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CD4-independent, CCR5-dependent infection of brain capillary endothelial cells by a neurovirulent simian immunodeficiency virus strain

AIMEE L. EDINGER*, JOSEPH L. MANKOWSKI†‡, BENJAMIN J. DORANZ*‡, BARRY J. MARGULIES†‡, BENHUR LEE*‡, JOSEPH RUCKER*, MATTHEW SHARRON*, TREVOR L. HOFFMAN*, JOANNE F. BERSON*, M. CHRISTINE ZINK†, VANESSA M. HIRSCH§, JANICE E. CLEMENTS†, AND ROBERT W. DOMS*¶

*Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104; †Division of Comparative Medicine, Johns Hopkins School of Medicine, Baltimore, MD 21205; and §National Institute of Allergy and Infectious Disease, National Institutes of Health, Rockville, MD 20852

Edited by Anthony S. Fauci, National Institute of Allergy and Infectious Disease, Rockville, MD, and approved October 21, 1997 (received for review September 16, 1997)

ABSTRACT **Brain capillary endothelial cells (BCECs) are targets of CD4-independent infection by HIV-1 and simian immunodeficiency virus (SIV) strains *in vitro* and *in vivo*. Infection of BCECs may provide a portal of entry for the virus into the central nervous system and could disrupt blood–brain barrier function, contributing to the development of AIDS dementia. We found that rhesus macaque BCECs express chemokine receptors involved in HIV and SIV entry including CCR5, CCR3, CXCR4, and STRL33, but not CCR2b, GPR1, or GPR15. Infection of BCECs by the neurovirulent strain SIV/17E-Fr was completely inhibited by aminooxypentane regulation upon activation, normal T cell expression and secretion in the presence or absence of ligands, but not by eotaxin or antibodies to CD4. We found that the envelope (env) proteins from SIV/17E-Fr and several additional SIV strains mediated cell–cell fusion and virus infection with CD4-negative, CCR5-positive cells. In contrast, fusion with cells expressing the coreceptors STRL33, GPR1, and GPR15 was CD4-dependent. These results show that CCR5 can serve as a primary receptor for SIV in BCECs and suggest a possible CD4-independent mechanism for blood–brain barrier disruption and viral entry into the central nervous system.**

HIV-1 infection is often associated with the development of neurologic disease, including sensory, motor, and cognitive dysfunction, despite the fact that direct infection of neurons has not been shown (1, 2). Therefore, it seems that neurologic deficits result from the infection of nonneuronal cells associated with the central nervous system (CNS) such as microglia, endothelial cells, and astrocytes. A variety of macrophage-tropic HIV-1 strains can infect microglia, which express CD4 and the chemokine receptors CCR3 and CCR5 (3). Infection of microglia can be inhibited by ligands or antibodies to CCR3 or CCR5, indicating that either of these chemokine receptors can participate in virus infection (3). Brain capillary endothelial cells (BCECs) have also been shown to be permissive for HIV-1 and simian immunodeficiency virus (SIV) replication (4, 5). Infection of endothelial cells, a critical component of the blood–brain barrier, could play a role in AIDS dementia by facilitating the entry of virus or circulating toxins into the CNS. BCECs have been shown by immunohistochemistry to be infected by HIV-1 *in vivo*, and their infection *in vitro* has been demonstrated to occur by a CD4- and galactosylceramide-independent mechanism (4). Understanding the mechanisms by which brain endothelial cells are infected may help clarify the events that lead to the development of neurologic disease in infected individuals.

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Simian immunodeficiency virus infection of rhesus macaques results in the development of neurologic symptoms similar to those seen with HIV-1 infection in humans. Several neurovirulent SIV strains have been characterized. One such strain, the macrophage-tropic SIV/17E-Br, and a molecular clone (SIV/17E-Fr) derived from that strain have been shown to productively infect CNS endothelial cells *in vitro* and *in vivo*, whereas the parental T-tropic virus (SIVmac239) does not (5–7). Rhesus BCECs also lack CD4, and soluble CD4 does not inhibit infection of BCECs (5). The viral receptor SIV uses to enter these cells has not been determined.

