Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8575–8579, November 1989
Neurobiology

Isolates of human immunodeficiency virus type 1 from the brain may constitute a special group of the AIDS virus

(central nervous system infection/glioma/macrophage)

CECILIA CHENG-MAYER, CAROL WEISS, DEBORAH SETO, AND JAY A. LEVY

Department of Medicine, Cancer Research Institute, University of California School of Medicine, San Francisco, CA 94143-0128

Communicated by Maurice R. Hilleman, August 2, 1989

ABSTRACT **The biologic, serologic, and molecular properties of isolates of human immunodeficiency virus type 1 (HIV-1) from the central nervous system (CNS) were determined and compared to those of isolates from peripheral blood and lymph nodes. Among these were pairs of CNS and blood isolates obtained from six infected individuals. The data show that HIV-1 isolates from the CNS can be distinguished from peripheral blood isolates by their (*i*) relative inability to infect established T-cell lines, (*ii*) reduced cytopathogenicity, (*iii*) inability to modulate CD4 antigen expression on infected cells, (*iv*) efficient replication in peripheral blood macrophages, and (*v*) insensitivity to serum neutralization. Paired CNS and peripheral blood isolates from the same individual also display some differences in cellular tropism. The blood isolates replicate better in T-cell lines and glioma cell lines, whereas the paired CNS isolates replicate more efficiently in primary macrophages. These results suggest that viruses isolated from the CNS of infected individuals may represent a specific HIV-1 subgroup.**

Neurological syndromes have been described in many patients with the acquired immunodeficiency syndrome (AIDS) (1–3). These include dementia, subacute encephalitis, and vacuolar degeneration of the spinal cord. The isolation of infectious human immunodeficiency virus type 1 (HIV-1) from brain tissues and cerebrospinal fluid (CSF) of patients with the disease (4, 5) and the detection of the HIV-1 genome in the brain (6) strongly suggest that this virus is directly responsible for some of the neurological disorders found in patients with AIDS. The major cell type in the brain that shows infection by HIV-1 is the macrophage (7, 8), although other cell types such as endothelial cells and glial cells have also been reported to be infected (8–11). This apparent "neurotropism" of HIV-1 is not surprising, since infection by other members of the lentivirus family (e.g., visna virus) also leads to neurologic disorders. However, these observations raise important questions as to whether isolates from the brain of individuals with neurologic disease constitute a specific subgroup of HIV.

Our laboratory has been able to distinguish HIV-1 strains by their ability to infect a variety of different human cells, their capacity to replicate to high titers in these cells, and their ability to induce cytopathic effects that correlate with their induction of plaques in the MT-4 cell line (10, 12, 13). Moreover, differences in their susceptibilities to serum neutralization and in the sensitivities of their proviral DNA to restriction enzyme digestions have been observed (12, 14). In an attempt to identify a "neurotropic" subgroup of HIV-1, the biologic, serologic, and molecular properties of HIV-1 isolates from the central nervous system (CNS) and peripheral blood were determined and compared. These included pairs of CNS and blood isolates obtained from six different infected individuals. The data suggest that HIV-1 isolates from the CNS of individuals with neurologic diseases exhibit several distinguishing properties that may reflect a specific subgroup of HIV.

MATERIALS AND METHODS

Subjects Studied. The isolates used in this study (Tables 1 and 2) were initially obtained by cocultivation of peripheral blood mononuclear cells (PBMCs), lymph node, CSF, or brain biopsy specimens from HIV-1-positive patients with mitogen-stimulated PBMCs from seronegative donors, as described (4, 15). The blood isolates examined came from randomly selected patients with different stages of HIV-1 infection. The CNS isolates were obtained from neurologic patients referred to our laboratory from 1984 to 1988 for study of HIV infection of the CNS.

Viruses and Cell Lines. All HIV-1 isolates were grown to high titers in PBMCs [i.e., reaching levels of reverse transcriptase (RT) activity of $>10^6$ cpm/ml] and then frozen in 1-ml aliquots at −70°C for future studies. The established T-cell lines HuT 78, CEM, and Jurkat and the monocyte/macrophage cell line U-937 were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, and antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml) (16). Primary monocytes were obtained from Ficoll/Hypaque gradient-purified PBMCs by the plastic-adherence technique (12, 17). To allow for differentiation into macrophages, these cells were cultured for 10–12 days in RPMI 1640 medium supplemented with 10% FBS, 5% heat-inactivated human serum, and antibiotics before infection with HIV-1. The glioma cell lines (U251, U343MGA), obtained from M. Rosenblum (University of California, San Francisco), were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and antibiotics (18).

