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Lymphadenopathy Associated Virus Infection of a Blood Donor–Recipient Pair with Acquired Immunodeficiency Syndrome

Abstract. *A retrovirus isolated from three patients with the acquired immunodeficiency syndrome (AIDS) in the United States was morphologically and antigenically identical to lymphadenopathy associated virus isolated in France. Two of these isolates were from a blood donor–recipient pair, each of whom developed AIDS. Lymphadenopathy associated virus was isolated from the blood donor's lymphocytes 12 months after his onset of AIDS symptoms and from the blood recipient's lymphocytes 1 month after her onset of AIDS symptoms. Two isolates from the blood donor–recipient pair and an isolate from an epidemiologically unrelated homosexual man were examined by competitive radioimmunoassay to determine their antigenic relatedness to each other and to other human retroviruses. The major core proteins (p25) of the isolates were antigenically identical and all three isolates were identical to prototype lymphadenopathy associated virus isolated in France.*

Human retroviruses have been implicated as etiologic agents of the acquired immunodeficiency syndrome (AIDS). Evidence to support this causal association includes (i) the detection in blood from patients with AIDS and lymphadenopathy syndrome (LAS) of antibodies to membrane antigens of human T-cell lymphotropic virus, HTLV (*1*); (ii) the isolation in France from lymphocytes from a homosexual man with lymphadenopathy of a retrovirus called lymphadenopathy associated virus (LAV) (*2*); (iii) the detection of antibodies to membrane antigens of HTLV in serum from donors who gave blood to patients who subsequently developed transfusion-associated AIDS (*3*); and (iv) the documentation of infection in patients with AIDS, AIDS-related diseases, and their contacts by a retrovirus called HTLV-III (*4*). We now report the isolation and characterization of a retrovirus from blood from three AIDS patients and compare its antigenic relatedness to HTLV-I, HTLV-II, and LAV.

Whole blood for virus isolation and serologic testing was collected from a blood donor–recipient pair, each member of which had developed AIDS. The blood recipient was a 38-year-old woman who was well until she developed uterine bleeding necessitating surgery. With surgery she received 2 units of packed red blood cells obtained from two separate donors. Two weeks after surgery she developed a mononucleosis-like syndrome, which gradually disappeared. Two months after surgery one of the two blood donors to this woman was identified as a 24-year-old homosexual man who had been hospitalized with oral thrush and *Pneumocystis carinii* pneumonia. His ratio of T-helper to T-suppressor cells at that time was 0.02. The second blood donor to this woman was a healthy man with no risk factors for AIDS.

After the donor's history became known, the cellular immune status of the recipient was tested. She was found to have lymphopenia and a decreased ratio of T-helper to T-suppressor cells 7 months after surgery. Thirteen months after surgery she was hospitalized with *Pneumocystis carinii* pneumonia; her ratio of T-helper to T-suppressor cells was 0.46. She had no other known risk factors for AIDS and denied intravenous drug use, sexual contact with any members of groups with an increased incidence of AIDS, and other exposures to blood or blood products within the preceding 5 years.

The blood specimens used in this study were drawn 12 months after the onset of AIDS symptoms in the blood donor and 1 month after the onset of AIDS symptoms in the recipient. Blood was obtained from a third AIDS patient 20 months after the onset of symptoms; this patient was a homosexual man who had had generalized lymphadenopathy for 20 months before the onset of multiple severe opportunistic infections. He was not epidemiologically connected to the blood donor–recipient pair.

