Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3526–3530, May 1987
Neurobiology

Human immunodeficiency virus can productively infect cultured human glial cells

(acquired immunodeficiency syndrome/host-range differences/CD4 antigen)

CECILIA CHENG-MAYER*†, JAMES T. RUTKA‡, MARK L. ROSENBLUM‡, THOMAS MCHUGH§, DANIEL P. STITES§, AND JAY A. LEVY*†

*Cancer Research Institute, Departments of †Medicine, ‡Neurological Surgery, and §Laboratory Medicine, University of California, School of Medicine, San Francisco, CA 94143

Communicated by Robert J. Huebner, February 9, 1987

ABSTRACT **Six isolates of the human immunodeficiency virus (HIV) showed differences in their ability to productively infect glioma-derived cell lines and early-passage human brain cell cultures. Susceptibility to HIV infection correlated well with the expression of the astrocyte marker glial fibrillary acidic protein. The CD4 molecule was expressed on some, but not all, of the brain-derived cells; however, no correlation was observed between CD4 protein expression and susceptibility to virus infection. The results show that HIV can productively infect human brain cells, particularly those of glial origin, and suggest that these cell types in the brain can harbor the virus.**

The disease acquired immunodeficiency syndrome (AIDS) is characterized by helper-T-cell depletion, depressed immune function, opportunistic infections, and neoplasms, particularly Kaposi sarcoma and B-cell lymphomas (1). Neurological syndromes, including subacute encephalitis and vacuolar degeneration of the spinal cord, have also been described in AIDS patients (2–4). The human immunodeficiency virus (HIV) has been isolated from patients with AIDS and shown to be associated with the disease (5–8). The detection of HIV DNA in the brain and the recovery of infectious virus from cerebrospinal fluid and brain tissues of patients with AIDS (9–11) have strongly suggested that HIV is also directly responsible for some of the neurological manifestations found in these individuals. To investigate the possible neurotropism of certain virus isolates and the cell type(s) in the brain susceptible to HIV infection, we attempted to infect brain-derived cell cultures with various HIV isolates. The results show that the AIDS retrovirus can productively infect glioma-derived cell lines and normal brain cell cultures, particularly those that express the astrocyte-specific marker glial fibrillary acidic protein (GFAP). Furthermore, the results suggest that a receptor other than the CD4 molecule may govern viral tropism in the brain.

MATERIALS AND METHODS

Cells and Cell Culture. The established cell lines of primary glial tumors either were derived at the Brain Tumor Research Center, University of California, San Francisco (SF210) or were a gift from J. Ponten, University of Uppsala, Sweden (U343MG, U343MGA, U251MG) (Table 1). The glial cell origin of all four cell lines has been well-documented (12–14). These cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (20%), glutamine (2 mM), penicillin (100 units/ml), and streptomycin (100 μg/ml).

The early-passage cell cultures were established from samples minced and treated with an enzyme mixture (0.02% DNase/0.05% Pronase/0.02% collagenase) for 30 min at 37°C and filtered through an 80-μm mesh. The filtered fluids were then centrifuged, and the cell pellets were resuspended in DMEM with 20% fetal bovine serum plus glutamine and antibiotics. SF609, SF611, and SF612 were derived from human fetal brain specimens; SF407 was obtained from a cerebellar glioblastoma in a child, and SF514P was derived from normal adult human leptomeninges (pia mater) (15).

Human peripheral blood mononuclear cells (PBMCs), obtained from healthy HIV-seronegative individuals, were prepared on Ficoll-Hypaque gradients as described (7) and propagated in RPMI-1640 medium containing 10% heat-inactivated (56°C, 30 min) fetal bovine serum and 10% interleukin 2 (Cellular Products, Buffalo, NY).

