Macrophage Tropism of Human Immunodeficiency Virus Type 1 Isolates from Brain and Lymphoid Tissues Predicts Neurotropism Independent of Coreceptor Specificity

PAUL R. GORRY, GREG BRISTOL, JEROME A. ZACK, KIMBERLY RITOLA, RONALD SWANSTROM, CHRIS J. BIRCH, JEANNE E. BELL, NORBERT BANNERT, KEITH CRAWFORD, HUI WANG, DOMINIQUE SCHOLS, ERIK DE CLERCQ, KEVIN KUNSTMAN, STEVEN M. WOLINSKY, AND DANA GABUZDA*

Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Department of Pathology, Pediatrics, and Neurology, Harvard Medical School, and Center for Blood Research, Boston, Massachusetts; Division of Hematology-Oncology, UCLA School of Medicine and UCLA AIDS Institute, Los Angeles, California; University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; Victorian Infectious Diseases Reference Laboratory, Victoria, Australia; Department of Pathology, University of Edinburgh, Edinburgh, United Kingdom; Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; and Department of Medicine, Northwestern University Medical School, Chicago, Illinois

Received 27 February 2001/Accepted 18 July 2001

The viral determinants that underlie human immunodeficiency virus type 1 (HIV-1) neurotropism are unknown, due in part to limited studies on viruses isolated from brain. Previous studies suggest that brain-derived viruses are macrophage tropic (M-tropic) and principally use CCR5 for virus entry. To better understand HIV-1 neurotropism, we isolated primary viruses from autopsy brain, cerebral spinal fluid, blood, spleen, and lymph node samples from AIDS patients with dementia and HIV-1 encephalitis. Isolates were characterized to determine coreceptor usage and replication capacity in peripheral blood mononuclear cells (PBMC), monocyte-derived macrophages (MDM), and microglia. EnV1/V2 and V3 heteroduplex tracking assay and sequence analyses were performed to characterize distinct variants in viral quasispecies. Viruses isolated from brain, which consisted of variants that were distinct from those in lymphoid tissues, used CCR5 (R5), CXCR4 (X4), or both coreceptors (R5X4). Minor usage of CCR2b, CCR3, CCR8, and Apj was also observed. Primary brain and lymphoid isolates that replicated to high levels in MDM showed a similar capacity to replicate in microglia. Six of 11 R5 isolates that replicated efficiently in PBMC could not replicate in MDM or microglia due to a block in virus entry. CD4 overexpression in microglia transduced with retroviral vectors had no effect on the restricted replication of these virus strains. Furthermore, infection of transfected cells expressing different amounts of CD4 or CCR5 with M-tropic and non-M-tropic R5 isolates revealed a similar dependence on CD4 and CCR5 levels for entry, suggesting that the entry block was not due to low levels of either receptor. Studies using TAK-779 and AMD3100 showed that two highly M-tropic isolates entered microglia primarily via CXCR4. These results suggest that HIV-1 tropism for macrophages and microglia is restricted at the entry level by a mechanism independent of coreceptor specificity. These findings provide evidence that M-tropism rather than CCR5 usage predicts HIV-1 neurotropism.

Human immunodeficiency virus type 1 (HIV-1) infects macrophages and microglia in the central nervous system (CNS) and frequently causes dementia and other neurological disorders in AIDS patients (65, 87). CNS infection can cause HIV-1 encephalitis, which is characterized by reactive astrocytes, myelin pallor, microglial nodules, perivascular inflammation, multinucleated giant cells, and neuronal loss. Neuroinvasion by HIV-1 occurs through trafficking of infected monocytes and possibly lymphocytes across the blood-brain barrier (87). Infection of macrophages and microglia in the brain represent a significant cellular reservoir for long-term viral persistence (reviewed in references 83 and 93). Other tissues that harbor persistently infected macrophages include lung, lymph node, spleen, and bone marrow. Macrophages are less susceptible to the cytopathic effects of HIV-1 than CD4+ T cells (37, 38, 48, 70), so they may continue to shed virus for the duration of their normal life span. Most drugs used in highly active antiretroviral therapy have relatively poor CNS penetration (83, 97). Therefore, CNS infection is a major barrier to effective antiviral therapy.

The tropism of HIV-1 is determined by the interaction of the HIV-1 envelope glycoprotein with CD4 and a particular coreceptor. Macrophage-tropic (M-tropic) HIV-1 isolates primarily use CCR5 (R5) as a coreceptor (these are referred to as R5 viruses) (2, 12, 17, 26, 27), whereas T-cell line-tropic HIV-1 isolates use CXCR4 (X4) (33). Dualtropic viruses (R5X4) use both coreceptors. A subset of viruses can also use alternative coreceptors, including CCR3, CCR2b, CCR8, Apj, Strl33 (BONZO), Gpr1, Gpr15 (BOB), CX3CR1 (V28), ChemR23, and RDC1 (11–13, 18, 26, 29, 31, 50, 53, 64, 89, 90, 96), but the role of these coreceptors in vivo is unknown. In some patients, disease progression is associated with a general broadening of virus tropism by expansion of coreceptor usage (14). HIV-1 enters the CNS in the early stages of infection.
However, it is late in the course of disease progression, when X4 and R5X4 isolates emerge, that neurological symptoms such as dementia typically arise.

CCR5 is the major coreceptor for HIV-1 infection of macrophages and microglia (1, 36, 41, 42, 45, 95). Furthermore, previous studies suggest that CCR5 is the principal coreceptor used by HIV-1 isolated from brain (1, 12, 45, 62, 95, 101). Most laboratory-adapted X4 viruses, such as IIIB and NL4-3, do not replicate efficiently in macrophages and microglia (19, 45, 60, 81, 91, 103, 107). However, macrophages and microglia can support efficient replication by a subset of primary X4 viruses (46, 81, 98, 99, 105). CCR3 is expressed on microglia and may facilitate infection by certain HIV-1 strains (45). Apj, CCR8, Gpr15, and Strl33 can be used by some brain-derived viruses at low efficiency (1, 45, 95), but the role of these coreceptors in mediating infection of macrophages and microglia is unknown.