The discovery that certain chemokine receptors, in conjunction with CD4, are used by HIV-1, HIV-2, and SIV strains for cellular entry affords new opportunities to study viral pathogenesis at the molecular level (reviewed in refs. 8–10). SIV strains have been shown to use CCR5 and the orphan seven-transmembrane domain receptors STRL33/Bonzo, GPR15/BOB, and GPR1 as coreceptors, but not CXCR4, suggesting that the determinants of macrophage and T cell tropism differ between SIV and HIV-1 (11–15). Interestingly, chemokine receptors can sometimes support viral entry in the absence of CD4; laboratory-adapted HIV-2 and FIV strains have been shown to infect cells productively in a CD4-independent, CXCR4-dependent manner (16–18). Seven-transmembrane domain receptors were thus likely candidates for the primary SIV receptor in BCECs. We determined that rhesus macaque BCECs expressed mRNA for CCR5, CCR3, CXCR4 and STRL33 and found that expression of CCR5 in the absence of CD4 supported SIV envelope-mediated cell–cell fusion and virus infection for a number of SIV strains, including SIV/17E-Fr. Furthermore, infection of rhesus BCECs was abolished by the CCR5 ligand, AOP-RANTES, but not by eotaxin or antibodies to CD4. These results show that CCR5 can serve as a primary receptor for several SIV strains and that rhesus BCECs can be infected by a neurovirulent SIV strain through a CD4-independent, CCR5-dependent pathway.

MATERIALS AND METHODS

Plasmids and Vaccinia Viruses. Rhesus CCR5, human CCR5, STRL33, GPR15, and CD4 were expressed using the pcDNA3 vector (Invitrogen). GPR1 was expressed from pRC/CMV (In-

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Abbreviations: CNS, central nervous system; BCECs, brain capillary endothelial cells; SIV, simian immunodeficiency virus; RT-PCR, reverse transcription–PCR; env protein, envelope protein; AOP-RANTES, aminooxypentane regulated upon activation, normal T cell expressed and secreted in the absence or presence of ligand.
‡These authors contributed equally to this work.
¶To whom reprint requests should be addressed at: Department of Pathology, University of Pennsylvania, 806 Abramson, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104. e-mail: doms@mail.med.upenn.edu.

vitrogen) (provided by Brian O'Dowd). Plasmids were used to express the envelope proteins of SIV/17E-Fr (pcDNA3), SIVmac1A11 (pCR3.1, M. Endres), SIVDeltaB670 clones 3 and 12 (pcDNA3, M. Murphey-Corb), SIVagmSab1.4 (pCR2.1, B. Hahn), SIVagm9063-2, SIVsm62A, SIVsm62B, SIVsm62D, SIVsmE543-3, SIVsmE543-B10, SIVsmPBj6.6 (all SIVsm isolates in pGEM5Zf), SIV CP-MAC (pCR3.1, M. Endres), HIV-1 89.6 (pSC65), HIV-1 JR-FL (pSC59) [C. Broder (19)], and HIV-2 ST (pCR3.1, M. Endres). SIVmac251 (v194), SIVmac239 (vCB74), SIVmac316 (vCB75), SIVmac316 mut (vCB76), HIV-1 89.6 (vBD3), and HIV-1 JR-FL (vCB28) envelopes were expressed in effector HeLa cells through infection with recombinant vaccinia viruses. vTF1.1 was used to express T7 RNA polymerase off of the vaccinia late promoter (20).

Reverse Transcription–PCR (RT-PCR). Total cellular RNA (5 μg) from cells derived from macaque BCECs or rhesus PBMCs was isolated by an RNEasy kit (Qiagen, Chatsworth, CA), treated with RNase-free DNase (RQ1; Promega), and reverse-transcribed in a 20-μl reaction with random hexamers, oligo(dT)$_{15}$ (Pharmacia), and Superscript II (Life Technologies, Gaithersburg, MD) under recommended reaction conditions. Reverse-transcribed RNA was used as template in a hot-started PCR reaction containing *Taq* polymerase (Perkin–Elmer) and *pfu* polymerase (Stratagene) (21). For CCR2b, CCR3, CCR5, CXCR4, STRL33, GPR15, and GPR1, 5′ primers were synthesized to correspond to the region of the human gene containing the ORFs initiating ATG and sequences immediately 3′ to that, and 3′ primers contained the endogenous stop codon of the human gene and sequences immediately 5′ to that. Detection of CXCR4 was accomplished with two primers designed to detect internal sequences of the human gene (22). Samples were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide, and photographed with an Eagle Eye Storage Device (Stratagene).

Cell–Cell Fusion Assay. Cell–cell fusion was determined with a gene reporter assay (23, 24). Briefly, effector cells expressing HIV or SIV envelope (env) proteins and T7 RNA polymerase were mixed with quail QT6 target cells transiently expressing the desired chemokine receptor with or without CD4 and luciferase under the control of the T7 promoter. Cytoplasmic mixing after membrane fusion allows transcription of luciferase which is quantified in cell lysates with a luminometer. Envelopes were expressed in HeLa cells via vaccinia or by transfection in 293T cells. Blocking experiments were conducted as above except that target cells were incubated with the indicated concentration of antibody for 30 min before effector cell addition and fusion was quantified at 5 rather than 8 h after cell–cell mixing.