HIV Isolates. The viruses were chosen because of their origin and different host-range specificities for established human T-cell lines (ref. 16 and unpublished data). HIV_{SF2} (formerly known as the AIDS-associated retrovirus ARV-2) was recovered from the PBMCs of a homosexual man who presented with oral candidiasis and progressed rapidly into frank AIDS and died within 1 year. This virus has been molecularly cloned and sequenced (17). HIV_{SF117} was recovered from the PBMCs of a Kaposi sarcoma patient with a normal CD4/CD8 ratio. HIV_{SF128A} was derived by cocultivation of phytohemagglutinin (PHA)-stimulated normal human PBMCs with spinal cord tissue of a patient who died with symptoms of neurological disease; his spinal cord showed vacuolar myelopathy. HIV_{SF178} was derived from infection of normal PBMCs with the cerebrospinal fluid of a patient with neurologic findings. HIV_{SF161B} was obtained from the cerebrospinal fluid of a HIV-seropositive patient who did not show any neurologic symptoms. HIV_{SF301A} was recovered by cocultivation of PBMCs with tissues from the cerebral cortex of a patient who died of AIDS. This virus was in culture for only 12 days.

Virus Infection and Detection. For infection with HIV, 10^5 cells from each cell line were plated in 30-mm plastic tissue culture dishes 18 hr before infection. Cells were treated with DEAE-dextran (25 μg/ml) for 30 min at 37°C, washed, and then infected with 0.5 ml of virus-containing fluids obtained from infected human PBMCs (reverse transcriptase activity $> 10^6$ cpm/ml, assayed as in ref. 18). After 24 hr, the infected cells were washed three times and refed with fresh medium. Culture fluids of the infected cells were changed every 3–4 days, and when the cells reached confluence (3–4 days postinfection), they were removed from the culture dish with 0.25% trypsin and plated onto 60-mm dishes. Cocultivation

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AIDS, acquired immunodeficiency syndrome; GFAP, glial fibrillary acidic protein; HIV, human immunodeficiency virus; IFA, indirect immunofluorescence assay; PBMC, peripheral blood mononuclear cell.

with normal PBMCs and measurement of HIV replication was carried out as described in the legend of Fig. 2. The reverse transcriptase activity (18) reflects the amount of replicating virus in the culture supernatants of the cocultivated PBMCs. The results were graded + and ± for reverse transcriptase activities >50,000 cpm/ml and 10,000–30,000 cpm/ml above background (<1000 cpm/ml), respectively. Presence of HIV was confirmed in the PBMCs by an indirect immunofluorescence assay (IFA) using a positive anti-HIV antiserum (7).

Detection of the CD4 Protein. The presence of surface and intracellular CD4 protein was examined by IFA. For detection of surface CD4, suspensions of cells were prepared by treating the monolayer cells with phosphate-buffered saline containing 2% EDTA. The cells were incubated for 30 min at 37°C with monoclonal antibody against CD4 (anti-Leu-3a+3b; Becton Dickinson) or isotype-matched mouse monoclonal anti-keyhole limpet hemocyanin (KLH) as a control for nonspecific staining. The cells were washed and then incubated with biotinylated goat $F(ab')_2$ anti-mouse IgG (Tago, Burlingame, CA) followed by streptavidin conjugated with phycoerythrin (Becton Dickinson) (19). The cells were fixed in 1% paraformaldehyde and analyzed on a fluorescence-activated cell sorter.

For detection of intracellular CD4 antigen, cells were fixed on slides with acetone and treated with anti-Leu-3a+b or mouse anti-KLH for 30 min at 37°C. Slides were then washed, treated with fluorescein-isothiocyanate-conjugated goat $F(ab')_2$ anti-mouse IgG and examined by microscopy. The HUT78 cell line (CD4-positive) (16) was used as a control.