The genetic evolution of HIV-1 within the brain is distinct from that in lymphoid tissues and other organs (9, 24, 39, 51, 58, 94, 104, 106). Specific sequences within Env, particularly the V3 region, are associated with brain infection (51, 58, 80, 81, 107). However, primary HIV-1 isolates show preferential tropism for microglia compared to blood monocyte-derived macrophages (MDM) (103), other studies suggest that the tropisms of HIV-1 isolates for microglia and macrophages are similar (41, 46). Thus, specific determinants that underlie HIV-1 neurotropism remain unresolved.

Relatively few brain-derived HIV-1 isolates from neurologically well-characterized patients are available to study HIV-1 neurotropism (39, 40, 62, 63, 69, 79, 101). To better understand HIV-1 neurotropism, we isolated and characterized primary viruses from autopsy brain, cerebrospinal fluid (CSF), spinal cord, blood, spleen, and lymph node samples from AIDS patients with dementia and HIV-1 encephalitis. We found that CCR5 usage was neither necessary nor sufficient for M-tropism. However, M-tropism, irrespective of coreceptor usage, predicted the ability of primary HIV-1 strains from brain and other tissues to replicate in microglia. These findings suggest that M-tropism rather than CCR5 usage predicts HIV-1 neurotropism.

### MATERIALS AND METHODS

**Subjects.** Autopsy brain, CSF, peripheral blood, lymph node, or spleen samples were collected from 18 patients who died of AIDS and were stored at −80°C. Clinical characteristics (risk factor for acquiring HIV-1 infection, last CD4 cell count, use of antiretroviral therapies, and clinical history of dementia) of these patients are described in Table 1. None had evidence of a CNS opportunistic infection or neoplasm at autopsy except for patient CB3, who was diagnosed with primary CNS lymphoma. Sixteen patients were male and two (UK1 and UK3) were female. Patients MACS1 through MACS12 were participants in the Chicago component of the Multicenter AIDS Cohort Study (MACS). Tissue samples from patients UK1 through UK4 were obtained from the Edinburgh Brain Bank. Patients CB1 and CB3 were treated at Fairfield Hospital, Victoria, Australia. Brain tissue samples were obtained from the frontal lobe. Additional samples from basal ganglia were obtained for the UK patients.

**Cells.** Peripheral blood mononuclear cells (PBMC) were purified from blood of healthy HIV-1-negative donors by Ficoll-Hypaque density gradient centrifugation, stimulated with 2 µg of phytohemagglutinin (PHA) (Sigma, St. Louis, Mo.) per ml for 3 days, and cultured in RPMI 1640 medium supplemented with 10% (vol/vol) fetal bovine serum (Mediatech, Herndon, Va.), 100 µg of penicillin and streptomycin per ml, and 20 U of interleukin-2 (IL-2) (Boehringer, Mannheim, Germany) per ml. PD8* T cells were depleted by magnetic separation with anti-CD8-conjugated magnetic beads (Miltenyi Biotech, Auburn, Calif.). MDM were purified from PBMC by plastic adherence and cultured for 5 days in RPMI 1640 medium supplemented with 10% (vol/vol) human AB+ serum (Nabi, Boca Raton, Fla.), 100 µg of penicillin and streptomycin per ml, and 12.5 ng of macrophage colony-stimulating factor (M-CSF) per ml. Primary human fetal brain cultures which contain a mixture of astrocytes, neurons, and microglia were prepared as previously described (81) and cultured in Dulbecco modified Eagle medium supplemented with 10% (vol/vol) bovine calf serum (HyClone, Logan, Utah), 100 µg of penicillin and streptomycin per ml, 2 mM l-glutamine, 1 mM sodium pyruvate, and 5 µg of M-CSF per ml. The protocol for tissue procure-

### TABLE 1. Clinical history and neuropathology of study subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Risk factor</th>
<th>Last CD4 count (cells/µl)</th>
<th>Antiretroviral(s)</th>
<th>Clinical dementia</th>
<th>HIV-1 encephalitis</th>
<th>Giant cells</th>
<th>HIV-1 isolation from brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACS1</td>
<td>MH</td>
<td>2</td>
<td>None</td>
<td>Yes</td>
<td>Severe</td>
<td>++ + + +</td>
<td>+</td>
</tr>
<tr>
<td>MACS2</td>
<td>MH</td>
<td>52</td>
<td>AZT</td>
<td>Yes</td>
<td>Moderate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MACS3</td>
<td>MH</td>
<td>95</td>
<td>None</td>
<td>Yes</td>
<td>Moderate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MACS4</td>
<td>MH</td>
<td>8</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MACS5</td>
<td>MH</td>
<td>14</td>
<td>AZT</td>
<td>Yes</td>
<td>None</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MACS6</td>
<td>MH</td>
<td>3</td>
<td>Indinavir</td>
<td>Yes</td>
<td>Mild</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MACS7</td>
<td>MH</td>
<td>44</td>
<td>AZT</td>
<td>No</td>
<td>None</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MACS8</td>
<td>MH</td>
<td>36</td>
<td>NA*</td>
<td>No</td>
<td>None</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MACS9</td>
<td>MH</td>
<td>4</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MACS10</td>
<td>MH</td>
<td>70</td>
<td>None</td>
<td>No</td>
<td>None</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MACS11</td>
<td>MH</td>
<td>7</td>
<td>D4T*</td>
<td>No</td>
<td>None</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MACS12</td>
<td>MH</td>
<td>149</td>
<td>None</td>
<td>Yes</td>
<td>None</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>UK1</td>
<td>IVDU</td>
<td>87</td>
<td>ddi (1 mo)</td>
<td>Yes</td>
<td>Moderate</td>
<td>++ + + +</td>
<td>+</td>
</tr>
<tr>
<td>UK2</td>
<td>HET</td>
<td>297</td>
<td>None</td>
<td>No</td>
<td>Mild</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>UK3</td>
<td>IVDU</td>
<td>137</td>
<td>AZT, ddi (6 wk)</td>
<td>Yes</td>
<td>Severe</td>
<td>++ + + +</td>
<td>+</td>
</tr>
<tr>
<td>UK4</td>
<td>MH</td>
<td>8</td>
<td>D4T (1 mo)</td>
<td>No</td>
<td>Mild</td>
<td>++ + + +</td>
<td>+</td>
</tr>
<tr>
<td>CB1</td>
<td>MH</td>
<td>10</td>
<td>ddi (prior AZT)</td>
<td>Yes</td>
<td>Severe</td>
<td>NA -</td>
<td>+</td>
</tr>
<tr>
<td>CB3</td>
<td>MH</td>
<td>5</td>
<td>ddi (prior AZT and dDC)</td>
<td>Yes</td>
<td>Severe</td>
<td>NA -</td>
<td>+</td>
</tr>
</tbody>
</table>

* Tissue samples were collected at autopsy from 18 patients who died of AIDS. The presence of clinical dementia and HIV-1 encephalitis was determined by the clinical history and neuropathological examination at the time of autopsy. Brain tissue samples were collected from frontal lobe (MACS and CB patients), or frontal lobe and basal ganglia (UK patients).