HIV-1 Infection. The T-cell and monocytic cell lines were infected with HIV-1 isolates, as previously described (16). For infection of peripheral blood macrophages, the cells were treated with Polybrene (2 μg/ml) for 30 min at 37°C and exposed to virus-containing fluids for 1 hr at 37°C. Cells were then washed three times and maintained in culture medium. Infection of glioma cell lines was performed as described (18). In brief, the monolayer cells were treated with 2 ml of DEAE-dextran (25 μg/ml) for 30 min at 37°C. The treated cells were then washed and incubated with virus for 1 hr at 37°C, washed three times, and refed with medium. The infected cells were passaged twice with trypsinization before cocultivation with PBMCs from seronegative donors. After 3 days of cocultivation, the PBMCs were removed and maintained as separate cultures. Culture supernatants of these

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: HIV, human immunodeficiency virus; CNS, central nervous system; PBMC, peripheral blood mononuclear cell; CSF, cerebrospinal fluid; RT, reverse transcriptase.

Table 1. Isolation of HIV-1 strains

HIV-1_{SF}	Clinical state*
Isolates from blood (PBMCs)	
2	Oral thrush
4	Kaposi sarcoma
33	Thrombocytopenia
66	Kaposi sarcoma
113	Asymptomatic
117	Kaposi sarcoma
Isolates from brain (CSF†)	
98	AIDS, VZV meningitis
161	PCP, cryptococcal meningitis
162	Toxoplasmosis, acute meningitis
178	AIDS, headache, confusion
301a	Lymphadenopathy, CMV pneumonia

*VZV, varicella–zoster virus; PCP, *Pneumocystis carinii* pneumonia; CMV, cytomegalovirus.
†Except for 301a, which was obtained at autopsy from cerebral cortex tissue of a patient without neurologic symptoms; the specimen was contaminated with blood at time of HIV isolation.

cocultivated cells were harvested at 3- to 4-day intervals and assayed for RT activity (19). The presence of HIV-1 was confirmed by identification of viral antigens in infected cells by an indirect immunofluorescence assay (20) or immunoblot analysis (21) or by a p24 HIV-1 core antigen ELISA (DuPont). CD4 expression on the surface of infected and uninfected cells was measured by flow cytometry (16).

Serum Neutralization. Serum neutralization was performed as described (14). In brief, serial dilutions of the heat-inactivated (56°C, 30 min) HIV-1 antibody-positive serum were incubated with equal amounts of virus-containing fluids (RT activity, $\approx 10^5$ cpm) for 1 hr at room temperature. The mixture was then inoculated onto PBMCs that were pretreated with Polybrene (2 μg/ml). Control cultures received virus incubated with sera from antibody-negative healthy donors. Culture supernatant fluids were assayed for RT activity at 3- to 4-day intervals. A 67% reduction in RT level, as compared to control cultures, was considered indicative of neutralization.

Southern Blot Analysis. High molecular weight DNA from 10^8 HIV-infected PBMCs was prepared (22) and digested with restriction enzymes under conditions specified by the supplier (Boehringer Mannheim). The restricted DNA fragments were separated by electrophoresis in 0.8% agarose and blotted onto nitrocellulose membranes, and the viral species were detected by hybridization with a ^{32}P-labeled probe representing the entire HIV-1_{SF2} genome (12).

Table 2. CNS and blood isolates of HIV-1 recovered from the same individual

Subject	HIV-1_{SF}*	Source	Clinical state
A	128A	Spinal cord	AIDS, vacuolar
	128B	Lymph node	myelopathy
B	185A	CSF	AIDS, subacute
	185B	PBMCs	encephalitis
C	921A	CSF	AIDS, PML†
	921B	PBMCs	
D	695A	Brain biopsy	Non-Hodgkin lymphoma
	695B	PBMCs	of the brain
E	689A	Brain biopsy	Non-Hodgkin lymphoma
	689B	PBMCs	of the brain
F	781A	CSF	Peripheral lymphoma, no
	781B	PBMCs	neurologic symptoms

*A, CNS isolate; B, blood isolate.
†Progressive multifocal leukoencephalopathy.