RESULTS

Derivation and Characterization of Glioma-Derived Cell Lines and Early-Passage Brain Cells. The relevant antigenic expression(s) in the four glioma-derived cell lines used are summarized in Table 1. GFAP is a 50-kDa intracytoplasmic filamentous protein that is structurally homologous to other intermediate filament proteins. First isolated from white-matter plaques of patients with long-standing multiple sclerosis (20, 21), GFAP is a specific marker for astrocytes, a subclass of glial cells (Fig. 1) (15). Two of the established lines, U343MGA and U251MG, expressed high levels of GFAP as detected by IFA (Fig. 1, Table 1). Neither SF210 nor U343MG (parental line of U343MGA) had the GFAP antigen. All the cell lines were negative when immunostained for factor VIII-related antigen (RAg, marker for endothelial cells) or procollagen III, (marker for fibroblasts) (15). Macrophage-specific antigens (Leu-M3) and neurofilament peptides characteristic of neurons were also not present on the cells.

Table 1. Immunocytochemical characterization of glioma-derived cell lines and their susceptibility to HIV

	SF210	U343MG	U343MGA	U251MG
	Antigen expression			
GFAP	−	−	100%	90%
Factor VIII RAg	−	−	−	−
Procollagen III	−	−	−	−
Surface CD4	−	−	−	−
Cytoplasmic CD4	−	−	−	−
	Virus replication			
HIV_{SF2}	−	−	−	+
HIV_{SF117}	−	−	+	±
HIV_{SF128A}	−	−	±	+
HIV_{SF178}	−	+	−	−
HIV_{SF161B}	−	−	−	−
HIV_{SF301A}	−	−	+	−

The established glioma-derived cell lines were examined by IFA for GFAP, factor VIII-related antigen (RAg, marker for endothelial cells), and procollagen type III (a fibroblast marker) as described (15); − indicates absence of markers, percentages indicate percent positive cells. For detection of virus replication, cells were infected with different HIV isolates and virus production in PBMCs cocultivated with infected cells was measured by the presence of particle-associated reverse transcriptase activity in the culture supernatant (18). +, reverse transcriptase activity >50,000 cpm/ml; ±, 10,000–30,000 cpm/ml; −, <5,000 cpm above background (1000 cpm/ml).

The early-passage human brain cell cultures, although highly enriched in GFAP-positive cells, were heterogeneous. They contained some GFAP-negative and procollagen III-positive cells, but no endothelial cells were detected (Table 2). Furthermore, in electron microscopic analysis of these cell cultures, only 0.1% of the cells could be identified as macrophages and 0.5–1% as neuronal cells.

Infection of Glioma-Derived Cell Lines. All the glioma-derived cell lines except SF210 could be infected by at least one of the six HIV isolates (Table 1). GFAP expression correlated well with susceptibility to HIV replication. For example, U343MGA and U251MG, with a high percentage of GFAP-positive cells (100% and 90%, respectively; Table 1), could be infected by three HIV isolates. U343MG, which is GFAP-negative, was infected by only one isolate (HIV_{SF178}). The titer of virus produced by most of the infected cell lines was very low. Detection of infectious virus progeny required cocultivation with normal human PBMCs. A rapid increase in reverse transcriptase activity in the culture supernatants was then noted (Fig. 2), and expression of HIV antigens in the PBMCs was detected by IFA (7). On occasion, the U343MG line infected with HIV_{SF178} produced high-titered virus without the addition of PBMCs (reverse transcriptase activity >10,000 cpm/ml of culture supernatant).

We concluded that the viruses recovered resulted from productive infection of the glioma-derived cells and not from an infection of normal PBMCs by residual input virus for the following reasons. (*i*) Before cocultivation, infected cultures were passaged twice in the presence of trypsin, which inactivates retroviruses (22). (*ii*) In most experiments, infected glial cultures were cocultivated with PBMCs at 7–10 days and at 11–14 days postinfection with the same results. The amount of residual infectious virus expected to be present even in unpassaged cultures at 11 days postinfection is negligible (unpublished observation). (*iii*) Two HIV_{SF128A} isolates established in culture at different times were used to infect the glial cultures. Both isolates replicated in the same cell lines, demonstrating the specificity of their infection. (*iv*) Different HIV isolates showed different host-range specificities.

