be a considerable challenge. A recent study indicating the presence of HTLV-III in brain tissue of AIDS patients (*32*) further links this virus with lentiviruses (*3*).

MATTHEW A. GONDA*
Laboratory of Cell and Molecular Structure, Program Resources, Inc., NCI–Frederick Cancer Research Facility, Frederick, Maryland 21701
FLOSSIE WONG-STAAL
ROBERT C. GALLO
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205
JANICE E. CLEMENTS
OPENDRA NARAYAN
Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 and *National Cancer Institute*
RAYMOND V. GILDEN
Program Resources, Inc., NCI–Frederick Cancer Research Facility

References and Notes

1. F. Fenner, *Intervirology* **6**, 1 (1975–76); *Acta Virol. (Praha)* **20**, 170 (1976); R. E. Matthews, *Intervirology* **12**, 234 (1979).
2. B. Sigurdsson, *Brit. Vet. J.* **110**, 225 (1954); _____, P. A. Paulsson, H. Grimsson, *J. Neuropathol. Exp. Neurol.* **16**, 389 (1957); B. Sigurdsson and P. A. Paulsson, *J. Exp. Neurol.* **39**, 519 (1958).
3. T. B. Crawford, D. S. Adams, W. P. Cheevers, L. C. Cork, *Science* **207**, 997 (1980); D. S. Adams, T. B. Crawford, P. Kelvjer-Anderson, *Am. J. Pathol.* **99**, 257 (1980); L. C. Cork, W. J. Hadlow, J. R. Gorham, R. C. Piper, T. B. Crawford, *J. Infect. Dis.* **129**, 134 (1974); L. C. Cork and O. Narayan, *Lab. Invest.* **42**, 596 (1980).
4. M. J. Van Der Maaten, A. D. Boothe, C. L. Seger, *J. Natl. Cancer Inst.* **49**, 1649 (1972); A. D. Boothe and M. J. Van Der Maaten, *J. Virol.* **13**, 197 (1974).
5. T. B. Crawford, W. P. Cheevers, P. Klevjer-Anderson, T. C. McGuire, in *Persistent Viruses, ICN/UCLA Symposium on Molecular and Cellular Biology*, J. Stevens, G. Todaro, C. F. Fox, Eds. (Academic Press, New York, 1978), vol. 11, pp. 727–749; Y. Kono, K. Kobayashi, Y. Fukunaga, *Arch. Virol.* **34**, 202 (1971); *ibid.* **41**, 1 (1973).
6. A. T. Haase, *Curr. Top. Microbiol. Immunol.* **72**, 101 (1975); D. Narayan, D. E. Griffin, A. M. Silverstein, *J. Infect. Dis.* **135**, 800 (1977); R. C. Kennedy, E. M. Eklung, C. Lopez, W. J. Hadlow, *Virology* **35**, 483 (1968); H. Thormar and P. A. Paulsson, *Perspect. Virol.* **5**, 291 (1967); O. Narayan *et al.*, *J. Gen. Virol.* **59**, 345 (1982).
7. For a brief review, see M. G. Sarngadharan *et al.*, in *Human Carcinogenesis*, C. C. Harris and H. H. Autrup, Eds. (Academic Press, New York, 1983), p. 679; R. C. Gallo, in *Cancer Surveys*, L. M. Frank, J. Wyke, R. A. Weiss, Eds. (Oxford Univ. Press, Oxford, 1984), vol. 3, pp. 113–159; F. Wong-Staal, *Blood*, in press.
8. V. S. Kalyanaraman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1653 (1982); M. Robert-Guroff *et al.*, *Science* **215**, 925 (1982); Y. Hinuma *et al.*, *Int. J. Cancer* **29**, 631 (1982); W. A. Blattner *et al.*, *ibid.* **30**, 257 (1982); J. Schüpbach, V. S. Kalyanaraman, M. G. Sarngadharan, Y. Nakao, R. C. Gallo, *Int. J. Cancer* **32**, 583 (1983); J. Schüpbach *et al.*, *Cancer Res.* **43**, 886 (1983); B. J. Poiesz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980); B. J. Poiesz, F. W. Ruscetti, M. S. Reitz, V. S. Kalyanaraman, R. C. Gallo, *Nature (London)* **294**, 268 (1981).
9. V. S. Kalyanaraman *et al.*, *Science* **218**, 571 (1982); A. Saxons, R. H. Stevens, D. W. Golde, *Ann. Intern. Med.* **88**, 323 (1978).
10. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **224**, 497 (1984); *ibid.*, p. 500; J. E. Groopman *et al.*, *ibid.* **226**, 447 (1984); D. Zagury *et al.*, *ibid.*, p. 449; D. D. Ho *et al.*, *ibid.*, p. 451.
11. R. C. Gallo *et al.*, *ibid.* **224**, 500 (1984).
12. J. Schüpbach *et al.*, *ibid.*, p. 503.
13. M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *ibid.*, p. 504 (1984).
14. F. Brun-Vézinet *et al.*, *ibid.* **226**, 453 (1984).
15. M. A. Gonda, S. Z. Salahuddin, M. Popovic, R. C. Gallo, R. V. Gilden, in preparation.
16. H. Thormar, *Virology* **14**, 463 (1961); J. E. Coward, D. H. Harter, C. Morgan, *ibid.* **40**, 1030 (1970); K. K. Takemoto, C. F. Mattern, L. B. Stone, J. E. Coe, G. Lavelle, *J. Virol.* **7**, 301 (1971).
17. M. A. Gonda, H. P. Charman, J. L. Walker, L. Coggins, *Am. J. Vet. Res.* **39**, 731 (1978).
18. G. Schidlovsky, in *Cancer Research: Cell Biology, Molecular Biology, and Tumor Virology*, R. C. Gallo, Ed. (CRC Press, Cleveland, 1977), pp. 189–245.
19. L. Montagnie *et al.*, *Science* **225**, 63 (1984); L. Montagnier *et al.*, in *Human T-Cell Leukemia Lymphoma Virus*, R. C. Gallo, M. E. Essex, L. Gross, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984).
20. S. Molineaux and J. E. Clements, *Gene* **23**, 137 (1983).
21. G. W. Shaw *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4544 (1984); G. W. Shaw *et al.*, *Acquired Immune Deficiency Syndrome*, M. S. Gottlieb and J. E. Groopman Eds. (Liss, New York, in press), vol. 16; E. P. Gelman, G. Franchini, V. Manzari, F. Wong-Staal, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 993 (1984); M. Clarke, E. P. Gelman, M. S. Reitz, *Nature (London)* **292**, 31 (1983).
22. B. H. Hahn *et al.*, *Nature (London)* **312**, 166 (1984); B. H. Hahn *et al.*, in preparation.
23. R. W. Davis and R. W. Hyman, *J. Mol. Biol.* **62**, 287 (1971).
24. N. R. Rice *et al.*, *Virology* **138**, 82 (1984); N. R. Rice, R. M. Stephens, A. Burny, R. V. Gilden, *Virology*, in press; S. Oroszlan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1291 (1982); T. D. Copeland, M. H. Morgan, S. Oroszlan, *FEBS Lett.* **156**, 37 (1983); T. D. Copeland, S. Oroszlan, V. S. Kalyanaraman, M. S. Sarngadharan, R. C. Gallo, *ibid.* **162**, 390 (1983); A. M. Schultz, T. D. Copeland, S. Oroszlan, *Virology* **135**, 417 (1984).
25. M. Seiki, S. Hattori, Y. Hirayama, M. Toshida, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3618 (1983).
26. D. J. Slamon, K. Shimotohno, M. J. Cline, D. W. Golde, I. S. Y. Chen, *Science* **226**, 61 (1984); T. H. Lee *et al.*, *ibid.*, p. 57 (1984); J. G. Sodroski, C. A. Rosen, W. A. Haseltine, *ibid.* **225**, 381 (1984); I. Chen, J. McLaughlin, D. W. Golde, *Nature (London)* **309**, 276 (1984).
27. J. Sodroski *et al.*, *Science* **227**, 171 (1985).
28. S. K. Arya *et al.*, *ibid.* **225**, 927 (1984).
29. J. A. Georgiades, A. Billiau, B. Vanderschueren, *J. Gen. Virol.* **38**, 375 (1978).
30. O. Narayan, D. E. Griffin, J. Chase, *Science* **197**, 376 (1977); O. Narayan, D. E. Griffin, J. E. Clements, *J. Gen. Virol.* **41**, 343 (1978); S. Payne, B. Parekh, R. C. Montelaro, C. J. Issel, *ibid.* **65**, 1395 (1984); J. E. Clements, N. D'Antonio, O. Narayan, *J. Mol. Biol.* **158**, 415 (1982).
31. P. Klevjer-Anderson and T. C. McGuire, *Infect. Immun.* **38**, 455 (1982); O. Narayan *et al.*, *J. Virol.* **49**, 349 (1984).
32. G. W. Shaw *et al.*, *Science* **227**, 77 (1985).
33. R. W. Davis, M. Simon, N. Davidson, *Methods Enzymol.* **21**, 413 (1971).
34. B. L. McConaughy, C. D. Laird, B. J. McCarthy, *Biochemistry* **8**, 3289 (1969).
35. P. W. J. Rigby, M. Diekmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
36. E. M. Southern, *ibid.* **98**, 503 (1975).
37. We thank K. Nagashima, J. Shumaker, and J. E. Elser for technical contributions and M. Fanning for assistance in preparing the manuscript. Supported by NCI under contract No. N01-CO-23910 with Program Resources, Inc. (M.A.G. and R.V.G.).
* To whom requests for reprints should be sent at Laboratory of Cell and Molecular Structure, Program Resources, Inc., NCI–Frederick Cancer Research Facility, P.O. Box B, Frederick, Md. 21701.