RESULTS

Isolation of HIV-1 Strains. Tables 1 and 2 summarize the origin of the HIV-1 isolates characterized in this study. The lymphocyte isolates (Table 1) were recovered from PBMCs of HIV-infected individuals without neurologic disorders. With the exception of HIV-1_{SF301a}, the CNS isolates were obtained from CSF of patients with various neurologic symptoms and disorders. HIV-1_{SF301a} was obtained at autopsy from the cerebral cortex tissue of a patient without neurologic symptoms. The sample was contaminated with blood at the time of virus isolation. Table 2 summarizes the origin of the paired brain and blood isolates obtained from six different individuals. Two of the individuals (subjects A and B) had neurological symptoms, two (D and E) had non-Hodgkin lymphoma of the brain, one (C) had progressive multifocal leukoencephalopathy, and one (F) had peripheral lymphoma with no neurologic findings at the time of virus isolation. Except for isolates from subject A (HIV-1_{SF128A} and HIV-1_{SF128B}), all other HIV-1 isolates were obtained from specimens collected during clinical examinations and from biopsies. HIV-1_{SF128A} and HIV-1_{SF128B} were isolated from specimens obtained at autopsy.

Characterization of HIV-1 Isolates from Peripheral Blood and CNS. The biologic and serologic properties of the HIV-1 isolates listed in Table 1 were determined by host range and serum neutralization studies. As shown in Table 3, five of the six PBMC isolates productively infected the human T-cell lines (HuT 78, Jurkat, CEM), whereas none of them replicated in primary macrophages. The isolates that replicated in T-cell lines caused cytopathic effects in the infected cells. Four of these five cytopathic HIV-1 strains induced plaques

Table 3. Comparison of biologic properties of HIV-1 recovered from blood and CNS

	Replication*					
HIV-1_{SF}	T cells	Mϕ	PF†	CPE‡	CD4 ↓§	SN¶
PBMCs						
2	+	−	−	+	+	≥100
4	+	−	+	++	+	≥100
33	+	−	+	++	+	≥100
66	+	−	+	++	+	≥100
113	−	−	−	−	−	≥100
117	+	−	+	++	+	≥100
CNS						
98	−	+	−	−	−	<10
161	−	−	−	−	−	<10
162	−	+	−	−	−	<10
178	−	+	−	−	−	<10
301a	+	−	−	+	+	≥100

*T-cell lines tested were HuT 78 and Jurkat; no difference in replication in these two lines was observed. Mϕ, primary macrophages. +, Particle-associated RT activity ($>5 \times 10^5$ cpm/ml) in the culture supernatant; −, absence of RT activity above background ($\approx$1000 cpm/ml).
†Plaque formation was assayed in MT-4 cells (13). +, $>10^3$ plaque-forming units/ml of culture supernatant; −, absence of plaque formation.
‡Cytopathic effects were assessed by the presence of cellular ballooning and syncytium formation in infected CD4$^+$ cells that were obtained by panning (23). −, Absence of CPE; +, $\approx$50% CPE in the culture; ++, >90% of infected cells showed CPE.
§CD4 antigen modulation was defined as the disappearance of the cell surface CD4 receptor molecule upon HIV-1 infection of purified CD4$^+$ peripheral blood lymphocytes as measured by flow cytometry (24). +, >70% CD4 down-modulation; −, <20% disappearance of CD4.
¶Serum neutralization was performed as described in *Materials and Methods*. Values represent the reciprocal of the highest serum dilution of three sera tested that caused a reduction of at least 67% in RT activity in the culture fluid as compared to control cultures.

in the MT-4 cells. Plaque-forming ability of an HIV-1 isolate has been shown to correlate with its ability to grow rapidly and to induce cytopathology in PBMCs and the MT-4 cell line (13). Further, all five of the cytopathic lymphocyte isolates reduced the expression of the surface CD4 receptor molecule on the infected cells. Finally, as previously reported (14), all the lymphocyte isolates were easily neutralized by sera from HIV-1 positive individuals at titers of ≥1:100.

