staining, sorting, and gene mapping will be useful (i) to locate random DNA fragments for linkage analysis with human disease loci and (ii) to improve flow karyotype diagnoses. Improved chromosome resolution will separate normal and abnormal chromosomes with increased purity to construct libraries from which linked DNA fragments can be chosen. The spot-blot method can then be used to determine the purity and subchromosomal location of any library fragment. Comparison of flow karyotypes of chromosomes stained with DIPI-chromomycin with those stained with Hoechst-chromomycin will detect differences in polymorphic chromosome bands to distinguish more reliably normal polymorphic variability from abnormal chromosomes.

ROGER V. LEBO
Howard Hughes Medical Institute and *Division of Genetics and Molecular Hematology, Department of Medicine, University of California, San Francisco 94143*

FREDRIC GORIN
ROBERT J. FLETTERICK
Departments of Biochemistry and Biophysics and *Neurology, University of California*

FA-TEN KAO
Eleanor Roosevelt Institute for Cancer Research, University of Colorado, Denver 80262

MEI-CHI CHEUNG
BARRY D. BRUCE
YUET WAI KAN
Howard Hughes Medical Institute and *Division of Genetics and Molecular Hematology, Department of Medicine, University of California*

References and Notes

1. A. V. Carrano *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1382 (1979).
2. R. V. Lebo, *Cytometry* **3**, 145 (1982).
3. C. Julien, D. Grunwald, J. L. Roti, G. Frelet, C. Rosenfeld, paper presented at Genes, Chromosomes and Flow Cytometry Conference, Santa Fe, N.M., October 1983.
4. R. V. Lebo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5804 (1979); R. V. Lebo *et al.*, *Hum. Genet.* **60**, 10 (1982).
5. K. E. Davies, B. D. Young, R. G. Elles, M. E. Hill, R. Williamson, *Nature (London)* **293**, 374 (1981); C. M. Disteche *et al.*, *Cytometry* **2**, 282 (1982).
6. A. Deisseroth *et al.*, *Cell* **12**, 205 (1977).
7. J. W. Gray, R. G. Langlois, A. V. Carrano, K. Burkhart-Schultz, M. A. van Dilla, *Chromosoma* **73**, 9 (1979).
8. R. V. Lebo and A. M. Bastian, *Cytometry* **3**, 213 (1982).
9. DIPI, 4′-6-bis[2′-imidazolinyl-4*H*,5*H*]-2-phenylindole [W. Schnedl, O. Dann, D. Schweizer, *Eur. J. Cell Biol.* **20**, 290 (1980); D. Schweizer, *Hum. Genet.* **57**, 1 (1981)].
10. R. V. Lebo, M.-C. Cheung, B. D. Bruce, Y. W. Kan, paper presented at Genes, Chromosomes and Flow Cytometry Conference, Santa Fe, N.M., October 1983; R. V. Lebo, O. Dann, B. D. Bruce, *ibid.*
11. B. McArdle, *Clin. Sci.* **10**, 13 (1951).
12. The National Foundation–March of Dimes, Paris Conference 1971, *Birth Defects: Original Article Series 5* (The National Foundation–March of Dimes, White Plains, N.Y., 1972), vol. 7.
13. *Human Genetic Mutant Cell Repository Catalog, NIH Publication 82-2011* (Institute for Medical Research, ed. 9, Camden, N.J., 1982).
14. B. D. Young, M. A. Ferguson-Smith, R. Sillar, E. Boyd, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7727 (1981).
15. A. Sakaguchi *et al.*, in preparation; J. M. Bishop provided cloned c-*sarc* DNA.
16. F. Gorin and R. Fletterick, in preparation; P. Hwang, M. Powers, R. Fletterick, in preparation.
17. J. F. Gusella *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2829 (1980).
18. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
19. A. Bernheim, P. Metezeau, G. Guellaën, M. Fellows, M. E. Goldberg, R. Berger, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7571 (1983).
20. F. H. Ruddle and R. P. Creagan, *Annu. Rev. Genet.* **9**, 431 (1981).
21. R. V. Lebo, unpublished results.
22. M. E. Chandler and G. F. Saunders, *Chromosoma* **83**, 431 (1981).
23. Supported in part by NIH grants AM32822 (R.J.F.) and HD02081 (F.T.K.). Y. W. Kan is an investigator of the Howard Hughes Medical Institute.