13 November 1984; accepted 6 December 1984

HTLV-III Infection in Brains of Children and Adults with AIDS Encephalopathy

Abstract. *Unexplained debilitating dementia or encephalopathy occurs frequently in adults and children with the acquired immune deficiency syndrome (AIDS). Brains from 15 individuals with AIDS and encephalopathy were examined by Southern analysis and in situ hybridization for the presence of human T-cell leukemia (lymphotropic) virus type III (HTLV-III), the virus believed to be the causative agent of AIDS. HTLV-III DNA was detected in the brains of five patients, and viral-specific RNA was detected in four of these. In view of these findings and the recent demonstration of morphologic and genetic relatedness between HTLV-III and visna virus, a lentivirus that causes a chronic degenerative neurologic disease in sheep, HTLV-III should be evaluated further as a possible cause of AIDS encephalopathy.*

The acquired immune deficiency syndrome, or AIDS, is frequently complicated by central nervous system (CNS) dysfunction (*1–5*). In some patients, this is due to well-defined focal lesions in the brain such as those resulting from toxoplasmosis or lymphoma. However, far more common than these focal disturbances is the development of a more generalized encephalopathy that includes dementia as a dominant feature (*1, 2*). Indeed, many adult AIDS patients eventually develop this encephalopathy which characteristically begins with impaired concentration and mild memory loss and progresses to severe global cognitive impairment. Motor signs, including generalized hyperreflexia and increased tone may accompany the dementia, and some patients develop a spastic-ataxic gait or frank paraparesis. These neurological symptoms and signs usually progress over a course of several weeks to months (*1, 2*). In children with AIDS, a similar constellation of neurologic abnormalities occurs (*3*). Although the prevalence of dementia or other unexplained generalized CNS abnormalities in AIDS is uncertain, it is believed to occur to some degree in a substantial number, if not the majority, of patients (*1–5*).