Virus Infections. Luciferase reporter viruses were prepared by transfecting 293T cells with the indicated envs and with the NL4-3 luciferase virus backbone (pNL-Luc-E$^-$R$^-$) (25, 26). Target cells for infection were 293T with CD4 and coreceptors introduced by calcium phosphate transfection. Similar results were obtained using the feline CCCS cell line. Cells were lysed 4 days postinfection by resuspension in 0.5% Nonidet P-40 in PBS and assayed for luciferase activity. Brain cells derived from microvessel endothelium were obtained as previously described (5). Sixth to eighth passage primary cells were grown in fibronectin-coated 48-well plates to ~70% confluence. Cells were preincubated for 1 h with either 400 ng/ml AOP-RANTES (a kind gift of Dr. Timothy Wells, Glaxo), 1 μg/ml eotaxin (R&D Systems), or Leu-3a, and then inoculated with 1.5×10^4 tissue culture 50% infective dose of SIV/17E-Fr. After 18 h, virus inoculum was removed, and cells were washed six times to remove cell-free virus before replenishing the medium. Medium containing the relevant blocking agent at one-half the concentration used for preincubation was added for the first 8 days postinfection, then medium without blocking agent was added to all wells. Culture supernatants were collected at 4-day intervals for 20 days and assayed for p27 by ELISA (Coulter). Mock-inoculated (medium alone) and virus-inoculated wells without blocking agent served as controls. All treatments were performed in triplicate.

RESULTS

Infection of Rhesus Brain Capillary Endothelial Cells. BCECs form a vital component of the blood–brain barrier and are targets of HIV-1 and SIV infection. SIV/17E-Br, a macrophage-tropic, neurovirulent strain derived from SIVmac239 by multiple intracerebral passages in macaques, can productively infect rhesus macaque BCECs *in vitro* by a CD4-independent mechanism (5–7). In addition, soluble CD4 does not inhibit infection of these cells (5). Recently, laboratory-adapted HIV-2 and feline immunodeficiency virus isolates have been shown to use the chemokine receptor CXCR4 as a primary virus receptor to infect CD4-negative cells (16–18). To determine whether infection of BCECs by neurotropic SIV isolates might involve CD4-independent utilization of a seven-transmembrane domain receptor, we examined rhesus BCECs for the presence of CCR2b, CCR3, CCR5, CXCR4, GPR1, GPR15, and STRL33 transcripts. As shown in Fig. 1*a*, BCECs expressed mRNA for CCR3, CCR5, CXCR4, and STRL33 but not CCR2b or GPR1. In addition, message for GPR15 was not detected (data not shown). Thus, macaque brain endothelial cells express two seven-transmembrane domain receptors that have been shown to serve as coreceptors for CD4-dependent SIV entry, CCR5 and STRL33 (11–14).

To determine whether infection of macaque BCECs is CCR5-dependent, we infected rhesus BCECs with SIV/17E-Fr (a molecular clone derived from SIV/17E-Br) in the presence or absence of ligands to CCR5 (AOP-RANTES) and CCR3 (eotaxin) or an antibody to CD4 (Leu-3a). We selected AOP-RANTES because it inhibits CCR5-dependent virus infection ~10-fold more effectively than wild-type RANTES and because it does not induce chemotaxis and induces a Ca^{2+} flux only in a limited number of cell types (27). Medium containing the relevant blocking agent at one-half the concentration used for preincubation was added for the first 8 days postinfection, after which medium without blocking agent was added to all wells. Culture supernatants were collected at 4-day intervals for 20 days and assayed for viral p27. AOP-RANTES completely inhibited infection of BCECs by SIV/17E-Fr in three independent experiments whereas eotaxin and Leu-3a had only a slight inhibitory effect (Fig. 1*b*). These results suggest that CD4-independent infection of BCECs is mediated by CCR5.

CD4-Independent, CCR5-Dependent Cell–Cell Fusion. To more directly examine the CD4-independent use of CCR5 by SIV, we evaluated the ability of the SIV/17E-Fr envelope to mediate cell–cell fusion in the absence of CD4. 293T cells were transfected with a plasmid encoding the SIV/17E-Fr env protein under control of the T7 and cytomegalovirus promoters and infected with a recombinant vaccinia virus expressing T7 polymerase. These cells were then mixed with quail QT6 cells expressing luciferase under the control of the T7 promoter and CD4 and CCR5 as indicated. The extent of cell–cell fusion was determined by measuring luciferase activity 7–8 h after cell mixing (23). Whereas the HIV-1 89.6 and JR-FL env proteins mediated cell–cell fusion only when CD4 and CCR5 were coexpressed, the SIV/17E-Fr env protein mediated fusion with cells expressing CCR5 alone at 30% the efficiency observed in the presence of CD4 (Fig. 2*a*). CD4-independent fusion was also observed when CCR5 was expressed in 293T, COS, or 3T3 cells, indicating that the results were not specific to QT6 cells. In addition, rhesus macaque CCR5 was capable of supporting CD4-independent membrane fusion as efficiently as human CCR5 (not shown).