Infection of Early-Passage Brain Cell Cultures. To demonstrate that the susceptibility of these human glioma-derived cells to HIV infection is not a unique property of the established cell lines, attempts were made to infect early-passage human brain cell cultures (passages 3–8) with the various virus isolates. Infection of these cells with the six HIV isolates showed that the cultures with GFAP staining replicated at least one HIV isolate (Table 2). Only SF514P, which lacked GFAP expression, was not susceptible to infection by any of the virus isolates tested.

Each HIV isolate showed a different pattern of infectivity in these human brain-derived cell cultures, as was noted in the human glioma-derived cell lines (Tables 1 and 2). HIV_{SF2} displayed the widest host range; it productively infected all but one (SF514P) of the early-passage cell cultures. However, it infected only one established line, U251MG. The HIV_{SF117} and HIV_{SF128A} isolates showed similar host-range patterns. They infected two of the early-passage cell cultures, SF407 and SF609 (Table 2), and the two GFAP-positive

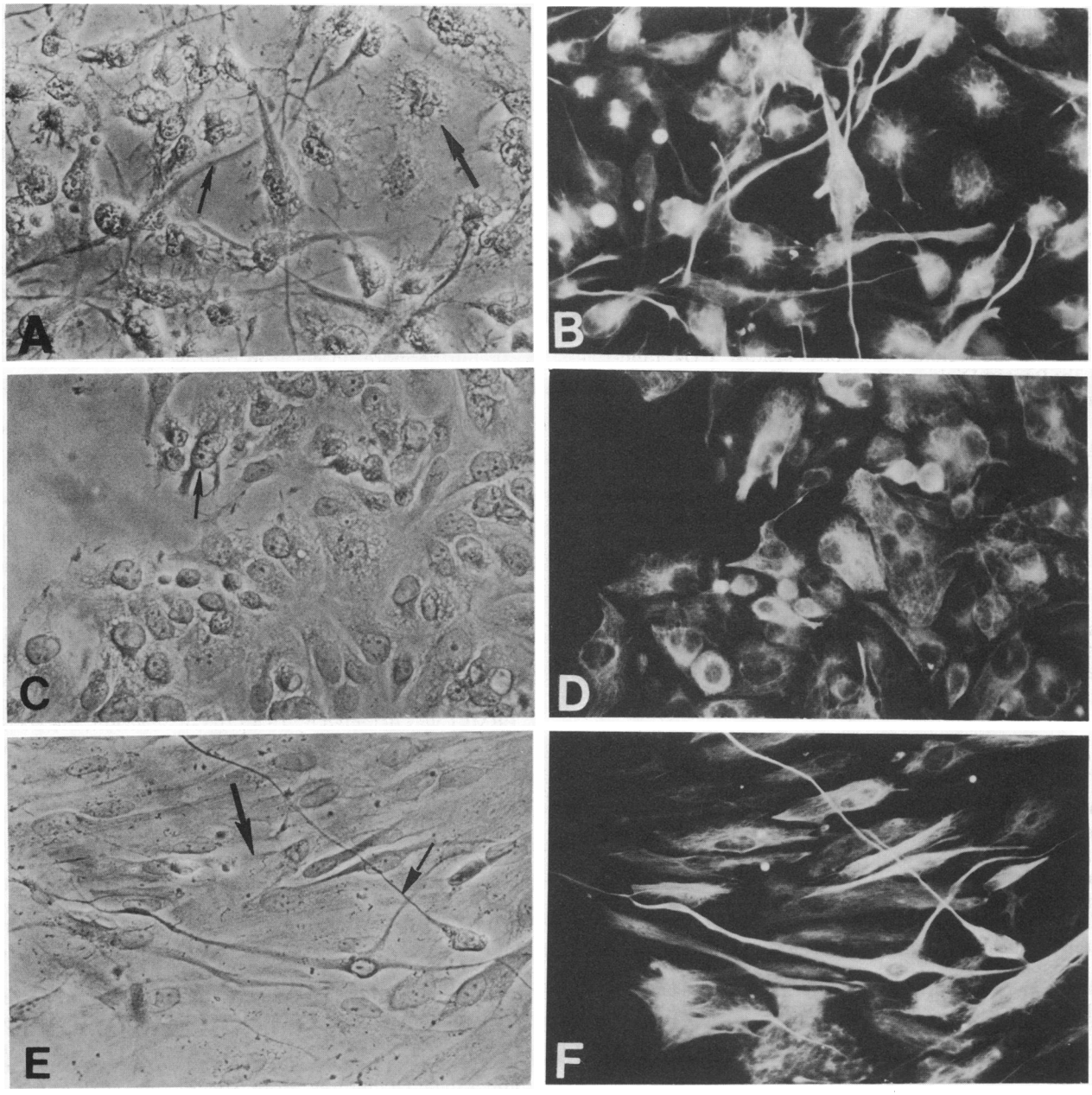

FIG. 1. Phase-contrast microscopy (*A*, *C*, and *E*) and IFA (*B*, *D*, and *F*) for GFAP in the human brain-derived cell cultures. IFA used rabbit antiserum to human GFAP (13) provided by L. F. Eng (Stanford, CA). Its specificity for GFAP was confirmed by immunoblot analysis. (*A*) U251MG malignant glioma cell line demonstrating heterogeneity in tumor-cell phenotype. Some cells have long, tapering cytoplasmic process (small arrow), while others are polygonal with an increased cytoplasmic/nuclear ratio (large arrow). (*B*) All U251MG cells show high intensity of staining for GFAP. (*C*) U343MGA malignant glioma cell line. The tumor cells are more uniform in size and shape than are the U251MG