have demonstrated that the V3 loop of HIV-1_{89,6} is an important determinant of its sensitivity to 12G5. This finding implies that this region of the envelope is involved in the interaction between gp120 and the CXCR-4 coreceptor, although direct binding was not demonstrated. Several studies (4, 19, 20) have similarly shown that the V3 loop plays a key role in the interactions between M-tropic viruses and CCR-5, although recombinants of a dual-tropic virus like HIV-1_{89,6} were not used in those experiments. Given the diversity of the V3 loop among HIV strains, and the potential complexity of the CXCR-4 coreceptor, it is reasonable to postulate that different motifs are involved in the gp120–CXCR-4 interaction. HIV-1_{89,6}, a transitional virus that uses multiple coreceptors, may have been isolated and cloned at a point in its evolution where it had not yet developed the most efficient interaction with CXCR-4. This speculation suggests that the binding between HIV-1_{IIIB} and CXCR-4 may be more efficient than that between HIV-1_{89,6} and this coreceptor. Alternatively, the interaction between CD4 and the viral envelope may influence the relative binding requirement for the cofactor. As suggested by Kozak et al. (11) in a recent report, laboratory-adapted T-tropic viruses may have a greater affinity for CD4 compared with primary M-tropic patient isolates; the latter would then rely more on a coreceptor interaction in order to infect cells that express CD4 to lower levels. This question will need to be

resolved by further studies that analyze the relative binding kinetics and affinities of the different viral envelopes to CXCR-4.

Finally, we have shown that in microglial cells, which express both CXCR-4 and CCR5, MAb 12G5 was ineffective in inhibiting HIV-1_{89,6} growth. This result implies that CCR5 may be used more efficiently than CXCR-4 for infection by dual-tropic viruses of these primary cells.

This work was supported by PHS grants NS-27405 and NS-31066.

We thank Michael O'Connor (Thomas Jefferson University) for brain tissue, Ned Landau (Aaron Diamond AIDS Research Center) for the U87 cells and derivatives, and Robert Doms (Department of Pathology, University of Pennsylvania) for helpful discussions.

REFERENCES

- Alkhatib, G., C. Combadiere, C. C. Broder, Y. Feng, P. E. Kennedy, P. M. Murphy, and E. A. Berger. 1996. CC CKR5: a RANTES, MIP-1 α , MIP-1 β receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272**: 1955–1958.
- Berson, J. F., D. Long, B. J. Doranz, J. Rucker, F. R. Jirik, and R. W. Doms. 1996. A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains. *J. Virol.* **70**: 6288–6295.
- Bleul, C. C., M. Farzan, H. Choe, C. Parolin, I. Clark-Lewis, J. Sodroski, and T. A. Springer. 1996. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* **382**:829–833.
- Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P. D. Ponath, J. Wu, C. R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski. 1996. The β -chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**:1135–1148.
- Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau. 1996. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**:661–666.
- Doranz, B. J., J. Rucker, Y. Yi, R. J. Smyth, M. Samson, S. C. Peiper, M. Parmentier, R. G. Collman, and R. W. Doms. 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the β -chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* **85**:1149–1158.
- Endres, M. J., P. R. Clapham, M. Marsh, M. Ahuja, J. D. Turner, A. McKnight, J. F. Thomas, B. Stoeckenau-Haggerty, S. Choe, P. J. Vance, T. N. C. Wells, C. A. Power, N. R. Landau, and J. A. Hoxie. 1996. CD4-independent infection by HIV-2 is mediated by fusin. *Cell* **87**:745–756.
- Federspiel, B., I. G. Melhado, A. M. V. Duncan, A. Delaney, K. Schappert, I. Clark-Lewis, and F. R. Jirik. 1993. Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. *Genomics* **16**:707–712.
- Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**:872–877.
- He, J., Y. Chen, M. Farzan, H. Choe, A. Ohagen, S. Gartner, J. Busciglio, X. Yang, W. Hofmann, W. Newman, C. R. Mackay, J. Sodroski, and D. Gabuzda. 1997. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **385**:645–649.
- Kim, F. M., D. L. Kolson, J. W. Balliet, A. Srinivasan, and R. G. Collman. 1995. V3-independent determinants of macrophage tropism in a primary human immunodeficiency virus type 1 isolate. *J. Virol.* **69**:1755–1761.
- Kozak, S. L., E. J. Platt, N. Madani, F. E. Ferro, K. Peden, and D. Kabat. 1997. CD4, CXCR-4, and CCR-5 dependencies for infections by primary patient and laboratory-adapted isolates of human immunodeficiency virus type 1. *J. Virol.* **71**:873–882.
- Lapham, C. K., J. Ouyang, B. Chandrasekhar, N. Y. Nguyen, D. S. Dimitrov, and H. Golding. 1996. Evidence for cell-surface association between fusin and the CD4-gp120 complex in human cell lines. *Science* **274**:602–605.
- Lavi, E., J. M. Strizki, A. M. Ulrich, W. Zhang, L. Fu, Q. Wang, M. O'Connor, J. Hoxie, and F. Gonzalez-Scarano. Unpublished data.
- Loetscher, M., T. Geiser, T. O'Reilly, R. Zwahlen, M. Gaggiolini, and B. Moser. 1994. Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. *J. Biol. Chem.* **269**:232–237.
- McKnight, A., D. Wilkinson, G. Simmons, S. Talbot, L. Picard, M. Ahuja, M. Marsh, J. A. Hoxie, and P. R. Clapham. 1997. Inhibition of human immunodeficiency virus fusion by a monoclonal antibody to a coreceptor (CXCR4) is both cell type and virus strain dependent. *J. Virol.* **71**:1692–1696.
- Rucker, J., M. Samson, B. J. Doranz, F. Libert, J. J. Berson, Y. Yi, R. G. Collman, C. C. Broder, G. Vassart, R. W. Doms, and M. Parmentier. 1996. Regions in β -chemokine receptors CCR5 and CCR2b that determine HIV-1 cofactor specificity. *Cell* **87**:1–20.
- Stefano, K., R. Collman, D. Kolson, J. Hoxie, N. Nathanson, and F. Gonza-

- lez-Scarano.** 1993. Replication of a macrophage-tropic strain of human immunodeficiency virus type 1 (HIV-1) in a hybrid cell line, CEMX174, suggests that cellular accessory molecules are required for HIV-1 entry. *J. Virol.* **67**:6707–6715.
18. **Strizki, J. M., A. V. Albright, H. Sheng, M. O'Connor, L. Perrin, and F. Gonzalez-Scarano.** 1996. Infection of primary human microglia and monocyte-derived macrophages with human immunodeficiency virus type 1 isolates: evidence of differential tropism. *J. Virol.* **70**:7564–7662.
19. **Trkola, A., T. Dragic, J. Arthos, J. M. Binley, W. C. Olson, G. P. Allaway, C. Cheng-Mayer, J. Robinson, P. J. Maddon, and J. P. Moore.** 1996. CD4-dependent antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. *Nature* **384**:184–187.
20. **Wu, L., N. P. Gerard, R. Wyatt, H. Choe, C. Parolin, N. Ruffing, A. Borsetti, A. A. Cardoso, E. Desjardin, W. Newman, C. Gerard, and J. Sodroski.** 1996. CD4-induced interaction of primary HIV-1gp120 glycoproteins with the chemokine receptor CCR-5. *Nature* **384**:179–187.