EMBARGOED

Hold for Release: APR 23 1984

2:00 p.m. concurrent with the
Department of Health & Human
Services Press Conference

Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS

Abstract. *A cell system was developed for the reproducible detection of human T-lymphotropic retroviruses (HTLV family) from patients with the acquired immunodeficiency syndrome (AIDS) or with signs or symptoms that frequently precede AIDS (pre-AIDS). The cells are specific clones from a permissive human neoplastic T-cell line. Some of the clones permanently grow and continuously produce large amounts of virus after infection with cytopathic (HTLV-III) variants of these viruses. One cytopathic effect of HTLV-III in this system is the arrangement of multiple nuclei in a characteristic ring formation in giant cells of the infected T-cell population. These structures can be used as an indicator to detect HTLV-III in clinical specimens. This system opens the way to the routine detection of HTLV-III and related cytopathic variants of HTLV in patients with AIDS or pre-AIDS and in healthy carriers, and it provides large amounts of virus for detailed molecular and immunological analyses.*

Epidemiologic data suggest that the acquired immunodeficiency syndrome (AIDS) is caused by an infectious agent that is horizontally transmitted by intimate contact or blood products (*1–3*). Though the disease is manifested by opportunistic infections, predominantly *Pneumocystis carinii* pneumonia (*4*), and by Kaposi's sarcoma (*5*), the underlying disorder affects the patient's cell-mediated immunity (*6*), resulting in absolute lymphopenia and reduced subpopulations of helper T lymphocytes ($OKT4^+$). Moreover, before a complete clinical manifestation of the disease occurs, its prodrome, pre-AIDS, is frequently characterized by unexplained chronic lymphadenopathy or leukopenia involving helper T lymphocytes (*5, 6*). This leads to the severe immune deficiency of the patient and suggests that a specific subset of T cells could be a primary target for an infectious agent. Although patients with AIDS or pre-AIDS are often chronically infected with cytomegalovirus (*7*) or hepatitis B virus (*8*), for various reasons these appear to be opportunistic or coincidental infections. We have proposed that AIDS may be caused by a virus from the family of human T-cell lymphotropic retroviruses (HTLV) (*9*) that includes two major, well-characterized subgroups of human retroviruses, called human T-cell leukemia-lymphoma viruses, HTLV-I (*9–12*) and HTLV-II (*9, 11, 13*). The most common isolate, HTLV-I, is obtained mainly from patients with mature T-cell malignancies (*9, 12*). Seroepidemiological studies, the biological effects of the virus in vitro, and nucleic acid hybridization data indicate that HTLV-I is etiologically associated with the T-cell malignancy of adults that is endemic in certain areas of the south of Japan (*14*), the Caribbean (*15*), and Africa (*16*). HTLV-II was first isolated from a patient with a T-cell variant of hairy cell leukemia (*13*). To date, this is the only reported isolate of HTLV-II from a patient with a neoplastic disease. Virus isolation and seroepidemiological data show that both HTLV-I and HTLV-II can sometimes be found in patients with AIDS (*17*).

That a retrovirus of the HTLV family might be an etiological agent of AIDS was suggested by the findings (i) that another retrovirus, feline leukemia virus, causes immune deficiency in cats (*18*); and that (ii) retroviruses of the HTLV family are T-cell tropic (*12, 19*); (iii) preferentially infect helper T-cells ($OKT4^+$) (*12, 19*); (iv) have cytopathic effects on various human and mammalian cells, as demonstrated by their induction of cell syncytia formation (*20*); (v) can alter some T-cell functions (*21*); (vi) can in some cases selectively kill T-cells (*22*); and (viii) may be transmitted by intimate contact and blood products (*9*). Also consistent with an HTLV etiology were the results of Essex and Lee and their colleagues showing the presence of antibodies to cell membrane antigens of HTLV-infected cells in serum samples from more than 40 percent of patients with AIDS (*23*). This antigen has since been defined as part of the envelope of HTLV (*24*). The more frequent detection in AIDS patients of antibodies to a membrane protein rather than to HTLV-I internal structural core proteins (*25*), together with the low incidence of isolations of HTLV-I or HTLV-II from AIDS patients, also suggested that a new variant of HTLV might be present.

The original detection and isolation of HTLV-I were made possible by the discovery of T-cell growth factor (TCGF) (*26*), also called interleukin 2 (IL-2), which stimulates the growth of different subsets of normal and neoplastic mature T-cells (*27*), and by the development of sensitive assays for reverse transcriptase (RT), an enzyme characteristic of retroviruses (*28*). The procedures used previously for the transmission and continuous production of HTLV-I and -II were first worked out in mammalian cells transformed by avian sarcoma virus (*29*). These methods involved cocultivation of the transformed cells with cells permissive for the particular virus strain. Normal human T cells in cocultivation experiments preferentially yielded HTLV of both subgroups. Some of these viruses showed an immortalizing (transforming) capability for certain target T cells (*9, 12*). We thought that HTLV variants that have cytopathic effects on their target cells but do not immortalize them might be more important in the cause of AIDS. In fact, such variants were frequently but only transiently detected when normal T cells were used as targets in cocultivation or cell-free transmission experiments. This transience was our main obstacle to the isolation of these cytopathic variants of HTLV from patients with AIDS or pre-AIDS. We subsequently found a cell line that is highly susceptible to and permissive for cytopathic variants of HTLV. This cell line can grow permanently after infection with the virus. We report here the estab-

lishment and characterization of this new immortalized T-cell population and its use in the isolation and continuous high-level production of HTLV variants from patients with AIDS and pre-AIDS.

Several neoplastic human cell lines established in vitro were assayed for susceptibility to infection with HTLV-I and -II and with many of the more cytopathic retroviruses isolated from AIDS patients (*30*). One neoplastic aneuploid T-cell line, derived from an adult with lymphoid leukemia, was found to be susceptible to infection with the new cytopathic virus isolates. This cell line, termed HT, has produced HTLV-variants in sufficient quantities to permit the development of specific immunologic reagents and nucleic acid probes that can be used to characterize new isolates and compare them with HTLV-I and HTLV-II (*30*). These cytopathic variants differ from HTLV-I and -II not only in their biological effects but also in several immunological assays and in their morphology (*31*). They nevertheless have many properties similar to HTLV-I and -II. For example, they are T4 lymphotropic, they have a similar RT (*30*), they cross-react with several structural proteins in heterologous radioimmune assays with serum from AIDS patients and with antisera to the virus raised in animals (*31*), and they induce syncytia. These new HTLV isolates are collectively designated HTLV-III, although it is not yet proved that they are identical.

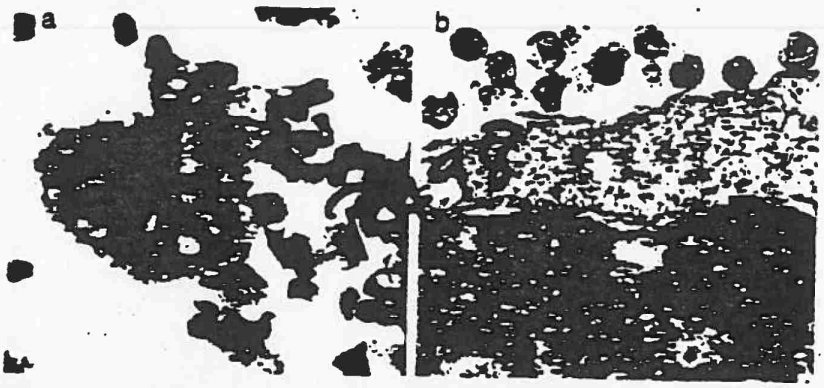

Fig. 1. Light and electron microscopic examination of clone H4/HTLV-III. (a) H4/HTLV-III cells were characterized by the presence of large multinucleated cells that showed, with Giemsa-Wright staining, a characteristic arrangement of their nuclei (×350). (b) Electron micrograph of the cells showing the presence of extracellular viral particles (×60,000).

Table 1. Response of cloned T-cell populations to infection with HTLV-III. Single-cell clones were isolated as described (*34*, *35*) from a long-term cultured aneuploid HT cell line exhibiting mature T-cell phenotype [OKT3$^+$ (62 percent), OKT4$^+$ (39 percent), and OKT8$^-$] as determined by cytofluorometry with a fluorescence-activated cell sorter. The cultures are routinely maintained in RPMI 1640 medium containing 20 percent fetal calf serum (FCS) and antibiotics. The terminal cell density of the parental cell culture, seeded at a concentration of 2×10^5 cells per milliliter of culture media, was in the range of 10^6 to 1.5×10^6 cells per milliliter after 5 days of culture.

Characteristics after infection	Clones*							
	H3	H4	H6	H9	H17	H31	H35	H38
Total cell number ($\times 10^6$)								
At 6 days	1	1.5	1.5	0.3	0.4	0.3	0.5	1.8
At 14 days	2.2	7.3	7.5	10	4.7	5.0	4.5	3.2
Multinucleated cells (%)*								
At 6 days	24	42	32	7	13	14	30	45
At 14 days	45	48	45	30	22	45	60	60
Immunofluorescence positive cells (%)†								
At 6 days								
Rabbit antiserum to HTLV-III	55	56	32	32	39	21	10	87
Patient serum (E.T.)	56	29	21	ND	ND	ND	ND	73
At 14 days								
Rabbit antiserum to HTLV-III	50	74	60	97	71	40	20	80
Patient serum	45	47	56	78	61	43	22	89
Reverse transcriptase activity ($\times 10^4$ cpm/ml)‡								
At 6 days	2.4	1.8	2.1	4.1	2.6	1.4	1.7	2.5
At 14 days	16.2	18.1	16.1	20.2	17.1	13.4	15.1	18.2

*Cell smears were prepared from cultures 6 and 14 days after infection and stained with Wright-Giemsa. Cells with more than five nuclei were considered to be multinucleated. Cloned cells from uninfected cultures also contained some multinucleated giant cells; however, the arrangement of the multiple nuclei in a characteristic ring formation (see Fig. 1a) was lacking and the number of these cells was much less (0.7 to 10 percent). †Cells were washed with phosphate-buffered saline (PBS) and resuspended in the same buffer at concentration 10^6 cells per milliliter. Approximately 50 μl of cell suspension was spotted on a slide, air dried, and fixed in acetone for 10 minutes at room temperature. Slides were stored at −20°C until use. Twenty microliters of either rabbit antiserum to HTLV-III (diluted 1:2000 in PBS) or serum from the patient (E.T.) diluted 1:8 in PBS was applied to cells and incubated for 30 minutes at 37°C. The fluorescein-conjugated antiserum to rabbit or human immunoglobulin G was diluted and applied to the fixed cells for 30 minutes at room temperature. Slides were then washed extensively before microscopic examinations. The uninfected parental cell line as well as the clones were consistently negative in these assays. ND, not done. ‡Virus particles were precipitated from cell-free supernatant as follows: 0.3 ml of 4*M* NaCl and 3.6 ml of 30 percent (weight to volume) polyethylene glycol (Carbowax 6000) were added to 8 ml of harvested culture fluids and the suspension was placed on ice overnight. The suspension was centrifuged in a Sorvall RC-3 centrifuge at 2000 rev/min at 4°C for 30 minutes. The precipitate was resuspended in 500 μl of 50 percent (by volume) glycerol (25 m*M* tris-HCl, *pH* 7.5, 5 m*M* dithiothreitol, 150 m*M* KCl, and 0.025 percent Triton X-100). Virus particles were disrupted by addition of 100 μl of 0.9 percent Triton X-100 to 1.5*M* KCl. Reverse transcriptase assays were performed as previously described (*10*, *28*) (see comments to Fig. 2b) and expressed in counts per minute per milliliter culture medium.