The histopathological substrate of AIDS encephalopathy has not been defined. Gross cerebral atrophy and scattered microglial nodules consisting of aggregates of microglia and astrocytes are the most common findings in these

Table 1. Fifteen patients who had AIDS and encephalopathy were evaluated for the presence of HTLV-III in brain tissue. Clinical findings were documented before death by the patients' physicians; histopathologic findings were confirmed by a neuropathologist; and Southern analyses and in situ hybridizations were performed as described (*30–32*). All brain specimens were coded and analyzed independently by Southern analysis and in situ hybridization in a blinded manner; N.D., not done. Abbreviations: KS, Kaposi's sarcoma; PCP, *Pneumocystis carinii* pneumonia; dCMV, disseminated cytomegalovirus infection; MAI, *Mycobacterium avium intracellulare*; cTOX, cerebral toxoplasmosis; NHL, non-Hodgkins lymphoma; ARC, AIDS-related complex; BTR, blood transfusion recipient, CMV, cytomegalovirus.

Patient	Age	Sex	Risk factors	Systemic diagnoses	Neurologic findings	Neuropathology	Detection of HTLV-III	
							Southern analysis	In situ
1	33 years	M	Homosexual	MAI, dCMV, PCP	Dementia (severe)	Microglial nodules (severe), CMV, gemistocytic astrocytosis (grey matter)	+	+
2	34 years	M	Homosexual	Oral *Candida*	Dementia (severe), paraparesis	Microglial nodules (severe), gemistocytic astrocytosis, perivascular lymphocytes and macrophages	+	+
3	4 months	M	Mother was intravenous drug abuser, BTR	PCP, oral *Candida*	Loss of developmental milestones, secondary microcephaly	Microglial nodules (mild), gross cerebral atrophy	+	+
4	6 years	M	Mother with ARC	dCMV, oral *Candida*	Loss of developmental milestones, hypertonia, ataxia, seizures	Gross cerebral atrophy, neuronal loss, microglial nodules	+	+
5	34 years	M	Homosexual	KS, PCP, MAI, dCMV	Dementia (moderate)	Microglial nodules (mild), gemistocytic astrocytosis (white matter), white matter vacuolation	+	–
6	44 years	M	Homosexual	KS, PCP, MAI, dCMV	Dementia (mild)	Microglial nodules (mild), vacuolar myelopathy	–	–
7	34 years	M	Homosexual	KS	Dementia (mild)	Healed toxoplasmosis	–	–
8	41 years	M	Homosexual	PCP, dCMV	Dementia (mild)	Microglial nodules (moderate)	–	–
9	37 years	M	Homosexual	MAI, dCMV, PCP	Dementia (severe), paraparesis	Normal brain, vacuolar myelopathy	–	–
10	8 months	F	Haitian parents	PCP, CMV, pneumonia	Loss of developmental milestones, seizures, hypertonia	Normal brain (terminal hypoxic change)	–	–
11	37 years	F	Intravenous drug abuser	PCP	Dementia, seizures	Cerebral atrophy	–	–
12	10 months	M	Mother was intravenous drug abuser, BTR	Oral *Candida*	Loss of developmental milestones, secondary microcephaly	Gross cerebral atrophy, perivascular lymphocytes	–	–
13	46 years	M	Homosexual	KS, PCP, dCMV	Dementia	Microglial nodules, CMV	–	N.D.
14	43 years	M	Homosexual	KS, NHL	Dementia	Demyelination inflammation (non-specific leukoencephalopathy)	–	N.D.
15	49 years	F	Sexual partner with ARC	KS, PCP, cTOX	Ataxia, seizures, dementia	Toxoplasmosis with necrosis and inflammation	–	N.D.

patients but are not present in every case (*1, 2*). Disseminated infection of brain by cytomegalovirus has been found in a minority of affected individuals (*1, 2*).

It is now generally accepted that a newly discovered human retrovirus, human T-cell leukemia (lymphotropic) virus type III (HTLV-III), is the causative agent of AIDS (*6–8*). HTLV-III possesses a variety of biological and physicochemical properties in common with HTLV types I and II, among which are distant nucleic acid similarities and a striking tropism for T lymphocytes (*6, 9, 10*). However, recent studies (*11*) indicate that the genome of HTLV-III is more closely related to visna virus, a lentivirus that causes a chronic degenerative neurologic disease in sheep (*12*), than it is to HTLV-I or HTLV-II (*9*). Because of the frequency of unexplained dementia or encephalopathy observed in patients with AIDS and because brain cells and T lymphocytes are known to share common cell surface antigens (*13–16*), we examined the brains of 15 AIDS patients for evidence of HTLV-III infection. This report shows that in five of these patients HTLV-III sequences were detectable in the brain.

Table 1 summarizes the clinical and pathological characteristics of the 15 AIDS patients. These patients were studied because they had evidence of dementia or encephalopathy before death. Their neuropathological findings were variable in nature and degree and included histologically normal brains, mild to severe microglial nodules, vacuolar myelopathy, and cytomegalovirus or *Toxoplasma gondii* infections. As shown here and elsewhere (*1, 2*), histologic abnormalities were not consistently correlated with the presence or degree of dementia in these patients.

We examined, by Southern blot hybridization, DNA samples obtained from the cerebral cortex (containing both grey and white matter) of each of the 15 patients. HTLV-III DNA sequences were detected in five of them (patients 1, 2, 3, 4, and 5), as shown in Table 1 and Fig. 1. HTLV-III was not detected in the brains of the other ten AIDS patients or in four other control patients who were either healthy or suffered from Alzheimer's disease (Fig. 1) (*17*). In each of the five positive brain samples, the restriction-enzyme Sst I generated one of two different hybridization patterns. For example, brain DNA from patients 1, 2, 3, and 5 contained HTLV-III sequences that gave two bands of 5.5 kb and 3.5 kb when digested with Sst I, whereas the viral sequences in patient 4 generated a single 9-kb band when digested with the same enzyme. These differences in restriction patterns result from restriction site polymorphisms and correspond to two highly related but distinct forms of HTLV-III which are similar to ones we previously identified, cloned, and characterized (*9, 18*). Both of these forms of the virus were also evident in DNA from the T-cell line H9/HTLV-III (*9, 18*), which was included in this experiment as a positive control (Fig. 1, lane j). The DNA samples were also digested with Bgl II and Hind III. These restriction enzymes generated many of the same bands found in other HTLV-III isolates (*18, 19*) as well as other bands that were different (for example, see the Hind III digestion of H9/HTLV-III DNA compared to brain DNA in Fig. 2). These findings showing different but highly related forms of HTLV-III in brain are in keeping with our previous demonstration that diversity in the viral genome is a characteristic feature of HTLV-III (*18–20*).