Two virus isolation techniques were used on specimens from these patients: (i) passage of cell-free supernatant fluids from primary patient lymphocyte cultures and (ii) cocultivation of patient lymphocytes with normal fetal cord blood lymphocytes. In both techniques lymphocytes were separated from fresh whole blood on Ficoll-Hypaque gradients and placed into culture with phytohemagglutinin (PHA). For cocultivation, fetal cord blood lymphocytes were added weekly to the patient's PHA-stimulated primary lymphocyte cultures to which interleukin-2 had been added. For the cell-free transmission assays, supernatant fluids were removed from the patient's PHA-stimulated primary lymphocyte cultures and added to cultures of fetal cord blood lymphocytes. Lymphocyte cultures were monitored for virus

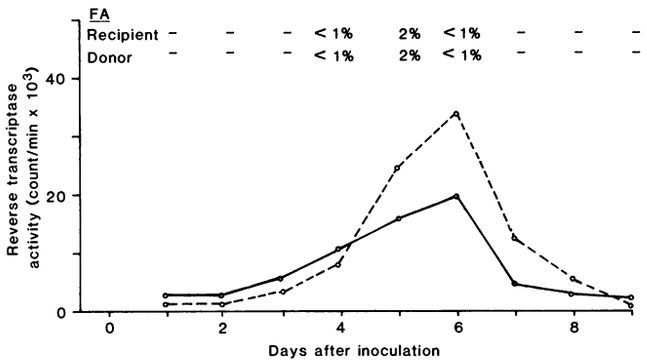

Fig. 1. Reverse transcriptase assays and immunofluorescence showing the dynamics of virus replication in fetal cord blood lymphocytes inoculated from material obtained from a blood donor and the recipient of his blood, both of whom developed AIDS. Supernatant fluids were removed from a 3-day donor cell culture and the 6-day recipient cell culture and after centrifugation (1000*g* for 15 minutes). Ten milliliters of each was inoculated onto human fetal cord blood lymphocyte cultures that had been treated with PHA for 3 days. Interleukin-2, antiserum to interferon (1:1000), and Polybrene (0.2 μg/ml) were added to the cultures. Samples of the cultured cells were examined daily by immunofluorescence (Fig. 2). Specimens of supernatant fluid were prepared daily for reverse transcriptase assay by centrifugation at 500*g* for 15 minutes. Supernatants were then pelleted (100,000*g* for 30 minutes) and assayed for particulate reverse transcriptase by using a template primer of $(A)_n$ $(dt)_{12-18}$ or $(dA)_n$ $(dT)_{12-18}$ in 7.5 m*M* Mg^{2+} or 0.1 m*M* Mn^{2+}.

replication with immunofluorescence and particulate reverse transcriptase assays. When evidence of infection was noted by these methods, cells were examined by electron microscopy.

Antibody to LAV p25 was assayed with an immunoprecipitation method (*5*). LAV (from the Institut Pasteur, Paris) was purified from supernatant fluids of infected primary lymphocyte cultures (*6*). Supernatant fluids were harvested at the time of peak reverse transcriptase activity and centrifuged at 100,000*g* for 60 minutes. The pellet was solubilized, centrifuged through a 30 percent sucrose cushion, and subjected to phosphocellulose chromatography. The purified p25 was labeled with ^{125}I (*7*). Serum that precipitated at least 15 percent of the ^{125}I label after the addition of gluteraldehyde-fixed *Staphylococcus aureus* Protein A was considered positive.

Both the AIDS patient who donated blood and the patient who received his blood had antibodies to LAV, as assayed by radioimmunoprecipitation. In addition, virus was isolated from both patients' peripheral lymphocytes. Cell-free supernatant fluids from primary lymphocyte cultures from both patients were found to be infectious when passaged into cultures of PHA-stimulated fetal cord blood lymphocytes. Significant elevations in particulate viral reverse transcriptase activity were observed after 4 days (Fig. 1). The reverse transcriptase activity was maximal when Mg^{2+} was used as the primary cation and when poly A/oligo dT was used as the template-primer. Little or no activity was found when Mn^{2+} was used as the cation or when poly dA/oligo dT was used as the primer. No reverse transcriptase activity was found in parallel uninoculated cultures of the same fetal cord blood lymphocytes.

When the inoculated cord blood lymphocyte cultures were examined by immunofluorescence with fluorescein isothiocyanate–conjugated human globulin fraction of antibody to LAV, antigen was distributed focally in the cytoplasm and as a rim beneath the plasma membrane of infected cells (Fig. 2). The proportion of cells with detectable antigen (about 2 percent) was maximal 1 day before maximum reverse transcriptase activity. As reverse transcriptase activity declined and the cells underwent cytopathic change, the immunofluorescence pattern became more diffuse throughout the cytoplasm and eventually was associated with cell debris.