The cell line HT was tested for HTLV before being infected in vitro and was negative by all criteria including lack of proviral sequences (*32*). Continuous production of HTLV-III was obtained after repeated exposure of parental HT cells (3×10^6 cells pretreated with polybrene) to concentrated culture fluids harvested from short-term cultures of T-cells (grown with TCGF) obtained from patients with AIDS or pre-AIDS. The concentrated fluids were first shown to contain particle-associated RT. When cell proliferation declined, usually 10 to 20 days after exposure to the culture fluids, the fresh (uninfected) HT cells were added to the cultures. Culture fluids from the infected parental cell line were positive for particulate RT activity, and about 20 percent of the infected cell population was positive in an indirect immune fluorescent assay (IFA) in which we used serum from a hemophilia patient with pre-AIDS (patient E.T.). Serum from E.T. also contained antibodies to proteins of disrupted HTLV-III (*33*) but did not react with proteins from cells infected with HTLV-I or HTLV-II.

The parental T-cell population was extensively cloned in order to select the most permissive clones that would preserve high rates of growth and virus production (for example, see clones 4 and 9 in Table 1). A total of 51 single-cell clones were obtained by both capillary (*34*) and limited dilution (*35*) techniques using irradiated mononuclear cells from peripheral blood of a healthy donor as a feeder. The clones were infected with HTLV-III by exposure to concentrated

virus (2×10^6 cells of each clone and 0.1 ml of virus). Then cell growth and morphology, expression of cellular viral antigens, and RT activity in culture fluids were assessed 6 and 14 days after infection. Results for eight of these clones are shown in Table 1. Although all of these clones were susceptible to and permissive for the virus, there were considerable differences in their ability to proliferate after infection. For example, the cell number decreased by 10 to 90 percent from the initial cell count within 6 days after infection. The percentage of T-cells positive for viral antigens ranged from 10 to 80 percent, as determined by immunofluorescence assays with serum from patient E.T. and with antiserum from rabbits infected repeatedly with disrupted HTLV-III. At 14 days after infection, the total cell number and the proportion of HTLV-III positive cells had increased in all eight clones. The virus positive cultures consistently showed a high proportion of round giant cells containing numerous nuclei (Fig. 1a). These cells resemble those induced by HTLV-I and -II (*9*) except that the nuclei exhibit a characteristic ring formation. Electron microscopic examinations showed that the cells released considerable amounts of virus (Fig. 1b).

Both virus production and cell viability of the infected clone H4 (H4/HTLV-III) were monitored for several months. Although virus production fluctuated (Fig. 2a), culture fluids harvested at approximately 14-day intervals consistently exhibited particulate RT activity which has been followed for over 5 months. The viability of the cells ranged from 65 to 85 percent and the doubling time of the cell population was approximately 30 to 40 hours (data not shown). Thus the data show that this permanently growing T-cell population can continuously produce HTLV-III.

The yield of virus from H4/HTLV-III cells was assessed by purification of concentrated culture fluids through a sucrose density gradient and assays of particulate RT activity in each fraction collected from the gradient. As shown in Fig. 2b, the highest RT activity was found at a density of 1.16 g/ml, which is similar to other retroviruses. The highest RT activity was found in the fractions with the largest amount of virus, as determined by electron microscopy. The actual number of viral particles determined by this method was estimated (*36*) to be about 10^{11} per liter of culture fluid.

We have used clones H4 and H9 for the long-term propagation of HTLV-III from patients with AIDS and pre-AIDS.

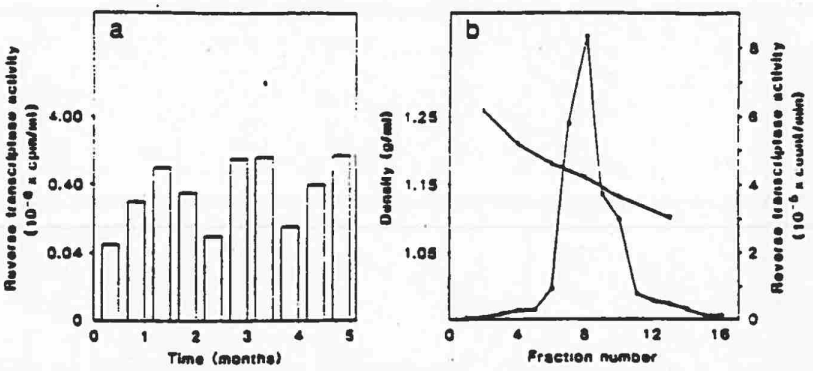

Fig. 2. (a) Continuous HTLV-III production from H4/HTLV-III in long-term culture was characterized by fluctuation in the amount of released virus as assessed by RT activity in the culture fluid (for details, see Table 1 and Fig. 2b). Viability of the infected cells was in the range of 60 to 90 percent. (b) Sucrose density gradient banding of HTLV-III showed the highest particulate RT activity at a density of 1.16 g/ml. A cell-free virus concentrate from a culture of H4/HTLV-III was layered on a 20 to 60 percent (by weight) sucrose gradient in 10 m*M* tris-HCl (*p*H 7.4) containing 0.1 *M* NaCl and 1 m*M* EDTA and centrifuged overnight at 35,000 rev/min in a Spinco SW47 rotor. Fractions of 0.7 ml were collected from the bottom of the gradient and portions were assayed for RT (●) with $(dT)_{15} \cdot (A)_n$ being used as the primer template and Mg^{2+} as the divalent cation according to the methods described earlier (*19, 28*). Density of sucrose (×) was determined by refractive index measurements.

HTLV-III was isolated from four patients by the cocultivation method and from one patient by cell-free infection of these T-cell clones (Table 2). The transmission was monitored by RT activity, electron microscopic examinations and expression of viral protein. When the H4 cells thus infected were fixed with acetone and tested with rabbit antiserum to HTLV-III and with serum from patients E.T., the percentage of positive cells was between 5 and 80 percent. HTLV-III has also been isolated in our laboratory from a total of 48 patients by the more conventional methods for isolation of HTLV (*30*). Some of these isolates have now successfully been transmitted to the HT clones for production and detailed analyses.

A few T-lymphocyte retroviruses that differed from HTLV-I and -II but were associated with lymphadenopathy syndrome were detected earlier (*37, 38*). One such virus, called LAV, was reported to be unrelated to HTLV-I or -II (*38*). Moreover, serum samples from 37.5 percent of patients with AIDS were found to react with it (*38*). In contrast, HTLV-III

Table 2. Isolation of HTLV-III from patients with AIDS and pre-AIDS.

Patient*	Diagnosis	Origin	Virus expression†			
			RT Activity (× 10^4 cpm)	Percent positive in indirect IFA with immune fluorescence assay		Electron microscopy
				Rabbit antiserum	Serum from E.T.	
R.F.	AIDS (heterosexual)	Haiti	0.25	80	33	ND
S.N.	Hemophiliac (lymphadenopathy)	United States	6.3	10	ND	–
B.K.	AIDS (homosexual)	United States	0.24	44	5	–
L.S.	AIDS (homosexual)	United States	0.13	64	19	–
W.T.	Hemophiliac (lymphadenopathy)	United States	3.2	69	ND	ND

*Cocultivation with H4 recipient T-cell clone was performed with fresh mononuclear cells from peripheral blood of patients R.F. and S.N., respectively. For patients B.K. and L.S. cocultivation was performed with T cells grown in the presence of exogenous TCGF (10 percent by volume) for 10 days. The ratio of recipient to donor (patient) cells was 1:5. The mixed cultures were maintained in RPMI 1640 medium containing 20 percent FCS and antibiotics) in the absence of exogenous TCGF. H9 cells were also infected by exposing the cells to concentrated culture fluids harvested from T-cell cultures of patient W.T. The cultures were grown in the presence of exogenous TCGF for 2 weeks before the culture fluids were harvested and concentrated. Cells of H9 clones were treated with polybrene (2 μg/ml) for 20 minutes and 2×10^6 cells were exposed for 1 hour to 0.5 ml of 100-fold concentrated culture fluids positive for particulate RT activity. †HTLV-III virus expression in cells infected by the coculture and cell-free methods was assayed approximately 1 month after cultivation in vitro. Note a considerable fluctuation in HTLV-III expression. For details of the RT and indirect immunofluorescence assays see Table 1.

is related to HTLV-I and -II (*31*, *39*) and, by all criteria, this new virus belongs to the HTLV family of retroviruses. In addition, more than 85 percent of serum samples from AIDS patients are reactive with proteins of HTLV-III (*33*). These findings suggest that HTLV-III and LAV may be different. However, it is possible that this is due to insufficient characterization of LAV because the virus has not yet been transmitted to a permanently growing cell line for true isolation and therefore has been difficult to obtain in quantity.

The transient expression of cytopathic variants of HTLV in cells from AIDS patients and the previous lack of a cell system that could maintain growth and still be susceptible to and permissive for the virus represented a major obstacle in detection, isolation, and elucidation of the precise causative agent of AIDS. The establishment of T-cell populations that continuously grow and produce virus after infection opens the way to the routine detection of cytopathic variants of HTLV in AIDS patients and provides the first opportunity for detailed immunological (*31*, *33*) and molecular analyses of these viruses.