Other SIV strains differed in their abilities to utilize CCR5 in the absence of CD4. Env proteins derived from SIVΔB670-Cl 12 [a neurotropic envelope clone (28, 29)], SIVΔB670-Cl 3, and SIVsm62B (30) all fused with cells expressing CCR5 alone as efficiently as with cells expressing CCR5 and CD4 together (Fig.

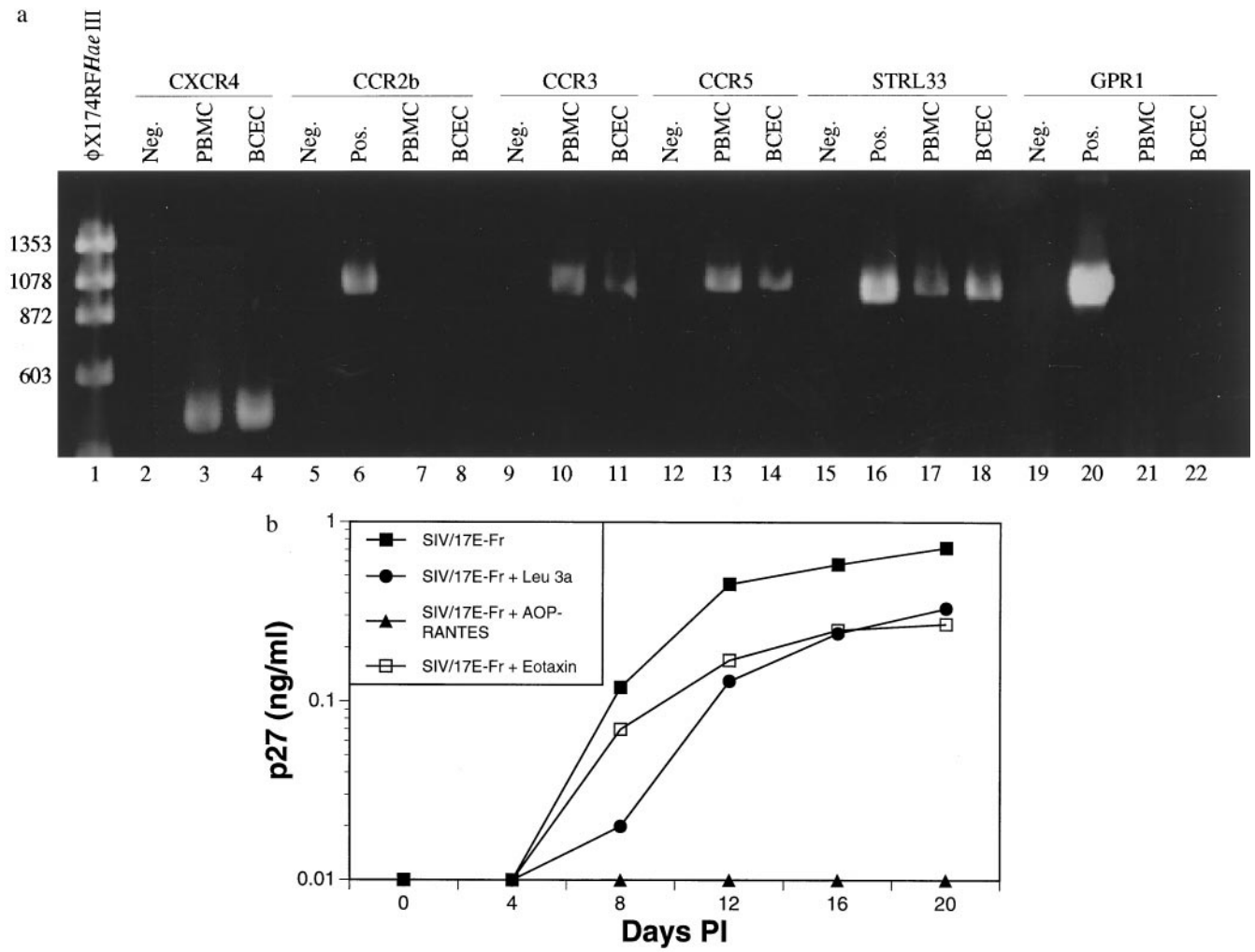

FIG. 1. (*a*) RT-PCR analysis of chemokine receptor expression in macaque BCECs. Rhesus macaque peripheral blood mononuclear cells and BCECs express mRNAs for the receptors CXCR4 (lanes 2–4), CCR3 (lanes 9–11), CCR5 (lanes 12–14), and STRL33 (lanes 15–18). mRNA for GPR15 was detected in peripheral blood mononuclear cells but not in BCECs (data not shown). Expression of CCR2b and GPR1 was not detectable in either cell type (lanes 5–8 and 19–22). Plasmids containing a cDNA for each human gene (Pos.) or water alone (neg.) were included in some reactions as sole templates, and ΦX174/*Hae*III was included as a marker (lane 1, sizes in bp at left). (*b*) Infection of macaque BCECs by SIV/17E-Fr. Purified macaque BCECs were infected with 1.5×10^4 tissue culture 50% infective dose of SIV/17E-Fr after a 1-h preincubation of cells with the indicated blocking agent. The virus inoculum was removed 18 h after infection, the cells were washed to remove cell-free virus, and medium containing the relevant blocking agent at one-half the concentration used for preincubation was added for the next 8 days postinfection. Culture supernatants were collected at 4-day intervals for 20 days and assayed for viral p27 by ELISA.

2*a* and Table 1). The SIVmac316, SIVmac251, SIVmac1A11, and SIVsm62A env proteins elicited fusion with cells expressing CCR5 alone less efficiently than in the presence of CD4. Other viral env proteins were either completely dependent on the presence of CD4 or could only fuse inefficiently with cells expressing CCR5 alone. These included SIVmac239, which does not infect BCECs (5), and several env proteins derived from African green monkey SIV strains (Fig. 2*a* and Table 1). Thus, the ability to utilize CCR5 for membrane fusion in the absence of CD4 was a property of many, but not all, SIV env proteins tested. In contrast, all 16 HIV-1 env proteins tested were CD4 dependent (Fig. 2*a* and data not shown).