In contrast, only one of the five CNS isolates (HIV-1_{SF301a}) replicated in the human T-cell lines, whereas three of the five isolates (HIV-1_{SF98}, HIV-1_{SF162}, HIV-1_{SF178}) replicated efficiently in primary macrophages. None of the isolates induced plaques in the MT-4 cell line and only HIV-1_{SF301a} produced cytopathic effects in infected $CD4^+$ lymphocytes concomitant with a reduction in surface CD4 antigen expression. The other brain isolates replicated substantially in $CD4^+$ cells, but there was no evidence of cytopathic effect in these cells nor a modulation in CD4 expression. All CNS isolates, except HIV-1_{SF301a}, were resistant to serum neutralization. HIV-1_{SF301a} was easily neutralized by HIV-1-positive sera at titers of >1:100.

Comparison of Blood and CNS Isolates Obtained from the Same Individual. To examine further the possibility that HIV brain isolates constitute a separate virus subgroup, HIV-1 strains recovered from the blood and CNS of the same individual were examined. Table 4 summarizes the biologic properties of these paired blood and CNS isolates. Three of the six paired blood isolates (from subjects C, D, and F) replicated in at least one of the T-cell lines, whereas none of the paired CNS isolates established a productive infection in these cells. When replication in macrophages was examined, all the isolates productively infected primary peripheral blood macrophages. However, the CNS isolates from subjects A–D replicated with faster kinetics and the virus titers from the infected macrophages were 5- to 10-fold higher than those obtained with their paired blood isolates (Fig. 1*A*).

Table 4. Comparison of the biologic properties of paired CNS and blood isolates

Subject	HIV-1_{SF}*	Replication		
		T cells†	Mϕ‡	Glioma cells§
A	128A	−	++	−
	128B	−	+	++
B	185A	−	++	+
	185B	−	+	+
C	921A	−	++	−
	921B	++	+	++
D	695A	−	+++	−
	695B	+++	+	+
E	689A	−	+	−
	689B	−	+	−
F	781A	−	+	−
	781B	+	+	−

*A, CNS isolate; B, blood isolate.

†For replication in T-cell lines (HuT 78, Jurkat, CEM): +++, presence of RT activity (>5 × 10^5 cpm/ml) in all three cell lines tested; ++, presence of RT activity in two of the three cell lines tested (Jurkat, CEM); +, presence of RT activity in one of the three cell lines tested (CEM); −, absence of RT activity above background (≤1000 cpm/ml).

‡For replication in primary macrophages (Mϕ): +, positive basal RT activity (≈10^5 cpm/ml); ++, activity 5-fold higher than basal; +++, activity 10-fold higher than basal.

§For detection of virus replication in the glial cells (U251 and U343MGA lines) cocultivation with PBMCs from seronegative individuals is necessary (18). −, Absence of RT activity above background (≤1000 cpm/ml) in cocultivated PBMCs; +, replication in only one of the cell lines (U251); ++, replication in both glioma cell lines tested as measured by RT activity (>10^5 cpm/ml) in the culture supernatant.

Moreover, only the CNS isolate from subject D productively infected the U-937 monocytic cell line (data not shown).

The ability of the paired isolates to infect two astrocytoma cell lines was also examined. In these cells, cocultivation of the infected glioma cells with PBMCs from seronegative individuals is necessary to detect HIV-1 replication (18). The blood isolates from subjects A, C, and D replicated more efficiently than their paired CNS isolates. This finding was again reflected both in the kinetics of replication and in titers of virus recovered (Fig. 1*B*). None of the blood or CNS paired isolates induced plaques in the MT-4 cell line, indicating that they are relatively noncytopathic (13). Further, these isolates did not modulate CD4 antigen expression on $CD4^+$ lymphocytes and were not sensitive to neutralization by the HIV-1 antibody-positive sera used in these studies. Moreover, in two cases in which autologous sera were available for serum neutralization (subjects A and D), no difference in sensitivity to serum neutralization of CNS and blood isolates was observed; both isolates were neutralized at serum titers of 1:10. Thus, the paired isolates appeared to be similar antigenically and resembled primarily HIV-1 isolates from patients with neurologic disorders (Table 3). Nevertheless, the data indicate that CNS and blood isolates obtained from the same individual can display differences in host range tropism.