16 February 1984; accepted 16 April 1984

Selective Tropism of Lymphadenopathy Associated Virus (LAV) for Helper-Inducer T Lymphocytes

Abstract. *Lymphadenopathy associated virus (LAV) has been isolated from patients with the acquired immunodeficiency syndrome (AIDS) or lymphadenopathy syndrome. Since the immune deficiency in AIDS seems to be primarily related to the defect of the helper-inducer T lymphocyte subset, the possibility that LAV is selectively tropic for this subset was investigated. Fractionation of T lymphocytes was achieved by cellular affinity chromatography with monoclonal antibodies. In a hemophilic patient who was a healthy carrier of LAV, reverse transcriptase activity and virus particles detected by electron microscopy were found only in cultures of helper-inducer lymphocytes. When infected with LAV in vitro, lymphocyte subsets from normal individuals yielded similar results. Virus production was associated with impaired proliferation, modulation of T3-T4 cell markers, and the appearance of cytopathic effects. The results provide evidence for the involvement of LAV in AIDS.*

Epidemiological data indicate that the acquired immunodeficiency syndrome (AIDS) is caused by an infectious agent, probably a virus (*1–3*). Since a qualitative and quantitative defect of the helper-inducer T cell subset ($T4^+$) is the major immunological abnormality of this disease (*4, 5*), one would expect such a causative agent to express a tropism for $T4^+$ lymphocytes, resulting in alteration of their function.

We recently discovered a new group of human retroviruses that differ from human T-cell leukemia virus (HTLV-I) (*6–10*). The first member of this group was isolated from a nonimmunodepressed homosexual patient with persistent lymphadenopathy, a syndrome considered to be related to AIDS (*11, 12*). This virus has tentatively been named lymphadenopathy associated virus (LAV). Similar viruses were found in patients with frank AIDS, including the only hemophiliac patient with AIDS reported in France (*7*). Specific antibodies against LAV have been detected in approximately 70 percent of patients with persistent lymphadenopathy and 40 percent of AIDS patients studied (*8, 13*). We have now investigated whether the tropism of LAV is selective for the $T4^+$ subset in vivo as well as in vitro.

The studies in vivo were conducted with the help of an asymptomatic virus carrier, patient E.L., and his brother, patient D.L. Both patients have B hemophilia (*7*). Patient D.L. recently developed AIDS as assessed by successive opportunistic infections accompanied by a profound alteration of immune functions in vivo and in vitro. By means of techniques already described (*6–8*), a retrovirus similar to LAV was isolated repeatedly from his peripheral blood lymphocytes (PBL). This virus differs from HTLV-I (*9, 14, 15*). When examined by an immunofluorescence technique with antibodies to the structural proteins p24 or p19 of HTLV-I, fixed LAV-producing cells (*6*) were negative. Similarly, [^{35}S]methionine-labeled p25 of LAV was not precipitated by the antibodies to HTLV-I. Moreover, no antibody to HTLV-producing cell lines (C10/MJ, C91/PL) could be detected in the patient's serum by immunofluorescence, nor to HTLV p24 by the commercial enzyme-linked immunosorbent assay (ELISA; Biotech) or by a radioimmunoassay with a monoclonal antibody to p24 (*7*). No antibodies against LAV or HTLV could be detected in serum samples from parents of the patients, but E.L., like his brother, had circulating antibodies against LAV. However, apart from having hemophilia, E.L. was healthy and displayed normal immune functions. Nevertheless, LAV was isolated three times at monthly intervals from his PBL. The fact that no difference could be found between the viruses isolated from the brothers by serological and morphological studies, as well as the similar history of factor IX transfusion,

strongly suggests that the viruses have a common origin (*7*). Since, contrary to AIDS patients, E.L. had normal numbers of helper-inducer lymphocytes, sufficient quantities of these cells could be purified and cultured to find out whether the virus displayed a selective tropism in vivo for any given blood cell population.

Preliminary experiments indicated that LAV can be propagated only in human T lymphocytes in vitro. We were unable to detect productive virus infection, as determined by reverse transcriptase (RT) activity, after in vitro infection of normal B lymphocytes, lymphoma cell lines (RAJI and MOLT4), or monocytes (*16*).

Peripheral blood lymphocytes of E.L. were fractionated into monocytes, B lymphocytes, and T lymphocytes. Helper-inducer ($T4^+$) and suppressor-cytotoxic ($T8^+$) fractions were then obtained by cellular affinity chromatography. This technique does not modify interleukin-2 (IL-2) production or proliferative or cytotoxic lymphocyte functions (*17*, and data not shown). Cells were then cultured as described (*6*, *8*) in the presence of phytohemagglutinin (PHA) followed by IL-2. Reverse transcriptase was then sequentially measured in each culture supernatant. When $T4^+$ or $T8^+$ lymphocytes from normal individuals are maintained under the same culture conditions, the baseline of RT remains negative. By comparison, a spontaneous increase of RT occurred as early as day 3 of culture in the $T4^+$ fraction from patient E.L. and reached a peak value of 700×10^3 count/min on day 10. It declined rapidly thereafter to less than 200×10^3 count/min on day 18 and remained low until the end of the culture period on day 45 (Fig. 1). During the same period, this activity was always below 4×10^3 count/min in the $T8^+$ culture, until day 60. Such a low degree of RT, compared to $T8^+$ cultures from normal noninfected lymphocytes ($< 2 \times 10^3$ count/min), probably reflects the small number of $T4^+$ contaminating cells. Moreover, when $T8^+$ lymphocytes were cocultivated with cord blood lymphocytes, in order to amplify the low virus production (*10*), no RT activity was detectable (data not shown).