MIKULAS POPOVIC
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205
M. G. SARNGADHARAN
Department of Cell Biology, Litton Bionetics, Inc., Kensington, Maryland 20895
ELIZABETH READ
ROBERT C. GALLO
Laboratory of Tumor Cell Biology, National Cancer Institute

References and Notes

1. Centers for Disease Control Task Force on Kaposi's Sarcoma and Opportunistic Infections, *N. Engl. J. Med.* **306**, 248 (1982).
2. J. P. Hanrahan, G. P. Wormser, C. P. Maguire, L. J. DeLorenzo, G. Davis, *ibid.* **307**, 498 (1982).
3. J. W. Curran *et al.*, *ibid.* **310**, 69 (9184).
4. "Pneumocystis pneumonia—Los Angeles," *Morbid. Mortal. Weekly Rep.* **30**, 250 (1981).
5. "Kaposi's sarcoma and pneumocystis pneumonia among homosexual men—New York City and California," *ibid.*, p. 305; A. E. Friedman-Klein *et al.*, *Ann. Int. Med.* **96**, 693 (1982).
6. M. Gottlieb *et al.*, *N. Engl. J. Med.* **305**, 1425 (1981); J. Masur *et al.*, *ibid.* **305**, 1431 (1981).
7. C. Urmacher, P. Myskowski, M. Ochoa, M. Kris, B. Safai, *Am. J. Med.* **72**, 569 (1982).
8. D. R. Francis and J. E. Maynard, *Epidemiol. Rev.* **1**, 17 (1979); N. Clumeck *et al.*, *N. Engl. J. Med.* **310**, 492 (1984).
9. R. C. Gallo, in *Cancer Surveys*, L. M. Franks, L. M. Wyke, R. A. Weiss, Eds. (Oxford Univ. Press, Oxford, in press).
10. B. J. Poiesz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980); M. Yoshida, I. Miyoshi, Y. Hinuma, *ibid.* **79**, 2031 (1982).
11. M. S. Reitz, M. Popovic, B. F. Haynes, S. C. Clark, R. C. Gallo, *Virology* **26**, 588 (1983).
12. M. Popovic *et al.*, *Science* **219**, 856 (1983).
13. V. S. Kalyanaraman *et al.*, *ibid.* **218**, 571 (1982).
14. Y. Hinuma *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6476 (1981); M. Robert-Guroff *et al.*, *Science* **215**, 975 (1982); V. S. Kalyanaraman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, (1982).
15. W. A. Blattner *et al.*, *Int. J. Cancer* **30**, 257 (1982).
16. W. C. Saxinger *et al.*, in *Human T-Cell Leukemia Viruses*, R. C. Gallo, M. Essex, L. Gross, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., in press).
17. R. C. Gallo *et al.*, *Science* **220**, 865 (1983); E. P. Gelmann *et al.*, *ibid.*, p. 862; M. Popovic *et al.*, in preparation.
18. M. Essex, W. D. Hardy, Jr., S. M. Cotter, R. M. Jakowski, A. Sliski, *Infect. Immun.* **11**, 470 (1975); W. D. Hardy, Jr., *et al.*, *Cancer Res.* **36**, 582 (1976); L. J. Anderson, O. Jarret, H. M. Laird, *J. Natl. Cancer Inst.* **47**, 807 (1971).
19. R. C. Gallo *et al.*, *Cancer Res.* **43**, 3892 (1983); F. Wong-Staal *et al.*, *Nature (London)* **302**, 626 (1983).
20. K. Nagy, P. Clapham, R. Cheinsong-Popov, R. A. Weiss, *Int. J. Cancer* **32**, 321 (1983).
21. M. Popovic *et al.*, in preparation.
22. H. Mitsuya, H. G. Guo, M. Megson, C. D. Trainor, M. S. Reitz, S. Broder, *Science*, **223**, 1293 (1984).
23. M. Essex *et al.*, *Science* **220**, 859 (1983).
24. J. Schüpbach, M. G. Sarngadharan, R. C. Gallo, *Science*, in press; T. H. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
25. M. Robert-Guroff *et al.*, in preparation.
26. D. A. Morgan, F. W. Ruscetti, R. C. Gallo, *Science* **193**, 1007 (1976).
27. F. W. Ruscetti, D. A. Morgan, R. C. Gallo, *J. Immunol.* **119**, 131 (1977); B. J. Poiesz, F. W. Ruscetti, J. W. Mier, A. M. Woods, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6134 (1980).
28. R. C. Gallo, F. W. Ruscetti, R. E. Gallagher, in *Hematopoietic Mechanisms*, B. Clarkson, P. A. Marks, J. Till, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1978), vol. 5, p. 671.
29. J. Svoboda, *Natl. Cancer Inst. Monogr.* **17**, 377 (1964); J. Svoboda and R. Dourmashkin, *J. Gen. Virol.* **4**, 523 (1969); M. Popovic, J. Svoboda, J. Suru, A. Vaheri, L. Ponten, *Int. J. Cancer* **19**, 834 (1977); M. Popovic, J. Svoboda, F. L. Kisselyov, K. Polakova, *Folia Biol.* **26**, 244 (1980).
30. R. C. Gallo *et al.*, *Science* **224**, 500 (1984).
31. J. Schüpbach, M. Popovic, R. V. Gilden, M. A. Gonda, M. G. Sarngadharan, R. C. Gallo, *Science* **224**, 503 (1984).
32. G. Shaw and F. Wong-Staal, unpublished data.
33. M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *Science* **224**, 506 (1984).
34. M. Popovic, M. Grofova, N. Valentova, D. Simkovic, *Neoplasma* **18**, 257 (1971).
35. H. F. Bach, B. J. Alter, B. M. Widmer, M. S. Segall, D. Dunlap, *Immunol. Rev.* **54**, 5 (1981).
36. J. Monroe and W. Brandt, *Appl. Microbiol.* **20**, 259 (1970).
37. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983).
38. L. Montagnier *et al.*, in *Human T-Cell Leukemia Viruses*, R. C. Gallo, M. Essex, L. Gross, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., in press).
39. S. Arya *et al.*, in preparation.
40. We thank B. Kramarsky for help in electron microscopic examination of HTLV-III infected cells, E. Richardson and R. Zicht for technical help, and A. Mazzuca for her editorial assistance.

30 March 1984; accepted 19 April 1984

Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS

Abstract. *Peripheral blood lymphocytes from patients with the acquired immunodeficiency syndrome (AIDS) or with signs or symptoms that frequently precede AIDS (pre-AIDS) were grown in vitro with added T-cell growth factor and assayed for the expression and release of human T-lymphotropic retroviruses (HTLV). Retroviruses belonging to the HTLV family and collectively designated HTLV-III were isolated from a total of 48 subjects including 18 of 21 patients with pre-AIDS, 3 of 4 clinically normal mothers of juveniles with AIDS, 26 of 72 adult and juvenile patients with AIDS, and from 1 of 22 normal male homosexual subjects. No HTLV-III was detected in or isolated from 115 normal heterosexual subjects. The number of HTLV-III isolates reported here underestimates the true incidence of the virus since many specimens were received in unsatisfactory condition. Other data show that serum samples from a high proportion of AIDS patients contain antibodies to HTLV-III. That these new isolates are members of the HTLV family but differ from the previous isolates known as HTLV-I and HTLV-II is indicated by their morphological, biological, and immunological characteristics. These results and those reported elsewhere in this issue suggest that HTLV-III may be the primary cause of AIDS.*

The acquired immunodeficiency syndrome known as AIDS was initially recognized as a separate disease entity in 1981 (*1*). Groups reported to be at risk for AIDS include homosexual or bisexual males (about 70 percent of reported cases), intravenous drug users (about 17 percent of cases), Haitian immigrants to the United States (about 5 percent of cases). Also at risk are heterosexual contacts of members of the highest risk group, hemophiliacs treated with blood products pooled from donors, recipients of multiple blood transfusions, and infants born of parents belonging to the high-risk groups (*2*). AIDS is diagnosed as a severe, unexplained, immune deficiency that usually involves a reduction in the number of helper T lymphocytes and is accompanied by multiple opportunistic infections or malignancies. A number of other clinical manifestations, when occurring in members of a group at risk for AIDS, are identified as its prodrome (pre-AIDS). These include unexplained chronic lymphadenopathy or leukopenia involving a reduction in the number of helper T lymphocytes (*1*, *2*). The increasing incidence of this disease, the types of patients affected, and other epidemiological data suggest the existence of an infectious etiologic agent that

can be transmitted by intimate contact or by whole blood or separated blood components (2). As indicated by Popovic *et al.* (3), we and others have suggested that specific human T-lymphotropic retroviruses (HTLV) cause AIDS (4, 5). Many properties of HTLV are consistent with this idea (6).

An association of members of the HTLV family with T lymphocytes from some AIDS or pre-AIDS patients was reported previously. For example, the first subgroup of HTLV to be characterized, HTLV-I, was isolated recently from T cells from about 10 percent of AIDS patients, and a virus related to HTLV-II was isolated from one AIDS patient (4). Another HTLV isolate was obtained from the lymph nodes of a patient with lymphadenopathy and at risk for AIDS (7). This isolate has been difficult to grow in quantities sufficient to permit its characterization. HTLV proviral DNA was detected in T lymphocytes from two additional AIDS patients (8) and HTLV-related antigens were found in another two patients (4). Studies in which disrupted HTLV-I or the purified structural proteins (p24 or p19) were used to detect antibodies in serum samples from patients with AIDS and pre-AIDS indicated that 10 to 15 percent of the patients had been exposed to HTLV-I (9). Essex and his co-workers, using HTLV-infected T-lymphocyte cultures to detect antibody in serum samples, found that about 35 percent of patients with AIDS and pre-AIDS had been exposed to HTLV (5). Further studies suggested that at least some of the antigens detected in this system were products of the genome of a member of the HTLV family (10), but it was not known whether the antibodies were specifically against HTLV-I, HTLV-II, or a virus of a different subgroup.

With the availability of large quantities of HTLV-III (3), it became possible to develop specific immunological reagents that would facilitate its characterization. HTLV-III was found to share many properties with other HTLV isolates (6), but it was morphologically, biologically, and antigenically distinguishable (3, 11). Here we describe the detection and isolation of HTLV-III from a large number of patients with AIDS and pre-AIDS.

For these studies we used cell culture conditions previously developed in our laboratory for the establishment of T lymphocytes in culture and for the detection and isolation of HTLV-I and HTLV-II from leukemic donors (12). Evidence for the presence of HTLV-III included: (i) viral reverse transcriptase (RT) activity (12) in supernatent fluids; (ii) transmission of virus by coculturing T cells with irradiated donor cells or with cell-free fluids (3, 13); (iii) observation of virus by electron microscopy (12, 13); and (iv) the expression of viral antigens in indirect immunofluorescence assays using serum from a patient positive for antibodies to HTLV-III as described (5, 11), or antisera prepared against purified, whole disrupted HTLV-III (11). Cells and supernatant fluids were also monitored for the expression of HTLV-I and HTLV-II by using antibodies to the viral structural proteins p19 and p24 and by indirect immunofluorescence and radioimmunoprecipitation procedures (14).

