Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation on a Ficoll-Hypaque gradient. PBMC (5×10^6) were then treated with PHA-P (10 μg/ml) and maintained on RPMI-1640 medium with 20 percent FCS and 10 percent interleukin-2. No abnormal cellular changes were observed by light microscopy throughout the culture period. However, after day 25, PBMC expressed HTLV-III antigens as detected by indirect immunofluorescence with a human serum positive for HTLV-III or with monoclonal antibodies to HTLV-III p15 and p24 (Table 1), but they were consistently negative for EBV, CMV, and HTLV-I antigens. The corresponding supernatant fluids showed significant RT activity on days 25 and 29 (Table 1). Furthermore, on day 12, addition of cell-free supernatant fluid (2 ml) from the PBMC culture to Polybrene-treated H9 cells (3×10^6) resulted in characteristic morphologic changes 14 days later. The CPE were similar to those induced by the mononuclear cells from the subject's semen described above. These H9 cells also become positive for HTLV-III antigens, and their supernatant fluids showed RT activity (0.4×10^5 to 4.8×10^5 cpm/ml).

That our isolates are HTLV-III is indicated by their (i) antigen expression, (ii) RT characteristics, (iii) morphology on electron microscopy, and (iv) ability to induce unique CPE in H9 cells (*7, 13*). That the isolates are not HTLV-I or HTLV-II is shown by the positive staining obtained with the HTLV-III–specific monoclonal antibodies (*14*) and the negative results with the HTLV-I–specific monoclonal antibodies.

HTLV-III was recovered from the mononuclear-cell fraction of semen. Attempts to find virus in the spermatozoa fraction of semen from 11 individuals have yielded negative results. Because of the toxic effects of semen on target cells used for these isolations, it is unclear whether there is also cell-free HTLV-III in seminal plasma. The coexistence of other viruses in semen, such as CMV (*16, 17*), may also interfere with the successful cultivation of HTLV-III.

The demonstration of HTLV-III in the semen of an asymptomatic individual who is at risk for AIDS supports epidemiologic data suggesting that AIDS can be sexually transmitted. It is unknown why one HTLV-III carrier remains well while another develops AIDS. Asymptomatic carriers should be closely followed for the possible development of AIDS. Recent surveys suggest that the prevalence of HTLV-III/LAV seropositivity in urban male homosexuals may be as high as 65 percent (*18*). Most of these men are healthy and without obvious immune deficits. The frequency of HTLV-III carriers in this population is unknown. These issues need to be addressed by careful prospective analyses of asymptomatic HTLV-III seropositive individuals.

DAVID D. HO
ROBERT T. SCHOOLEY
TERESA R. ROTA
JOAN C. KAPLAN
THERESA FLYNN
Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School, Boston 02114
SYED Z. SALAHUDDIN
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205
MATTHEW A. GONDA
Program Resources Inc., Frederick Cancer–Research Facility National Cancer Institute, Frederick, Maryland 21701
MARTIN S. HIRSCH
Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School

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Prevalence of Antibodies to Lymphadenopathy-Associated Retrovirus in African Patients with AIDS

Abstract. *The presence of antibodies to lymphadenopathy-associated retrovirus (LAV) was determined by a radioimmunoprecipitation assay and by an enzyme-linked immunosorbent solid assay of sera from Zairian patients with the acquired immune deficiency syndrome (AIDS) in 1983. Thirty-five of 37 patients (94 percent) and 32 of 36 patients (88 percent), respectively, were seropositive by the two tests. In a control group of 26 patients, six (23 percent) showed positive results in these tests. Of these six control patients, five had clinically demonstrable infectious diseases and a low ratio of T4 to T8 lymphocytes. In addition, sera collected from a control group of Zairian mothers in 1980 were positive for LAV in 5 of 100 cases. Other serologic data suggest that LAV was present as early as 1977 in Zaire.*

The isolation of a new lymphotropic retrovirus from cultured lymphocytes of a patient with lymphadenopathy syndrome (LAS) was reported in May 1983 (*1*). This virus, named lymphadenopathy-associated virus (LAV), differed from the previous isolates of human T-cell leukemia virus (HTLV-I) by the lack of antigenic relatedness of its major core protein (p25) to HTLV-I p24 and by a peculiar morphology of mature virions, which was similar to those of D type particles and equine infectious anemia virus (*2*). In addition, antibodies produced in horses infected by the latter virus precipitated the p25 of the human virus (*2*).