To estimate the relative abundance of HTLV-III DNA sequences present in brain compared to other body tissues, we examined DNA from spleen, lymph node, liver, lung, and brain of one patient (Fig. 2). As shown, very small amounts of viral DNA were detectable in DNA from lymph node and spleen, and the sizes of the bands, as expected, corresponded exactly to those generated by the same restriction enzyme digestion of brain DNA from this patient (see Fig. 1, lane a). When the same amount of DNA from brain and the other tissues from this patient were compared direct-

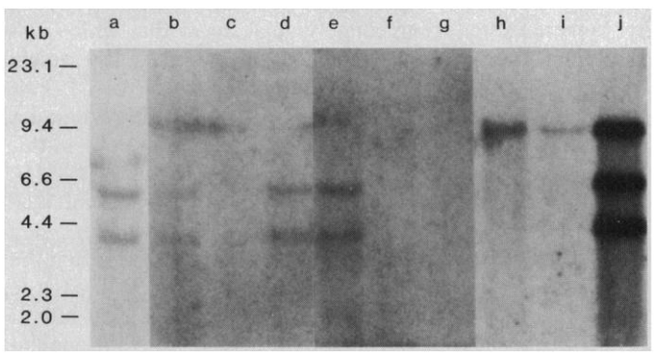

Fig. 1. Southern blot analysis of brain DNA from patients with AIDS. High molecular weight DNA was extracted, digested with Sst I, and blot-hybridized to an HTLV-III–specific probe as described (*30*). Lane a, patient 3; lane b, patient 1; lane c, patient 10; lane d, patient 5; lane e, patient 2; lane f, patient 8; lane g, patient 9; lanes h and i, the parietal and occipital cortex, respectively, of patient 4; lane j, HTLV-III–infected T-cell line (H9/HTLV-III) (*9, 18*). Not shown are the blot hybridizations of brain DNA from patients 6 and 11 to 15, and from four non-AIDS control individuals, all of which were negative. As shown, HTLV-III sequences were detected in brain DNA from patients 1 to 5. Two Sst I patterns were evident (5.5 kb and 3.5 kb in lanes a, b, d, and e and 9 kb in lanes h and i) which, as discussed in the text, correspond to two distinguishable but highly related forms of HTLV-III (*9, 18*). Both of these forms of the virus are evident in the DNA from H9/HTLV-III cells shown in lane j.

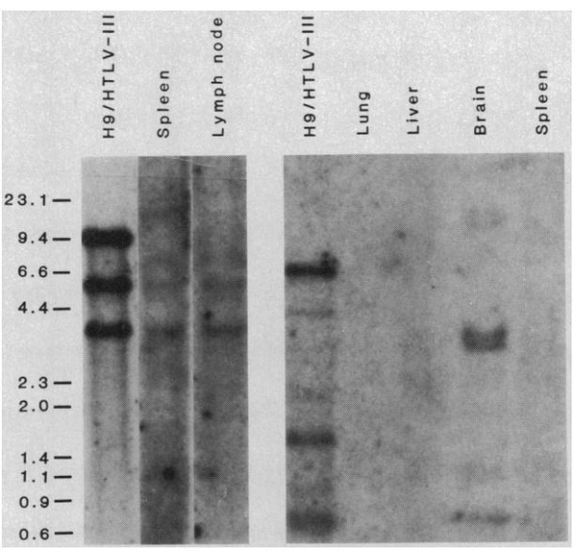

Fig. 2. Detection of HTLV-III DNA in brain and other tissues from patient 3 (see Table 1 and lane a in Fig. 1). DNA was extracted from brain, spleen, lymph node, liver, and lung, digested with the indicated restriction endonucleases, and blot-hybridized as described (*30*). DNA (30 μg) from each tissue was used so that the relative abundance of HTLV-III DNA in each could be ascertained. DNA (3 μg) from the H9/HTLV-III cell line was included as a positive control [note that H9/HTLV-III cells each contain many HTLV-III molecules (*9, 18*)]. The Sst I blot (left) was exposed to x-ray film for 7 days whereas the Hind III blot (right) was exposed for only 1 day. This blot hybridization demonstrates that the abundance of HTLV-III was much greater in brain than in any of the other tissues from this patient. It is also evident that the restriction pattern for Hind III is different in the HTLV-III isolate from this patient compared with those forms present in H9/HTLV-III cells (see text for discussion).

ly, it was apparent that brain was by far the most heavily infected with HTLV-III (Fig. 2).

We did not have the opportunity to examine other tissues besides brain from the remaining four positive cases in this study. However, from Fig. 2 and from an earlier survey of different lymphoid tissues from AIDS patients (*18*) we conclude that the abundance of HTLV-III in these brains samples was generally equivalent to, and sometimes greater than, that found in other tissues including peripheral blood, lymph node, spleen, and bone marrow of other AIDS and ARC patients.

To define further the nature of HTLV-III infection in brain, we used in situ hybridization to examine brain cells directly for viral-specific RNA. Frozen sections of brain from 12 of the 15 patients already evaluated by Southern analysis (Table 1 and Fig. 1) were examined in a coded fashion without knowledge of the results of the blot-hybridization. There was nearly complete agreement between the results obtained by the two techniques (Table 1). Four of five specimens positive for HTLV-III by Southern analysis were also positive by in situ hybridization, indicating that the HTLV-III genome was being expressed in these tissues. Similarly, all seven specimens negative for viral sequences by Southern analysis were also scored negative by in situ analysis. The discrepancy between the results of the Southern and in situ hybridizations in patient 5 could have resulted from degradation of RNA (which is more labile than DNA), a lack of expression of the viral genome in that sample, or the presence of virus in one region of the cerebral cortex but not others.