As summarized in Table 1, we found HTLV-III in 18 of 21 samples from patients with pre-AIDS, from three of four clinically normal mothers of juvenile

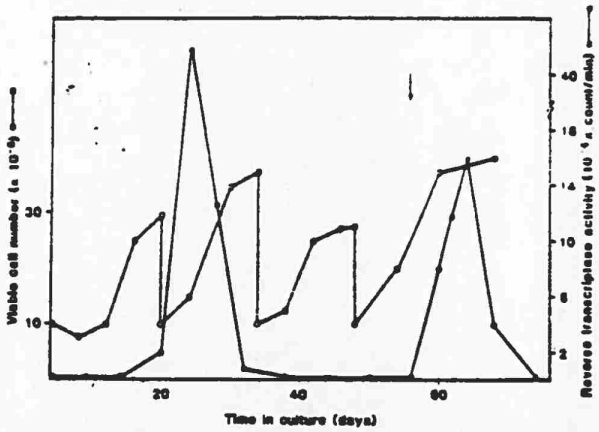

Fig. 1. Reverse transcriptase activity from lymphocytes established in cell culture from a patient with pre-AIDS. Viable cell number and Mg^{++}-dependent RT activity were determined by established procedures (13). Symbols: ○, viable cell number in 1.5 ml of growth medium; ●, RT in 5 μl of fivefold concentrated conditioned medium sampled at the indicated time. A sudden vertical drop in the dashed curve indicates the time of subculturing of cells to the indicated cell number. Arrow indicates the time of addition of rabbit antiserum to α-interferon to a portion of the cultured cells (also see legend to Table 1).

Fig. 2. Transmission electron micrographs of fixed, sectioned lymphocytes from a patient with pre-AIDS. (A) ×10,000 x; (B) ×30,000 x; (C and D) ×100,000 x.

AIDS patients, three of eight juvenile AIDS patients, 13 of 43 of adult AIDS patients with Kaposi's sarcoma, and 10 of 21 adult AIDS patients with opportunistic infections. Virus was detected in only one of 22 samples from clinically normal, nonpromiscuous homosexual males believed to be at only moderate risk for AIDS. It is interesting, however, that 6 months after these tests were conducted the one positive normal homosexual subject developed AIDS. In no instance, 0 of 115, was virus detected in or isolated from cells of the normal volunteers. Samples from 15 of these were tested under rigorously controlled conditions, which included addition of antibody to α-interferon.

Primary cells from patients usually produce virus for 2 to 3 weeks (Fig. 1). After this time the production of virus declines even though the culture may contain actively replicating cells that can be maintained for long periods in the presence of added T-cell growth factor (TCGF). In some instances virus release can be reinitiated by the addition of antibody to α-interferon (Fig. 1). The HTLV-III–producing cell cultures were characterized by established immunological procedures (*13*). They were predominantly T lymphocytes (E rosette receptor-positive, $OKT3^+$ and $Leu1^+$) with a helper-inducer (T4) phenotype ($OKT4^+$ and $Leu3^-$).

The fairly uniform morphological appearance of HTLV-III is shown in Fig. 2. The diameter of the virus is 100 to 120 nm, and it is produced in high numbers from infected cells by budding from the cell membrane. A possibly unique feature of this virus is the cylindrical shaped core observed in many mature virions.

The incidence of virus isolation reported here probably underestimates its true incidence since many tissue specimens were not received or handled under what we now recognize as optimal conditions (*15*). This is particularly so for the samples received from late-stage AIDS patients. Such samples usually contain many dying cells and very few viable T4 lymphocytes. However, a high proportion of patients with AIDS and pre-AIDS have circulating antibody to HTLV-III (*11*).

The HTLV-III produced by cultured T cells from patients with AIDS and pre-AIDS is highly infectious and can be readily transmitted to fresh umbilical cord blood and adult peripheral blood or bone marrow lymphocytes. The production of HTLV-III by these cells is transient, often declining to undetectable levels by 2 to 3 weeks after infection (data not shown). The transmission of HTLV-III to an established T-cell line (*3*), however, now makes possible its production in large quantities for detailed analyses and for development of reagents for its detection (*3*, *11*).

That the viruses we have named HTLV-III belong to the HTLV family is indicated by their T cell tropism, Mg^{++}-dependent RT of high molecular weight, antigenic cross-reactivity with HTLV-I and -II (*11*), cytopathic effects on T lymphocytes (*3*), and their morphological appearance in the electron microscope. HTLV-III also contains some structural proteins similar in size to those of other members of the HTLV family (*11*).

These studies of HTLV-III isolates from patients with AIDS and pre-AIDS and from some healthy individuals at risk for AIDS provide strong evidence of a causative involvement of the virus in AIDS.

ROBERT C. GALLO
SYED Z. SALAHUDDIN
MIKULAS POPOVIC
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205

GENE M. SHEARER
Immunology Branch, National Cancer Institute

MARK KAPLAN
Division of Infectious Diseases, North Shore University Hospital, Manhusset, New York 11030

BARTON F. HAYNES
THOMAS J. PALKER
Department of Medicine, Duke University School of Medicine, Durham, North Carolina 27710

ROBERT REDFIELD
Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20012

JAMES OLESKE
Division of Allergy, Immunology, and Infectious Disease, University of Medicine and Dentistry of New Jersey, Newark 07103

BIJAN SAFAI
Dermatology Service, Memorial Sloan Kettering Cancer Center, New York, New York 10021

GILBERT WHITE
PAUL FOSTER
Department of Medicine, University of North Caroiina, Chapel Hill 27514

PHILLIP D. MARKHAM
Department of Cell Biology, Litton Bionetics, Inc., Kensington, Maryland 20895

Table 1. Detection and isolation of HTLV-III from patients with AIDS and pre-AIDS. Peripheral blood leukocytes were banded in Ficoll-Hypaque, incubated in growth medium (RPMI-1640, 20 percent fetal bovine serum, and 0.29 mg of glutamine per milliliter) containing phytohemagglutinin (PHA-P; 5 μg/ml) for 48 hours at 37°C in a 5 percent CO_2 atmosphere. They were then refed with growth medium containing 10 percent purified T-cell growth factor (TCGF). Cells and conditioned media from these lymphocytes were assayed for the presence of HTLV-III. Samples exhibiting more than one of the following were considered positive: repeated detection of a Mg^{2+}-dependent reverse transcriptase activity in supernatant fluids; virus observed by electron microscopy; intracellular expression of virus-related antigens detected with antibodies from seropositive donors or with rabbit antiserum to HTLV-III; or transmission of particles, detected by RT assays or by electron microscopic observation, to fresh human cord blood, bone marrow, or peripheral blood T-lymphocytes. All isolates are distinguishable from HTLV-I or HTLV-II by several criteria and are classified as HTLV-III on the basis of similar morphological features observed by electron microscopy (Fig. 1); similar cytopathic effects (*3*); antigenic cross-reactivity (*11*); and nucleic acid analysis (*16*).

Diagnosis*	Number positive for HTLV-III	Number tested	Percent positive
Pre-AIDS	18	21	85.7
Clinically normal mothers of juvenile AIDS patients	3	4	75.0
Juvenile AIDS	3	8	37.5
Adult AIDS with Kaposi sarcoma	13	43	30.2
Adult AIDS with opportunistic infections	10	21	47.6
Clinically normal homosexual donors	1	22	4.5
Clinically normal heterosexual donors	0	115	0

*With the exception of the normal heterosexual donors and some of the clinically normal mothers of juvenile AIDS patients, all others belong to one of the groups of people identified as being at risk for AIDS (homosexual males, intravenous drug users, Haitian immigrants, heterosexual contacts of members of a group at risk, hemophiliacs treated with pooled blood products, recipients of multiple blood transfusions, and infants born of parents belonging to other groups at risk). Pre-AIDS includes patients with unexplained chronic lymphadenopathy and leukopenia, with an inverted T4 (helper)/T8 (suppressor) lymphocyte ratio. The clinically normal, nonpromiscuous, homosexual subjects are from Washington, D.C., and are believed to be at moderate risk. The clinically normal heterosexual donors include both male and female subjects believed not to be at risk for AIDS.

References and Notes

1. M. S. Gottlieb *et al.*, *N. Engl. J. Med.* **305**, 1425 (1981); H. Masur *et al.*, *ibid.* **305**, 1431 (1981); F. P. Siegal *et al.*, *ibid.* **305**, 1439 (1981).
2. Centers for Disease Control, *Morbid. Mortal. Week. Rpt.* **32**, 688 (1984); J. W. Curran *et al.*, *N. Engl. J. Med.* **310**, 69 (1984); G. B. Scott, B. E. Buck, J. G. Letterman, F. L. Bloom, W. P. Parks, *ibid.* **310**, 76 (1984); J. Oleske *et al.*, *J. Am. Med. Assoc.* **249**, 2345 (1983).
3. M. Popovic *et al.*, *Science* **224**, 497 (1984).
4. R. C. Gallo *et al.*, *ibid.* **220**, 865 (1983); M. Popovic and R. C. Gallo, in preparation.
5. M. Essex *et al.*, *Science* **220**, 859 (1983); M. Essex *et al.*, in *Human T-Cell Leukemia Viruses*, R. C. Gallo, M. Essex, L. Gross, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., in press).
6. R. C. Gallo *et al.*, *Cancer Res.* **43**, 3892 (1983); in *Cancer Surveys*, L. M. Franks, L. M. Wyke, R. A. Weiss, Eds. (Oxford Univ. Press, Oxford, in press).
7. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983).
8. E. P. Gelmann *et al.*, *ibid.*, p. 862.
9. M. Robert-Guroff *et al.*, in *Human T-Cell Leukemia Viruses*, R. C. Gallo, M. Essex, L. Gross, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., in press).
10. T. H. Lee *et al.*, personal communication; J. Schüpbach, M. G. Sarngadharan, R. C. Gallo, in preparation.
11. M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schupbach, R. C. Gallo, *Science* **224**, 506 (1984); J. Schupbach *et al.*, *ibid.*, p. 503.
12. 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); V. S. Kalyanaraman *et al.*, *Science* **218**, 571 (1982).
13. P. D. Markham *et al.*, *Int. J. Cancer* **31**, 413 (1983); P. D. Markham, S. Z. Salahuddin, B. Macchi, M. Robert-Guroff, R. C. Gallo, *ibid.* **35**, 13 (1984); S. Z. Salahuddin *et al.*, *Virology* **129**, 51 (1983).
14. M. Robert-Guroff and R. C. Gallo, *Blut* **47**, 1 (1983); V. S. Kalyanaraman, M. G. Sarngadharan, B. J. Poiesz, F. W. Ruscetti, R. C. Gallo, *J. Virol.* **31**, 906 (1981); C. Saxinger and R. C. Gallo, *Lab. Invest.* **49**, 371 (1983).
15. For virus isolation, samples of freshly drawn, heparinized peripheral blood or bone marrow, yielding a minimum of 10^7 viable cells (greater than 90 percent), are needed. These samples must contain the cells of interest, namely $OKT4^+$ T cells, which are frequently depleted in AIDS patients.
16. S. Arya *et al.*, in preparation.
17. We thank M. Gonda for electron microscopy and A. Patel, S. Roberson, A. Fladager, and E. Reid for technical assistance. We are also indebted to many clinical collaborators who provided patient materials.