tumor cells. The cells are largely polygonal in shape and demonstrate marked crowding. A binucleate cell is seen (arrow). Cytoplasmic extensions are not seen. (*D*) By IFA, all the U343MGA cells demonstrate a moderate intensity of staining for GFAP. (*E*) SF407 cerebellar glioblastoma early-passage cell culture. A variety of different cell types is seen. Some cells demonstrate marked cytoplasmic process formation (small arrow), whereas others are broadly bipolar (large arrow). (*F*) Not all SF407 cells are GFAP-positive, and the intensity of staining varies from cell to cell. (×170.)

glioma-derived cell lines, U343MGA and U251MG (Table 1). Nevertheless, in these lines, each showed a reproducible difference in their ability to replicate. HIV_{SF178} replicated in SF612 and was the only isolate that productively infected the U343MG line. Finally, HIV_{SF301A} infected SF612 and U343MGA, but HIV_{SF161B}, obtained from the cerebrospinal fluid of a seropositive patient, was unable to infect any of the cultured brain cells.

Expression of the CD4 Molecule. Based on observations with human hematopoietic cells (23–25), the sensitivity of the brain-derived cells to HIV infection could be associated with the presence of the CD4-complex protein on the cell surface. We, therefore, examined the expression of the CD4 antigen by use of monoclonal antibodies (19). The results showed that none of the established lines (Table 1) and only two early-passage cell cultures, SF609 and SF611, expressed detectable amounts of the CD4 antigen on their surface; 15% and 17% of the cells were positive in these cultures, respectively (Table 2). Cytoplasmic staining of CD4 was detected only in SF609 (Table 2). This cell culture was susceptible to infection by three HIV isolates (HIV_{SF2}, HIV_{SF128A}, and HIV_{SF117}); SF611 replicated only HIV_{SF2}.