To determine whether these paired isolates are genomically related or distinct viruses, restriction endonuclease digestion analysis was performed on infected cellular DNA. Fig. 2 shows Southern blot analyses of two pairs of blood and CNS isolates. The restriction enzyme digestion patterns of these paired isolates are very similar. Minor differences were observed with some restriction endonucleases, but these differences could be accounted for by the gain or loss of a restriction site. For example, a gain of a *Pvu* II site was seen in the HIV-1_{SF128B} strain as compared to HIV-1_{SF128A} for subject A (Fig. 2*A*); a gain of a *Sac* I site in HIV-1_{SF921B} was observed when compared to HIV-1_{SF921A} from subject C (Fig. 2*B*). Similar observations were made with restriction endonuclease studies performed on the other paired isolates (data not shown). The results show that blood and CNS isolates obtained from the same individual are genotypically related strains of HIV-1.

DISCUSSION

A set of biologic and serologic properties that distinguish individual HIV-1 strains was used to examine whether isolates from the CNS might constitute a distinct subgroup of HIV-1. Randomly selected peripheral blood isolates obtained from infected individuals without neurologic disorders and HIV-1 recovered from the CNS of patients referred for neurologic examinations (Table 1) were studied. In comparison to HIV-1 recovered from the blood, the CNS isolates are less cytopathic, do not replicate in established human T-cell lines, and display a greater tropism for primary peripheral blood macrophages (Table 3). That isolates from the CNS are less cytopathic and replicate better in primary monocyte/macrophages than do blood isolates has been reported by others (17, 25). In addition, we found that the CNS-derived HIV-1 strains do not reduce the expression of the CD4 receptor molecule on infected peripheral blood $CD4^+$ lymphocytes despite substantial replication in these cells, and these viruses are less susceptible to serum neutralization. Exceptions in our study were found for both a lymphocyte (HIV-1_{SF113}) and a CNS (HIV-1_{SF301a}) isolate. HIV-1_{SF113} was obtained from an asymptomatic individual. The biologic properties of this isolate are similar to those reported for HIV-1 strains recovered from other healthy seropositive individuals and for early isolates obtained from individuals who later progress in disease development (12). These viruses characteristically show a reduced capacity to infect