Similar isolates have been made from AIDS or LAS patients belonging to the groups that are at risk for the disease: four from homosexuals, two from two hemophiliac siblings, two from Haitians, and three from Zairians (*3–6*). Such viruses display selective tropism for the $T4^+$ subset of lymphocytes, both in vitro and in vivo, in which they induce a depression of cell growth and a cytopathic effect (*3, 7*) upon activation. A high prevalence of antibodies to viral structural proteins was found in AIDS and LAS patients hospitalized in France, including patients of African origin (*3, 8*). By contrast, only one of 330 controls (French blood donors, laboratory workers, and prisoners) was serologically positive for these antibodies (*6, 8*). These data suggest that such a group of viruses could play a role in the etiology of AIDS,

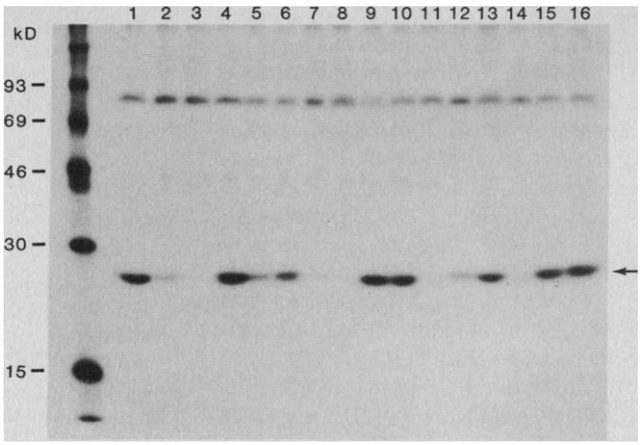

Fig. 1. Radioimmunoprecipitation assay of patient antibody by LAV p25. Virus produced by LCo cells was metabolically labeled with [^{35}S]methionine as described (*1*). The pelleted virus was not further purified and was lysed in RIPA buffer containing 5 percent Zymofren (Specia) and diluted in 50-μl portions of the same buffer. Each portion of viral lysate was incubated with 5 μl of serum to be tested for 1 hour at 37°C and 18 hours at 4°C. Immune complexes were adsorbed on protein A–Sepharose beads, washed, denatured, and subjected to electrophoresis in 12.5 percent polyacrylamide-sodium dodecyl sulfate slab gel as described (*1*). Numbers on the left are molecular size markers in kilodaltons. (Lanes 1, 4, 6, 9, 10, 13, 15, and 16) Highly positive sera; (lanes 2, 5, 12) weakly positive sera; and (lanes 3, 7, 8, 11, and 14) negative sera. In contrast to HTLV-III (*16*), the main protein specifically precipitated is p25. The 43-kD band and the 84-kD band are cellular contaminants that are immunoprecipitated in all the tested sera.

with the possible help of other antigenic stimuli activating latently infected lymphocytes (*3*, *4*).

We now report the results of a study to determine the presence of LAV antibodies in sera of AIDS patients diagnosed in 1983 in Zaire by an international team (*9*). The results show a high prevalence of antibodies in the AIDS group, as compared to control groups, and correlation with a decrease in the ratio of T4 to T8 cells and in the absolute number of T4 cells.

For antibody detection, two assays were used in parallel:

1) A radioimmunoprecipitation assay (RIPA), followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis, which detects immunoglobulin G antibodies to LAV p25 protein after metabolic labeling of the virus with [^{35}S]methionine (*3*, *8*).