Table 2. Immunocytochemical characterization of early-passage glioma- and normal brain-derived cells and their susceptibility to HIV

	SF407	SF514P	SF609	SF611	SF612
	Antigen expression				
GFAP	++	−	+	++	++
Factor VIII RAg	−	−	−	−	−
Procollagen III	±	+++	+	±	±
Surface CD4	−	−	15%	17%	−
Cytoplasmic CD4	−	−	±	−	−
	Virus replication				
HIV_{SF2}	+	−	+	+	+
HIV_{SF117}	+	−	+	−	−
HIV_{SF128A}	±	−	+	−	−
HIV_{SF178}	−	−	−	−	+
HIV_{SF161B}	−	−	−	−	−
HIV_{SF301A}	−	−	−	−	+

The characterization and infection of the early-passage cell cultures (passages 3–8) were carried out as described (Table 1, Figs. 1 and 2). The intensity of staining by IFA for GFAP, factor VIII-related antigen (RAg), and procollagen type III is expressed as follows: −, negative; +, minimal; ++, moderate; +++, marked. Virus replication was measured as described in the legend to Table 1.

DISCUSSION

These studies show that HIV can directly infect human glioma and normal brain-derived cells, in addition to their known ability to infect human hematopoietic cells (16). The fact that HIV_{SF301A}, an isolate that had been maintained in culture for only 12 days, infected the brain-derived cells indicates that HIV tropism for these cells is not a result of long-term growth of the virus in tissue culture. The results suggest that astrocytes (detected by GFAP staining) may be a subclass of glial cells particularly susceptible to productive HIV infection (Tables 1 and 2). However, low levels of HIV infection of other cell types (e.g., oligodendrocytes or neuronal cells) present in the early-passage brain cells cannot be excluded. The cell types in the brain that support HIV infection *in vivo* have been identified by others to be predominantly endothelial cells and cells of monocytic lineage (26, 27). Our results of HIV infection of glial cells *in vitro*—particularly of astrocytes—suggest that these cells may also be infected in the brain but below the detection limits of the immunocytochemistry and *in vivo* hybridization techniques used in the other studies (26, 27). HIV infection of cells with an astrocyte marker further links the virus to immune cells in the body. The astrocyte is the presumed antigen-presenting cell in the brain, corresponding in part to circulating macrophages (28, 29).

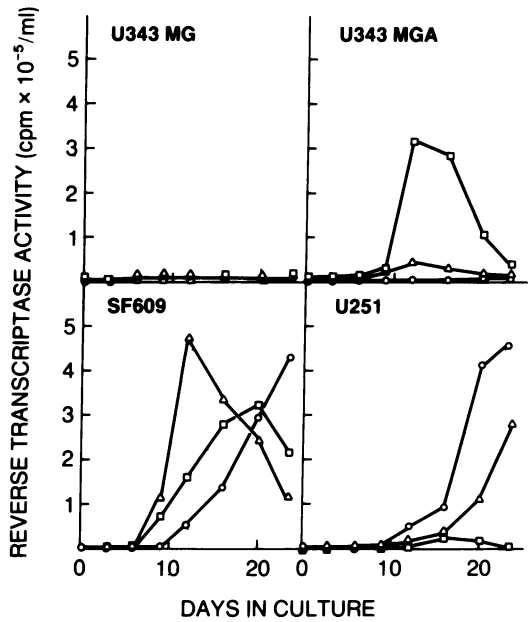

FIG. 2. Kinetics of reverse transcriptase activity in culture supernatant of PBMCs cocultivated with infected human brain-derived cells. Mitogen-stimulated normal human PBMCs (3×10^6 cells per culture) were added to the brain-derived cells 7–10 days after infection with HIV_{SF2} (○), HIV_{SF117} (□), or HIV_{SF128A} (△). After 3–5 days of cocultivation, the PBMCs were removed and maintained in separate cultures. The supernatants of these cultures were assayed for reverse transcriptase activity every 3–4 days (18), and the cells were examined for HIV antigens by IFA (7).