Figure 3, A and B, shows numerous silver grains representing HTLV-III viral RNA in the brain cells of two of the AIDS patients. Such grains were not present in brain from a control, non-AIDS patient (Fig. 3D). As an additional control, a probe lacking HTLV-III sequences was hybridized to sections of brain from each of the 12 AIDS patients; no positively hybridizing cells were found (Fig. 3C). The specimens shown in Fig. 3, A and C, were taken from a region of the cerebral cortex of patient 3 immediately adjacent to that which was used to make high molecular weight DNA for Southern analysis (Fig. 1, lane a). We estimate from both techniques that from 1 to 10 percent of cells in this specimen were infected with HTLV-III.

The specific type of brain cell infected with HTLV-III could not be determined from these studies, but it is clear that the positive hybridization was not due to the infiltration of brain by HTLV-III infected lymphocytes. We base this conclusion on (i) the histologic characteristics of the brain samples used for the Southern and in situ hybridizations (lymphocytes were scant or absent in all but one brain specimen), (ii) the cellular morphology of the virally infected cells identified by in situ hybridization (nuclear and cytoplasmic dimensions were not those of lymphocytes), and (iii) the fact that only very few lymphocytes in lymph node, spleen, and peripheral blood (less than one in ten) are infected with HTLV-III at any one time [see Fig. 2 and (*18, 21*)]. Further studies with the use of in situ hybridization and immunocytochemical techniques are needed to determine the cell type or types infected with HTLV-III, be they of neuronal, glial, or macrophage lineage.

The true incidence of HTLV-III brain infection in AIDS patients with encephalopathy is not yet known. Nor is it known whether HTLV-III brain infection may occur in patients with AIDS or AIDS-related complex (ARC) without encephalopathy. The brain specimens used in this study were taken from different cortical regions (for example, frontal, parietal, or occipital lobes) and, except for one patient (number 4), we were able to examine only a single region of each brain for HTLV-III sequences. Thus, although HTLV-III was detected in frontal (patient 2), parietal (patients 4 and 5), and occipital (patients 1 and 4) lobes, it is possible that viral infection could have been anatomically restricted at certain points in the disease course in some of the patients studied and thus were not detected by our limited sampling. Moreover, a number of brain specimens had been less than optimally preserved, so it is possible that low-level infection with HTLV-III could have gone undetected. Thus the cellular and anatomic distribution of the virus, its histopathological correlate, and its relation to the patients' clinical signs and symptoms, if any, remain to be determined.

Our finding of HTLV-III in brains of 5 out of 15 AIDS patients (33 percent) is comparable to results in lymphoid tissues examined by the same Southern blot technique (*18*). Moreover, the abundance of HTLV-III DNA in brain appears to be equal to, and sometimes greater than, that in lymphoid tissues (Fig. 2) (*18*). We have also observed that the relative abundance of HTLV-III RNA per cell as determined by in situ hybridization is generally greater in brain than in lymph node, peripheral blood, or bone marrow (*21*).

The finding of HTLV-III DNA and RNA in brain indicates that not only is the virus present in this tissue compartment but that it is being expressed there.

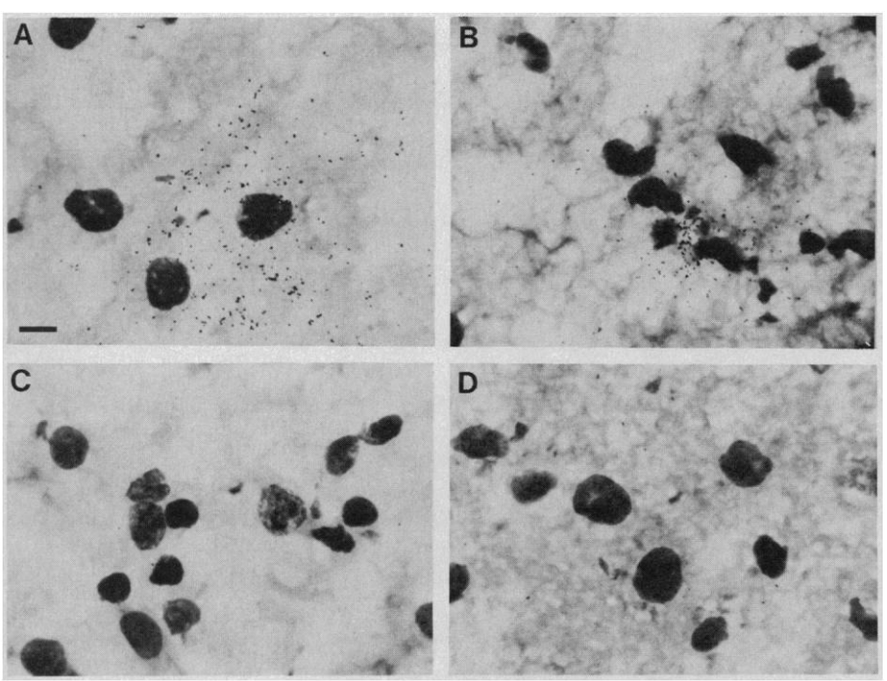

Fig. 3. Detection of HTLV-III viral RNA in the brains of AIDS patients by in situ hybridization. Brain section from (A) patient 3, hybridized with HTLV-III–specific probe, (B) patient 4, hybridized with HTLV-III–specific probe, (C) patient 3, hybridized with control phage λ-specific probe lacking HTLV-III sequences, and (D) non-AIDS (control) patient hybridized with HTLV-III–specific probe. In situ hybridization was carried out as described (*31*). Exposure, 2 days. Scale bar, 10 μm.