To evaluate whether SIV could utilize CCR5 in a CD4-independent manner when the molecules were coexpressed, we performed antibody blocking studies in the cell–cell fusion assay. In the presence of saturating concentrations of an antibody to CD4, fusion through the HIV-1 ADA env was completely inhibited, but fusion through the SIVmac251 env was only reduced to 40% of control (Fig. 2*b*). Cell–cell fusion mediated by the CD4-dependent SIVsmE543/B10 was completely inhibited by this antibody (not shown). These results indicate that CCR5 can serve as a primary receptor for SIV even in the presence of CD4.

The orphan seven-transmembrane domain receptors STRL33, GPR15, and GPR1 have been shown to serve as coreceptors for a number of SIV strains (11, 31). We found that the SIV/17E-Fr, SIV/B670-Cl 3, and SIVsmE543 env proteins could mediate fusion with cells expressing CD4 and either STRL33, GPR15, or GPR1; however, utilization of these receptors was strictly CD4-dependent (Fig. 3). These env proteins did not use CCR3 in the presence or absence of CD4 (data not shown). In summary: (*i*) diverse SIV env proteins mediated cell–cell fusion in a CD4-independent, CCR5-dependent fashion; (*ii*) CCR5 was able to mediate cell–cell fusion with cells expressing the SIVmac251 env in the presence of CD4 and saturating levels of an antibody against CD4; (*iii*) utilization of other coreceptors was CD4-dependent; (*iv*) both human and rhesus CCR5 supported CD4-independent membrane fusion; and (*v*) the presence of CD4 increased the efficiency of fusion for some SIV env proteins, but not others.

CD4-Independent Reporter Virus Infection. Although the cell–cell fusion assay described above generally reflects the ability of a virus to enter cells, results between cell–cell fusion and virus infection assays can sometimes differ. Therefore, we infected human 293T cells expressing CD4, CD4/CCR5, or CCR5 alone

