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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

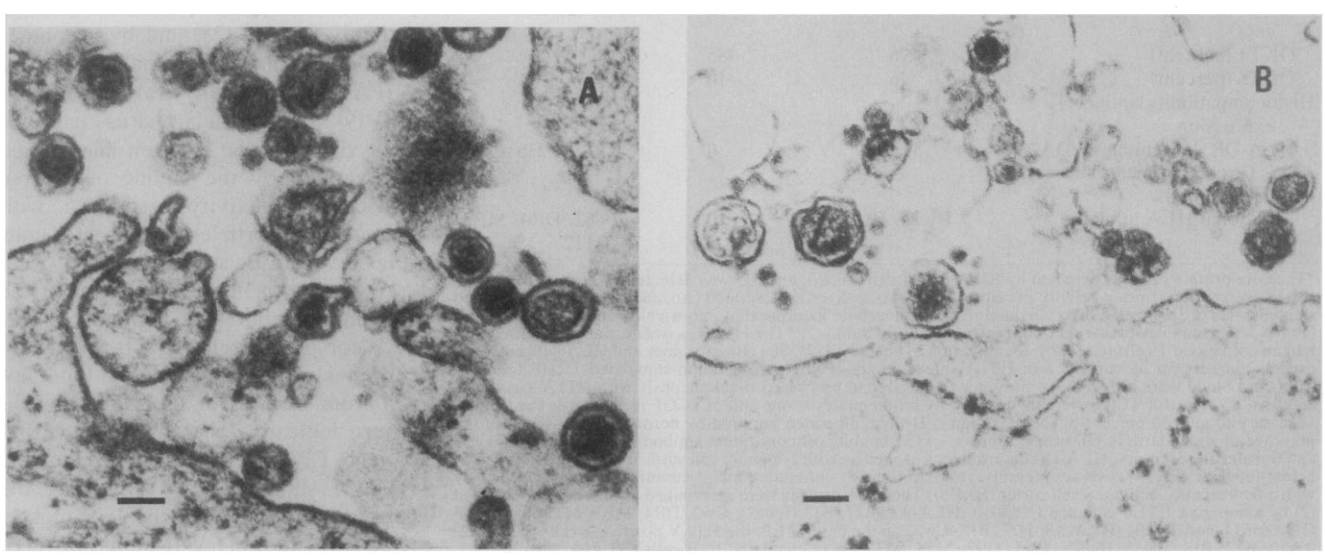

Fig. 1. Electron micrographs of (A) T cells from a patient (EP) with AIDS and (B) human umbilical cord blood T cells (C183/EP) cocultured with EP cells. Many mature type-C particles are visible in the extracellular space of both cell lines (scale bars, 100 nm).

cell-mediated immunity in patients with AIDS have demonstrated generalized impairment of T-lymphocyte functions, including lymphopenia, cutaneous anergy, and reduced helper T-lymphocyte ($OKT4^+$) subpopulations. This results in reversed ratios of helper to suppressor T-lymphocyte ($OKT4^+/OKT8^+$), poor lymphocyte responsiveness to mitogens, and, in some cases, decreased natural killer cell activity (*21*). The epidemiology of this syndrome—that is, the increasing incidence and clustering of cases, particularly in New York and California—suggests the involvement of a transmissible agent (*14, 15*). Patients with AIDS are often chronically infected with cytomegalovirus (*22*) or hepatitis B virus (*23*). Serum antibodies that react with membrane proteins from HTLV-infected cells have been found in many AIDS patients (*24*), and HTLV sequences have been found in DNA from two of 33 AIDS cases (*25*). Here we describe the isolation of HTLV from peripheral blood T lymphocytes from one U.S. patient with AIDS, and report briefly on the finding of HTLV antigens in T cells cultured from peripheral blood T lymphocytes of two cases of AIDS in France.

Peripheral blood T cells from the U.S. patient, EP, were grown in suspension culture in the presence of partially purified human T-cell growth factor (TCGF) (*26*) as described (*3, 26*). The morphology of the virus is shown in Fig. 1. Data on the identification of HTLV in the T cells are summarized in Table 1. The morphology was that of a type-C retrovirus, with the virus being indistinguishable from the earlier HTLV isolates. By using monoclonal antibodies and hyperimmune sera we determined that the T cells contained the HTLV core proteins p19 and p24, respectively. These results also showed that the new isolate belongs to the HTLV group.

We then transmitted the virus from the patient's peripheral blood lymphocytes into T cells from human umbilical cord blood (C183 cells). The donor (EP) cells were x-irradiated (6000 R) and then washed and cocultivated with recipient cord blood T cells of the opposite sex as described (*3*) (Table 1). Analyses of the sex chromosomes and HLA profiles (histocompatibility antigens) distinguished the recipient cord blood cells from the EP cells. Subsequent cell sorter analysis with monoclonal antibodies showed that both the donor EP cells and the recipient C183 cells were infected with the virus (C183/EP cells) and that cells in both lines were mature T cells. They reacted with monoclonal antibodies to $OKT3^+$ (Pan T), $OKT4^+$ (helper-inducer), $OKT8^+$ (cytotoxic-suppressor), and 9.6^+ (sheep red blood cell receptor). Both the EP and C183/EP cell lines had indefinite growth potential, cell surface alterations such as expression of inappropriate HLA antigens, and other properties similar to other HTLV-infected cord blood T cells (*27*).

We found HTLV antigens in both the EP and C183/EP cell lines by means of an indirect immunofluorescence assay (IFA) with a highly specific monoclonal antibody for p19 (*28*) and by a competition radioimmunoprecipitation assay (RIPA) for the major viral core protein p24 (*9*). Extracellular virus particles were visible in the electron microscope and verified by the finding of reverse transcriptase activity associated with extracellular particles in both cell lines (Fig. 1, A and B, and Table 1).