Furthermore, we have shown, by using restriction enzymes that cleave the viral genome only once or not at all, that in brain, as in other tissues (*18*), HTLV-III DNA occurs in substantial amounts in both chromosomally integrated and unintegrated forms (*17*). Since unintegrated DNA represents a replicative intermediate stage in the life cycle of retroviruses, this finding suggests that HTLV-III is actively replicating in the brains of these AIDS patients.

The finding of HTLV-III in brain and its apparent enrichment there is of considerable interest aside from its possible clinical implications. Certain surface antigens, such as Thy-1 (*13–16*), occur on both T lymphocytes and brain cells. The question of whether there are other shared antigens between lymphocytes and brain cells, both neuronal and glial, is controversial (*15, 16, 22–24*). It is possible that the dual tissue tropism of HTLV-III is the result of similarities in surface membrane determinants of T lymphocytes and certain brain cells, and the characterization of such common viral receptors on these cells could define further their antigenic relatedness.

This apparent tropism for brain by a retrovirus is not without precedence. Certain strains of murine leukemia virus induce both lymphoma and neurologic disease in wild mice (*25*). These animals experience a slowly progressive paralytic disease characterized pathologically by spongiform degeneration of the gray matter, particularly in the anterior horns of the spinal cord. As in AIDS encephalopathy, there is a characteristic absence of inflammatory reaction in the involved neural tissue.

Another retrovirus, visna, causes a chronic degenerative CNS disease in sheep (*12*). Like HTLV-III, visna virus infects both brain and lymphocytes (*12*), has pronounced cytopathic activity (*26*), has morphologic and genetic similarities with HTLV-III (*11*), and persists in cells in substantial amounts as unintegrated viral DNA (*27*). This last property is very unusual for retroviruses and is a particular feature of HTLV-III and other cytopathic retroviruses (*18, 28*). Unlike the noninflammatory lesions described in AIDS encephalopathy, the CNS disease resulting from visna virus is associated with an intense mononuclear cell inflammatory response (*29*). This difference between the histopathologic characteristics of visna infection and that of AIDS encephalopathy, however, must be considered in light of the profound cellular immunodeficiency that occurs in patients with AIDS but not in animals infected with visna virus.

Our results indicate that HTLV-III, in addition to its role in causing the immune deficiency of AIDS (*6–8*), may also have a role in the pathogenesis of AIDS encephalopathy. It will be important to determine which CNS cell types are infected with HTLV-III and how the virus affects these cells in vitro and in vivo. Finally, in attempting to develop therapeutic agents for the treatment of AIDS, investigators will now have to allow for the presence of HTLV-III within the sanctuary of the CNS.

GEORGE M. SHAW
MARY E. HARPER
BEATRICE H. HAHN
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205

LEON G. EPSTEIN
D. CARLETON GAJDUSEK
Laboratory of Central Nervous System Studies, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20205

RICHARD W. PRICE
BRADFORD A. NAVIA
CAROL K. PETITO
Departments of Neurology and Pathology, Cornell University Medical College, New York 10021

CARL J. O'HARA
JEROME E. GROOPMAN
Departments of Pathology and Medicine, New England Deaconess Hospital, Boston, Massachusetts 02215

EUN-SOOK CHO
JAMES M. OLESKE
Departments of Pathology and Pediatrics, University of Medicine and Dentistry of New Jersey, Newark 07103

FLOSSIE WONG-STAAL
ROBERT C. GALLO
Laboratory of Tumor Cell Biology, National Cancer Institute