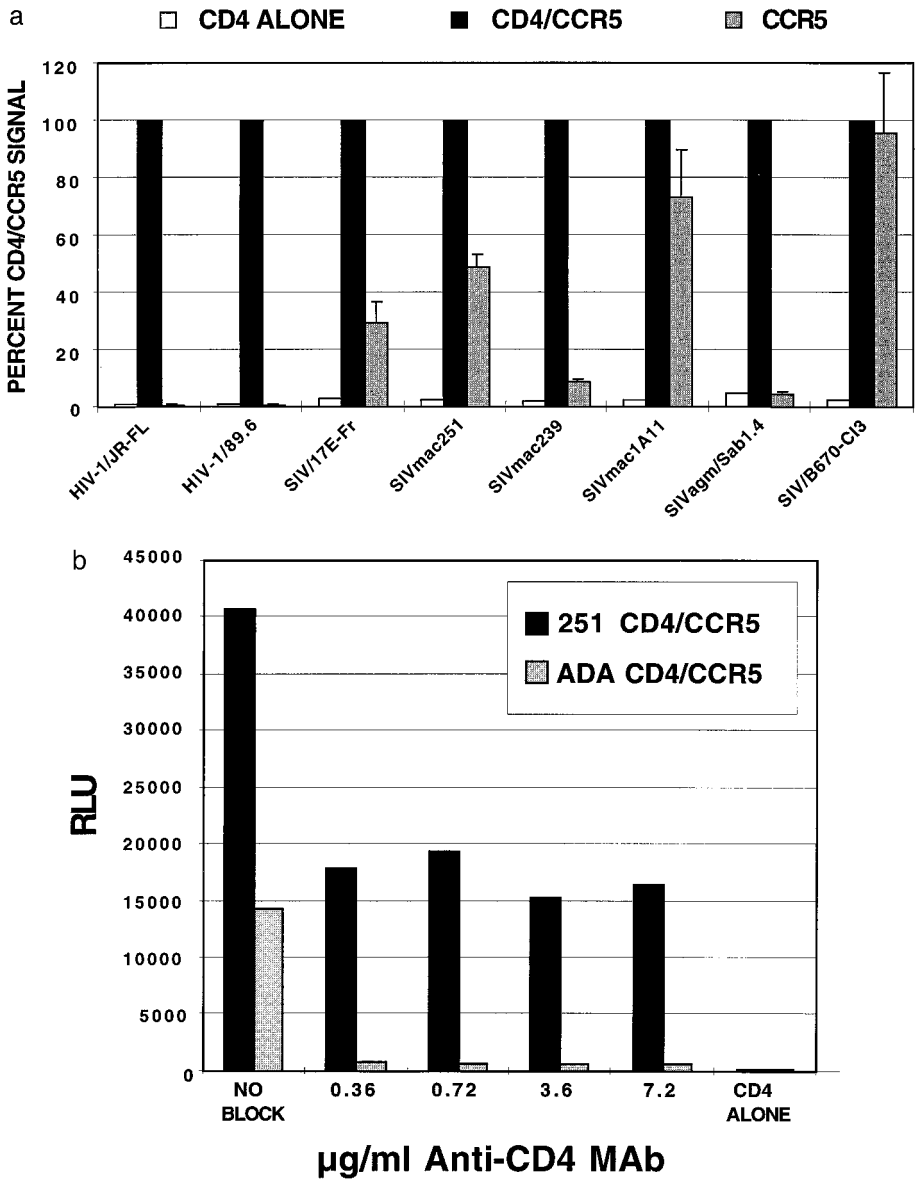

FIG. 2. (*a*) CD4-independent cell–cell fusion. Effector cells expressing the indicated viral envelope protein and T7 polymerase were mixed with target cells expressing luciferase under the control of the T7 promoter and CCR5 and CD4 as indicated. Values were normalized by expressing the signal as the percentage of signal obtained when CD4 and CCR5 were coexpressed (defined as 100% fusion) and represent the mean of three or four independent experiments with standard error bars shown. (*b*) Effects of an anti-CD4 MAb on cell–cell fusion. Cell–cell fusion was performed as in *a*. Effector cells expressing with the SIVmac251 or HIV-1 ADA env proteins were mixed with target cells expressing CD4 and CCR5 in the presence of MAb #19, a monoclonal antibody to CD4 that inhibits HIV-1 entry (16). The extent of cell–cell fusion was determined by measuring luciferase activity in relative light units (RLU) 5 h after cell mixing. The results are from a representative experiment.

with luciferase reporter viruses pseudotyped with different SIV or HIV env proteins. Whereas infection by HIV-1 ADA was strictly CD4-dependent, viruses with env proteins from several SIV strains could infect cells expressing CCR5 alone. Luciferase reporter viruses bearing the SIV/17E-Fr, SIVΔB670-Cl 3, and SIVΔB670-Cl 12 env proteins infected CD4-negative, CCR5-positive cells with efficiencies similar to that observed in the cell–cell fusion assays (Fig. 4). In contrast, SIV CP-MAC-infected cells only when CD4 and CCR5 were coexpressed.

DISCUSSION

The development of neurologic disorders is an important cause of morbidity and mortality for individuals with AIDS (1, 2). Neurologic symptoms are also a prominent feature in rhesus macaques after infection with pathogenic SIV strains (6, 32), although the mechanisms underlying neuropathogenesis are poorly understood. Generally, viruses isolated from the CNS are macrophage-tropic, consistent with the role of macrophage-derived microglia as a major cellular target for HIV and SIV infection *in vivo* (33, 34). Microglia express CD4 and the chemokine receptors CCR5 and CCR3, and ligands and antibodies to CCR3 and CCR5 can inhibit infection of microglia (3). However, not all macrophage-tropic viruses are neurotropic, suggesting that other factors besides macrophage tropism play an important role in CNS infection.