* MH male homosexual; IVDU, intravenous drug user; HET, heterosexual transmission; mo, months; wk, weeks; N/A, not available.

* Frequency of multinucleated giant cells observed in autopsy brain tissue sections. −, −, ++, and ++ + +, none, occasional, moderate, and high, respectively.

* NA, not available.

* d4T, stavudine.

Downloaded from http://jvi.asm.org/ on November 10, 2017 by guest
Isolation of HIV-1. Autopsty brain and lymphoid tissue samples (approximately 2 to 4 mm3) were rinsed three times in 2 ml of RPMI 1640 medium containing 100 μg of penicillin and streptomycin per ml, and 0.7 mg of G418 (Gibco BRL, Gaithersburg, Md.) per ml.

Isolation of HIV-1. Antiprosty brain and lymphoid tissue samples (approximately 2 to 4 mm3) were rinsed three times in 2 ml of RPMI 1640 medium containing 100 μg of penicillin and streptomycin per ml, 0.7 mg of G418 (Gibco BRL, Gaithersburg, Md.) per ml. 

Reaction mixtures were incubated at 42°C for 2 min.
primer binds to the 5' region of the LTR and has been described previously (108). The fluorescent probe ZXF (6FAM 5'-TCTGGACCTGTGTAACCTGAGATCTCCTCAGACC-3' TAMRA), used at 200 nM, corresponds to nucleotides 584 to 616 in the JR-CSF LTR. The oligonucleotides used to detect full-length (LTR-gag) HIV-1 reverse transcripts included the forward primer SR1 and the reverse primer M661 (5'-CTCGGCTCGAGAGGCCTGCTGG-3') at 150 nM. This primer binds to sequences in the beginning of gag and has been previously described (108). The probe for this condition was identical to that used for early HIV-1 reverse transcripts. Thus, the combination of primers SR1 and AA55 amplifies sequences in R-U5 of the LTR and detects early events in reverse transcription. The combination of SR1 and M667 amplifies the LTR-gag junction, which is formed near the completion of the reverse transcription process. The oligonucleotides used to detect the human beta-globin gene were as follows. The forward primer BGF1 (5'-CAACCTCAAACAGACCATGG-3') was used at a concentration of 300 nM. This primer corresponds to nucleotides 846 to 866 in the human beta-globin gene. Reverse primer BGR1 (5'-TCACGTTCACCTGCCCC-3') was used at 150 nM. BGR1 corresponds to nucleotides 911 to 928 in the human beta-globin sequence. Together these primers amplify a band of 83 nucleotides in length. The fluorescent probe BGX1 (6FAM 5'-CTCTGAGGAGAAGTCTGCCGTTACTGCC-3' TAMRA) was used at 200 nM. Probe BGX1 corresponds to nucleotides 677 to 903 in the human beta-globin sequence. Oligonucleotides were purchased from Applied Biosystems and Annovis. All amplifications were performed in parallel with a set of known quantitative standards. The standard curve used to determine HIV DNA levels ranged from 10 to 20,000 copies of cloned HIV DNA (108). The standard curve used to determine levels of beta-globin gene sequences consisted of DNA derived from 10 to 100,000 normal human peripheral blood lymphocytes.

Quantitation of HIV-1 sequences was achieved by extrapolation from these standard curves.

Retroviral transduction of microglia. The envelope-deficient simian-human immunodeficiency virus (SHIV) vectors pSIVc1LenGFP and pSIVc1LenhucCD4, used to transduce microglia with green fluorescent protein (GFP) or human CD4, respectively, have been described previously (6, 49). Viruses pseudotyped using for an additional 48 h. Transduced microglia were added to wells of 48-well immunode...
V3 HTA analysis demonstrated similar (MACS1-br and MACS1-spln) or distinct (MACS3-br) major variants in PBMC- compared to MDM-derived isolates. A minor variant not detected in the MDM-derived MACS1-spln isolate was present in the PBMC-derived isolate, consistent with the V1/V2 heteroduplex patterns. The differences in V1/V2 and V3 heteroduplex formation between PBMC- and MDM-derived isolates were consistent with amino acid sequence changes detected in the same isolates (Fig. 2). These results demonstrate that coculture of brain or spleen tissue with CD8-depleted PBMC or MDM results in a cell-dependent selection of distinct viral variants.

**Coreceptor usage.** To determine whether primary HIV-1 brain isolates from patients with HIV-1 dementia exhibit particular patterns of chemokine coreceptor usage, viruses isolated by coculture with CD8-depleted PBMC were characterized for the ability to use CCR5, CXCR4, or alternative coreceptors for virus entry using Cf2-Luc cells (Fig. 3). The X4, R5, and R5X4 strains NL4-3, ADA, and 89.6, respectively, were used as positive controls. NL4-3 used CXCR4 and Apj; ADA used CCR3, CCR5, CCR8, CX3CR1, Strl33, Gpr15, Gpr1, and Apj; and 89.6 used CCR2b, CCR3, CCR5, CXCR4, and Apj as coreceptors for virus entry, as described in previous studies (11, 12, 17, 18, 26–29, 31, 64, 89). The use of Gpr1 by ADA was not detected in most of these studies but has occasionally been observed at low levels, particularly in fusion assays (28, 81). These differences may reflect the particular assay system used. Brain-derived HIV-1 isolates used CCR5 (four of six isolates), CXCR4 (one of six isolates), or both CXCR4 and CCR5 (one of six isolates) as principal coreceptors for virus entry. Minor usage of CCR2b, CCR3, CCR8, or Apj was demonstrated for two brain isolates. The pattern of coreceptor usage was similar between isolates from brain and other tissue compartments for three patients (CB3, MACS1, and MACS2) but was different for two patients (CB1 and MACS3). Thus, primary brain HIV-1 isolates from patients with dementia and HIV-1 encephalitis displayed diverse patterns of coreceptor usage and were not restricted to the use of CCR5 for virus entry.