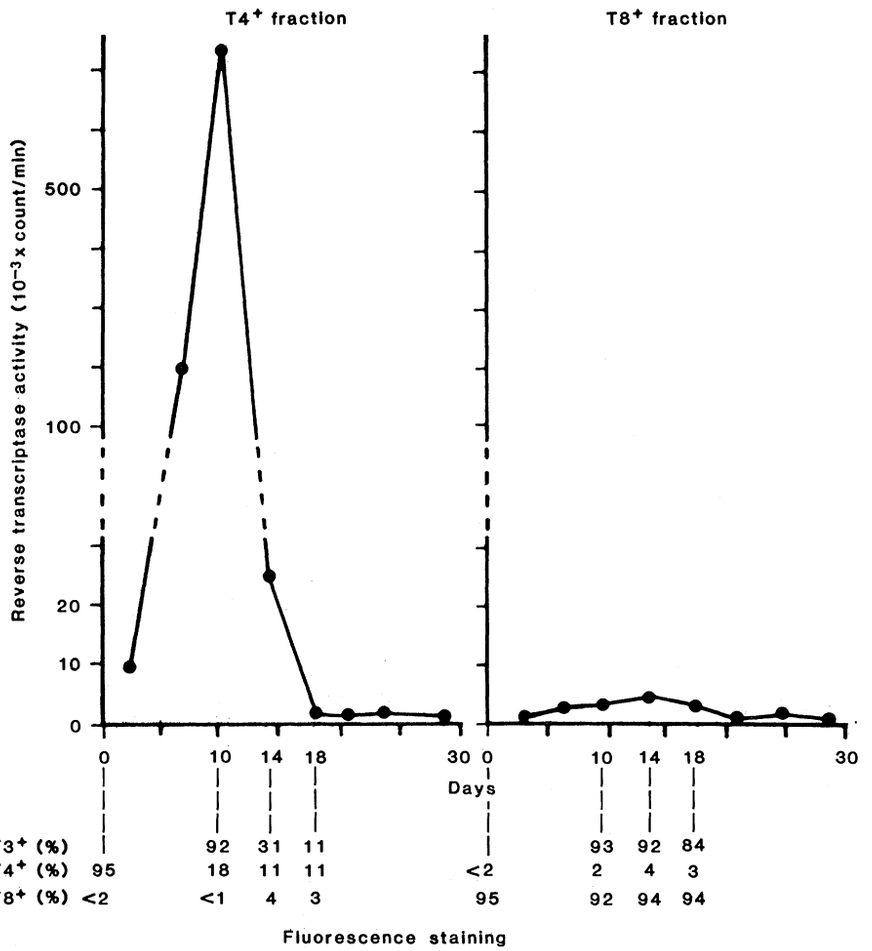

Fig. 1. Virus production and phenotype of cultured lymphocyte subsets. $T4^+$ and $T8^+$ fractions were purified by cellular affinity chromatography. A suspension of enriched T lymphocytes was obtained after depletion of monocytes by plastic-adherence, and by rosetting with aminoethylisothiouranium-treated sheep erythrocytes followed by centrifugation on a Ficoll-Hypaque gradient. The suspension usually contained 95 percent of T lymphocytes, the contaminating cells being mainly B lymphocytes. Approximately 6×10^7 T-enriched lymphocytes were incubated for 15 minutes at room temperature with 40 μl of OKT8 antibody (Ortho Diagnostic System) in 2 ml of RPMI 1640 medium supplemented with 5 percent autologous serum and 1 percent antibiotics. After two washes, cells were resuspended in 1 ml of medium and placed in a plastic column (Pharmacia K9/15) containing 5 ml of Sepharose 6-MB gel coupled with the 7*S* fraction of a goat antiserum to mouse immunoglobulin (Nordic). This gel (Pharmacia) was prepared following the manufacturer's specifications with 10 mg of proteins for 1 ml of gel. The antibody-labeled T lymphocytes were incubated in the gel for 15 minutes at room temperature. The $T8^-$ population was recovered by washing the column with 30 ml of medium at a rate of 1 ml per minute. The $T8^+$ fraction was then eluted by gentle mechanical agitation and washing with medium that had been warmed to 37°C. The efficiency of this method was checked by indirect immunofluorescence staining (*4*). All these procedures were performed under sterile conditions and the cell viability was excellent, as judged by trypan blue exclusion. Reverse transcriptase activity in culture was determined every 3 to 4 days, at the time of medium change (*6*). The cell suspension phenotypes were determined on days 0, 10, 14, and 18 after infection.