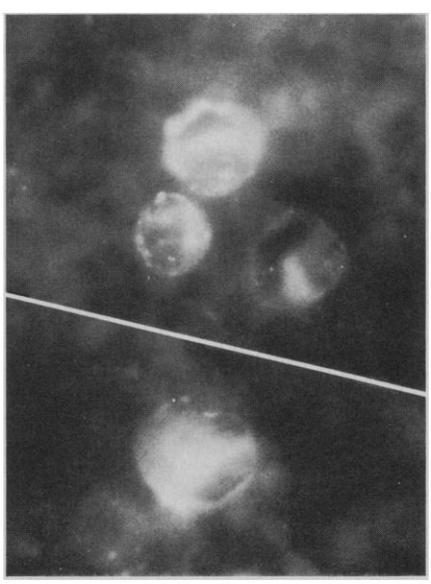

Fig. 2. Infected cultured lymphocytes from a blood donor (top) and the recipient of his blood (bottom) treated with one of two fluorescein isothiocyanate conjugates prepared from the immunoglobulin G fraction of the serum of two individuals with high titers of antibody to LAV. When used at a dilution of 1:100, both conjugates were unreactive on cells infected with HTLV-I, HTLV-II, herpes simplex virus types 1 and 2, cytomegalovirus, Epstein-Barr virus, and adenovirus (group reactivity). Cultured lymphocytes from the patients reacted with the conjugates to a dilution of 1:400.

The serum of the epidemiologically unrelated homosexual AIDS patient was negative for antibody to LAV p25. However, cells infected with material from his blood exhibited the same patterns of reverse transcriptase and immunofluorescence as found for the other patients.

Retrovirus particles were seen by electron microscopy in all cultures derived by cocultivation or infection with cell-free supernatant fluids from all three AIDS patients (Fig. 3). These particles budded from the plasma membranes of infected cells without involvement of any preformed intracytoplasmic structure. Nascent budding particles and immature particles had lucent centers; mature particles had small eccentric dense cores and usually an irregular shape. These virus particles were indistinguishable from those depicted in the original characterization of LAV (*2*, *6*) but were different from the typical morphology of HTLV-I and -II.

Competitive radioimmunoassays were used to compare the major core proteins of the viruses isolated from the three AIDS patients with the analogous proteins of other human retroviruses. Virus from all three patients was separated by ultracentrifugation from lymphocyte culture supernatant fluids. These virus preparations were used as competitive antigens in homologous radioimmunoassays for HTLV-I p24, HTLV-II p24, and LAV p25 (Fig. 4). The three isolates from the AIDS patients did not compete in the homologous HTLV-I p24 or HTLV-II p24 assays. In contrast, each of the three isolates competed in the homologous LAV p25 assay. The quantitative pattern of this competition, as reflected by the shape and slope of the curves, indicates that their major core proteins were identical.

The ultimate proof that LAV or any other virus is the cause of AIDS requires studies that cumulatively fulfill the modern equivalent of Koch's postulates. That is, an indicator (virus, viral protein, or viral nucleic acid) of a specific viral infection must be found in all or nearly all patients with AIDS or with signs or symptoms that frequently precede AIDS; antibody to the same virus must be shown to develop in constant temporal association with the development of

AIDS; and transmission of the same virus to a previously uninfected experimental animal or to a human must be demonstrated with subsequent development of the disease. Progress toward fulfilling the first two postulates has been substantial; the findings reported here contribute to fulfillment of the third. The isolation of the same retrovirus from both a blood donor and the recipient of that donor's blood, a person with no other known source of infection, and the subsequent development of AIDS in this recipient suggests that this virus is the etiologic agent of AIDS. Isolating LAV

