velopment of lymphoma, especially those of T-cell origin. Our results also suggest that HTLV should, along with cytomegalovirus and other agents, be studied to determine what role, if any, it might play in the development of AIDS.

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Proviral DNA of a Retrovirus, Human T-Cell Leukemia Virus, in Two Patients with AIDS

Abstract. *The acquired immune deficiency syndrome (AIDS) is characterized by T-lymphocyte dysfunction and is frequently accompanied by opportunistic infections and Kaposi's sarcoma. Human T-cell leukemia virus (HTLV) is associated with T-cell malignancies and can transform T lymphocytes in vitro. In an attempt to find evidence of HTLV infection in patients with AIDS, DNA from samples of peripheral blood lymphocytes from 33 AIDS patients was analyzed by Southern blot-hybridization with a radiolabeled cloned HTLV DNA probe. Analysis of DNA from both the fresh (uncultured) lymphocytes and from T cells cultured with T-cell growth factor revealed the presence of integrated HTLV proviral sequences in lymphocytes from two of the patients, both of whom had antibody to HTLV. The proviral sequences could not be detected in blood samples obtained from these individuals at a later date, consistent with the possibility that the population of infected cells had become depleted.*

Acquired immune deficiency syndrome (AIDS) is a new disease whose incidence in the United States has increased steadily since 1979 (*1*). The disorder was first noted in male homosexuals who presented with opportunistic infections, predominantly *Pneumocystis carinii* pneumonia (*2*), or with Kaposi's sarcoma (*3*). The syndrome has recently been found in other groups including intravenous drug users (*4*), Haitian immigrants to the United States (*5*), hemophiliacs (*6*), and inmates at a New York state prison (*7*). Although patients with AIDS usually come to medical attention because of Kaposi's sarcoma or opportunistic infections, the underlying disorder affects the patients' cell-mediated immunity (*8*). The T-cell dysfunction is often marked by an absence of delayed hypersensitivity, an absolute lymphopenia, and reversal of the usual ratio of phenotypic T-helper ($OKT4^+$) to T-suppressor ($OKT8^+$) cells whereby the latter come to predominate among circulating lymphocytes (*8*). Although the epidemiologic data suggest an infectious, possibly viral, etiology for AIDS, no agent has been linked etiologically to the disease. Many of the patients have chronic infection with cytomegalovirus (*9*) or hepatitis B virus, but the presence of these agents may be characteristic of the social history of the patients and may have preceded the disease or may represent infections permitted by the immune deficit.

We have been testing the hypothesis that AIDS is caused by a human retrovirus related to the human T-cell leukemia virus, HTLV (*10*). Retrovirus infection, known to cause leukemias, lymphomas, and solid tumors in several species of animals and T-cell malignancies in man, has also been shown to result in immune deficiency in some cats infected with feline leukemia virus (*11*). The target cell for the putative AIDS agent may be the T cell or a T-cell subset. Since HTLV is a T-cell tropic retrovirus (*12*), it can be linked hypothetically to other human T-cell disorders. The finding of AIDS in Haitians who may not have been exposed to other risk factors for the disease may be important since HTLV appears to be endemic in the West Indies (*13*). The search for evidence of retrovirus infection poses many problems. Several of the hallmarks of such infection, namely virus production, complete provirus integration into the host DNA, and antibody response to viral antigens have not been found in retrovirus-induced neoplasms. In a disease characterized by cellular depletion, as AIDS appears to be, it may be difficult to sample a pa-

tient's cells during the period of virus replication before the affected cell population becomes too small to detect. Since patients with AIDS show depressed antibody formation in response to some new stimuli (*14*), their antibody response to the putative infectious agent may also be depressed. In several patients with leukemia, the presence of HTLV was documented by finding the virus in cultured lymphocytes (*15*) or by identifying proviral sequences in fresh leukemic cells (*16*), but serum antibodies to HTLV proteins were not detected. Conversely, antibodies to HTLV have been found in a small number of healthy people (*17*), especially in individuals who have been in close contact with HTLV positive patients (*18*).