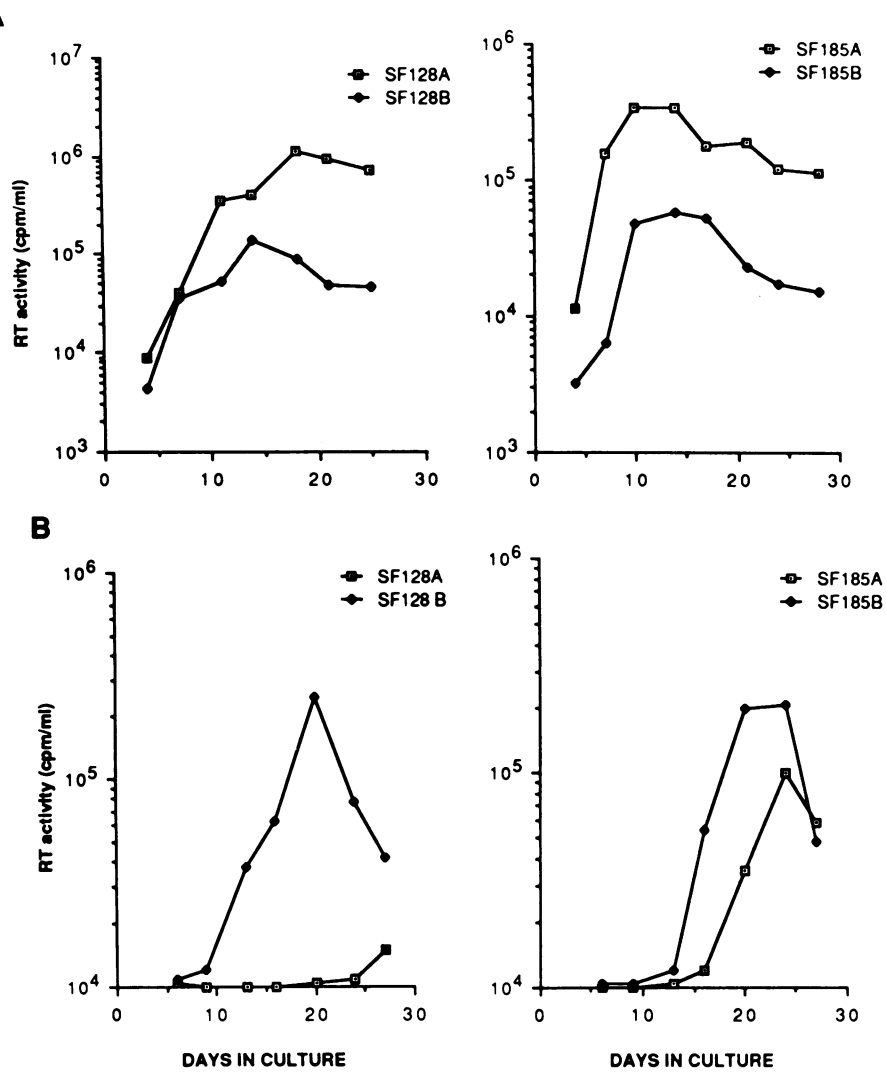

FIG. 1. Kinetics of replication of paired blood and brain isolates in primary peripheral blood macrophages (*A*) and the glioma cell line U251 (*B*). Primary macrophages prepared from PBMCs of seronegative individuals were infected with two pairs of blood and brain isolates obtained from the same individuals (HIV-1_{SF128A}, HIV-1_{SF128B} and HIV-1_{SF185A}, HIV-1_{SF185B}). Culture supernatants were assayed for RT activity at 3- to 4-day intervals. For detection of HIV infection of glioma cells, mitogen-stimulated normal human PBMCs (3×10^6 cells per culture) were added to the HIV-infected monolayer cells 7–10 days after infection and trypsinization as described (18). After 3 days of cocultivation, the PBMCs were removed and maintained in separate cultures. The supernatants of these cultures were assayed for RT activity at 3- to 4-day intervals.

established T-cell lines and are noncytopathic. HIV-1_{SF301a} displayed features similar to those of other lymphocyte isolates. Since HIV-1_{SF301a} was recovered from a brain biopsy of a patient without neurologic symptoms, these results suggest that HIV-1_{SF301a} is an isolate from blood that contaminated the cerebral cortex tissue at the time of isolation.

Results with paired CNS and PBMC isolates obtained from the same individual support these conclusions on distinguishing properties of HIV-1 strains recovered from the blood and brain. Differences both in kinetics of replication and in levels of virus production were noted in the ability of paired isolates to infect primary peripheral blood macrophages, T-cell lines, and glioma cell lines bearing glial fibrillary acid protein, a marker specific for astrocytes (18) (Table 4). The CNS isolates replicated better in primary peripheral blood macrophages, whereas the blood isolates infected T-cell lines and glial cells more efficiently. Since the paired isolates appeared to be genomically related (Fig. 2), these findings suggest that in individuals with neurologic disorders, the presence of variants of one HIV-1 strain with different biologic properties is likely. Similar differences in cellular tropism have been reported for HIV-1 isolates obtained from the CSF and brain tissues of the same individual (26). The isolate from the brain efficiently infected primary peripheral blood monocyte/macrophages, whereas the virus from the CSF productively infected a glioma cell line. These isolates, however, were genotypically distinct virus strains (26).