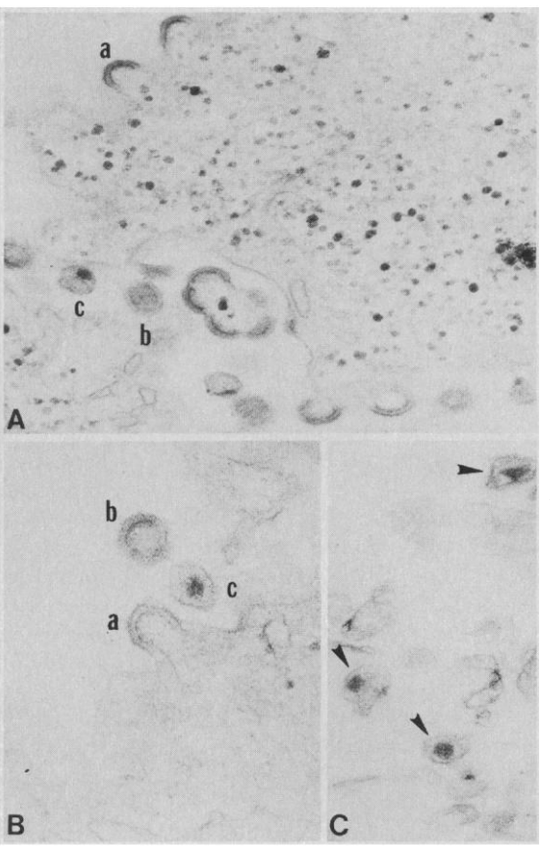

Fig. 3. Retrovirus particles in cocultivated lymphocytes from a blood donor–recipient pair, both of whom developed AIDS. (A) Donor lymphocyte culture with budding (*a*), immature (*b*), and mature (*c*) virus particles. (B) Recipient lymphocyte culture with virus particles in the morphogenic stages. (C) Recipient lymphocyte culture with mature virus particles having dense, eccentric, and bar-shaped nucleoids similar to those described for LAV. Lymphocytes from the blood donor and recipient were separated by Ficoll-Hypaque gradient centrifugation and placed in RPMI 1640 medium supplemented with 10 percent fetal calf serum, PHA (final concentration, 1:500), and antibiotics. On day 2 one lymphocyte culture from each patient was cocultivated with fresh human fetal cord lymphocyte cells (1×10^7) in 5 percent interleukin-2. Additional fresh fetal cord lymphocytes were added to the culture on days 10 and 17. Four days after the last addition of fetal cord lymphocytes, cultures were prepared by standard methods for thin-section electron microscopy.