At the beginning of the culture, each cellular fraction was enriched to more than 95 percent and contained less than 2 percent of cells of the opposite phenotype. This phenotype remained unchanged throughout the period of culture in the $T8^+$ fraction with always less than 5 percent of contaminating cells. In contrast, a progressive disappearance of the markers identified by the monoclonal antibodies OKT3 and OKT4 was observed in the $T4^+$ cell fraction. Thus, the level of T4 expressing cells dropped to less than 20 percent as early as on day 6, when RT was already at 200×10^3 count/min. However, at that time, such cells continued to express OKT3 antigen, but half of them already showed an unusual weak and thin staining of the cell membrane compared to the normal patchy staining of the other half. Later on, percentages decreased to about 10 percent of $T3^+$ and $T4^+$ cells, whereas $T8^+$ cells never exceeded 4 percent in this fraction (Fig. 1). Since the disappearance of T4 antigens was not accompanied by the appearance of the T8 antigen, this phenomenon could not be related to a switch between these two membrane antigens. Such a switch has been

observed only during culture of cloned cells, but not in bulk culture of either $T4^+$ or $T8^+$ purified cells (*18, 19*). Furthermore, the kinetics of the disappearance of T3 and T4 markers at the cell membrane was related to the kinetics of virus production and could also be observed during infection of normal lymphocytes in vitro. This intriguing phenomenon may be due to virus-induced modulation of the expression of the T3-T4 molecules at the cell membrane, or by steric hindrance of the antibody binding site. Modifications in the expression of other membrane markers are currently under study.

After fixation (*6*), each cell fraction was also examined by indirect immunofluorescence with serum samples from the patient himself, his brother, his parents, or patients from whom similar viruses had been isolated. Both $T4^+$ and $T8^+$ fractions were strictly negative when tested with serum from the parents or other normal individuals with no antibodies against LAV. In contrast, when serum samples from E.L., D.L., or other patients were tested (all containing antibodies to LAV), a positive specific staining was observed in the $T4^+$ fraction and never in the $T8^+$ one. However, even at the peak of virus production, the percentage of stained cells in the $T4^+$ fraction only reached 10 percent. We suggest that the low percentage of $T4^+$ cells expressing viral antigens is due to the fact that only a fraction of $T4^+$ lymphocytes is susceptible to virus infection, indicating a heterogeneity in the $T4^+$ subset. It is also possible that the majority of the cells were infected but only a few were virus producers. The availability of molecular or immunological probes will be useful in discriminating between these possibilities.

Since virus production by infected cells is dependent on their proliferation (*8*), we verified that both fractions were able to proliferate under our culture conditions. Indeed, cell growth was followed every 3 or 4 days by counting these cells before they were systematically readjusted to 1×10^6 per milliliter. Proliferation of $T8^+$ cells was always greater than that of $T4^+$ lymphocytes as confirmed by [^{3}H]thymidine incorporation (Fig. 3). Thus, the absence of virus production in the $T8^+$ fraction could not be related to lower proliferation. Furthermore, in this experiment, as in others in which normal lymphocytes were infected in vitro by LAV, an early decrease of the $T4^+$ lymphocyte multiplication was noted at the same time that virus was being produced. The appearance of giant cells was also observed, indicating a cytopathic effect of the virus (Fig. 4).

To confirm these findings in vivo, we also investigated which lymphocyte populations from normal individuals were sensitive to infection by LAV or related retroviruses in vitro. We obtained PBL from healthy Caucasian volunteers who had no antibodies against retroviruses and separated them into T, $T4^+$, and $T8^+$ fractions. Half of each fraction was separately infected with virus-containing cell-free supernatant. Reverse transcriptase was sequentially measured in each infected and uninfected culture. In three different experiments, using LAV-1 or virus isolated from E.L., we found that infected $T4^+$ lymphocytes displayed virus production, as assessed by an increase in RT which reached 30×10^3 to 100×10^3 count/min at the peak value and by electron microscopy. On the contrary, uninfected cultures of $T4^+$ or $T8^+$ cells as well as infected $T8^+$ fractions expressed less than 2×10^3 count/min throughout the culture period. When the $T4^+$ and $T8^+$ fractions were mixed, RT production was similar to that of the original unfractionated population, demonstrating that the technique used to separate the T cell subsets did not interfere with virus production (data not shown). Immunofluorescence staining showed no modification of the surface phenotype of the uninfected $T4^+$ or $T8^+$ cultured cells. In contrast, in the infected $T4^+$ fractions, a decrease of T4 and T3 markers was observed, but only when a high level of RT was produced in the culture.