We next determined whether the pattern of coreceptor usage differed between HIV-1 isolates recovered by coculture with CD8-depleted PBMC versus MDM as target cells (Table...
2). The levels of CCR5-mediated virus entry by the MACS1-br, MACS1-spln, and MACS3-br viruses isolated using MDM were lower than, equivalent to, or higher than, respectively, those by viruses isolated from the same tissues using CD8-depleted PBMC. In contrast, the levels of CXCR4-mediated virus entry by the MACS1-br and MACS1-spln viruses isolated using MDM were much lower than those by viruses isolated from the same tissues using PBMC. In contrast to viruses isolated using PBMC, usage of CCR3 (MACS3-br), CCR8 (MACS3-br), and Apj (MACS1-spln) was not detected for viruses isolated using MDM. These findings indicate that the pattern of coreceptor usage by viruses isolated using CD8-depleted PBMC was more diverse than that by viruses isolated using MDM. Furthermore, coculture with CD8-depleted PBMC allowed recovery of virus from more tissue samples. Therefore, viruses isolated by coculture with CD8-depleted PBMC were used for all subsequent experiments.

**Replication in PBMC.** We examined the capacity of primary brain HIV-1 isolates to replicate in PBMC compared to viruses isolated from CSF, spinal cord, or lymphoid tissue from the same patient (Fig. 4). The ADA, NL4-3, and 89.6 positive control viruses replicated to high levels. The brain isolates replicated to variable levels. Two of six (MACS1-br and CB1-br) reached peak levels of replication similar to those of the control viruses, three (MACS2-br, MACS3-br, and UK1-br) replicated at moderate levels, and one (CB3-br) replicated at lower levels.

**FIG. 2. V1/V2 and V3 amino acid sequence analysis.** The amino acid sequences were obtained from RT-PCR-amplified HIV-1 Env V1/V2 and V3 regions as described in Materials and Methods. V1/V2 and V3 alignments are compared to the clade B consensus sequence. Dots indicate residues identical to the clade B consensus, dashes indicate gaps, and X indicates uncertainty at the nucleotide level.
low levels. The viruses derived from CSF, spinal cord, and lymphoid tissue also replicated to variable levels. Three of eight viruses (CB1-CSF, CB1-PBMC, and MACS1-spln) replicated at high levels, four viruses (CB3-CSF, CB3-PBMC, the spinal cord isolate from patient CB3 [CB3-SC], and MACS2-LN) replicated at moderate levels, and one virus (MACS3-LN) replicated at low levels. Replication capacity in PBMC was similar between viruses isolated from brain and other tissue compartments for three patients (CB1, MACS1, and MACS2) but was different for two patients (MACS3 and CB3). The brain-derived isolate from patient MACS3 reached higher peak levels of replication than viruses isolated from lymph node, whereas the virus isolated from brain from patient CB3 replicated at lower levels than virus isolated from CSF, PBMC, or spinal cord. Thus, the replication capacity of brain-derived isolates in PBMC was diverse.
Replication in MDM. HIV-1 isolates from brain are typically M-tropic (36–38, 62, 63, 79). To determine the relationship between coreceptor usage and M-tropism, replication kinetics were analyzed in MDM (Fig. 5). The ADA strain, used as a positive control, replicated to high levels. Four of six viruses isolated from brain and five of eight viruses isolated from CSF or lymphoid tissue were replication competent in MDM. However, only 2 of 11 R5 viruses (MACS2-br and UK1-br) and 2 of 2 R5X4 viruses (MACS1-br and MACS1-spln) replicated to high levels. In contrast, nine R5 viruses replicated at low levels or showed no evidence of productive infection. The X4 CB1-br isolate also showed no evidence of productive infection. These results indicate that only a subset of primary R5 isolates that replicate in PBMC can productively infect MDM, suggesting that CCR5 usage is not sufficient for M-tropism.

Replication in microglia. To determine the relationship between coreceptor usage, M-tropism, and neurotropism, we analyzed replication kinetics in microglia (Fig. 6). The R5 ADA and BAL control viruses replicated at high levels, whereas the R5X4 89.6 virus replicated at moderate levels. The capacity of primary HIV-1 viruses to replicate in microglia was generally similar to that demonstrated in MDM (Table 3). However, three isolates (CB3-PBMC, MACS1-br, and MACS3-br) replicated at lower levels in microglia than in MDM. Two of 11 R5 isolates (MACS2-br and UK1-br) replicated to high levels, three (CB1-PBMC, CB3-CSF, and CB3-SC) replicated to moderate or low levels, and six (CB1-CSF, CB3-br, CB3-PBMC, MACS2-LN, MACS3-br, and MACS3-LN) showed no evidence of productive infection. Both R5X4 viruses (MACS1-br and MACS1-spln) replicated in microglia, but in contrast to replication in MDM, only MACS1-spln replicated to high levels. All three viruses that replicated to high levels in microglia (MACS1-spln, MACS2-br, and UK1-br) replicated to high levels in MDM. Surprisingly, two of these viruses (MACS2-br and UK1-br) replicated inefficiently in PBMC (Fig. 4 and Table 3). Together, these data indicate that M-tropism rather than CCR5 usage predicts the ability of HIV-1 isolates to replicate in microglia. Thus, CCR5 usage is not sufficient for microglia tropism.

Sensitivity to CCR5 and CXCR4 inhibitors in microglia. The sensitivity of primary M-tropic HIV-1 viruses to coreceptor-targeted inhibitors was tested in microglia by treating pri-

---

**TABLE 2. Coreceptor usage by HIV-1 viruses isolated using CD8-depleted PBMC versus MDM as target cells**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Target cells used for isolation</th>
<th>Coreceptor usagea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CCR2b</td>
</tr>
<tr>
<td>MACS1-br</td>
<td>MDM</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>MACS1-spln</td>
<td>MDM</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

a The pattern of coreceptor usage of viruses isolated using CD8-depleted PBMC or MDM as target cells was determined in C2-Luc cells, as described for Fig. 3. Control cells were transfected with CD4 alone. Entry levels were scored as +++ (>50,000 luciferase activity units), ++ (between 30,000 and 50,000 luciferase activity units), + (between 10,000 and 30,000 luciferase activity units), +/- (between 5,000 and 10,000 luciferase activity units), or – (<5,000 luciferase activity units).