A sensitive and perhaps more specific indicator of active retrovirus infection is the presence of the integrated proviral genome in the host cell chromosomal DNA. As part of their normal replicative cycle, retroviruses transcribe a DNA copy of their RNA genome. This copy is inserted colinearly into the host cell DNA and is then replicated during cell division and passed to daughter cells. This proviral DNA serves as a template for copies of new RNA genomes for virus production and may alter the expression of cellular genes near the chromosomal insertion site. We have tested fresh peripheral blood lymphocytes from AIDS patients for the presence of integrated HTLV provirus and now report that such viral sequences were present in two cases of AIDS.

The patients were diagnosed as having AIDS by the clinical presentation of opportunistic infection or Kaposi's sarcoma and by an immunologic profile as described above. Lymphocytes were isolated from 50 ml of peripheral blood by Ficoll-Hypaque gradient and immediately treated with sodium dodecyl sulfate and proteinase K, after which high-molecular-weight DNA was prepared by standard techniques. The DNA samples (5 μg) were treated with a restriction endonuclease, usually Eco RI, and analyzed by Southern blot hybridization (*19*) to radiolabeled cloned HTLV DNA (*20*). The viral probe, pCR_{CH}, represents 2.4 kb of the HTLV genome, predominantly the *env* region. Under stringent conditions of hybridization (see Fig. 1 legend), the probe hybridized only to DNA from virus-infected cells. In surveys of leukemic cell DNA for HTLV we have used Eco RI, which does not cleave the viral DNA, to excise a single restriction fragment, usually larger than 10 kb, which contains the integrated provirus and can be detected by the pCR_{CH} probe (*16*). Positive findings were confirmed by using the restriction enzyme Bam HI to cleave the sample DNA to yield a 1.05-kb fragment from proviral DNA which is also internal to the pCR_{CH} fragment and is easily seen on a Southern blot when it is hybridized with that probe.

Patient 1 was a 32-year-old black male from New York City. He had intermittent fevers, lymphadenopathy, and *Pneumocystis carinii* pneumonia, and showed loss of weight. A blood count revealed the following: white cells, 4500 cell/mm^3; lymphocytes, 765 cell/mm^3; and an OKT4$^+$/OKT8$^+$ lymphocyte ratio of 0.2. Interviews with the patient and his family indicated that there was no history of malignancy. The patient's only travel outside the United States was for a year's military service in Vietnam; he was an admitted homosexual and denied that he had ever received a blood tranfusion or used intravenously administered illicit drugs.

When peripheral blood lymphocytes from this patient were cultured in the presence of human T-cell growth factor (TCGF) (*21*), a line of TCGF-dependent cells became established. These cells contained HTLV viral antigens, p19 (*22*) and p24 (*23*), produced reverse transcriptase activity in the culture fluid (*10*), showed the presence of type-C virus particles when thin sections were examined by electron microscopy, and contained integrated HTLV proviral DNA (*24*).

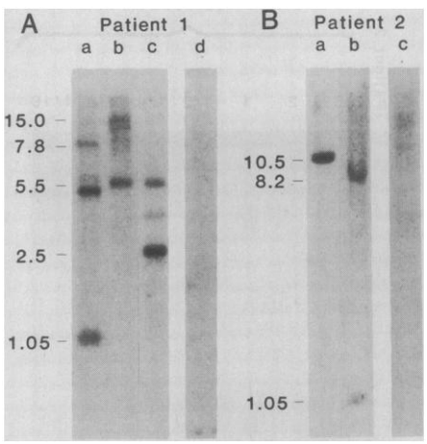

Fig. 1. Southern blot hybridization of the HTLV probe pCR_{CH} to restriction enzyme–digested lymphocyte DNA from AIDS patients. Five micrograms of high-molecular-weight DNA were digested with the designated restriction enzyme under conditions described by the supplier. The DNA was subjected to electrophoresis on 0.8 percent agarose and blotted on nitrocellulose filters. The filters were heated in a vacuum at 80°C for 2 hours, and the DNA was then hybridized to ^{32}P-labeled pCR_{CH} at 37°C in 0.45*M* NaCl, 50 percent formamide, and 10 percent dextran sulfate, overnight. The filters were washed in 0.15*M* NaCl at 60°C and autoradiographed overnight. (A) Patient 1. Digests of DNA from cultured T cells with (lane a) Bam HI, (lane b) Eco RI, and (lane c) Pst I. Lane d contains Eco RI–digested fresh peripheral blood lymphocyte DNA samples 4 months after the cells were taken for culture. (B) Patient 2. Digests of fresh peripheral blood lymphocyte DNA: (lane a) 5 μg of DNA digested with Eco RI; (lane b) 3 μg of DNA digested with Bam HI; (lane c) 5 μg of DNA, sampled 2 months after the DNA obtained for (lanes a and b), digested with Eco RI.