30 March 1984; accepted 19 April 1984

Serological Analysis of a Subgroup of Human T-Lymphotropic Retroviruses (HTLV-III) Associated with AIDS

Abstract. *The two main subgroups of the family of human T-lymphotropic retroviruses (HTLV) that have previously been characterized are known as HTLV-I and HTLV-II. Both are associated with certain human leukemias and lymphomas. Cell surface antigens (p61 and p65) encoded by HTLV-I are frequently recognized, at low titers, by antibodies in the serum of patients with acquired immuno deficiency syndrome (AIDS) or with signs or symptoms that precede AIDS (pre-AIDS). This suggests an involvement of HTLV in these disorders. Another subgroup of HTLV, designated HTLV-III, has now been isolated from many patients with AIDS and pre-AIDS. In the studies described in this report, virus-associated antigens in T-cell clones permanently producing HTLV-III were subjected to biochemical and immunological analyses. Antigens of HTLV-III, specifically detected by antibodies in serum from AIDS or pre-AIDS patients and revealed by the Western blot technique, are similar in size to those found in other subgroups of HTLV. They include at least three serologically unrelated antigenic groups, one of which is associated with group-specific antigens (p55 and p24) and another with envelope-related (p65) proteins, while the antigens in the third group are of unknown affiliation. The data show that HTLV-III is clearly distinguishable from HTLV-I and HTLV-II but is also significantly related to both viruses. HTLV-III is thus a true member of the HTLV family.*

Members of the family of human lymphotropic retroviruses (HTLV) have the following features in common: a pronounced tropism for $OKT4^+$ lymphocytes (*1*), a reverse transcriptase (RT) with a high molecular weight (100,000) and a preference for Mg^{2+} as the divalent cation for optimal enzymatic activity (*2*, *3*), and the capacity to inhibit T cell function (*4*) or, in some cases, kill T cells (*5*). Many HTLV also have the capacity to transform infected T-cells (*1*). The two major subgroups that have been characterized (*6*) are HTLV-I, which is causatively linked to certain adult T-cell malignancies (*7*), and HTLV-II, which was first identified in a patient with hairy cell leukemia (*8*).

Viruses of the HTLV family have been detected in some patients with the acquired immuno deficiency syndrome (AIDS) (*9*) or with pre-AIDS, a condition frequently progressing to AIDS (*10*). A high proportion of patients with AIDS or pre-AIDS, as well as a significant number of hemophiliacs, have antibodies in their serum that recognize a cell surface glycoprotein (gp61) that is present on certain human T cells infected with HTLV-I (*11*). Gp61 and p65, a slightly larger protein that is a homolog of gp61 and occurs in another cell line producing HTLV-I, were subsequently shown to be related to the HTLV viral glycoprotein (*12*, *13*). Studies of blood transfusion recipients who later developed AIDS and of their blood donors have revealed the presence, in the blood of the donors, of antibodies to a retrovirus of the HTLV family (*14*). These findings suggest an involvement of viruses of the HTLV family in the cause of AIDS and pre-AIDS. An involvement of HTLV-I alone appeared doubtful, however, because antibody titers to gp61 of HTLV-I in these patients are generally very low, and antibodies to the structural proteins of HTLV, notably p24 and p19 (*15*), are not detectable in most AIDS patients (*16*). Instead, it seemed likely that another member of the HTLV family might be involved in the etiology of AIDS. Here we describe our studies of a group of cytopathic viruses (collectively designated HTLV-III) isolated from patients with AIDS or pre-AIDS. Isolation of these viruses was achieved by means of a novel system permitting the continuous growth of T-cell clones infected with the cytopathic types of HTLV found in these disorders (*17*). We show that antigens associated with human cells infected by HTLV-III are specifically recognized by antibodies in serum from AIDS and pre-AIDS patients, and present a preliminary biochemical and immunological analysis of these antigens.

Lysates of two immortalized and infected human T-cell clones, H4/HTLV-III and H17/HTLV-III (*17*), were tested with samples of human serum in a strip radioimmunoassay (RIA) based on the Western blot technique (*18*). The sera were from patients with AIDS or pre-AIDS, from contacts of such patients, and from homo- or heterosexual male controls. Sera from the same patients were also tested by the enzyme-linked immunosorbent assay (ELISA) with purified HTLV-III as part of a larger, systematic serologic study of the prevalence of antibodies to HTLV-III in AIDS and pre-AIDS patients (*19*).

Representative results are shown in Fig. 1. Sera from patients with AIDS or pre-AIDS, and from some homosexuals and heroin-addicts, recognized a number of specific antigens not detected by sera from heterosexual subjects. The most prominent reactions were with antigens of the following molecular weights: 65,000, 60,000, 55,000, 41,000, and 24,000. Antigens with molecular weights of approximately 88,000, 80,000, 39,000, 32,000, 28,000, and 21,000 gave less prominent reactions. The reaction with the antigen of 55,000 (p55) only occurred in sera that also recognized p24, suggesting a relation between the two.

The specificity of these reactions was studied by comparing lysates of H4/

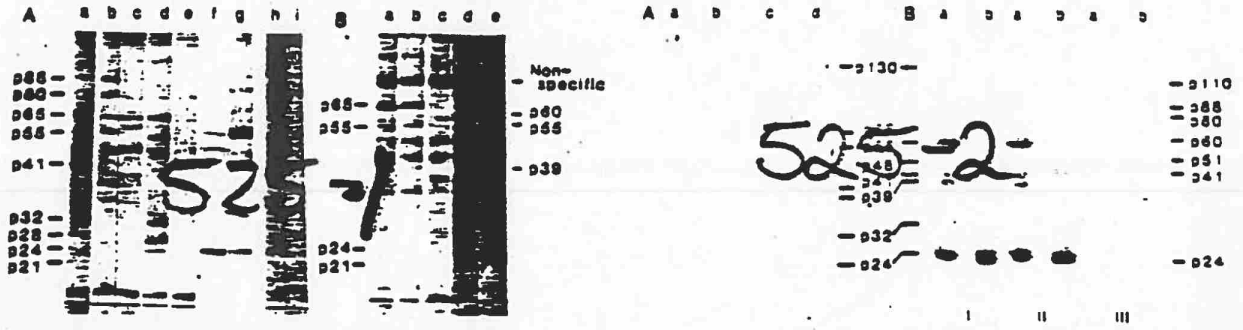

Fig. 1. Serologic detection of antigens in HTLV-III producer cell clones. Strip RIA were performed with human serum as described elsewhere in detail (*21*). Briefly, lysates of HTLV-III producer cell clones were subjected to electrophoresis under reducing conditions on preparative sodium dodecyl sulfate (SDS)-polyacrylamide slab gels, and electroblotted to nitrocellulose sheets (*18*). The sheets were cut into strips. These were incubated with human serum diluted 1:100. After three thorough washings, bound antibodies of immunoglobulin G (IgG) and immunoglobulin M (IgM) classes were made visible with radiolabeled, affinity-purified goat antiserum to human IgG and IgM (H-chain specific) and autoradiography. (A) Analysis with H4/HTLV-III cells. (Lanes a, d, and g) U.S. patients with AIDS; (lane b) a French heterosexual male who developed AIDS after receiving a blood transfusion in Haiti (*24*); (lane c) an AIDS patient from Switzerland; (lane e) a normal heterosexual control; (lane f) a French pre-AIDS patient (*24*); (lane h) a Swiss heterosexual drug addict; (lane i) a normal homosexual control. (B) Analysis with H17/HTLV-III cells. (Lane a) An infant with AIDS whose mother is a prostitute; sera from both are highly positive for antibodies to the HTLV membrane antigen (*11*, *25*) and in our ELISA with disrupted HTLV-III (*19*); (lane b) same serum as in (A), lane d; (lane c) normal heterosexual control; (lane d) another Swiss AIDS patient; (lane e) a Swiss heterosexual male intravenous drug abuser with generalized lymphadenopathy and thrombocytopenic purpura (pre-AIDS). Fig. 2. (A) Specificity of the antigens recognized. Lysates of cloned cells before and after infection with HTLV-III were analyzed by the Western blot technique (*18*) with a 1:500 dilution of the serum shown in Fig. 1B, lane e. (Lane a) The H17 clone before and (lane b) the same clone after infection (H17/HTLV-III); (lane c) the H4 clone before and (lane d) the same clone after infection (H4/HTLV-III). All reactive antigens are virus-related with the exception of that with a molecular weight of 80,000 in H17 cells; this antigen binds antibodies from all human sera investigated. Normal human serum did not bind to any of the virus-related bands (not shown). (B) Comparison of antigens in (lanes a) cells and (lanes b) virus. Lysates of H4/HTLV-III (250 μg per lane) or virus purified from the cell culture fluids (*19*) (5 μg per lane) were analyzed with 1:500 dilutions of human sera. (Panel I) Same serum as in Fig. 2A; (panel II) serum of a Swiss male homosexual with fatigue and generalized lymphadenopathy (pre-AIDS); (panel III) serum from same AIDS patient as in Fig. 1B, lane d. An antigen with a 110,000 and p41, p39, and p24 are enriched in the virus preparation [see (*20*)]. The serum in panel III recognized a subset of the antigens recognized by the sera used in panels I and II.

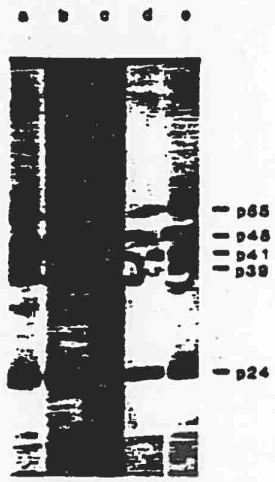

Fig. 3. Relation between HTLV-III and HTLV-II. Serum of an AIDS patient at a dilution of 1:500 was tested in a competition RIA on strips (*20*) prepared with H4/HTLV-III cells. (Lane a) The human serum was added directly to the strip (uncompeted control); (lanes b to e) the serum was first absorbed for 3 hours at 37°C with 1 mg of cellular extract. In (b) the absorption was with uninfected H4 cells (not producing virus); in (c) the absorption was with H4/HTLV-III cells producing HTLV-III (positive control); in (d) the absorption was with C3/44 cells (*26*) producing HTLV-II; in (e) the absorption was with HUT 102 cells producing HTLV-I (*2*).