---

**FIG. 4. Replication kinetics in PBMC.** PBMC were infected with equivalent amounts of each virus, as described in Materials and Methods, and cultured for 18 days. Virus production in culture supernatants was measured by RT assays. Values shown are means from duplicate infections. Error bars represent standard deviations. Results are representative of two independent experiments using cells obtained from different donors.
mary brain cultures with monoclonal antibodies against CCR5 or CXCR4 (2D7 and 12G5, respectively) or with small-molecule inhibitors of CCR5 or CXCR4 (TAK779 and AMD3100, respectively) (4, 25, 92) (Fig. 7). The R5 ADA, R5X4 89.6, and X4 SG3 (43) isolates were used as positive controls. 2D7 or TAK779 completely abolished infection of microglia by ADA and reduced infection by 89.6 by approximately 50%. 12G5 or AMD3100 completely abolished infection by SG3 and reduced infection by 89.6 by approximately 70%. Combinations of 2D7 and 12G5 or TAK779 and AMD3100 completely abolished infection by 89.6. 2D7 or TAK779 completely abolished infection by the R5 viruses MACS2-br and UK1-br but had no effect (MACS1-spln) or a minimal effect (MACS1-br) on the R5X4 viruses MACS1-br and MACS1-spln. In contrast, 12G5 or AMD3100 completely inhibited infection by MACS1-br and MACS1-spln but had no effect on the R5 viruses, indicating that these primary R5X4 viruses principally use CXCR4 for entry in microglia. Combinations of 2D7 and 12G5 or TAK779 and AMD3100 completely abolished infection by all primary M-tropic viruses tested, indicating that coreceptors other than CCR5 and CXCR4 are not used by these isolates for infection of microglia. The results further suggest that infection of microglia is highly sensitive to inhibition by coreceptor-targeted inhibitors.

Analysis of HIV-1 entry in macrophages by real-time PCR. To determine whether the inability of certain R5 isolates to replicate in MDM and microglia was due to a block at the entry or postentry level, real-time PCR was used to quantify HIV-1 DNA sequences that appear early and late in the reverse transcription process (Fig. 8). To detect early events in reverse transcription, primers that span the first region of the viral genome involved in reverse transcription, the R-U5 region of

FIG. 5. Replication kinetics in MDM. MDM were infected with equivalent amounts of each virus, as described in Materials and Methods, and cultured for 27 days. Virus production in culture supernatants was measured by RT assays. Results are representative of two independent experiments using cells obtained from different donors.

FIG. 6. Replication kinetics in microglia. Mixed brain cell cultures containing microglia were infected with equivalent amounts of each virus as described in Materials and Methods and cultured for 28 days. HIV-1 production in culture supernatants was measured by p24 antigen (Ag) ELISA (NEN). Data are represented as means from duplicate infections. Error bars represent standard deviations. Results are representative of three independent experiments using cells obtained from different donors.
the LTR, were used. To detect late stages of reverse transcription, primers that span the LTR-gag junction were used (see Materials and Methods). MDM infected with ADA or heat-inactivated ADA served as positive and negative controls, respectively. Viruses capable of productively infecting MDM produced high levels of fully reverse-transcribed viral DNA. In contrast, viruses that did not productively infect these cells showed no evidence of early or late reverse transcription products at 18 h postinfection.

To exclude the possibility that this block to reverse transcription was due to slow kinetics of the reverse transcription process in macrophage lineage cells (80), we similarly assessed the levels of viral DNA at 36 h postinfection. No additional DNA sequences in cells originally negative for viral DNA at 18 h

---

**TABLE 3. Summary of viral phenotypes**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Coreceptor usage b</th>
<th>PBMC</th>
<th>Macrophages</th>
<th>Microglia</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACS1-br</td>
<td>CXCR4, CCR5</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MACS1-spin</td>
<td>CXCR4, CCR5 (Apj)</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MACS2-br</td>
<td>CCR5</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>MACS2-LN</td>
<td>CCR5</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>MACS3-br</td>
<td>CCR5, (CCR3, CCR8)</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>MACS3-LN</td>
<td>CCR5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>UK1-br</td>
<td>CCR5</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>CB1-br</td>
<td>CXCR4, (CCR2b, Apj)</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CB1-CSF</td>
<td>CCR5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CB1-PBMC</td>
<td>CCR5</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>CB3-br</td>
<td>CCR5</td>
<td>+/−</td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td>CB3-CSF</td>
<td>CCR5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CB3-PBMC</td>
<td>CCR5</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>CB3-SC</td>
<td>CCR5</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
</tr>
</tbody>
</table>

a Viruses isolated by coculture with CD8-depleted PBMC were characterized for coreceptor usage and the ability to replicate in PBMC, MDM, and microglia.

b Minor usage of coreceptors by HIV-1 isolates, where entry levels were <10-fold above background, is shown in parentheses.

c For replication in PBMC, virus levels were scored as +++ (>25,000 cpm/ml), ++ (between 10,000 and 25,000 cpm/ml), + (between 5,000 and 10,000 cpm/ml), +/- (between 1000 and 5000 cpm/ml), or − (<1000 cpm/ml). For replication in MDM, virus levels were scored as +++ (>100,000 cpm/ml), ++ (between 50,000 and 100,000 cpm/ml), + (between 10,000 and 50,000 cpm/ml), +/- (between 1000 and 10,000 cpm/ml), or − (<5000 cpm/ml). For replication in microglia, virus levels were scored as +++ (>25 ng/ml p24), ++ (between 10 and 25 ng/ml p24), + (between 5 and 10 ng/ml p24), +/- (between 0.5 and 5 ng/ml p24), or − (<0.5 ng/ml p24).