All these observations strongly suggest that HIV-1 strains recovered from the CNS can be distinguished from the HIV-1 strains predominantly found in the blood. Whether the brain-associated HIV-1 is present in an individual at the time of infection, perhaps at low level in the blood, or evolves with time in the individual requires further study. In any case, this biologically distinct type of HIV-1 appears to grow selectively in the CNS compartment of individuals with neurological findings. How these biologic features correlate with the pathology in the brain has not been established. The reduced killing of cultured $CD4^+$ lymphocytes by the CNS isolates could reflect the less prominent loss of $CD4^+$ cells observed in some patients with neurologic diseases (10). The macrophage tropism of the isolates from brain supports the *in situ* hybridization and immunohistochemical staining data indicating the macrophage as a major cell type in the brain showing HIV-1 infection (7, 8). This observation has led some investigators to cite the macrophage as a source of entry and pathogenic effects of HIV in the CNS (1, 7, 27, 28). However, *in situ* hybridization and immunocytochemical studies of brain tissues from infected individuals, together with *in vitro* infection studies, suggest that HIV-1 also infects endothelial and glial cells (8–11, 18, 29–31). Further, our observation that the paired blood isolates infected glial cells more efficiently than CNS isolates raises the possibility that blood-borne HIV-1 strains enter the brain through infection of these cell types. Subsequently, from the glial cells, these HIV-1 could spread or generate variants that infect other

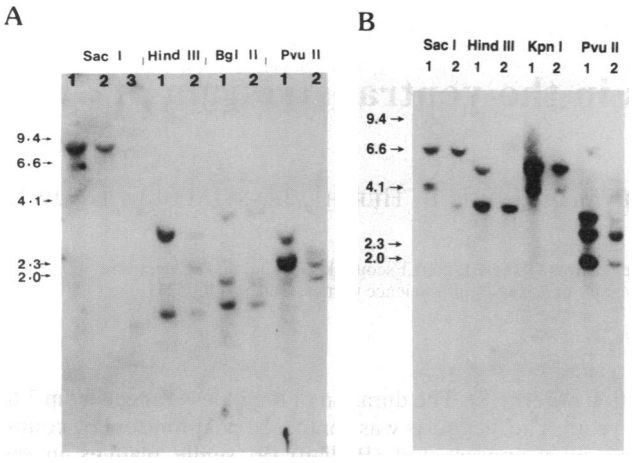

FIG. 2. Southern blot analyses of genomic structures of paired blood and brain isolates. High molecular weight whole-cell DNA was prepared for normal human PBMCs infected with blood and brain isolates from subjects A and C. Samples (15 μg) of each DNA were digested with the restriction enzymes indicated and blotted as described in *Materials and Methods*. (*A*) HIV-1_{SF128A} (lanes 1) and HIV-1_{SF128B} (lanes 2) from subject A; uninfected cell DNA as control (lane 3). (*B*) HIV-1_{SF921A} (lanes 1) and HIV-1_{SF921B} (lanes 2) from subject C.

cells in the CNS. Thus, dissemination of virus from the bloodstream to glial cells with subsequent killing or interference with the normal functions of these cells could be considered a factor in HIV neuropathogenesis.

In summary, our data suggest that CNS isolates obtained from individuals with neurologic disorders share properties defined by cell tropism, cytopathology, and antigenicity that distinguish them from the predominant HIV-1 strains derived from blood of individuals without neurologic disease. This conclusion is supported as well by relative differences in cellular tropism observed in blood and CNS isolates obtained from the same individual with neurologic disorders. How this subgroup of HIV-1 is generated or emerges in the host merits further investigation.

We thank Dr. M. Rosenblum for the CSF, brain tissues, and blood samples from the patients with non-Hodgkin and peripheral lymphomas; M. Tateno for performing the MT-4 plaque assay; and D. Wong for preparation of the manuscript. This research was supported by grants from the National Institutes of Health (R29 NS25007 and R01 AI24499).