One established cell line, U343MG, which lacked GFAP expression, replicated HIV_{SF178}; moreover, it was the only line in which virus production was sufficient to be directly detected in the cell culture fluid. The cell type of this particular line is difficult to prove conclusively by present characterization techniques, but it is most likely of glial type. U343MG is the parental line of U343MGA, and an inverse correlation exists between anaplasia and GFAP expression (21, 30). Therefore, U343MG may represent astrocytoma cells that have become markedly anaplastic and have lost the ability to synthesize GFAP in culture.

What receptor governs HIV infection of glial and other brain-derived cells is not known. Our results show that some cultured human brain cells can express the helper/inducer CD4 protein. Moreover, CD4 mRNA has been detected in one out of three established cell lines examined (U343MG but not U251MG or U343MGA; D. Littman, personal communication). These findings may explain the susceptibility of some but not all brain cells to HIV infection. Most of the susceptible lines had no detectable CD4 antigen on their cell surface, and our assay has been optimized for detection of low levels of antigen (as few as 1000 molecules of fluorochrome per cell) (19). Furthermore, the difference in tropism displayed by the individual HIV isolates for the glioma and normal brain-derived cell cultures resembles results obtained with infection of established lines of human T cells; there was a lack of correlation of susceptibility to HIV replication with CD4 protein expression (16, 31). Therefore, we favor the conclusion that the CD4 molecule is not the only surface receptor(s) responsible for HIV infection of cells, particularly from the brain. The reason why some HIV isolates replicate in one cell line and not in others is not clear. Whether a virus-coded gene product governs the host-range specificity of HIV or whether intracellular regulation exists remains to be determined. Finally, the observation that HIV isolates, whether isolated from brain tissues or PBMCs, can infect the brain-derived cell cultures suggests that a single subtype of the virus that is only neurotropic is unlikely.

We thank Mary Whalen and Harold Legg for technical assistance and Mary Runyan for preparation of the manuscript. Funds for this research were provided by grants from the State of California Universitywide Task Force on AIDS, the National Cancer Institute (CA31882), and the Preuss Foundation. C.C.-M. was supported by National Institutes of Health Training Grant CA09043; J.T.R. was supported by the Medical Research Council of Canada.

1. Reichert, C. M., O'Leary, T. J., Levens, D. L., Simrell, C. R. & Marcher, A. M. (1983) *Am. J. Pathol.* **112**, 357–382.
2. Nielsen, S., Petito, C. K., Urmacher, C. D. & Posner, J. B. (1984) *Am. J. Clin. Pathol.* **82**, 678–682.
3. Petito, C. K., Navia, B. A., Cho, E.-S., Jordan, B. D., George, D. C. & Price, R. N. (1985) *N. Engl. J. Med.* **312**, 874–879.
4. Levy, R. M., Bredesen, D. E. & Rosenblum, M. L. (1985) *J. Neurosurg.* **62**, 475–495.
5. Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre,