Figure 1 shows the results obtained by the Southern blot hybridization technique. The two bands of higher molecular weight cleaved by Eco RI in Fig. 1A (lane b) indicate the presence of two complete proviral copies. The 5.5-kb band represents a partial provirus. The extra proviral copies may have arisen in culture, since increased copies of provirus and defective viral genomes occur commonly when HTLV-infected T cells are passed in vitro for prolonged periods of time (*25*). Digestion with Bam HI gave the 1.05-kb internal fragment and Pst I cleaved a 2.5-kb internal fragment. This analysis did not reveal any differences between this HTLV isolate, HTLV-I_{CR}, and other HTLV-I isolates derived from malignant T cells (*26*).

Although this patient's fresh lymphocytes were not assayed for viral sequences at the time his cells were cultured, he did possess circulating antibodies to HTLV core proteins p24 and p19 (*27*). One year after he first became ill the patient developed a cerebral lymphoma (cell type undetermined). At that time no HTLV sequences could be found in his circulating lymphocytes (Fig. 1A, lane d).

Patient 2 was a 48-year-old black male resident of Philadelphia. He became ill with extensive perianal *Herpes simplex* and two nodular lesions of Kaposi's sarcoma. A blood count showed the following: white cells, 2400 cells/mm^3; total lymphocytes, 432 cell/mm^3; and an OKT4$^+$/OKT8$^+$ ratio of 0.1. From interviews with the patient and his family it was learned that he was homosexual, there was no history of malignancy, and that he had lived the first 12 years of his life in Alabama and then moved to Philadelphia. His only travel outside the United States had been to Canada, and he had never had a blood transfusion or used intravenously administered illicit drugs.

Fresh, uncultured, peripheral blood lymphocytes from this patient were analyzed by the Southern blot hybridization technique (Fig. 1B). A single-copy HTLV genome was seen in the Eco RI digestion. Digestion with Bam HI confirmed the presence of the viral genome

by cleaving the internal 1.05-kb restriction fragment. To determine what fraction of the patient's peripheral blood lymphocytes were infected with HTLV, we compared the intensity of hybridization of the patient's DNA to dilutions of cloned viral DNA, that is, the CR_{CH} insert, mixed with salmon sperm DNA as a carrier. The amount of cloned CR_{CH} insert added to each lane was calculated to represent the density of viral copies per cell as shown in Fig. 2. By including on the Southern blot a known amount of lymphocyte DNA from patient 2 and comparing the autoradiographic intensity of the HTLV sequences in this DNA (arrow) with the intensity of the dilutions of this cloned insert, we were able to estimate that between 1/8 and 1/16 (about 10 percent) of the patient's peripheral lymphocytes contained HTLV provirus at the time of sampling. A second analysis of the patient's peripheral blood lymphocytes obtained 2 months after the first showed that the HTLV provirus could no longer be detected (Fig. 1B, lane c). At both times this patient had circulating antibodies to disrupted whole HTLV (*28*).

We also used the Southern blot technique to test DNA randomly sampled from peripheral blood lymphocytes of 31 other individuals with AIDS. No HTLV sequences were detected.

That the viral genome of HTLV was found to be integrated into peripheral blood lymphocyte DNA in 2 of 33 AIDS patients tested thus far may reflect an etiologic association that is detectable by our methods only in a small percentage of cases. Because of the depressed cell population numbers and the possible toxicity of the virus to the host cell it may be necessary to develop alternative sampling techniques. A baseline for the presence of HTLV DNA sequences in the peripheral blood of healthy patients is unknown. We have thus far tested only 25 healthy homosexual males, none of whom had detectable virus. That both of the positive cases in our study are black is important since blacks may have a predisposition for HTLV expression; HTLV-induced lymphocytic malignancies appear to be endemic in the Caribbean basin area (*13*). A disproportionate number of the HTLV lymphomas studied in our laboratory occurred in blacks (*13*). The only large survey for nonleukemic HTLV-positive individuals in the United States was a screening of stored serum samples from patients attending a venereal disease clinic in the southeastern United States. Three percent of the samples, predominantly from black people, reacted positively with HTLV antigen (*29*).