BCECs form a critical component of the blood–brain barrier and can be infected by HIV-1 and SIV *in vivo* and *in vitro* by a CD4-independent mechanism (4–6, 35). We found that primary macaque BCECs expressed mRNA for CCR5, CCR3, CXCR4, and STRL33 (Fig. 1*a*). Because CCR5 and STRL33 have been previously shown to function as SIV coreceptors (11–14), we determined whether these molecules could also serve as a primary virus receptor in the absence of CD4. We found that

Table 1. Ability of SIV strains to use CCR5 in the absence of CD4 for cell–cell fusion

Virus	CD4 alone	CD4/CCR5	CCR5 (S.E.)	Isolate tropism/origin
SIVmac316	2	100	30 (12)	M-tropic* derivative of SIVmac239
SIVmac316mut	2	100	12 (1)	T-tropic derivative of SIVmac316
SIV/B670-Cl 12	2	100	120 (16)	Variant found in brain, crosses placenta
SIVagm9063-2	6	100	3 (1)	Dual tropic, pathogenic, vervet origin
SIVsm62A	1	100	74 (10)	TCL, monkey PBMC, not pathogenic
SIVsm62B	5	100	131 (16)	Restricted to human PBMC
SIVsm62D	1	100	5 (1)	Dual tropic, mildly pathogenic
SIVsmE543-3	1	100	7 (1)	Dual tropic; pathogenic
SIVsmE543/B10	1	100	4 (0)	Brain isolate
HIV-2/ST	4	100	4 (1)	Noncytopathic HIV-2

Effector cells expressing the viral envelope protein and T7 polymerase were mixed with target cells expressing luciferase under the control of the T7 promoter and CCR5 and CD4 as indicated. Values are normalized by expressing the signal as percent of the signal when CD4 and CCR5 are coexpressed. Values represent the mean of three to five experiments.
*M-tropic, macrophage-tropic; TCL, tissue culture lines; PBMC, peripheral blood mononuclear cells.

the env protein of a number of SIV strains could mediate cell–cell fusion and infection through CCR5 in both the presence and absence of CD4 in several cell types. In contrast, utilization of the orphan receptors GPR1, GPR15, and STRL33 was CD4-dependent. These results, coupled with the ability of AOP-RANTES to inhibit infection of BCECs by SIV/17E-Fr, indicate that CCR5 can serve as a primary receptor in these cells.

No simple relationship between CD4 independence and viral tropism was observed. Notably, two neurotropic SIV strains, SIV/17E-Fr and SIVΔB670-Cl 12, readily infected CD4-negative, CCR5-positive cells. Interestingly, the uncloned B670 swarm and the uncloned strain SIVmac251 are able to replicate in rhesus brain capillary endothelial cells, whereas other strains such as $SIV_{mac}239$ are not, consistent with CD4-independent usage of CCR5 as a determinant of endothelial cell tropism. In contrast, the env clone B10 of the neurotropic strain SIVsm543 was completely CD4-dependent in these assays. Clearly, all viruses found in the CNS are not CD4-independent. However, this property may facilitate the development of neurologic disease if the CD4-independent infection of endothelial cells negatively affects the integrity of the blood–brain barrier by allowing the entry of CD4-dependent viruses or blood-borne toxins into the CNS. It will be important to determine whether HIV-1 strains can evolve to infect CD4-negative, CCR5-positive cells. In the case of SIV infection of macaques, CD4-independent viruses clearly do arise under the selection pressures which exist *in vivo* as SIV/17E-BR, B670 (clones 3 and 12), 62A, and 62B are primary isolates. Whether this CD4-independent phenotype is exhibited by SIV isolates in their natural, nonhuman primate hosts remains to be determined. Strikingly, none of the HIV-1 strains we have examined, including some neurotropic isolates, can utilize CCR5 independently of CD4. Given the small number of amino acid changes required to render a virus CD4-independent (there are nine changes between SIVmac239 and SIVmac316, for example), the existence of such HIV-1 virus strains would not be unexpected. However, if viruses are isolated by coculture with peripheral blood mononuclear cells or $CD4^+$ cell lines, CD4-independent viruses may not be fairly sampled because CD4-dependent viruses may possess a relative growth advantage under these conditions.