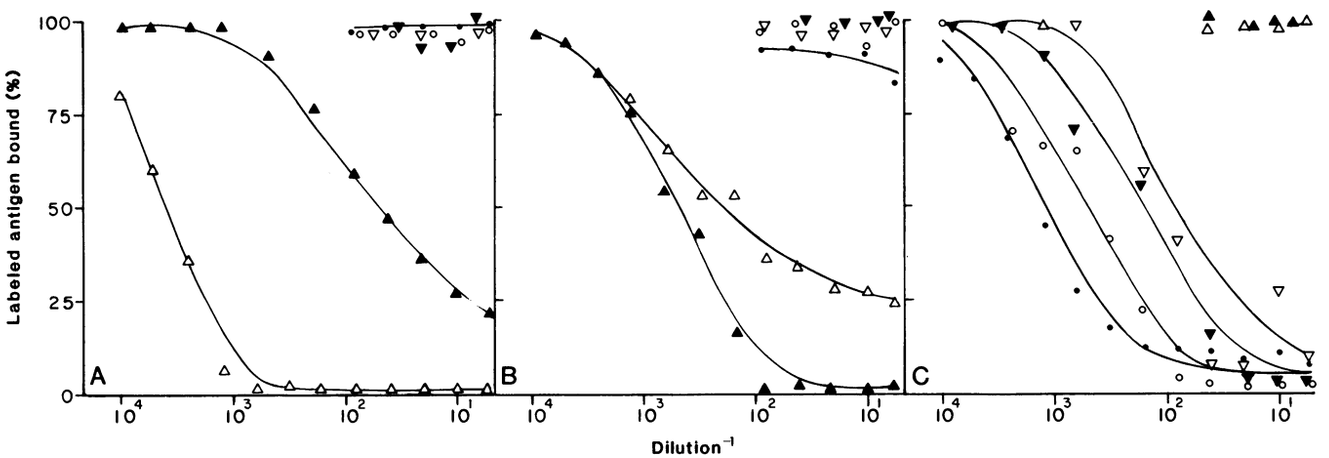

Fig. 4. Homologous competition radioimmunoassay of HTLV-I p24, HTLV-II p24, and LAV p25. Competition radioimmunoassays were carried out with ^{125}I-labeled HTLV-I p24, HTLV-II p24, LAV p25 and limiting dilution of hyperimmune rabbit antibody to HTLV-I (1:2000) or serum from patients M.O. (HTLV-II) (1:2000) and B.R.U. (LAV) (1:600) (*2*). Serial dilutions (100 μl) of solubilized virus (initial concentrate, 10 μg) in buffer 1 (20 m*M* Na_2HPO_4; *p*H 7.6), 200 m*M* NaCl, 1 m*M* EDTA, 0.3 percent Triton X-100, 0.1 m*M* phenylmethylsulfonyl fluoride, and bovine serum albumin (2 mg/ml) were incubated with the appropriate serum for 1 hour at 37°C. Labeled HTLV-I p24, HTLV-II p24, or LAV p25 (8000 count/min in 50 μl of buffer 1) was then added and the mixture was further incubated at 37°C for 2 hours and at 4°C overnight. A 20-fold excess of goat antiserum to rabbit immunoglobulin G or goat antiserum to human immunoglobulin G was then added and the volume was made up to 1 ml in buffer 1. The samples were further incubated at 37°C for 1 hour and at 4°C for 2 hours and then centrifuged at 2500 revolutions per minute for 20 minutes. The supernatants were aspirated and the radioactivity in the sediment was determined in a gamma counter. (A) Competition radioimmunoassay with rabbit antiserum to HTLV-I and ^{125}I-labeled HTLV-I p24. (B) Competition radioimmunoassay with serum from M.O. and ^{125}I-labeled HTLV-II p24. (C) Competition radioimmunoassay with serum from B.R.U. and ^{125}I-labeled LAV p25. Virus extracts used for competition were as follows: (●) LAV; (△) HTLV-I; (▲) HTLV-II; (○) supernatant (homosexual man, LAV/CDC-151); (▼) supernatant (blood donor, LAV/CDC-228); and (▽) supernatant (blood recipient, LAV/CDC-230).

from other AIDS patients and identifying LAV-specific antibodies in the serum of AIDS patients, including the donor-recipient pair reported here, but not in serum from persons in groups with low AIDS incidence (*4*, *8*), provide further evidence for such an etiologic association.

An etiologic association between HTLV-III and AIDS was reported recently (*4*). The most likely explanation for the parallel evidence for HTLV-III and LAV being the cause of AIDS is that the two viruses are the same.

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Altered Transcription of the c-*abl* Oncogene in K-562 and Other Chronic Myelogenous Leukemia Cells

Abstract. *Expression of the cellular* abl *(c-*abl*) oncogene was studied in K-562 and other chronic myelogenous leukemia (CML) cells and cell lines by means of Northern blot hybridization. In contrast to non-CML cells, which contained 7.4- and 6.8-kilobase* abl*-related transcripts, the CML cells contained a predominant and novel 8.2-kilobase* abl*-related RNA. In addition, the levels of* abl*-related message were up to eight times higher in CML cell lines from patients at the blast crisis stage of the disease compared with CML cells obtained during the chronic phase and with non-CML cells.*