2) An enzyme-linked immunosorbent assay (ELISA) for LAV antibodies which has been described in detail (*3*, *8*). Each serum was analyzed in duplicate at a 1:40 dilution. In order to eliminate nonspecific binding of immunoglobulins, which often occurs in the sera of AIDS patients, we performed a control adsorption on lysates of uninfected lymphocytes at the same protein concentration for each serum. To improve the sensitivity of the test, we introduced the following modifications: The virus was purified three times in sucrose gradients and disrupted with 1 percent Triton and 0.1 percent sodium deoxycholate in RIPA buffer (*1*) lacking SDS. Two hundred nanograms of virus-associated proteins were coated in each well. Only when the optical density difference between control and virus was higher than 0.4 was a response considered positive.

As the source of virus, the original strain of LAV (LAV-1) grown on stimulated T lymphocytes of an adult healthy donor (F.R.) was used at the beginning of these studies. Later, virus was produced in a more convenient way from a lymphoblastoid cell line from the same donor, persistently infected with the virus. Although the original strain of LAV did not grow on normal B cells, we found that LAV, after being passaged several times on cultured lymphocytes, could grow readily on a lymphoblastoid line (FR8) obtained by transformation of FR's B lymphocytes with Epstein-Barr virus (*10*). The virus grown on this line (B-LAV-1) has retained the original tropism of LAV for T4 lymphocytes, although it is also able to grow in some other lymphoblastoid lines, including one derived from umbilical cord (LCo). It could not be distinguished from LAV-1 by ultrastructural morphology and its major proteins. The validity of using B-LAV-1 antigens for detection of antibodies to LAV type viruses was assessed with RIPA and ELISA by comparing results obtained on the same sera with the antigens of LAV grown on T cells, and those with the antigens of B-LAV grown on the lymphoblastoid line FR8. In the ELISA against B-LAV, the control for nonspecific binding was performed with a cytoplasmic lysate of the uninfected FR8 line. No contamination by Epstein-Barr virus antigens (EA and VCA) could be detected by precipitation of gradient-purified B-LAV with the corresponding specific antisera. Without exception, results obtained with both viral strains were the same. The sensitivity of ELISA was within the same range as that of RIPA, whether lysed LAV-1 or B-LAV-1 was used.

The criteria, both clinical and immunological, for AIDS diagnosis in the Zaire patients are described in detail elsewhere (*9*). Briefly, a patient with AIDS was a previously healthy adult under 60 years of age, who had evidence of an opportunistic infection or disseminated Kaposi's sarcoma, no underlying history of immunosuppressive disease or immunosuppressive drug use, and in addition fulfilled at least two of the following three immunologic criteria: skin test anergy to multiple antigens, an absolute number of helper T lymphocytes (OKT4) less than 400 per cubic millimeter and a ratio of helper to suppressor T cells (OKT4/OKT8) less than 0.7. Most of the Zaire patients were diagnosed in the Mama Yemo Hospital and the University Hospital. Sera from 37 of the 38 patients who were diagnosed as having AIDS during a 3-week period were available for testing. In addition, 26 sera from a control group of concurrently hospitalized patients with other diagnoses were also screened for LAV antibodies. Of these control patients, 14 had noninfectious diseases, eight had tuberculosis, three had acute malaria, and one had acute nonbacterial meningoencephalitis. For each case, the absolute number of leukocytes and T lymphocytes and the T4/T8 ratio were determined. The coded sera were tested independently by RIPA (L.M. and S.C.) and ELISA (C.R. and F.B.-V.).

The high percentage of LAV seropositivity (94 percent) was demonstrated in the AIDS patients (Table 1). A few sera were weakly positive by RIPA (as exemplified in Fig. 1) and borderline by ELISA, and they were not considered positive in the latter test. One serum (patient 27) was frankly positive by ELISA, and negative by RIPA. In addition, sera from four patients with AIDS-related complex were also positive. Sera from two women who were sexual partners of men who died of AIDS were also weakly positive. Both women were healthy, but one women had a T4/T8 ratio of 0.11 with 122 T4 cells per cubic millimeter whereas the other woman had a ratio of 1.39.