Fig. 4. Electron microscopy of thin sections of cells producing HTLV-I, -II, and -III. (Top) HUT 102 cells producing HTLV-I (*2*). (Middle) Cells from an AIDS patient (J.P.) producing HTLV-II (*24*). (Bottom) Cells from a patient [described in (*27*)] with pre-AIDS, producing HTLV-III. (Panels a) Virus particles budding from the cell membrane. (Panels b) Free particles have separated from the membrane. (Panels c) Free particles sectioned in a different plane. Note the dense, cylindrical core region of HTLV-III.

HTLV-III and H17/HTLV-III with lysates of the same cell clones. H4 and H17, before viral infection (Fig. 2A). No antigen from the uninfected clones reacted with the sera, with the exception of protein with a molecular weight of 80,000 in H17 which bound antibodies from all of the human serum samples tested (see Fig. 1B) but not from rabbit or goat serum. Antigens newly expressed after viral infection and recognized by the human serum used for this analysis included p65, p55, p41, p39, p32, and p24. A large protein with a molecular weight of approximately 130,000 and a protein of 48,000 were also detected. With this serum, p55 consistently appeared as a doublet of bands of similar intensity. With normal human serum, none of the antigens was detected (not shown). These results show clearly that the antigens detected after virus infection are either virus-coded proteins or cellular antigens specifically induced by the infection.

The antigens of H4/HTLV-III were also compared with antigens from virus purified from the culture fluids of H4/HTLV-III (Fig. 2B). Extensive accumulation of p24 and p41 [see (*20*)] occurred in the virus preparation (Fig. 2B, panels I and II). Protein stains showed that these molecules are the major components of the virus preparation (*19*). P24 and p41 may therefore be considered viral structural proteins. Furthermore, an antigen with a molecular weight of approximately 110,000 was detected in the virus preparation but was below limit of detection in the cells. Also, p39 [see (*20*)] was present in the virus preparation. It is interesting that p24 in the virus preparation consistently appeared as a doublet (p24/p23), whereas in the cells it appeared as p24 alone. The significance of this is under investigation. P55 was not detected in the virus; however, the intensity of the p55 band in the cells (Fig. 2B, lanes a) appeared to correlate with the intensity of p24/p23 in the virus preparation (Fig. 2B, lanes b), thus again suggesting a relation between these antigens. The p55 is probably a precursor of p24, since a group-specific antigen of similar size (Pr 54^{gag}) in HTLV-I–infected cells is the precursor of p24 and the other *gag*-coded proteins (*21*). Occasionally an additional set of antigens was recognized by a serum (Fig. 2B, Panel III) but their relation to the antigens described above is unclear.

Thus we have shown that viral or virus-induced antigens in cloned human T cells infected with HTLV-III are specifically recognized by antibodies in the serum of patients with AIDS or pre-AIDS. The detection of p65 by many of the serum samples is of special interest. We have tested these sera on strips prepared from lysates of cells producing HTLV-I or -II. Some of these cells produce a p65 that has been shown (*13*) to be coded for by the *env* gene of HTLV-I and to be the homolog of the gp61 described by others (*11, 12*). Many of the sera recognizing p65 in HTLV-III–infected cells also recognized, though somewhat faintly, p65 in cells producing HTLV-I or -II, and some of them also recognized *gag*-related antigens (data not shown). In addition, the reaction of some human sera with virus-related antigens of HTLV-III–infected cells could be partially inhibited by large amounts of extracts of cells producing HTLV-II (Fig. 3). When a human serum not recognizing p65 was used, the antigens for which there was competition included p55, p48, p41, p39, and p24. These results were confirmed by the demonstration that a rabbit antiserum raised against purified HTLV-III showed some reactivity with antigens of HTLV-II and, to a lesser extent, with HTLV-I. In contrast, antiserum to HTLV-II recognized both HTLV-I and -III antigens, and an antiserum to HTLV-I reacted well with HTLV-II, but only faintly with HTLV-III (*22*). Moreover, nucleotide sequences of HTLV-III have been found to be related to HTLV-I and -II (*23*). Although the morphology of HTLV-III particles appears to be somewhat different from the morphology of HTLV-I and -II (Fig. 4), and although some differences are also found in the protein patterns of purified virus preparations (*19*), these immunological and nucleic acid data clearly indicate that HTLV-III is a true member of the HTLV family and that it is more closely related to HTLV-II than to HTLV-I.

JÖRG SCHÜPBACH
MIKULAS POPOVIC
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205

RAYMOND V. GILDEN
MATTHEW A. GOND
Program Resources, Inc. NCI–Frederick Cancer Research Facility, Frederick, Maryland 21701

M. G. SARNGADHARAN
Department of Cell Biology, Litton Bionetics, Inc., Kensington, Maryland 20895

ROBERT C. GALLO
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205

4 MAY 1984

References and Notes

1. M. Popovic, P. S. Sarin, M. Robert-Guroff, V. S. Kalyanaraman, D. Mann, J. Minowada, R. C. Gallo, *Science* 219, 856 (1983); P. D. Markham, S. Z. Salahuddin, V. S. Kalyanaraman, M. Popovic, P. Sarin, R. C. Gallo, *Int. J. Cancer* 31, 413 (1983); S. Z. Salahuddin, P. D. Markham, F. Wong-Staal, G. Franchini, V. S. Kalyanaraman, R. C. Gallo, *Virology* 129, 51 (1983).
2. B. J. Poiesz, F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* 77, 7415 (1980).
3. H. M. Rho, B. Poiesz, F. W. Ruscetti, R. C. Gallo, *Virology* 112, 355 (1981); M. Seiki, S. Hattori, Y. Hirayama, M. Yoshida, *Proc. Natl. Acad. Sci. U.S.A.* 80, 3618 (1983).
4. M. Popovic *et al.*, in preparation.
5. H. Mitsuya, H. G. Guo, M. Megson, C. O. Trainor, M. S. Reitz, S. Broder, *Science* 223, 1293 (1984).
6. 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.
7. V. S. Kalyanaraman, M. G. Sarngadharan, Y. Nakao, Y. Ito, T. Aoki, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* 79, 1653 (1982); M. Robert-Guroff *et al.*, *Science* 215, 975 (1982); Y. Hinuma *et al.*, *Int. J. Cancer* 29, 631 (1982); W. A. Blattner *et al.*, *ibid.* 30, 257 (1982); J. Schupbach, V. S. Kalyanaraman, M. G. Sarngadharan, Y. Nakao, R. C. Gallo, *ibid.* 32, 583 (1983); J. Schüpbach, V. S. Kalyanaraman, M. G. Sarngadharan, W. A. Blattner, R. C. Gallo, *Cancer Res.* 43, 886 (1983).
8. V. S. Kalyanaraman *et al.*, *Science* 218, 571 (1982); E. P. Gelmann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 81, 993 (1984).
9. R. C. Gallo *et al.*, *Science* 220, 865 (1983); E. P. Gelmann *et al.*, *ibid.*, p. 862.
10. F. Barré-Sinoussi *et al.*, *ibid.*, p. 868.
11. M. Essex *et al.*, *ibid.*, p. 859; M. Essex *et al.*, *ibid.* 221, 1061 (1983).
12. T. H. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
13. J. Schüpbach, M. G. Sarngadharan, R. C. Gallo, *Science*, in press.
14. H. W. Jaffe *et al.*, *Science* 223, 1309 (1984).
15. V. S. Kalyanaraman, M. G. Sarngadharan, P. A. Bunn, J. D. Minna, R. C. Gallo, *Nature (London)* 294, 271 (1981); V. S. Kalyanaraman, M. Jarvis-Morar, M. G. Sarngadharan, R. C. Gallo, *Virology* 132, 61 (1984).
16. M. Robert-Guroff *et al.*, in *Cancer Cells, Vol. 3: Human T-Cell Leukemia Viruses*, R. C. Gallo and M. Essex, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, in press).
17. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* 224, 497 (1984).
18. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* 76, 4350 (1979).
19. M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schupbach, R. C. Gallo, *Science* 224, 506 (1984).
20. Although in Fig. 2B the p41 in the virus preparation appears to be larger than the p41 in cells, the two molecules are of the same size. During application of the lysates to the gel in another experiment, a small amount of cellular lysate was spilled into the neighboring lanes and the cellular p41 moved with the same velocity as the viral p41. A connecting band was thus formed between the p41 in the lane containing the cells and the p41 in the lane with the virus. The same situation occurred with p39 in cells and virus.
21. J. Schüpbach, V. S. Kalyanaraman, M. G. Sarngadharan, R. C. Gallo, in preparation.
22. M. G. Sarngadharan *et al.*, in preparation.
23. S. Arya *et al.*, in preparation.
24. J. B. Brunet *et al.*, *Lancet* 1983-I, 700 (1983).
25. M. Essex, personal communication.
26. M. Popovic, V. S. Kalyanaraman, D. L. Mann, E. Richardson, P. S. Sarin, R. C. Gallo, in *Cancer Cells, Vol. 3: Human T-Cell Leukemia Viruses*, R. C. Gallo and M. Essex, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, in press).
27. R. C. Gallo *et al.*, *Science* 224, 500 (1984).

28. We thank J. Ahmat for technical assistance and R. Luthy and M. Vogt, Division of Infectious Diseases, Department of Medicine, University Hospital, Zurich, and O. Haller, Institute for Immunology and Virology, University of Zurich, Zurich, Switzerland, for making some sera from AIDS and pre-AIDS patients available and for providing clinical information. J.S. is a Fogarty International Fellow of the National Cancer Institute.

30 March 1984; accepted 19 April 1984

Antibodies Reactive with Human T-Lymphotropic Retroviruses (HTLV-III) in the Serum of Patients with AIDS

Abstract. *In cats, infection with T-lymphotropic retroviruses can cause T-cell proliferation and leukemia or T-cell depletion and immunosuppression. In humans, some highly T4 tropic retroviruses called HTLV-I can cause T-cell proliferation and leukemia. The subgroup HTLV-II also induces T-cell proliferation in vitro, but its role in disease is unclear. Viruses of a third subgroup of human T-lymphotropic retroviruses, collectively designated HTLV-III, have been isolated from cultured cells of 48 patients with acquired immunodeficiency syndrome (AIDS). The biological properties of HTLV-III and immunological analyses of its proteins show that this virus is a member of the HTLV family and that it is more closely related to HTLV-II than to HTLV-I. Serum samples from 88 percent of patients with AIDS and from 79 percent of homosexual men with signs and symptoms that frequently precede AIDS, but from less than 1 percent of heterosexual subjects, have antibodies reactive against antigens of HTLV-III. The major immune reactivity appears to be directed against p41, the presumed envelope antigen of the virus.*

The incidence of the acquired immunodeficiency syndrome (AIDS) in homosexual men with multiple sexual partners, intravenous drug abusers, hemophiliacs, blood transfusion recipients, and close heterosexual contacts of members of these high-risk groups (*1–7*) strongly suggests that the disease spreads by the transmission of an infectious agent (*8, 9*). The agent's primary targets within the body appear to be specific subpopulations of T cells. The severe immune deficiency of AIDS patients results from an unusually low proportion of helper T lymphocytes ($OKT4^+$) and a resulting lack of many helper functions, including production of antibodies by B cells (*1, 3*).