It seems clear that the virus can be produced in vivo only by the $T4^+$ cells of the healthy carrier and that this selective tropism for $T4^+$ lymphocytes is identical to that observed after the in vitro infection of lymphocytes of healthy blood donors. It remains to be determined whether this phenomenon is due to a lack of recognition of specific receptors at the cell membrane of $T8^+$ cells or to a lack of expression of the proviral DNA. Data published by Gallo and co-workers (*10*) have suggested that HTLV shows a similar tropism in transformed cell lines derived from leukemic patients or immortalized after infection in vitro. In both situations, most cell lines were $T4^+$, but some others have been described as being $T4^+$ and $T8^+$ or $T4^-$ and $T8^-$ (*10, 14, 15, 20*) or even B cells (*21*). Tropism for T4 cells has also been suggested for a new HTLV isolate which resembles LAV, called HTLV-III (*22*).

That LAV was found in a "healthy" carrier whose brother had AIDS does

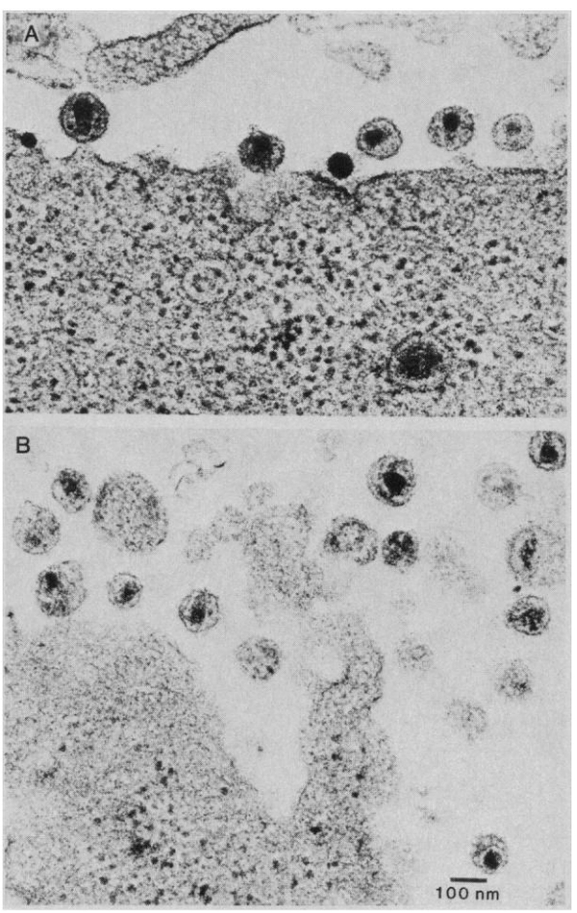

Fig. 2. Electron microscopy of viral particles in ultrathin section. (A) Portion of a T4 lymphocyte from a healthy donor, which had been infected by the virus isolated from patient E.L. Note the numerous mature particles with eccentric dense core in the external space. These particles are morphologically similar to D particles such as those found in Mason-Pfizer virus or the virus recently isolated from simian AIDS (*27, 28*). However, no antigenic relatedness was found between the p25 of the human virus and the p27 of Mason-Pfizer virus (*29*). (B) Portion of a T4 lymphocyte from patient E.L. at the time of virus production. Note the abundant mature virions around a piece of altered cytoplasm.

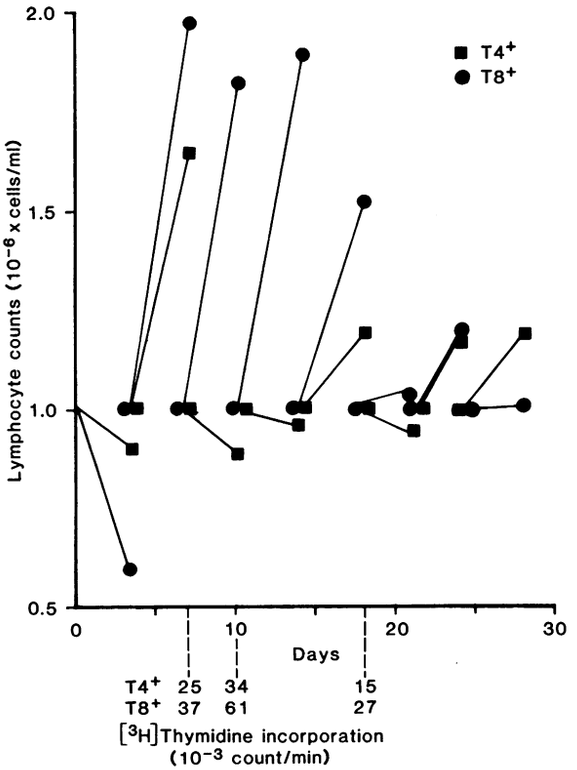

Fig. 3. Cell growth as followed by cell counting and [^{3}H]thymidine incorporation. Every 3 to 4 days, at the time of medium change, cells were counted and adjusted at 10^6 per milliliter. On days 7, 10, and 18, portions of cell suspension were labeled with [^{3}H]thymidine for 12 hours and material precipitated in trichloroacetic acid was counted in a liquid scintillation spectrometer.