---

**FIG. 7.** Inhibition of HIV-1 infection in microglia by CCR5 and CXCR4 inhibitors. Mixed brain cell cultures containing microglia were treated with monoclonal antibody 2D7 (10 μg/ml), 12G5 (10 μg/ml), or both or treated with TAK779 (100 nM), AMD3100 (1.2 μM), or both for 1 h prior to infection. Untreated cells contained no antibody or inhibitor. Cells were infected with neurotropic primary isolates MACS1-br (R5X4), MACS1-spin (R5X4), MACS2-br (R5), and UK1-br (R5) or with control viruses 89.6 (R5X4), SG3 (X4), and ADA (R5) individually in the presence of each antibody or inhibitor as described in Materials and Methods and cultured for 28 days. HIV-1 production in culture supernatants was measured by p24 antigen (Ag) ELISA (NEN). Data are represented as means from duplicate infections. Error bars represent standard deviations. Results are representative of two independent experiments using cells obtained from different donors. The in vitro coreceptor usage phenotype for each virus determined with transfected CI2-Luc cells (Fig. 3) is shown in parentheses.
were seen. Thus, there is a complete absence of reverse transcription products in these cells. Similar results were obtained in mixed brain cultures, but the levels of reverse transcription products were 10- to 20-fold lower than those in MDM due to the low percentage of microglia in these cultures (data not shown). These experiments demonstrate that a subset of primary R5 isolates (hereafter termed non-M-tropic R5 isolates) cannot replicate in MDM and microglia due to a block in virus entry or an early step prior to reverse transcription.

**Effect of CD4 and CCR5 levels on infection by primary R5 isolates.** The expression of CD4 on MDM and microglia is significantly lower than that on primary T cells (61, 82). Furthermore, at low concentrations of CD4, relatively high levels of CCR5 are required for entry by some primary R5 viruses (84). Therefore, we investigated whether M-tropic R5 viruses could utilize lower levels of CD4 and/or CCR5 than non-M-tropic R5 viruses. C8-Luc cells were transfected with different amounts of CD4- and CCR5-expressing plasmid, ranging from 0.05 to 10 μg, and analyzed by flow cytometry to measure the relative levels of cell surface expression of each receptor (Fig. 9A). A close relationship was found between the levels of CD4 and CCR5 expression and the amount of CD4- and CCR5-expressing plasmid used for transfection. However, this relationship was linear only for transfections of 0.05 to 5 μg of either plasmid. At low, medium, or high levels of CD4 (0.05, 0.5, and 5 μg of CD4-expressing plasmid, respectively), all R5 isolates except UK1-br utilized low, medium, or high levels of CCR5 (0.05, 0.5, and 5 μg of CCR5-expressing plasmid, respectively) with similar efficiency (Fig. 9B). UK1-br entered cells more efficiently than the other viruses, even in cells expressing low levels of CD4 or CCR5, suggesting that UK1-br has reduced dependence on CD4 and CCR5. These findings demonstrate a similar dependence on CD4 and CCR5 levels for entry between M-tropic (e.g., ADA and MACS2-br) and non-M-tropic (e.g., MACS2-LN, MACS3-br, and MACS3-LN) R5 viruses. Therefore, the entry block to infection of MDM and microglia by non-M-tropic R5 viruses is not due to an intrinsic inability to use low levels of CD4 or CCR5 for virus entry.

**Effect of CD4 overexpression on HIV-1 replication in microglia.** Recent studies have shown that overexpression of CD4 on rhesus macrophages can rescue productive infection by M-tropic HIV-1 or T-tropic simian immunodeficiency virus (SIV) strains (6, 73). To determine whether low levels of CD4 expression on microglia account for the entry block of non-M-tropic R5 viruses, microglia were transduced with the SHIV vector pSIvec1ΔenvCD4 to overexpress CD4 and then infected with HIV-1 isolates. To control for the effect of SHIV vector elements on HIV-1 replication, control cultures were similarly transduced with pSIvec1ΔenvGFP. Twenty-four percent of microglia were effectively transduced, as determined by GFP expression (Fig. 10A). Transduction of cells by CD4 resulted in a greater-than-2-fold increase in the number of CD4-positive cells and a 20-fold increase in the mean fluorescence intensity of CD4 (Fig. 10B). The levels of CCR5 expressed on untransduced and CD4-transduced microglia were similar (data not shown). CD4 overexpression was not able to restore virus infection by primary non-M-tropic R5 viruses (e.g., MACS2-LN, MACS3-br, MACS3-LN, and CB1-CSF) and did not enhance replication of M-tropic viruses (e.g., ADA and MACS1-spln) (Fig. 10C and D). Replication kinetics in GFP- and CD4-transduced microglia were similar to those in untransduced cells (data not shown). These results suggest that the inability of non-M-tropic R5 isolates to replicate in microglia is not due to insufficient cell surface expression of CD4.

**DISCUSSION**

In this study, we isolated and characterized primary viruses from CNS, peripheral blood, and lymphoid tissue from six AIDS patients with dementia and HIV-1 encephalitis. Brain-derived viruses, which consisted of variants that were distinct from those in lymphoid tissues, exhibited diverse patterns of coreceptor usage and used CCR5 (four of six isolates), CXCR4 (one of six isolates), or both CCR5 and CXCR4 (one of six isolates) as primary coreceptors for virus entry. Additional minor usage of CCR3, CCR2b, Apj, or CCR8 was demonstrated for two of six viruses isolated from brain. Previous studies have suggested that brain-derived HIV-1 viruses principally use CCR5 for virus entry (1, 41, 42, 45, 62, 95, 101). However, relatively few brain-derived viruses have been isolated and characterized. Our studies show that CXCR4 can
mediate efficient virus entry into microglia and suggest that CXCR4 usage by viruses in brain may be more prevalent than originally thought. Moreover, we demonstrate an association between the abilities of primary viruses to replicate in MDM and microglia irrespective of coreceptor usage. In other studies, we cloned full-length HIV-1 \textit{env} genes directly from brain and showed that a subset could use both CCR5 and CXCR4 for virus entry (A. Ohagen, A. Devitt, K. J. Kunstman, P. R. Gorry, P. Rose, B. Korber, J. Taylor, R. Levy, R. Murphy, S. Wolinsky, and D. Gabuzda, unpublished data). These findings suggest that M-tropism rather than CCR5 usage predicts HIV-1 neurotropism. Previous studies frequently defined HIV-1 viruses in the brain as M-tropic based on genetic analysis of gp120 Env sequences in the V3 region (20, 39, 51, 58, 85, 88). However, recent studies suggest that the V3 net charge does not predict coreceptor usage or M-tropism (62, 100; Ohagen et al., unpublished data). These findings indicate that determinants of M-tropism are more complex than coreceptor usage or predictions based on V3 sequences. When the full spectrum of determinants that underlie M-tropism is taken into account, M-tropism of HIV-1 or SIV may indeed predict neurotropism in vivo.