Retrovirus infections are known to lead to depressed immune functions in animal systems. For example, in cats, a major result of infection with feline leukemia virus (FeLV) is loss of normal immune function. More FeLV-infected cats die from consequences of this immune dysfunction than from the leukemia itself (*10*). FeLV provides an example of a single T-cell tropic retrovirus that causes both target cell proliferation (leukemia) and depletion (immunosuppression). By analogy, a human retrovirus with a tropism for T cells should be considered a serious candidate in the etiology of human AIDS. Two subgroups of a family of human T-lymphotropic retroviruses (HTLV) have been isolated and characterized (*11*). The first, HTLV-I, was isolated from a black American with an aggressive form of T-cell lymphoma (*12*) and has been etiologically linked to the pathogenesis of adult T-cell leukemia-lymphoma (ATL) (*13–15*). Infection with HTLV-I in vitro can alter T-cell function (*16*) and, in some cases, lead to T-cell death (*17*). HTLV-II was isolated from a patient with a T-cell variant of hairy cell leukemia (*18*).

Although there are distinct differences between HTLV-I and HTLV-II, they have the following common features: a tropism for $OKT4^+$ lymphocytes (*19*); a Mg^{++}-dependent reverse transcriptase (RT) of high molecular weight (100,000) (*20*); some antigenic cross-reactivity in their proteins (*18*); a novel set of nucleotide sequences called pX at the 3′ end of the viral genome; a limited amount of nucleic acid homology in their genomes (*21*); and similar morphology. Both HTLV-I and HTLV-II have been isolated from cultured T cells of patients with AIDS (*22, 23*). Another retrovirus was isolated from a homosexual patient with chronic generalized lymphadenopathy (*24*), a syndrome that often precedes AIDS and is therefore referred to as pre-AIDS. Proviral DNA of HTLV-I was detected in the cellular DNA of two AIDS patients (*25*), and serum samples from some patients were shown to react with antigens of HTLV-I (*26*). A larger proportion of the sera reacted with a cell membrane antigen specific to HTLV-I–infected cells (*27*). This antigen has since been identified as a precursor of the envelope glycoprotein, gp46, of HTLV-I (*28, 29*). However, the correlation between AIDS and serum antibodies to HTLV-I protein (including the cell membrane antigen, p61) is weak.

These results are consistent with the idea that the primary cause of AIDS is another member of the HTLV family with limited cross-reactivities with the known HTLV subgroups. Sera with high titers of antibodies to the AIDS-specific virus might show a detectable reaction with antigens of HTLV-I and HTLV-II, whereas the reaction of sera with low titers might be too weak to recognize in such a cross-reactive system. Our attempts to isolate other retroviruses from AIDS patients resulted in the identification of a number of HTLV isolates that are similar to each other but are distinguishable from HTLV-I and HTLV-II. These new isolates are designated HTLV-III and are described in the ac-

Table 1. Antibodies to HTLV-III in serum samples from patients with AIDS and pre-AIDS and from control subjects. Wells of 96-well Immulon plates were coated overnight with a lysate of density-banded HTLV-III (*30*) at 0.5 μg protein per well in 100 μl 50 m*M* sodium bicarbonate buffer, *p*H 9.6. The wells were washed with water and incubated for 20 minutes with 100 μl of 5 percent bovine serum albumin in phosphate buffered saline (PBS). The wells were washed again in water, and then 100 μl of 20 percent normal goat serum in PBS were added to each well, followed by 5 or 10 μl of the test sera. These were allowed to react for 2 hours at room temperature. The wells were washed three times with 0.5 percent Tween-20 in PBS and incubated for 1 hour at room temperature with peroxidase-labeled goat antiserum to human immunoglobulin G at a dilution of 1:2000 in 1 percent normal goat serum in PBS. The wells were successively washed four times with 0.05 percent Tween-20 in PBS and four times with PBS and reacted with 100 μl of the substrate mixture containing 0.05 percent orthophenylene diamine and 0.005 percent hydrogen peroxide in phosphate-citrate buffer, *p*H 5.0. The reactions were stopped by the addition of 50 μl of 4*N* H_2SO_4, and the color yield was measured with a Dynatech ELISA reader. Assays were done in duplicate and absorbance reading greater than three times the average of four normal negative control readings was taken as positive.

Subjects	Number positive for antibodies to HTLV-III	Number tested	Percent positive
Patients with AIDS	43	49	87.8
Patients with pre-AIDS	11	14	78.6
Intravenous drug users	3	5	60
Homosexual men	6	17	
Sexual contact of AIDS patient	1	1	
Persistent fatigue	1	1	
Other	4	15	26.6
Other controls	1	186	0.5
Normal subjects	1	164	0.6
Patients with hepatitis B virus infection	0	3	
Patient with rheumatoid arthritis	0	1	
Patients with systemic lupus erythematosus	0	6	
Patients with acute mononucleosis	0	4	
Patients with lymphatic leukemias	0	8	

companying reports (*30–32*). Here we describe the use of HTLV-III in an immunological screening of serum samples from patients with AIDS and pre-AIDS and from individuals at increased risk for AIDS.

The virus was purified from supernatants of cell cultures supporting the continuous production of HTLV-III (*30*). The virus showed a difference in the makeup of its protein components as revealed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of a sucrose density banded preparation (Fig. 1, lane 2). Like HTLV-I (lane 1), and unlike common mammalian retroviruses (for example, Rauscher murine leukemia virus, lane 3), HTLV-III (lane 2) has a major group-specific antigen (*gag* protein) with a molecular weight of 24,000 (p24). It has an RT with a molecular weight of about 100,000, another protein with a molecular weight of 41,000 (presumably the envelope glycoprotein), and shows a tropism for $OKT4^+$ lymphocytes. However, it lacks the band separating at a molecular weight of 19,000 (p19). Instead, it has a smaller band that is missing in HTLV-I. Immunological studies presented in an accompanying report (*32*) also indicate that HTLV-III is antigenically different from HTLV-I and -II, but that it also shares a variety of antigenic determinants with them, especially with HTLV-II. This relatedness has also been confirmed by comparison of nucleotide sequences of the three types of HTLV (*33*).

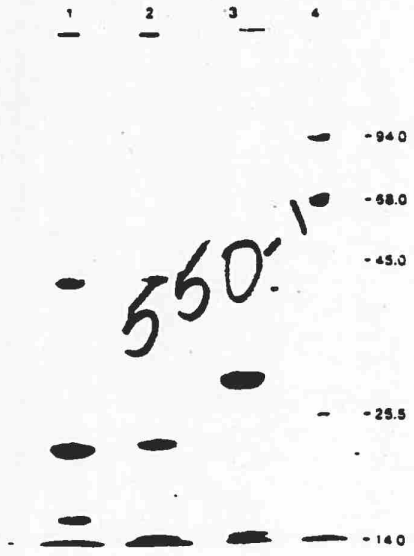

Fig. 1. Comparison of the SDS-polyacrylamide gel profile of HTLV-III with profiles of HTLV-I and Rauscher murine leukemia virus (R-MuLV). Lane 1, HTLV-I; lane 2, HTLV-III; lane 3, R-MuLV; lane 4, molecular weight standards: phosphorylase b (94,000), bovine serum albumin (68,000), ovalbumin (45,000), chymotrypsinogen (25,500), and lysozyme (14,000).

Serum samples were obtained from patients with clinically documented AIDS, Kaposi's sarcoma, sexual contacts of AIDS patients, intravenous drug abusers, homosexual men, and heterosexual subjects. These sera were tested for their reactivity to HTLV-III by means of the enzyme-linked immunosorbent assay (ELISA) (*34*). Lysates of sucrose density banded HTLV-III were coated on 96-well microtiter plates. The test sera were diluted with normal goat serum, added to the wells, and allowed to react for 2 hours or overnight at room temperature. The primary immune complex formed with the antibodies in the human sera was detected by adding peroxidase-labeled goat antiserum to human immunoglobulins and assaying for a colored peroxidase reaction product (*34*). The results are presented in Table 1. Of 49 clinically diagnosed AIDS patients, 43 (88 percent) showed serum reactivity in this assay. Two of the subjects whose serum reacted positively with the HTLV preparation had developed AIDS after receiving blood transfusions, one in Haiti and the other in Aruba. Of 14 homosexual men with pre-AIDS, 11 (79 percent) were positive. Of 17 homosexual men with no clinical symptoms of AIDS, seven were positive. At least one of these was known to be a long-time sexual partner of a patient with clinically diagnosed AIDS. Another had persistent fatigue and possibly other early symptoms of AIDS. Because these 17 men had been seeking medical assistance, they are not a representative sample of the homosexual population, and the high incidence of HTLV-III-specific antibodies in their sera may not reflect the true incidence in the homosexual population. One of the three intravenous drug abusers that were positive for serum antibodies to HTLV-III was also a homosexual. Serum samples from only one of 186 control subjects reacted positively in this test. These control subjects included three with hepatitis B virus infection, one with rheumatoid arthritis, six with systemic lupus erythematosus, four with acute mononucleosis, and eight with various forms of lymphatic leukemias and lymphomas, some of whom were positive for HTLV-I. The rest were normal donors of unknown sexual preference including laboratory workers ranging in age from 22 to 50.