Our results, if they do reflect an etiologic role for HTLV in AIDS, suggest a possible mechanism of disease induction. Restriction enzyme identification of two integration sites in one patient's cells and a single site in another implies a mono- or oligoclonal proliferation of infected cells. After exposure to the virus one or a few infected cells proliferated until they represented at least 10 percent of the circulating lymphocytes, a level enabling us to detect the sequence. HTLV is a T-cell tropic virus that apparently chiefly infects the $OKT4^+$ subset (*25*). The two patients with HTLV studied here had been ill for some months before we sampled their infected lymphocytes. Since $OKT4^+$ cells are markedly reduced in AIDS, it is less likely that these cells were infected with the virus. It seems more likely that the infected cells were suppressor T cells whose function was in some way altered by virus infection. Virus-induced proliferation of a T-suppressor clone may be an initiating step in AIDS. Studies of the HTLV integration sites in the DNA of these two patients, of the possible transmission of AIDS to animals, of transfusion recipients who contract AIDS, and of the genome of the HTLV isolated from AIDS patients compared to that of HTLV from T-cell neoplasias may shed further light on the involvement of HTLV in AIDS.

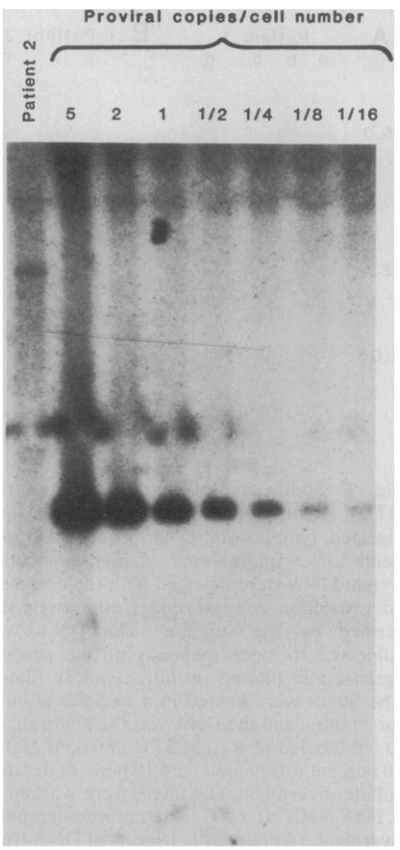

Fig. 2. Determination of the density of HTLV proviral copies in fresh peripheral blood lymphocytes of patient 2. Southern blot hybridization was performed as in Fig. 1. The first lane contains 3 μg of Eco RI–digested DNA from patient 2. The lanes to the right contain different amounts of the pCR_{CH} cloned fragment added to 3 μg of salmon sperm DNA. The ratio of pCR_{CH} insert to carrier salmon sperm DNA is calculated to represent the ratio of viral copy to cell number listed at the top of each lane.

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Isolation of Human T-Cell Leukemia Virus in Acquired Immune Deficiency Syndrome (AIDS)

Abstract. *Several isolates of a human type-C retrovirus belonging to one group, known as human T-cell leukemia virus (HTLV), have previously been obtained from patients with adult T-cell leukemia or lymphoma. The T-cell tropism of HTLV and its prevalence in the Caribbean basin prompted a search for it in patients with the epidemic T-cell immune deficiency disorder known as AIDS. Peripheral blood lymphocytes from one patient in the United States and two in France were cultured with T-cell growth factor (TCGF) and shown to express HTLV antigens. Virus from the U.S. patient was isolated and characterized and shown to be related to HTLV subgroup I. The virus was also transmitted into normal human T cells from umbilical cord blood of a newborn. Whether or not HTLV-I or other retroviruses of this family with T-cell tropism cause AIDS, it is possible that patients from whom the virus can be isolated can also transmit it to others. If the target cell of AIDS is the mature T cell as suspected, the methods used in these studies may prove useful for the long-term growth of these cells and for the identification of antigens specific for the etiological agent of AIDS.*