M-tropic HIV-1 viruses typically utilize CCR5 for entry in primary CD4$^+$ cells. However, our studies showed that CCR5 usage is neither necessary nor sufficient for M-tropism. We found that 6 of 11 primary R5 isolates could not replicate in MDM and microglia. Furthermore, inhibition of CXCR4 by 12G5 or AMD3100 abolished virus replication in microglia by the highly M-tropic R5X4 isolates MACS1-br and MACS1-spln, indicating that these isolates entered cells primarily via CXCR4. These findings are consistent with previous studies that failed to establish a strict correlation between CCR5 usage and M-tropism (10, 15, 21, 52, 55) and showed a lack of M-tropism by some R5 HIV-1 clones obtained from brain, lymph node, spleen, and lung tissue (22). Thus, the terms M-tropism and CCR5 usage cannot be used interchangeably.

We quantified early and late viral transcripts by real-time PCR and determined that non-M-tropic R5 isolates were unable to replicate in MDM and microglia due to a block in an early step prior to reverse transcription, presumably at the

FIG. 9. Effect of CD4 and CCR5 levels on infection by R5 viruses. (A) Cf2-Luc cells were transfected with 0.05, 0.5, 5, or 10 μg of CD4- or CCR5-expressing plasmid and analyzed for surface expression of CD4 or CCR5 by flow cytometry. The total amount of DNA in each transfection was adjusted to 10 μg with pCDNA3. (B) Cotransfected Cf2-Luc cells expressing low, medium, or high levels of CD4 (0.05, 0.5, and 5 μg of CD4-expressing plasmid, respectively) and low, medium, or high levels of CCR5 (0.05, 0.5, and 5 μg of CCR5-expressing plasmid, respectively) were infected with equivalent amounts of M-tropic (ADA, MACS2-br, and UK1-br) or non-M-tropic (MACS2-LN, MACS3-br, and MACS3-LN) R5 virus as described in Materials and Methods. Cell lysates were prepared at 48 h postinfection and assayed for luciferase activity. Data are expressed as means from duplicate infections. Error bars represent standard deviations. Similar results were obtained in two independent experiments.
level of virus entry. Entry and postentry restrictions to replication in MDM have been described for some R5 and X4 HIV-1 viruses. Several studies have demonstrated an entry block in MDM for some R5 viruses (34, 52, 62, 74, 75). Our findings suggest that a similar block to virus entry exists in microglia. Arthos et al. (3) showed that a subset of R5 viruses can enter MDM and synthesize early viral transcripts but are blocked at the postentry level due to an inability to induce signaling via CCR5. Postentry blocks have also been demonstrated for some laboratory-adapted X4 HIV-1 and SIV strains, possibly occurring during or after nuclear translocation of viral DNA (56, 91). In contrast to these studies, our findings suggest that non-M-tropic R5 isolates may be restricted by entry rather than postentry blocks. However, the possibility of a defect in early postpenetration steps prior to reverse transcription (i.e., uncoating) cannot be excluded, and further studies are required to determine whether the restriction is at the entry or postentry step.

Recent studies demonstrated that low levels of CD4 expressed on macrophages from rhesus macaques account for the lack of infection by M-tropic HIV-1 or T-tropic SIV strains (6, 73). Based on these and other studies (19, 60, 84), it has been proposed that low levels of CD4 expression restrict entry in MDM and microglia for some HIV-1 strains. However, we found that CD4 overexpression in microglia was not sufficient to rescue infection by non-M-tropic primary R5 isolates. Furthermore, M-tropic and non-M-tropic R5 viruses showed a similar dependence on CD4 and CCR5 levels for entry in transfected CI2-Luc cells. Interestingly, we found that UK1-br, a virus isolated from the brain of an intravenous drug user, showed an increased capacity to infect cells expressing low levels of CD4 or CCR5 compared to other primary isolates. Further studies are required to determine whether this results from higher affinity for these receptors, increased exposure of the CCR5 binding site, or yet another mechanism (23). The Env amino acid sequence of UK1-br revealed loss of a potential N-linked glycosylation site at asparagine 76 in the V1/V2 stem region, which corresponds to position 197 in HXB2 (Fig. 2). The elimination of a glycosylation site at this position is sufficient for CD4-independent infection by HIV-1 ADA (57).

FIG. 10. Effect of high CD4 expression on virus replication in microglia. Microglia were untreated or transduced with retroviral vectors to express GFP (A) or CD4 (B). Microglia expressing high levels of CD4 (D) or GFP as a control (C) were infected with equivalent amounts of each HIV-1 isolate as described in Materials and Methods and cultured for 28 days. Virus production in culture supernatants was measured by p24 antigen (Ag) ELISA (NEN). Results are representative of those from two independent experiments with cells obtained from different donors. Percentages represent the proportion of cells expressing GFP or CD4. MCF, mean cell fluorescence.
Studies to determine whether this amino acid change enhances CCR5 affinity and/or reduces CD4 dependence of UK1-br are in progress. Together, these results suggest that the inability of non-M-tropic R5 isolates to replicate in MDM and microglia is not due to insufficient levels of CD4 or CCR5 or to an intrinsic inability to interact efficiently with these receptors. The mechanisms that underlie the restriction to MDM and microglia infection remain to be determined, but possibilities include differences in CCR5 conformation (23, 47) and/or posttranslational modifications such as sulfation (32) or O-linked glycosylation (8, 32, 35) that exist between macrophages, resting T cells, and activated T cells. Other cell-specific factors (23), as well as viral factors such as Nef, could also influence M tropism (5, 72).

We demonstrated an association between the abilities of primary HIV-1 viruses to replicate in MDM and microglia. However, a few viruses replicated slightly (CB3-PBMC and MACS3-br) or moderately (MACS1-br) better in MDM than in microglia (Table 3). This discrepancy most likely reflects the greater numbers and high purity of MDM used as target cells compared to the microglia in mixed brain cell cultures. Other explanations include possible differences in native CD4 and/or coreceptor density or conformation (reference 23 and references therein). Our results are consistent with studies that demonstrated similar tropism of HIV-1 strains for replication in MDM and microglia (41, 46), but they contrast with a study that showed an increased ability of some viruses to replicate in microglia compared to MDM (103). One factor that may explain these discrepancies is the use of different cell culture systems. Our studies and those by Ghorpade et al. (41) used fetal microglia cultured under similar conditions with medium containing M-CSF, whereas Strizki et al. (103) used adult microglia cultured in medium containing giant cell tumor supernatant. Further studies that directly examine HIV-1 replication in fetal versus adult microglia using similar culture conditions may help to elucidate any intrinsic differences in susceptibility to HIV-1 infection that may exist between these two tissue sources. One brain isolate (CB1-br) that principally used CXCR4 for entry (Fig. 3) could not replicate in MDM or microglia (Fig. 5 and 6), thus bringing its neurotropism into question. This isolate might be a minor X4 variant that is not representative of the predominant viral quasispecies, possibly a blood contaminant.