The observation that a large number of SIV primary isolates are capable of CD4-independent entry has implications beyond resolving issues associated with neurotropism. The CD4-independent viruses SIV/17E-Fr and SIVΔB670-Cl 12 are selectively transmitted transplacentally (29, 36). Whether CCR5 plays a role in this process is not known but will warrant further investigation given that this would have significant implications

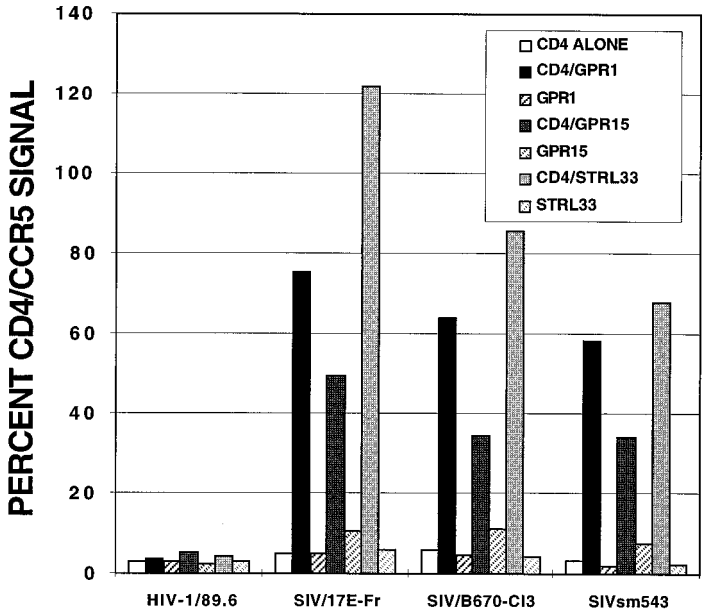

FIG. 3. CD4-dependent cell–cell fusion with the orphan receptors GPR1, GPR15, and STRL33. Effector cells expressing the indicated env proteins were mixed with cells expressing combinations of CD4 and GPR1, GPR15, or STRL33. The extent of cell–cell fusion was determined 8 h after cell mixing by measuring luciferase activity as in Fig. 2. Values represent the mean of two or three independent experiments.

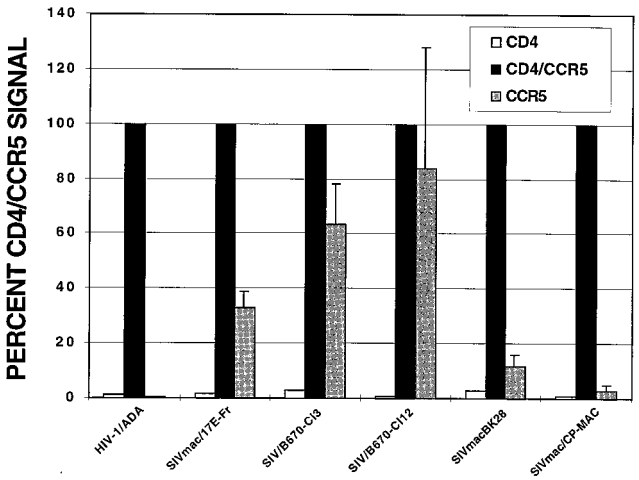

FIG. 4. CD4-independent virus infection. Luciferase reporter viruses were made bearing the envelope proteins of the listed viruses and used to infect 293T cells transfected with CD4 and CCR5 as indicated. Values were normalized by expressing as the percentage of the signal when CD4 and CCR5 are coexpressed and are the mean of three or four independent experiments with standard error bars shown.

for maternal–fetal virus transmission. In addition to the SIV strains reported here, two laboratory-adapted HIV-2 strains as well as feline immunodeficiency virus have been shown to use CXCR4 as a primary virus receptor in the absence of CD4 (16–18). Thus, the acquisition of CD4 as the primary HIV-1 receptor may have occurred evolutionarily quite late, and CCR5 may be the primordial receptor for primate lentiviruses.

In summary, we have shown that a number of pathogenic and virulent isolates of SIV are able to use the chemokine receptor CCR5 as a primary viral receptor. This property may have significant ramifications for understanding neurotropism, maternal–fetal transmission, and viral evolution and may also have implications in the evaluation of pathogenic chimeric simian–human immunodeficiency viruses and the use of the SIV system as a model for HIV-1 and the development of AIDS. Other significant differences exist with regard to how SIV and HIV-1 use chemokine receptors for virus infection. For example, CXCR4 is not used, or is used rarely, by SIV strains (11–15). Whether or not CD4-independent usage of CCR5 or other chemokine receptors represents an important part of the HIV life cycle *in vivo* will require further investigation, particularly of CNS-derived isolates which have not been passaged *in vitro*.

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