By contrast, only 23 percent of patients in the control group showed positive results for LAV (Table 1; $P = 0.0001$); of the six patients with LAV antibodies, five had a T4/T8 ratio

less than 0.7 (three with lung tuberculosis and two with acute falciparum malaria), whereas only one of the 18 patients with T4/T8 > 1.0 had antibodies to LAV ($P = 0.02$ by Fisher's exact test). The mean T4/T8 ratio of the six controls with LAV antibodies was 0.37 (range, 0.09 to 0.42), compared to 1.79 (range, 0.6 to 5.4) for the 20 controls lacking antibodies ($P < 0.001$, Student's t-test). The LAV-positive control patient with a T4/T8 ratio of 1.05 was a 20-year-old woman suffering from arterial hypertension and recent weight loss. Seronegative patients included 14 with noninfectious diseases, five with pulmonary tuberculosis, and one with meningoencephalitis of probable viral origin.

Titers of antibody to LAV in both groups were measured by ELISA. A control positive serum was tested at four dilutions (1/40 to 1/320) on each plate, and the standard curve obtained was used to determine the antibody titer of the patients' sera, according to a computerized program (*8*). Of the 32 AIDS patients, 14 had titers higher than 1/400, seven had titers between 1/100 and 1/400, and 11 had titers between 1/40 and 1/100. The latter were weakly positive by RIPA. In the control group, two had a titer higher than 1/400, including the patient with arterial hypertension, and three had titers between 1/40 and 1/100.

Sera of African patients hospitalized in Belgium from 1977 until 1983 were also found to have LAV antibodies. These include three Zairians and three Rwandeses with AIDS, and two Zairians and one Rwandese with LAS. Two additional control groups were sampled in 1980 and 1983. Sera from 100 healthy mothers living in Kinshasa, Zaire, were collected in April to July 1980 for hepatitis B studies; five of these were found to have LAV antibodies by ELISA (Table 1). In another group of 100 patients with noninfectious disease treated at the Ngaliema Hospital in Kinshasa in 1983, seven had positive results for LAV (Table 1).

One case deserves particular notice, since it shows that LAV-associated AIDS was already present in Zaire in 1977, and it suggests familial transmission. The patient was a Zairian mother who died in 1978 and whose clinical signs of illness in 1977 were retrospectively considered to fulfill the criteria of AIDS (*11*). Her serum from 1977 contained antibodies to LAV. She had a female infant who, throughout her first year of life, had recurrent extensive candidiasis and depressed mitogen responses. The girl has completely recovered and is now a healthy 7-year-old living in Belgium. Her serum taken at age 6 years was weakly positive by RIPA for LAV antibody.

Table 1. LAV antibodies in Zairian groups.

Subjects	Positive for LAV antibodies	
	RIPA	ELISA
1983 (Mama Yemo and University hospitals, Kinshasa)		
AIDS patients*	35/37	32/36
AIDS-related complex	4/4	4/4
Sexual partners of AIDS patients	2/2	2/2
Controls (Mama Yemo and University hospitals, Kinshasa)	6/26	5/26
T4/T8 < 0.7†	5/8	4/8
T4/T8 > 0.7	1/18	1/18
T4 cells < 400/mm^3‡	4/8	
T4 cells > 400/mm^3	2/18	
1980 Controls (mothers)	N.D.	5/100
1983 Controls (hospitalized patients, Ngaliema Hospital)	N.D.	7/100

*Association between presence of LAV antibodies and AIDS (1983), $P = 0.0001$ (χ^2 test). †Association of LAV antibodies with lower T4/T8 ratio in the control group, $P = 0.02$ (Fisher test). ‡Association of LAV antibodies with absolute T4 number < 400/mm^3 in control group, $P = 0.03$ (Fisher's exact test).