Human T-cell leukemia-lymphoma virus, HTLV, was first discovered in and isolated from mature T cells associated with certain T-cell malignancies in adults in the United States (*1*). Since the isolation and characterization of the first two isolates, HTLV-I_{CR} and HTLV-I_{MB} (*1, 2*), several new isolates of HTLV were obtained in our laboratory from patients and in a few instances from clinically healthy individuals in the United States, Israel, the West Indies, and Japan (*3*). Recently, a new human retrovirus, called HTLV-II, was identified in (*4*) and isolated from (*5*) cultured cells from a patient with hairy cell leukemia; this isolate is related to but quite distinct from all other HTLV isolates. Independent detection and isolation of HTLV have now also been reported from Japan, Europe, and other laboratories in the United States (*6*). Detailed characterization of the HTLV isolates indicates that all but HTLV-II are very similar to each other (*3, 7*); none are endogenous in man (*2*); and all are readily distinguishable from the known animal retroviruses by nucleic acid hybridization (*8*), immunological assays of structural proteins (*9*), and reverse transcriptase (*10*). Specific antibodies to HTLV proteins have been found in serum samples from adults with mature T-cell leukemia (*11*), and the data from serological and epidemiological studies indicate that HTLV is endemic in certain regions of the world, particularly the Caribbean region and southern Japan (*12*). We are testing the possibility that HTLV is associated with the newly described acquired immune deficiency syndrome (AIDS) (*13*). This disease, which has been described with increasing frequency since the first case reports (*14, 15*), is suspected of being caused by a transmissible agent (*16*).

Patients with AIDS are now known to include male homosexuals (*13*), intravenous drug users (*17*), Haitian immigrants to the United States (*18*), and hemophiliacs (*19*). Clinical signs of the disease include opportunistic infections, predominantly *Pneumocystis carinii* pneumonia (*13*), and Kaposi's sarcoma (*20*) in previously healthy persons. Studies of

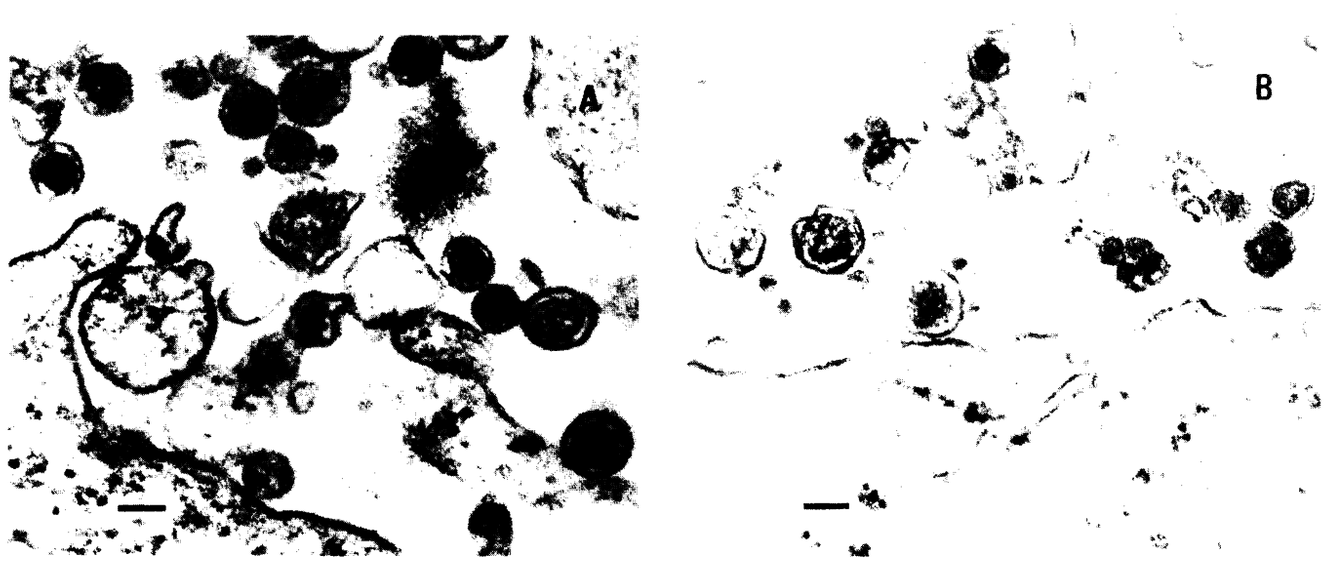

Fig. 1. Electron micrographs of (A) T cells from a patient (EP) with AIDS and (B) human umbilical cord blood T cells (C183/EP) cocultured with EP cells. Many mature type-C particles are visible in the extracellular space of both cell lines (scale bars, 100 nm).