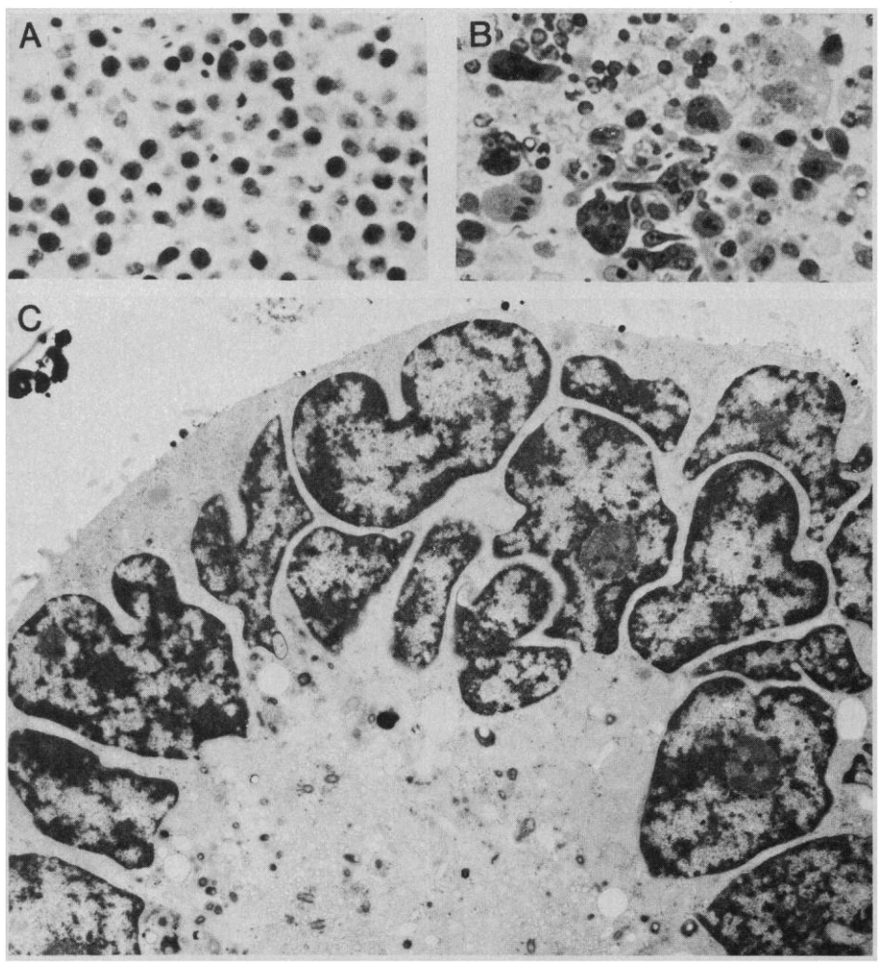

Fig. 4. (A and B) Light microscope picture of thin sections of cultured T4$^+$ lymphocytes from a healthy donor. Staining by toluidine blue. (A) Uninfected culture. (B) LAV-infected culture at the peak of virus production. Note the enlarged and giant cells. (C) Electron microscopy picture of a giant cell of (B). Note the numerous nuclei arising by cell fusion and the viral particles at the cell surface (×8000).

not provide evidence against its causal role in the disease. First, the incubation period after virus infection may vary from one individual to another. Indeed, 3 months after these experiments were carried out, E.L.'s T4$^+$ lymphocytes decreased although he still showed no clinical symptoms (*23*). Second, individual variations in susceptibility (genetic background or natural history) to virus infection are well documented in man and mice (*24–26*) and, with respect to AIDS, are suggested by epidemiological data on regular sexual partners of AIDS patients. Third, since LAV infection of T4$^+$ cells impairs their proliferative capacity and causes cytopathogenic effects, the present results support the hypothesis that LAV is involved in AIDS and point to its possible interference with T-cell differentiation.

DAVID KLATZMANN
Laboratoire d'Immunologie Néphrologique et de Transplantation, UER Pitié-Salpétrière, 75634 Paris Cédex 13, France
FRANCOISE BARRÉ-SINOUSSI
MARIE THÈRÉSE NUGEYRE
CHARLES DAUGUET
Département de Virologie, Institut Pasteur, 75724 Paris Cédex 15
ETIENNE VILMER
CLAUDE GRISCELLI
Département de Pédiatrie, Hôpital des Enfant Malades, Paris
FRANCOISE BRUN-VEZINET
CHRISTINE ROUZIOUX
Laboratoire de Virologie, Hôpital Claude Bernard, Paris
JEAN CLAUDE GLUCKMAN
Laboratoire d'Immunologie Nephrologique et de Transplantation, UER Pitié-Salpétrière
JEAN-CLAUDE CHERMANN
LUC MONTAGNIER
Département de Virologie, Institut Pasteur