References and Notes

1. W. D. Snider *et al.*, *Ann. Neurol.* **14**, 403 (1983); B. D. Jordan, B. A. Navia, C. Petito, E-S. Cho, and R. W. Price, *Front. Radiat. Ther. Oncol.*, in press.
2. B. A. Navia *et al.*, in preparation.
3. L. G. Epstein *et al.*, *Ann. Neurol.*, in press.
4. K. Welch *et al.*, *J. Am. Med. Assoc.* **252**, 1152 (1984).
5. P. Gapen, *ibid.* **248**, 2941 (1982).
6. M. Popovic *et al.*, *Science* **224**, 497 (1984).
7. R. Gallo *et al.*, *ibid.* **224**, 500 (1984); J. Schüpbach *et al.*, *ibid.* **224**, 503 (1984); M. G. Sarngadharan *et al.*, *ibid.* **224**, 506 (1984); B. Safai *et al.*, *Lancet* **1984-I**, 1438 (1984); J. Groopman *et al.*, *N. Engl. J. Med.* **311**, 1419 (1984).
8. S. Z. Salahuddin *et al.*, in preparation.
9. B. H. Hahn *et al.*, *Nature (London)* **312**, 166 (1984).
10. R. C. Gallo, *Cancer Surv.* **3**, 114 (1984); R. C. Gallo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4680 (1982).
11. M. A. Gonda *et al.*, *Science* **227**, 173 (1985).
12. J. E. Clements *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4454 (1980); J. E. Clements, O. Narayan, L. C. Cork, *J. Gen. Virol.* **50**, 423 (1980); O. Narayan *et al.*, *ibid.*, p. 69.
13. A. E. Reif and J. M. V. Allen, *J. Exp. Med.* **120**, 413 (1964); M. C. Raff, *Nature (London)* **224**, 378 (1969); H. G. Thiele, R. Stark, D. Keese, *Eur. J. Immunol.* **2**, 424 (1972); W. Stohl and N. K. Gonatas, *ibid.* **119**, 422 (1977); J. McKenzie and J. W. Fabre, *Brain Res.* **230**, 307 (1981).
14. R. C. Seeger *et al.*, *J. Immunol.* **128**, 983 (1982).
15. S. Szuchet, J. Antel, B. G. W. Arnason, *Nature (London)* **295**, 66 (1982).
16. J. A. Garson *et al.*, *ibid.* **298**, 375 (1982).
17. G. Shaw *et al.*, unpublished data.
18. G. M. Shaw *et al.*, *Science* **226**, 1165 (1984).
19. F. Wong-Staal *et al.*, in preparation.
20. The ability to detect proviral DNA integrated into the host cells' genome at multiple sites depends on the generation of internal viral restriction fragments of the same size that appear as discreet bands on Southern blots. Enzymes that cut the provirus only once, or not at all, will not generate such discreet bands. Given the recognized diversity, or polymorphism, in the restriction patterns of different HTLV-III isolates (*18*), the number of cleavage sites for any particular restriction enzyme could not be known a priori. Thus, to diminish the likelihood of false-negative hybridization results, we digested each of the 15 DNA samples described in this report with two different restriction enzymes (Sst I alone and Bgl II alone) that had been found to generate internal fragments in every HTLV-III isolate previously tested (*18, 19*). As an additional control, the quality of the DNA extracted from each brain sample and the completeness of each restriction enzyme digestion were both assured by rehybridizing the filters to HLA class II probes and demonstrating the expected bands. Conversely, to ensure that the observed bands in the DNA from positive brains were not due to contaminating phage or pBR322, filters were also hybridized to each of these vector DNA's and shown to be negative. Moreover, we could be sure that the positive brain specimens were not contaminated accidentally with cloned HTLV-III DNA, since the viral sequences in the different specimens of brain differed from each other and from other isolates and clones of HTLV-III (*18*) by at least some restriction sites.
21. M. E. Harper, unpublished observations.
22. M. Hirayama *et al.*, *Nature (London)* **301**, 152 (1983).
23. U. Traugott, E. L. Reinherz, C. S. Raine, *J. Neuroimmunol.* **3**, 365 (1982); S. L. Hauser *et al.*, *ibid.* **5**, 197 (1983).
24. C. S. Raine, personal communication.
25. M. B. Gardner *et al.*, *J. Natl. Cancer Inst.* **51**, 1243 (1973); M. B. Gardner, *Curr. Top. Microbiol. Immunol.* **79**, 216 (1978).
26. B. Sigurdsson, H. Thormar, P. A. Palsson, *Arch. Ges. Virusforsch.* **10**, 368 (1960).
27. J. E. Clements and O. Narayan, *Virology* **113**, 412 (1981); S. Molineaux and J. E. Clements, *Gene* **23**, 137 (1983).
28. E. Keshet and H. M. Temin, *J. Virol.* **31**, 376 (1979); S. K. Weller, A. E. Joy, H. M. Temin, *ibid.* **33**, 494 (1980).
29. G. Petursson *et al.*, *Lab. Invest.* **35**, 402 (1976).
30. Specimens of brain were obtained from 15 AIDS patients (see Table 1) either at necropsy or open biopsy and were frozen at −70°C for subsequent analysis. All specimens were from cerebral cortex containing grey and white matter, but in only some instances was the exact region of the cerebrum known. Frozen brain tissue (approximately 1 g) was pulverized under liquid nitrogen, made up to 20 ml in tris (20 m*M*, *p*H 7.4), EDTA (5 m*M*) sodium dodecyl sulfate (5 mg/ml), and proteinase K (100 μg/ml), and incubated at 50°C for 3 hours. The DNA was then adjusted to 0.3*M* Na^+ with sodium acetate (*p*H 6.0), extracted three times with a solution of phenol and chloroform (1:1 by volume) saturated with 50 m*M* tris (*p*H 9.0), extracted once with chloroform alone, and then precipitated with two volumes of absolute ethanol. High molecular weight DNA was dissolved in TE buffer (20 m*M* tris, *p*H 7.4; and 1 m*M* EDTA). The DNA (20 μg) was digested with 150 units of Sst I for 8 hours at 37°C and then subjected to electrophoresis through 0.7 percent agarose slab gels. Gels were blotted in 10× SSC onto 0.1 μm nitrocellulose filters (Schleicher and Schuell). Hybridizations were performed at 37°C for 18 hours in 2.4× SSC, 40 percent formamide, 10 percent dextran sulfate, 1 mg/ml each of bovine serum albumin, polyvinylpyrollidone, and Ficoll, and 20 μg of transfer RNA per milliliter. Filters were washed for 3 hours at 65°C in 1 × SSC. The probe used was the 8.9-kb-long Sst I–Sst I insert from λBH-10 (*9*), 10×10^6 dpm/ml (approximately 2×10^8 dpm/μg). Blots were exposed to Kodak XAR-5 film for 3 days.
31. In situ hybridization was carried out as described by Harper *et al.* (*32*). Briefly, frozen

sections were prepared from OCT-embedded brain tissue, mounted on microscope slides, fixed in 4 percent paraformaldehyde, and stored in 70 percent ethanol at 4°C until hybridized. Slide preparations were then acetylated, treated with 0.1*M* tris-HCl (*p*H 7.0) and 0.1*M* glycine, and hybridized with 10^6 count/min of ^{35}S-labeled RNA transcribed from *p*BH10-R3 [8.9-kb-long Sst I–Sst I viral insert from HTLV-III clone λBH-10 (*9*) subcloned into pSP64]. Hybridization was performed in 50 percent formamide, 2× SSC, and 10 m*M* DTT for 3 hours at 50°C. Slides were rinsed in 50 percent formamide, 2× SSC at 52°C, treated with ribonucleases A and T_1 for 30 minutes, and dehydrated in ethanol. Preparations were autoradiographed with Eastman NTB2 emulsion, exposed at 4°C for 2 days, developed in Dektol developer, and stained with Wright stain. For these in situ hybridization studies, sections were not subjected to DNA strand separation (denaturation), thereby ensuring that hybridization of the HTLV-III probe was to viral RNA and not DNA.