The prediction that a single infectious agent is at the origin of AIDS implies that all those with proven AIDS show signs of infection. Failures to show infection by the agent should be rare or must be reasonably attributed to lack of sensitivity for demonstrating virus or antibody. In the case of a lymphotropic, lymphocytolytic agent such as LAV, failure to show antibody may also be due to eventual depletion of cells that are a necessary link in immune reaction. Evidence for secondary antibody failure in AIDS was presented earlier (*4*). The prediction does not imply that all those infected by the agent proceed to clinical AIDS but, unless additional factors outweigh the direct role of the agent in the causation of AIDS, it does imply that the agent is relatively infrequent in the healthy population at risk for AIDS, and the frequency of infection in that population parallels, at a lower level, the frequency of AIDS cases.

The incidence of AIDS in Zaire has recently been found to be very high in Kinshasa, ranging from 15 to 20 cases per 100,000 population (*9*). Our data, showing LAV infection in 94 percent of Zairian AIDS cases and in at least 5 percent of control populations, support the hypothesis that retroviruses of the LAV type are universally involved in this disease. The finding of seropositivity in healthy individuals in 1980 (5 percent in Zairian mothers) suggests that almost as many individuals were carrying LAV in 1980 as in 1983 (7 percent). Indeed this rate in Zaire is much higher than that observed in European countries, which have a lower incidence of AIDS (0.3 percent positive for antibody to LAV in healthy French control populations in 1983). However, LAV-associated AIDS in Africa is not solely restricted to Zaire, but it extends to other countries of equatorial or subequatorial Africa. We and others (*12*) have also found a high degree of LAV seropositivity in AIDS patients in Rwanda and the Central African Republic.

Further evidence of the causal relationship between LAV and AIDS is the high prevalence of LAV antibody in AIDS among Caucasian homosexuals, hemophiliacs, parenteral drug users, and Haitians, and its rarity in control groups (*8, 13*). In addition, viruses with characteristics similar to LAV have also been isolated from lymphocytes from each of the above risk groups, as well as from Zairian AIDS patients diagnosed in France and in Kinshasa (*13–15*).

The observation of LAV seropositivity in heterosexual partners and children of AIDS patients is consistent with earlier observations of heterosexual and familial (vertical) transmissions of AIDS in Zairian patients (*9*). These epidemiologic features are different from those observed in the United States and Europe and will require further studies for confirmation.

Finally, the significance of the higher rate of LAV seropositivity in hospitalized patients without AIDS but with tuberculosis or malaria and the significance of the correlation of seropositivity with lowered T4/T8 ratios and decreased T4 lymphocytes are still unknown. One possibility is that these patients may have had early clinical manifestations of AIDS, but which did not fulfill our strict definition of AIDS. Larger prospective studies, including culture and serology for LAV, will be required to confirm these associations, to examine the high rates of infection in various populations in Africa, to identify groups at special

risk, and to determine the modes of LAV and AIDS transmission in Africa, which may be more diverse than those observed in Europe and the United States.

F. BRUN-VÉZINET
C. ROUZIOUX
Hopital Claude Bernard, Paris, France
L. MONTAGNIER
S. CHAMARET
J. GRUEST
F. BARRÉ-SINOUSSI
D. GEROLDI
J. C. CHERMANN
Viral Oncology Unit, Institut Pasteur, Paris, France
J. MCCORMICK
S. MITCHELL
Centers for Disease Control, Atlanta, Georgia 30333
P. PIOT
H. TAELMAN
Institute of Tropical Medicine, Antwerp, Belgium
KAPITA BILA MIRLANGU
Mama Yemo Hospital, Kinshasa, Zaire
ODIO WOBIN, MBENDI N.
MAZEBO P., KAYEMBE KALAMBAYI
University Hospital, University of Kinshasa, Zaire
C. BRIDTS
University of Antwerp, Antwerp, Belgium
J. DESMYTER
Rega Institute, University of Leuven, Leuven, Belgium
F. M. FEINSOD
T. C. QUINN
National Institute of Allergy and Infectious Disease, Bethesda, Maryland 20205

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Noninvasive Three-Dimensional Computer Imaging of Matrix-Filled Fossil Skulls by High-Resolution Computed Tomography