References and Notes

1. E. Dournon *et al.*, *Lancet* **1983-I**, 1040 (1983).
2. J. B. Brunet *et al.*, in *Epidemic Kaposi's Sarcoma and Opportunistic Infections in Homosexual Men: Expression of Acquired Immunoregulatory Disorder*, A. E. Friedman Kein, Ed. (Masson, New York, in press).
3. Centers for Disease Control, *Mortal. Morbid. Weekly Rep.* **31**, 305 (1982).
4. D. Klatzmann *et al.*, *Ann. N.Y. Acad. Sci.*, in press.
5. R. W. Schroff *et al.*, *Clin. Immunol. Immunopathol.* **27**, 300 (1983).
6. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983).
7. For the clinical details of these patients, see E. Vilmer *et al.*, *Lancet* **1984-I**, 753 (1984).
8. L. Montagnier *et al.*, in *Human T-Cell Leukemia Lymphoma Viruses*, R. C. Gallo, M. E. Essex, L. Gross, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in press).
9. B. J. Poiesz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980).
10. M. Popovic *et al.*, *Science* **219**, 856 (1983).
11. C. E. Metroka *et al.*, *Ann. Int. Med.* **99**, 585.
12. H. Kornfeld *et al.*, *N. Engl. J. Med.* **307**, 729 (1982).
13. F. Brun-Vézinet *et al.*, *Lancet*, in press.

14. P. D. Markham *et al.*, *Int. J. Cancer* **31**, 413 (1983).
15. M. Popovic *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5402 (1983).
16. J.-C. Chermann *et al.*, *Antibiot. Chemother.* **32**, in press.
17. S. Chouaib *et al.*, *J. Immunol.* **132**, 1851 (1984).
18. G. F. Burns *et al.*, *Cell. Immunol.* **71**, 12 (1982).
19. D. Zagury *et al.*, *Int. J. Cancer* **31**, 705 (1983).
20. F. W. Ruscetti *et al.*, *ibid.*, p. 171.
21. N. Yamamoto *et al.*, *Nature (London)* **299**, 367 (1982).
22. M. Popovic *et al.*, *Science* **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500.
23. E.L.'s lymphocyte phenotypes and functions were determined in August 1983 and January and February 1984. No lymphopenia was observed and the responses to mitogenic or allogenic stimulation were normal. The percentages of T-cell subsets were, respectively: T3: 70, T4: 33, T8: 27 in August 1983; T3: 52, T4: 5, T8: 52 in January 1984; T3: 65, T4: 14, T8: 50 in February 1984. The fractionation experiment reported here was performed in September 1983.
24. J. L. Virelizier *et al.*, *Arch. Virol.* **50**, 279 (1976).
25. F. B. Bang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **46**, 1065 (1960).
26. D. T. Purtillo *et al.*, *Lancet* **1976-I**, 882 (1976).
27. M. D. Daniel *et al.*, *Science* **223**, 602 (1984).
28. P. A. Marx *et al.*, *ibid.*, p. 1083.
29. L. Montagnier *et al.*, *Ann. Virol. (Inst. Pasteur)*, **135E**, 119 (1984).
30. We thank C. Gazengel for providing the patient's sera, F. Chapuis, F. Rey, C. Axler, J. Gruest, and S. Chamaret for technical assistance, and E. Martin for typing the manuscript. We are indebted to G. Janossy (Royal Free Hospital, London) for information about the cellular affinity chromatography technique. This work was supported by grants from the Ministère de l'Industrie et de la Recherche (contracts 82 L 13.13., 83 C 14.11. and 83 C 14.14), Centre National de la Recherche Scientifique (CNRS), and Ligue Nationale contre le Cancer. F. Barré-Sinoussi, J. C. Chermann, and L. Montagnier are also members of the research team No. 147 of CNRS.

1 May 1984; accepted 29 May 1984

Adaptation of Lymphadenopathy Associated Virus (LAV) to Replication in EBV-Transformed B Lymphoblastoid Cell Lines

Abstract. *A strain of lymphadenopathy associated retrovirus (LAV) passaged in vitro was used to infect a lymphoblastoid cell line obtained by transformation with Epstein-Barr virus of B lymphocytes from a healthy donor. The virus produced from this line (B-LAV) was also able to grow at a high rate in some other lymphoblastoid lines and in a Burkitt lymphoma line. This adapted strain retained the biochemical, ultrastructural, and antigenic characteristics of the original strain, as well as its tropism for normal T4$^+$ lymphocytes. It is thus possible to grow LAV in large quantities that can be used for the preparation of diagnostic reagents. The interaction between such a human retrovirus and Epstein-Barr virus, a DNA virus, may have some implication for the pathology of the acquired immunodeficiency syndrome and related diseases.*

Lymphadenopathy associated virus (LAV) is a human T-cell lymphotropic retrovirus that was first isolated from the cells of a lymph node of a homosexual man with lymphadenopathy syndrome (LAS) (*1*). Similar isolates were made from several patients with the acquired immunodeficiency syndrome (AIDS), including a B hemophiliac (*2, 3*), a Zairian woman (*2*), a Zairian couple (*4*), and a Haitian man (*2*).