We isolated virus from 6 of 18 autopsy brain tissue samples from patients with AIDS. All patients with positive isolations of virus from brain had dementia and were diagnosed with HIV-1 encephalitis at autopsy. In contrast, brain-derived viruses were not recovered from patients without HIV-1 encephalitis. These findings suggest that a higher local burden of virus in the brain (44) increased the chance of successful virus isolation. Another factor that probably enhanced the success of our virus isolations was the lack of antiviral therapies or use of single agents. Consistent with previous studies (37, 39, 69, 78), the frequency of successful virus isolation from brain was much lower than that of isolation from peripheral blood or lymphoid tissue. Presumably, this discrepancy is due to higher viral loads in peripheral blood and lymphoid compartments compared to brain (7, 24, 44). Sampling different regions of brain might increase the frequency of virus isolation and/or recovery of additional viral variants (101).

V1/V2 and V3 HTA and sequence analyses of viruses isolated from brain revealed major viral species that were distinct from those in viruses isolated from spleen or lymph node of the same patients. The V1/V2 probe was more sensitive than the V3 probe for detecting distinct viral variants, most likely due to greater variability of V1/V2 sequences compared to V3 sequences. Clustered mutations, deletions, or insertions are necessary for changes in heteroduplex mobility. The predicted V1/V2 and V3 amino acid sequences of brain and lymph node isolates from patient MACS3 were identical, despite a shift in mobility of the major V1/V2 heteroduplexes. The difference in V1/V2 heteroduplex mobility for these two isolates most likely resulted from several silent changes in the V1/V2 nucleotide sequences (GenBank accession numbers AF414888 to AF414912). These results demonstrate distinct viral variants in brain compared to lymphoid tissues from the same patients, consistent with previous studies (9, 24, 39, 51, 58, 94, 104, 106), and provide further evidence for tissue-specific compartmentalization of HIV-1.

We found a higher prevalence of brain-derived viruses that used CXCR4 as a coreceptor for entry than in previous studies (1, 37, 39, 95). One difference between our study and previous reports that may explain this finding is the use of CD8-depleted PBMC rather than MDM to isolate viruses. We used this method because it has been shown to be the most sensitive method for virus isolation from lymphocytes and monocytes (16, 59, 102). As expected, we found that coculture with CD8-depleted PBMC was more sensitive for isolation of HIV-1 from brain and lymphoid tissue samples than coculture with MDM. Chemokine receptor expression on PBMC is more heterogeneous than that on MDM. In addition, CD4 expression is higher on PBMC than on MDM (61, 82). Therefore, PBMC would be expected to be more permissive than MDM when used for isolation of primary HIV-1 viruses and thereby would allow the recovery of more diverse strains. Indeed, we found that compared to CD8-depleted PBMC, MDM exerted a selection bias that favored isolation of R5 HIV-1 strains and restricted recovery of variants that used CXCR4 or alternative coreceptors (Table 2). Consistent with this finding, V1/V2 and V3 HTA and sequence analysis of viruses isolated from the same tissues by both methods revealed a cell-dependent selection of distinct viral species.

Our studies suggest that M-tropism, irrespective of coreceptor usage, predicts HIV-1 neurotropism. Consistent with this model, a SHIV (chimeric SIV-HIV) variant that is neurotropic and neurovirulent in rhesus macaques in vivo contains the env gene from the T-cell line-tropic HIV-1 IIIB strain that was adapted for growth in MDM and uses only CXCR4 for virus entry (66). Furthermore, recent studies using SCID mice inoculated intracebrally with HIV-1-infected MDM demonstrated a relationship between neuropathological changes and the level of virus replication in MDM (A. Nukuna, H. E. Gendelman, J. Limoges, L. Poluektova, J. Rasmussen, A. Ghorpade, and Y. Persidsky, submitted for publication). Neurotropic HIV-1 or SIV isolates are not necessarily neurovirulent (54, 67, 68, 86). In vitro studies have shown that X4 viruses induce neuronal apoptosis more frequently than R5 viruses (81, 109, 110). Similarly, X4 viruses are generally more cytotoxic than R5 viruses for cells of the immune system. High-level replication of X4 viruses in MDM and microglia may
represent a pathogenic phenotype in the CNS that contributes to neurodegenerative mechanisms in HIV-1 dementia. Understanding the role of strain variability in HIV-1 neurotropism and neurovirulence may advance the development of therapeutics to inhibit CNS infection and prevent neurologic injury in AIDS patients.

ACKNOWLEDGMENTS

We thank H. Naif for assistance with HIV-1 isolation protocols; A. Mele for sequence analysis; A. Ohagen, J. Wang, M. Farzan, J. Sodroski, S. Gartner, and C. Wood for helpful discussions; and J. Brannan for management of the Edinburgh HIV Brain Bank. We are also grateful to J. Sodroski and B. Etemad-Moghadam for providing C2-Luc cells, J. Sodroski for providing SHIV vectors, J. Moore for providing TAK-779, and J. Sodroski, R. Doms, and S. Peiper for coreceptor plasmids.

This work was supported by NIH grants NS37277 and NS35734 to D.G., AI36059 and AI36554 to J.A.Z., AI44667 to R.S., and DA13127 to J.B. We also acknowledge support from the Multicenter AIDS Cohort Study (U01 AI35039), a NIDA supplement, and the G. Harold and Leila Mailer Charitable Foundation. Core facilities were supported by Center for AIDS Research grants (AI28691, AI28697, HD37260, and CA79488) and DFCI/Harvard Center for Cancer Research grants. The Edinburgh Brain Bank is supported by UKMRC SPG0708080. K.R. was supported in part by NIH training grant T32-AI07419. K.C. was supported in part by the Robert Wood Johnson Foundation. D.G. and J.A.Z. are Elizabeth Glaser Scientists supported by the Pediatric AIDS Foundation.

REFERENCES