M. T., Chamaret, S., Gruest, J., Dauget, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1985) *Science* **220**, 868–871.
6. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. & Markham, P. D. (1984) *Science* **224**, 500–503.
7. Levy, J. A., Hoffman, A. D., Kramer, S. M., Landis, J. A., Shimabukuro, J. M. & Oshiro, L. S. (1984) *Science* **225**, 840–842.
8. Coffin, J., Haase, A., Levy, J. A., Montagnier, L., Oroszlan, S., Teich, N., Temin, H., Toyashima, K., Varmus, H., Bogt, P. & Weiss, R. (1986) *Science* **232**, 697.
9. Shaw, G. M., Harper, M. E., Hahn, B. H., Epstein, L. G., Gadusek, C. D., Price, R. W., Navia, B. A., Petito, C. K., O'Hara, C. J., Cho, E.-S., Oleske, J. M., Wong-Staal, F. & Gallo, R. C. (1985) *Science* **227**, 177–182.
10. Levy, J. A., Shimabukuro, J., Hollander, H., Mills, J. & Kaminsky, L. (1985) *Lancet* **ii**, 586–588.
11. Ho, D. D., Rota, T. R., Schooley, R. T., Kaplan, J. C., Allan, J. D., Groopman, J. E., Resnick, L., Felsenstein, D., Andrews, C. A. & Hirsch, M. D. (1985) *N. Engl. J. Med.* **313**, 1493–1497.
12. Bigner, D. D., Digner, S. H., Ponten, J., Westermark, B., Mahaley, M. S., Ruoslahti, E., Herschman, H., Eng, L. F. & Wiksstrand, J. (1981) *J. Neuropathol. Exp. Neurol.* **40**, 201–229.
13. Carlsson, J. (1983) *Int. J. Cancer* **31**, 523–533.
14. Rutka, J. T., Giblin, J. R., Dougherty, D. V., Liu, H. S., McCulloch, J. R., Bell, C. W., Stern, R. S., Wilson, C. B. & Rosenblum, M. L. (1987) *Acta Neuropathol.*, in press.
15. Rutka, J. T., Giblin, J., Dougherty, D. V., McCulloch, J. R., DeArmond, S. J. & Rosenblum, M. L. (1986) *J. Neuropathol. Exp. Neurol.* **45**, 285–303.
16. Levy, J. A., Shimabukuro, J., McHugh, T., Casavant, C., Stites, D. & Oshiro, L. (1985) *Virology* **147**, 441–448.
17. Sanchez-Pescador, R., Power, M. D., Barr, P. J., Steimer, K. S., Stempten, M. M., Brown-Shimer, S. L., Gee, W. W., Renard, A., Randolph, A., Levy, J. A., Dina, D. & Luciw, P. A. (1985) *Science* **227**, 484–492.
18. Hoffman, A. D., Banapour, B. & Levy, J. A. (1985) *Virology* **147**, 326–335.
19. Vernon, T. O., Glazer, A. N. & Stryer, L. (1982) *J. Cell Biol.* **93**, 981–986.
20. Eng, L. F., Vanderhaegan, J. J., Bignami, A. & Gerostl, B. (1971) *Brain Res.* **23**, 351–354.
21. Deck, J. H. N., Eng, L. F., Bigbee, J. & Woodcock, S. M. (1978) *Acta Neuropathol.* **42**, 183–190.
22. Levy, J. A. & Rowe, W. P. (1971) *Virology* **45**, 844–847.
23. Dalgleish, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. H. (1984) *Nature (London)* **312**, 763–767.
24. Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercent, T., Gluckman, J.-C. & Montagnier, L. (1984) *Nature (London)* **312**, 767–768.
25. McDougal, J. S., Kennedy, M. S., Sligh, J. M., Cort, S. P., Mawle, A. & Nicolson, J. K. A. (1986) *Science* **231**, 382–385.
26. Wiley, C., Shrier, R. D., Nelson, J. A., Lampert, P. W. & Oldstone, M. B. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7089–7093.
27. Koenig, S., Gendelman, H. E., Orenstein, J. M., Dal Canto, M. C., Pezeskpour, G. H., Youngbluth, M., Janotta, F., Aksamit, A., Martin, M. A. & Fauci, A. S. (1986) *Science* **233**, 1089–1093.
28. Fontana, A., Fierz, W. & Wekerle, H. (1984) *Nature (London)* **307**, 273–276.
29. Massa, P. T., Dorries, R. & ter Meulen, V. (1986) *Nature (London)* **320**, 543–546.
30. Jacques, C. M., Kujas, M. & Poreau, A. (1979) *J. Natl. Cancer Inst.* **62**, 479–483.
31. Kikukawa, R., Koyanaji, Y., Harada, S., Kobayachi, N., Hatanaha, H. & Yamamoto, N. (1986) *J. Virol.* **57**, 1159–1162.