All viral isolates have in common a major, antigenically related protein with a molecular weight of 25,000 (p25), a Mg^{2+}-dependent reverse transcriptase (RT), mature virions with a morphology similar to that of equine infectious anemia virus (EIAV), and D type particles. The p25 of LAV is immunoprecipitated by serum from horses infected with EIAV, suggesting antigenic similarity with the core protein of this virus (*5*). In contrast, the p25 of LAV and LAV-related viruses is not antigenically related to the p24 of human T-cell leukemia virus type I (HTLV-I), the core proteins of Mason-Pfizer virus (*5*) or similar viruses isolated from simian AIDS (*6*), or to other known animal retroviruses (*5*). An important biological feature of members of the LAV family is their specific tropism for activated T4$^+$ (helper) lymphocytes, in which they induce a cytopathic effect without giving rise to immortalized lines (*1, 2, 7*). Initially, we found that LAV could not be grown in B lymphocytes, Raji cells, MOLT/4, or in several established fibroblastic lines (*1*).

We report here the successful adaptation of LAV to persistent infection of several lymphoblastoid cell lines obtained by the transformation of B lymphocytes with Epstein-Barr virus (EBV) as well as in one Burkitt lymphoma cell line. Thus, it is now possible to grow LAV on a large scale in permanent cell lines. The growth of LAV in EBV-transformed cells may have important implications for the pathogenic role of such viruses in AIDS and other diseases.

The first LAV isolate was initially propagated in cord blood lymphocytes and then in lymphocytes from several adult donors whose sera contained no antibody against LAV and whose lym-

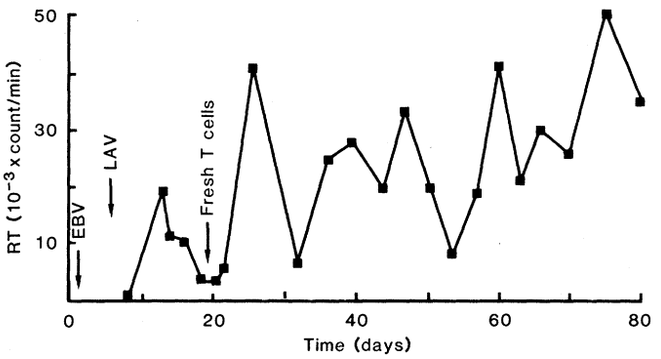

Fig. 1 (above). EBV and LAV co-infection of lymphocytes from a blood donor (F.R.). The culture medium (RPMI 1640 with 10 percent fetal calf serum) included TCGF, antiserum to interferon α, and Polybrene, as described (*1*). Production of LAV was determined by assaying RT activity in 1-ml portions of pelleted virus (*1*).

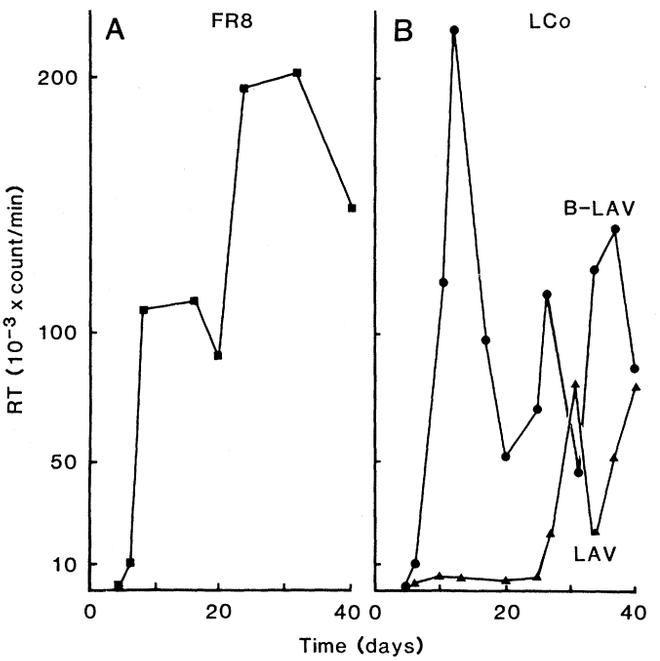

Fig. 2 (right). (A) Virus production by the established FR8 lymphoblastoid line. The cells were infected with LAV at time zero as described (*1*). (B) Virus production by the LCo lymphoblastoid line after infection by LAV and B-LAV. Same medium as in Fig. 1, except that TCGF was omitted.