To understand the molecular nature of the antigens recognized by ELISA, we conducted the following experiment. A lysate of HTLV-III was fractionated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose sheet by the electrophoretic blotting (Western) technique of Towbin *et al.* (*35*). The nitrocellulose sheet was cut into 0.5-cm strips and reacted with samples of the human sera. Antigen-antibody complex-

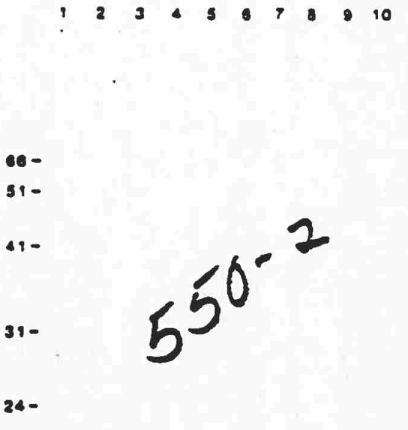

Fig. 2. Identification of HTLV-III antigens recognized by sera of AIDS patients. HTLV-III was lysed and fractionated by electrophoresis on a 12 percent polyacrylamide slab gel in the presence of SDS. The protein bands on the gel were electrophoretically transferred to a nitrocellulose sheet according to the procedure of Towbin *et al.* (*35*). Strip solid-phase radioimmunoassays were then performed as described (*36*). The sheet was incubated at 37°C for 2 hours with 5 percent bovine serum albumin in 10 m*M* tris-HCl, *p*H 7.5 containing 0.9 percent NaCl and cut into 0.5-cm strips. Each strip was incubated for 2 hours at 37°C and 2 hours at room temperature in a screw cap tube containing 2.5 ml of buffer-1 (20 m*M* tris-HCl, *p*H 7.5, 1 m*M* EDTA, 0.2*M* NaCl, 0.3 percent Triton X-100, and 2 mg of bovine serum albumin and 0.2 mg of human Fab per milliliter). Test sera (25 μl) were then added to individual tubes containing the strips and incubation was continued for 1 hour at room temperature and overnight in the cold. The strips were washed three times with a solution containing 0.5 percent sodium deoxycholate, 0.1*M* NaCl, 0.5 percent Triton X-100, 1 m*M* phenylmethylsulfonyl fluoride, and 10 m*M* sodium phosphate, *p*H 7.5. The strips were incubated for 1 hour at room temperature with 2.4 ml of buffer-1 and 0.1 ml of normal goat serum. Affinity-purified and ^{125}I-labeled goat antiserum to human immunoglobulin (μ chain and Fc fragment) (1.25×10^6 count/min) were added to the reaction mixture and the incubation was continued for 30 minutes at room temperature. The strips were washed as described, dried, mounted, and exposed to x-ray film. Strip 1, adult T-cell leukemia; strip 2, normal donor; strip 3, mother of a child with AIDS; strips 4 and 6 to 10, AIDS patients; and strip 5, patient with pre-AIDS.

es formed were detected by autoradiography after incubation of the strips with ^{125}I-labeled goat antibody to human immunoglobulin. Figure 2 shows that the antigen most prominently and commonly detected among all of the sera from AIDS patient had a molecular weight of 41,000 (p41). This corresponds to one of the major proteins of the virus (Fig. 1) and is presumably the envelope protein. Strip 7 shows the result obtained with serum from an AIDS patient that reacted negatively in the ELISA but in this more sensitive strip assay it gave a low, but definitely positive, result. Reactivity to p24 of the virus was generally very weak and was clear only in two cases (strips 4 and 5). This may be a reflection of the relative titer toward different antigens. One would expect the highest antibody titer against the envelope of the infecting agent, especially if the infection causes a pronounced immune deficiency and decreased capacity to make antibodies in response to subsequent antigenic challenge. Additional reactivities against antigens with molecular weights of 66,000 and 51,000 were seen in some sera. In strip 8 the serum reacted with an additional antigen that has a molecular weight of 31,000. These additional antigens appear to be related to those detected by sera from the same patients in HTLV-III–producing cells (*33*). Strips 1 and 2 show that sera from a patient with ATL who was positive for HTLV-I and from a normal subject do not react with the antigens of HTLV-III.

Of particular interest is the finding that among the serum samples that reacted positively with HTLV-III two were from young children (ages 7 months and 2 years). These children were free of known opportunistic infections including cytomegalovirus, Epstein-Barr virus, *Pneumocystis carinii*, and fungus. The mother of one of them was positive in both tests described here. The children presumably acquired the infection in utero, by their mother's milk, or by another route.

Among the positive serum samples from AIDS patients there appears to be a wide variation in antibody titer to HTLV-III. Generally, the titers in sera from patients with advanced AIDS are significantly lower than those in sera from newly diagnosed patients and patients with pre-AIDS. This is consistent with the idea that HTLV-III infection causes an initial lymphoid proliferation but eventually causes death of the target lymphocytes (OKT4$^+$) leading to the abnormal T4$^+$ T8$^+$ ratios and loss of helper T-cell functions including antibody production by B cells. Therefore, the low or negative result in the ELISA of sera from some cases of advanced AIDS may be a consequence of the natural course of the disease. To prove this it will be necessary to study antibody titers in sera obtained at intervals from subjects at risk for the disease. The serum of one AIDS patient showed a low positive titer, but serum from his homosexual partner with no symptoms of AIDS had a significantly higher antibody titer. It is interesting that the serum of one AIDS patient that was negative in the ELISA did show a definite but low positive reaction with p41 in the more sensitive Western blot assay (Fig. 2, strip 7). The ELISA with purified p41 might prove to be even more sensitive. It is significant that although HTLV proviral sequences were clearly detected in DNA from cell samples obtained from two AIDS patients early in the course of their disease, these sequences could not be detected in cells obtained after 1 year in one case and 2 months in the second case (*25*). It is conceivable that the subset of T lymphocytes that forms the target of the provirus had been depleted before the second samples were obtained in each case.

In conclusion, we have shown a high incidence of specific antibodies to HTLV-III in patients with AIDS and pre-AIDS. Among the antibody-positive cases reported here a few are of particular importance with respect to the transmission of the disease. For example, the mother of the baby with AIDS was positive for HTLV-III as was a long-term sexual partner of a homosexual with AIDS. Recipients of blood products originating from individuals at risk for AIDS were also positive for HTLV-III and, as described in an accompanying report (*31*), the virus has been isolated from several children with AIDS as well as from their mothers. The data presented here and in the accompanying reports (*30–32*) suggest that HTLV-III is the primary cause of AIDS.

M. G. SARNGADHARAN
Department of Cell Biology, Litton Bionetics, Inc., Kensington, Maryland 20895

MIKULAS POPOVIC
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205

LILIAN BRUCH
Department of Cell Biology, Litton Bionetics, Inc.

JÖRG SCHÜPBACH
ROBERT C. GALLO
Laboratory of Tumor Cell Biology, National Cancer Institute

References and Notes

1. M. S. Gottlieb, R. Schroff, H. M. Schanker, J. D. Weisman, P. T. Fan, R. A. Wolf, A. Saxon, *N. Engl. J. Med.* **305**, 1425 (1981).
2. H. Masur, M. A. Michelis, J. B. Greene, *ibid.* **305**, 1431 (1981).
3. F. P. Siegal *et al.*, *ibid.*, p. 1439.
4. M. Poon, A. Landay, E. F. Prashofer, S. Stagno, *Ann. Int. Med.* **98**, 287 (1983).
5. *Mortal. Morbid. Weekly Rep.* **31**, 697 (1983).
6. B. Moll, E. E. Emerson, C. B. Small, G. H. Friedland, R. S. Klein, I. Spigland, *Clin. Immunol. Immunopathol.* **25**, 417 (1982).
7. J. W. Curran *et al.*, *N. Engl. J. Med.* **310**, 69 (1984).
8. Centers for Disease Control, Task Force on Kaposi's Sarcoma and Opportunistic Infections, *ibid.* **306**, 248 (1982).
9. J. P. Hanrahan, G. P. Wormser, C. P. Maguire, L. J. De Lorenzo, G. Davis, *ibid.* **307**, 498 (1982).
10. M. Essex, W. D. Hardy, Jr., S. M. Cotter, R. M. Jakowski, A. Sliski, *Inject. Immun.* **11**, 470 (1975); W. D. Hardy, Jr., P. W. Hess, E. G. MacEwen, A. J. McClelland, E. E. Zuckerman, M. Essex, S. M. Cotter, *Cancer Res.* **36**, 582 (1976); L. J. Anderson, O. Jarrett, H. M. Laird, *J. Natl. Cancer Inst.* **47**, 807 (1971).
11. R. C. Gallo, in *Cancer Surveys*, L. M. Franks, L. M. Wyke, R. A. Weiss, Eds. (Oxford Univ. Press, Oxford, in press).
12. B. J. Poiesz, F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980).
13. V. S. Kalyanaraman, M. G. Sarngadharan, Y. Nakao, Y. Ito, T. Aoki, R. C. Gallo, *ibid.* **79**, 1653 (1982).
14. W. A. Blattner, V. S. Kalyanaraman, M. Robert-Guroff, T. A. Lister, D. A. G. Galton, P. Sarin, M. H. Crawford, D. Catovsky, M. Greaves, R. C. Gallo, *Int. J. Cancer* **30**, 257 (1982).
15. R. C. Gallo *et al.*, *Cancer Res.* **43**, 3892 (1983).
16. M. Popovic, N. Flomberg, D. Volkman, D. Mann, A. S. Fauci, B. DuPont, R. C. Gallo, in preparation.
17. H. Mitsuya, H. G. Guo, M. Megson, C. D. Trainor, M. S. Reitz, S. Broder, *Science* **223**, 1293 (1984).
18. V. S. Kalyanaraman, M. G. Sarngadharan, M. Robert-Guroff, I. Miyoshi, D. Blayney, D. Golde, R. C. Gallo, *ibid.* **218**, 571 (1982).
19. M. Popovic, unpublished observation.
20. H. M. Rho, B. J. Poiesz, F. W. Ruscetti, R. C. Gallo, *Virology* **112**, 355 (1981).
21. E. P. Gelmann, G. Franchini, V. Manzari, F. Wong-Staal, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 993 (1984).
22. R. C. Gallo *et al.*, *Science* **220**, 865 (1983).
23. M. Popovic *et al.*, in preparation.
24. F. Barre-Sinoussi *et al.*, *Science* **220**, 868 (1983).
25. E. P. Gelmann, M. Popovic, D. Blayney, H. Mazur, G. Sidhu, R. E. Stahl, R. C. Gallo, *ibid.* p. 862.
26. M. Robert-Guroff *et al.*, unpublished results.
27. M. Essex, M. F. McLane, T. H. Lee, L. Falk, C. W. S. Howe, J. I. Mullins, C. Cabradilla, D. P. Francis, *Science* **220**, 859 (1983).
28. J. Schüpbach, M. G. Sarngadharan, R. C. Gallo, *ibid.*, in press.
29. T. H. Lee, J. E. Coligan, T. Homma, M. F. McLane, M. Tschipana, M. Essex, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
30. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **224**, 497 (1984).
31. R. C. Gallo *et al.*, *ibid.*, p. 500.
32. J. Schüpbach *et al.*, *ibid.* p. 503.
33. S. Arya *et al.*, in preparation.
34. C. Saxinger and R. C. Gallo, *Lab Invest.* **49**, 371 (1983).
35. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
36. J. Schüpbach, V. S. Kalyanaraman, M. G. Sarngadharan, R. C. Gallo, in preparation.
37. We thank K. L. Arnett for technical assistance and R. Luthy and M. Vogt, Division of Infectious Diseases, Department of Medicine, University Hospital, Zurich, and O. Haller, Institute for Immunology and Virology, University of Zurich, Zurich, Switzerland, for providing us with sera from AIDS and pre-AIDS patients and for clinical information. J.S. is a Fogarty International Fellow of the N.C.I.

30 March 1984; accepted 19 April 1984