1. Price, R., Drew, B., Sidtis, J., Rosenblum, M., Scheck, A. & Cleary, P. (1988) *Science* **239,** 586–592.
2. Rosenblum, M. L., Levy, R. M. & Bredesen, D. E. (1988) *AIDS and the Nervous System* (Raven, New York), pp. 1–13.
3. Dalakas, M., Wichman, A. & Sever, J. (1989) *J. Am. Med. Assoc.* **261,** 2396–2399.
4. Levy, J. A., Shimabukuro, J., Hollander, H., Mills, J. & Kaminsky, L. (1985) *Lancet* **ii,** 586–588.
5. Ho, D. D., Rota, T. R. & Hirsch, M. S. (1985) *N. Engl. J. Med.* **313,** 1493–1497.
6. Shaw, G. M., Harper, M. E., Hahn, B. H., Epstein, L. G., Gajdusek, D. C., Price, R. W., Navia, B. A., Petito, C. K., O'Hara, C. J., Groopman, J. E., Cho, E.-S., Oleske, J. M., Wong-Staal, F. & Gallo, R. C. (1985) *Science* **227,** 177–182.
7. Koenig, S., Gendelman, H. E., Orenstein, J. M., Dal Canto, M. C., Pezeshkpour, G. H., Yungbluth, M., Janotta, F., Aksamit, A., Martin, M. A. & Fauci, A. S. (1986) *Science* **233,** 1089–1093.
8. Wiley, C. A., Schrier, R. D., Nelson, J. A., Lampert, P. W. & Oldstone, M. B. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7089–7093.
9. Gyorkey, F., Melnick, J. L. & Gyorkey, P. (1987) *J. Infect. Dis.* **155,** 870–876.
10. Levy, J. A., Evans, L. A., Cheng-Mayer, C., Pan, L.-Z., Lane, A., Staben, C., Dina, D., Wiley, C. & Nelson, J. (1987) *Ann. Inst. Pasteur* **138,** 101–111.
11. Michaels, J., Sharer, L. R. & Epstein, L. G. (1988) *Immunodeficiency Rev.* **1,** 77–104.
12. Cheng-Mayer, C., Seto, D., Tateno, M. & Levy, J. A. (1988) *Science* **240,** 80–82.
13. Tateno, M. & Levy, J. A. (1988) *Virology* **167,** 299–301.
14. Cheng-Mayer, C., Homsy, J., Evans, L. A. & Levy, J. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2815–2819.
15. Hollander, H. & Levy, J. A. (1987) *Ann. Int. Med.* **106,** 692–695.
16. Evans, L. A., McHugh, T. M., Stites, D. P. & Levy, J. A. (1987) *J. Immunol.* **138,** 3415–3418.
17. Gartner, S., Markovits, P., Mackovitz, D. M., Kaplan, M. H. & Popovic, M. (1986) *Science* **233,** 215–219.
18. Cheng-Mayer, C., Rutka, J., Rosenblum, M., McHugh, C., Stites, D. & Levy, J. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3526–3530.
19. Hoffman, A. D., Banapour, B. & Levy, J. A. (1985) *Virology* **147,** 626–632.
20. Kaminsky, L. S., McHugh, T., Stites, D., Volberding, P., Henle, G., Henle, W. & Levy, J. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5535–5539.
21. Pan, L.-Z., Cheng-Mayer, C. & Levy, J. A. (1987) *J. Infect. Dis.* **155,** 626–632.
22. Luciw, P. A., Oppermann, H., Bishop, M. & Varmus, H. E. (1984) *Mol. Cell. Biol.* **4,** 1260–1269.
23. Wysocki, L. J. & Sato, V. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2844–2848.
24. Evans, L. A., Moreau, J., Odehouri, K., Legg, H., Barboza, A., Cheng-Mayer, C. & Levy, J. A. (1988) *Science* **240,** 1522–1525.
25. Anand, R. (1988) *Ann. Neurol.* **23,** Suppl. s27–s33.
26. Koyanagi, Y., Miles, S., Mitsuyasu, R. T., Merrill, J. E., Vinters, H. V. & Chen, I. S. Y. (1987) *Science* **236,** 819–822.
27. Gabuzda, D. H., Ho, D. D., de la Monte, S. M., Hirsh, M. S., Rota, T. R. & Sobel, R. A. (1986) *Ann. Neurol.* **20,** 89–95.
28. Eilbott, D. J., Peress, N., Burger, H., LaNeve, D., Orenstein, J., Gendelman, H. E., Siedman, R. & Weiser, B. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3337–3341.
29. Pumarola-Sune, T., Navia, B. A., Cordon-Cardo, C., Cho, E.-S. & Price, R. W. (1987) *Ann. Neurol.* **21,** 490–496.
30. Chiodi, F., Fuerstenberg, S., Gidlund, M., Asjo, B. & Fenyo, E. M. (1987) *J. Virol.* **61,** 1244–1247.
31. Dewhurst, S., Sakai, K., Bressner, J., Stevenson, M., Evinger-Hodges, M. J. & Volsky, D. J. (1987) *J. Virol.* **61,** 3774–3782.