32. M. E. Harper *et al.*, in preparation.

33. We thank L. Rankin and A. B. Minnefor for providing clinical data and postmortem material, L. Marselle for technical assistance, and R. Singer for discussions. G.M.S. was funded through the Intergovernmental Personnel Act of the National Institutes of Health in conjunction with the Ohio State University College of Medicine, and B.H.H. was supported by the German Science Foundation and the Fogarty Center for International Studies. R.W.P. was supported, in part, by New York AIDS institute grant number A0159. L.G.E. is an assistant professor of Neuroscience and Pediatrics at the University of Medicine and Dentistry of New Jersey and is currently a visiting scientist at the NIH.

13 November 1984; accepted 6 December 1984

Targeted Modulation of Acute Inflammation

Abstract. *Localized inflammation of the lungs was induced with the neutrophil chemoattractant,* N*-formylmethionylleucylphenylalanine (FMLP) in combination with magnetically responsive albumin microspheres, a drug carrier that provides efficient, extremely rapid localization in tissue. Intravenous targeting to rat lungs was accomplished by means of an external thoracic magnet. This caused progressive local accumulation of neutrophils, extravascular cell migration, and acute tissue injury. Microscopic findings favored chemotaxis as the principal mechanism of cell accumulation. This system provides a new experimental model for acute alveolar damage, a rapid in vivo assay for drugs that modulate neutrophil chemotaxis, and a new therapeutic approach to focusing inflammation in patients with chemotactic defects.*

Targeted modulation of acute inflammation requires the combination of a potent neutrophil attractant and a drug carrier that provides efficient intravascular targeting, rapid carrier transport across vascular endothelium, and controlled drug release within tissues. The neutrophil chemotactic peptide, *N*-formylmethionylleucylphenylalanine (FMLP) is an ideal local biomodulator because of its high affinity of binding to specific neutrophil receptors and its chemical stability (*1, 2*). However, it is also extremely toxic or lethal when administered in a freely circulating form. This limits the choice of carriers to ones that encapsulate the biomodulator (preventing intravascular availability) and become localized within several minutes of injection. The only carrier that has both of these properties is the magnetically responsive albumin microsphere (*3*). Such spheres localize up to 80 percent of entrapped drug in target tissues when captured with an 8000-gauss magnetic field (*3, 4*).

FMLP spheres were prepared by emulsifying an aqueous mixture of the chemoattractant, human serum albumin, and fluidic magnetite (Fe_3O_4) in cottonseed oil; sonicating to produce particles smaller than 1 μm; and heating at 135°C to obtain controlled FMLP release (*5, 6*). The efficiency of peptide entrapment was 11 percent, and the resulting FMLP and magnetite contents were 0.65 and 20 percent (by weight), respectively. The preparation had a mean particle diameter of 612 nm and a shelf life of more than 1 year. The half-time for release in vitro of [^{3}H]FMLP was 20 minutes (*6, 7*). This was optimal for triggering acute tissue changes. All of the FMLP released between 0 and 24 hours was biologically active, as evaluated by neutrophil chemiluminescence assays (*8, 9*).

An initial bioassay was performed in vivo, without magnetic targeting, by injecting FMLP spheres (20 mg/kg) intravenously into Sprague-Dawley rats. Spheres localized spontaneously in the spleens, and targeting was maximal isotopically in 5 minutes. This was followed by a progressive local accumulation of neutrophils (Table 1). On the basis of these results, a targeted bioassay was designed to test if a lower dose of FMLP spheres (6.6 mg/kg) could be magnetically localized to produce inflammation in a nonreticuloendothelial organ, namely, lung. The dose was selected to produce lung concentrations of total (entrapped plus free) FMLP in the range of $2.9 \times 10^{-6}M$ [which induced a 75 percent maximal chemiluminescence response in vitro (*9*)] and concomitantly to maintain plasma concentrations of free FMLP at levels of $1.9 \times 10^{-8}M$ (which induced <10 percent of maximal chemiluminescence). Spheres were injected intravenously and captured in the right lung with a 5500-gauss gradient (300 gauss per 0.1 cm) magnetic field oriented perpendicular to the right lateral thorax. Lungs were removed 0 to 70 minutes later, and histologic sections were stained by a newly devised method that allows both neutrophil esterase (*10*) and microsphere magnetite iron (*11*) to be visualized in the same tissue section.

Localization of [^{3}H]FMLP spheres occurred during the first circulatory pass and was maximal in the outer 0.5 cm of lung (*12*). The concentration of total FMLP in target tissue reached maximal levels within 5 minutes at $2.9 \times 10^{-6}M$ and remained unchanged over the next 70 minutes. By 70 minutes, 71 percent of this FMLP was estimated (from in vitro kinetics) to be released as free tissue

Table 1. Neutrophil accumulation in rat target tissues after intravenous injection of saline or microspheres.

Treatment	Minutes after injection	Magnet	Neutrophils per ×50 field*
Spleen			
Saline	70	−	46.6 ± 6.0†
Placebo spheres (20 mg/kg)	70	−	49.2 ± 5.2†
FMLP spheres (spheres, 20 mg/kg; FMLP, 47.6 μg/kg)	5	−	49.0 ± 4.0†
	20	−	92.6 ± 14.0‡
	40	−	164.0 ± 17.0‡
	70	−	254.4 ± 18.2‡
Lung			
Saline	70	+	43.4 ± 4.6†
Placebo spheres (6.6 mg/kf)	70	+	40.8 ± 3.8†
FMLP spheres (spheres, 6.6 mg/kg; FMLP, 15.7 μg/kg)	70	−	48.0 ± 4.7†
	5	+	42.4 ± 4.4†
	20	+	74.5 ± 7.7‡
	40	+	122.5 ± 12.9‡
	70	+	163.8 ± 13.5‡

*×50 fields are 800 × 500 μm in area and 5 μm thick; $n = 5$. Data are reported as mean ± 1 standard deviation. †Groups do not differ at $P = 0.05$ (Newman-Keuls multiple comparison test). ‡Each group differs significantly from all others of the respective target tissue at $P < 0.001$ (Newman-Keuls multiple comparison test).