Chronic myelogenous leukemia (CML) is a pluripotent stem cell disease characterized in over 90 percent of cases by the presence of the Philadelphia chromosome (Ph^1) (*1*). A number of recent findings suggest that the human cellular homolog (c-*abl*) of the tranforming sequences of the Abelson murine leukemia virus (v-*abl*) may be involved in the pathogenesis of CML: the c-*abl* gene, normally located on chromosome 9, is translocated to the Philadelphia chromosome in cases of CML (*2*); moreover, in at least one case of CML the chromosomal break point on chromosome 9 is in close proximity to the c-*abl* locus, mapping within 14 kb 5′ of the c-*abl* gene (*3*); in addition, c-*abl* is amplified some four to eight times in the K-562 CML cell line, and this cell line exhibits increased expression of *abl*-related RNA (*4*). Here we demonstrate in three Ph^1 positive CML cell lines and in a fresh CML cell sample the presence of an 8.2-kb c-*abl*–related messenger RNA that is absent from cells of non-CML origin. We also show that the expression of *abl*-related messenger RNA is increased in CML cell lines obtained from patients during blast crisis compared to fresh CML cells obtained during the chronic phase and to non-CML cell lines.

Northern blot analysis of polyadenylated RNA derived from various human cell lines of non-CML origin and hybridized to a mouse v-*abl*–specific probe

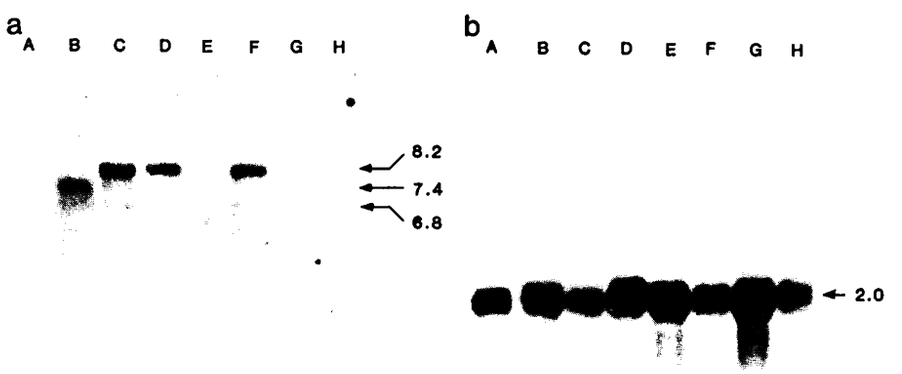

Fig. 1. (a) C-*abl*–related RNA transcripts in CML and non-CML cell lines. Cells were homogenized in guanidine thiocyanate (Eastman-Kodak) and subjected to ultracentrifugation through a cesium chloride cushion as described by Chirgwin *et al.* (*17*). The RNA pellet was suspended in 0.3 ml of water and precipitated twice with ethanol. The pellet was resuspended in 0.3 ml of water. Total RNA was enriched for polyadenylate-containing RNA by one cycle of oligo dT cellulose affinity chromatography (*18*). Portions (5 μg) of polyadenylated RNA were subjected to electrophoresis in formaldehyde gels and transferred to nitrocellulose filters as described (*19*). The filter was baked and then hybridized to the v-*abl*–specific pABlsub9 probe (*6*) that was radioactively labeled with ^{32}P by nick translation (*20*). Prehybridization, hybridization, wash, and exposure conditions were as described (*4*). Exposure time was 6 days. Sizes in kilobases were determined by comparison with 28*S* (5.0 kb) and 18*S* (2.0 kb) ribosomal RNA bands visualized by ethidium bromide staining of a marker lane excised from each gel before nitrocellulose transfer. Cells and cell lines are as follows: A, HL-60 promyelocytic leukemia (*21*); B, normal peripheral blood cells transformed with EBV; C, K-562; D, EM-2; E, Raji (Burkitt lymphoma); F, KCL-22; G, peripheral blood cells from a patient with Ph^1-positive CML; H, KG-1 myelogenous leukemia (*22*). (b) Transcripts of β-actin–related RNA in CML and non-CML cell lines. The same samples as in (a), after being allowed to undergo several half-lives of ^{32}P decay of the initial v-*abl* probe, were hybridized to the chick pAl β-actin complementary DNA probe (*23*) under the same conditions as for (a). Exposure time was 5 hours.