Abstract. *A noninvasive computer imaging technique allows three-dimensional images of fossil skulls to be generated from two-dimensional serial computerized tomographic scan data. The computer programs can "dissect" the skull in different planes by making portions of it and any obstructing matrix transparent in order to reveal intracranial morphology. The computer image is geometrically precise so that linear distances, angles, areas, volumes, and evaluations of symmetry can be determined.*

There has not been a satisfactory method for noninvasively visualizing intracranial morphology in fossil skulls in more than two dimensions. Skulls subjected to such paleontological study are normally "dissected" by sectioning the skull in the desired plane or by piecemeal removal of calvarial fragments. Conventional skull x-ray radiographs and computerized tomography (CT) scans are abstract, two-dimensional representations that fail to reveal important three-dimensional relations among intracranial structures. These limitations to morphologic analysis are particularly severe in paleoanthropology because most fossil skulls are too precious to be physically "invaded" by scientists wishing to examine intracranial structure (*1–3*).

For fossil crania filled with hardened matrix (*4–6*), noninvasive examination of intracranial three-dimensional morphology or direct measurement of intracranial dimensions and volume has not been possible without matrix removal. If attempted, such direct measurements would necessarily damage the original fossil. Thus, some potentially important fossil specimens lie unprepared on museum shelves with hardened matrix obscuring anatomical information.

New computer imaging techniques now enable the viewing and analysis of fossil specimens filled with matrix. The techniques involve computer imaging capabilities that produce three-dimensional images from two-dimensional CT data. The three-dimensional images are formed from a series of high-resolution, transaxial CT scans of the skull taken at 2-mm intervals. The method has already been used to examine over 500 patients with craniofacial deformities, facial trauma, neoplastic disease of the head and neck, intracranial soft-tissue abnormalities, and musculoskeletal diseases (*7–9*).

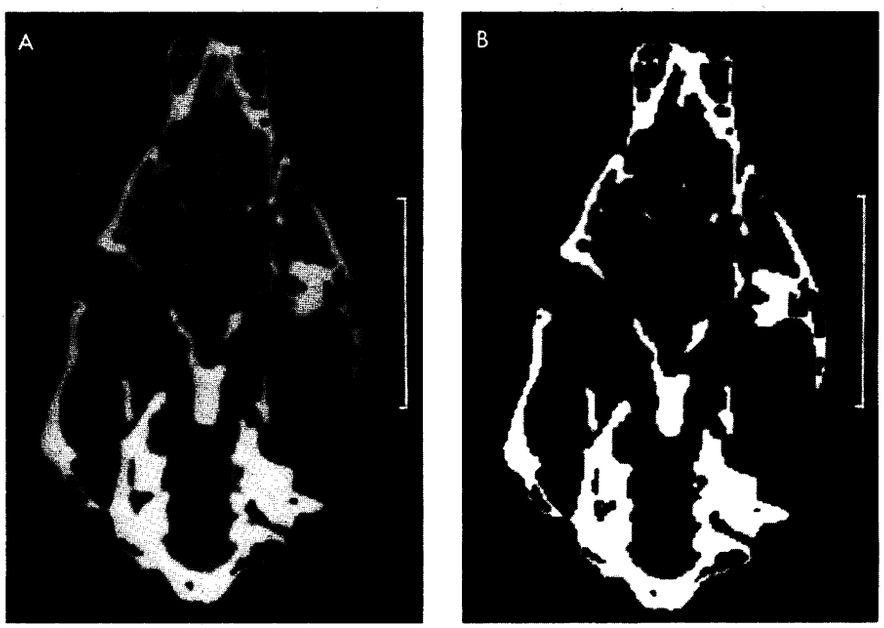

Fig. 1. (A) A 2-mm slice through the cranial and nasal cavities showing the CT scanner's ability (in extended bone range) to clearly distinguish mineralized fossil bone from the hardened sandstone matrix within these cavities. (B) The osseous contours are separated from the sandstone matrix in each CT slice by setting the window width to 0 and the window level to the threshold value representing the CT attenuation that distinguishes mineralized bone from the particular matrix. The computer program repeats this procedure for each CT slice to produce the three-dimensional images. Scale bars, 5 cm.