As shown in Fig. 2, the slopes of the competition curves in homologous RIPA's for p24 in cell extracts from both cell lines were very similar to those for p24 from isolates of the HTLV-I subgroup, indicating that the isolate from patient EP is closely related to the HTLV-I subgroup. By this assay the new isolate appears to be less closely related to the HTLV-II subgroup (*29*). We call this isolate $HTLV\text{-}I_{EP}$. Analysis of the nucleic acids of the HTLV isolated

Table 1. Detection and isolation of HTLV in peripheral blood T cells of an AIDS patient and isolation of the virus by transmission into T cells from umbilical cord blood obtained from newborns. The T-cell line was derived from the peripheral blood of patient EP, a 32-year-old black male homosexual from New York City who had *Pneumocystis carinii* pneumonia with abnormal lymphocyte function characteristic of AIDS (*23*). N.D., not done; M, male; F, female.

Characteristics of the cultured T cells	Source of T cells		
	AIDS patient (EP)	Normal cord blood T cells (C183)	Cord blood (C183) T cells infected with $HTLV_{EP}$
HTLV expression*			
p24 (ng/mg)	40	0	500
p19 (percent positive cells)	9	0	37
Reverse transcriptase activity (pmole/ml extract)	81	0	21
Virus particles (electron microscopy)	+	N.D.	+
Sex chromosome†	M	F	F
Growth potential/morphology	Indefinite/uniform (few binucleated cells)	Limited/uniform (normal)	Indefinite/polymorph (bi- and multinucleated cells)
TCGF requirements (units)‡	< 0.7	1	< 0.1
TCGF receptors (TAC) (percent/mean fluorescence units)§	77/475	20/150	42/1255
Transferrin receptors (5E9) (percent/mean fluorescence units)§	12/19	N.D.	28/1015
Lymphoid surface phenotype§			
Pan T (OKT3, 3A1, 9.6) (percent)	45 to 95	95	21 to 61
OKT4 (percent)	76	85	40
OKT8 (percent)	46	10	20
Histocompatibility antigen expression			
HLA-DR determinants (DA2, 3.1) (percent/mean fluorescence units)§	59/236	0	56/2363
Additional HLA antigens	Bw35, Bw62	0	Bw62, DR4, MB3, MT2

*The core protein p24 was detected by RIPA in cell extracts (*9*), and p19 was detected by IFA on fixed cells (*28*). Reverse transcriptase activity in culture fluids was assayed as described (*10*), and the activity expressed as picomoles of ^3H-labeled deoxythymidine monophosphate incorporated into trichloracetic acid–precipitable DNA per milliliter of 30 times concentrated culture fluid. †The $HTLV_{EP}$ isolate was transmitted into human cord blood T cells (C183) by cocultivation as described (*3*). Sex chromosomes and HLA antigens were used as markers for identification of HTLV-infected cells. Phytohemagglutinin-stimulated T cells (control) from cord blood were grown in the presence of TCGF and processed simultaneously with HTLV positive T cells. ‡Values for TCGF were determined by probit analysis; one unit of TCGF is defined as the amount necessary to give 50 percent of the maximum [^3H]thymidine incorporation by normal T cells (C183) in the presence of a standard TCGF preparation. §The binding of monoclonal antibodies reacting with TCGF and transferrin receptors, HLA-DR determinants as well as with lymphoid cell surface antigens using Pan T, helper-induced (OKT4), and suppressor-cytotoxic (OKT8) antibodies was determined by cytofluorometry with a fluorescence-activated cell sorter (FACS). The HLA profiles were determined as previously described (*2*) by comparing HTLV-infected C183/EP [HLA-A3, B27, B12 (Bw62), Cw2, DR4, MB3, MT2] to normal C183 cord blood T cells (HLA-A3, B27, B12, Cw2); in the case of EP, the HTLV positive T cells [HLA-A2, Aw30, B17, B18 (Bw35, Bw62), Cw5, DR2, MT1, MT2] were compared to B cells (HLA-A2, Aw30, B17, B18, Cw5, DR2, MT1, MT2) from the patient. The full identity in HLA profiles was found in both cases; however, as previously demonstrated (*2*), the expression of additional HLA antigens associated with HTLV infection was also detected.

from EP also indicated its similarity to viruses of the HTLV-I subgroup (*29*).

The AIDS patients in France were female. One was a Haitian, the other a was a Caucasian who had visited Haiti. Peripheral blood T cells from both patients were cultured with partially purified TCGF and, by means of hyperimmune sera and monoclonal antibodies, were shown to express HTLV antigen and the core proteins p19 and p24. Present data indicate that the virus can be transmitted to normal cord blood T lymphocytes by the cocultivation technique. We have not yet determined whether these isolates from France belong to subgroup I or II of HTLV or to a new subgroup.

Antibodies specifically reactive with internal structural proteins of HTLV have been found in the serum of AIDS patients more frequently than in the normal population, but less than in serum from patients with HTLV-positive T-cell leukemia (*30*). The evidence from immunological, epidemiological, and molecular biological studies (*31*) for an association between HTLV and certain adult T-cell leukemias and lymphomas is much stronger. The less consistent detection of the virus in AIDS patients may reflect a depletion in the number of available target T cells in their peripheral blood, a hypothesis supported by the results of serial analyses of DNA peripheral blood lymphocytes of AIDS patients (*25*). These results showed that patients initially positive for HTLV sequences may be negative with subsequent repeated blood samples.

Since HTLV is endemic in southern Japan, the apparent lack of epidemic AIDS in this region may argue against a causative role for HTLV in AIDS. However, the absence of an AIDS epidemic in the area might also be explained by a recent genomic modification in some HTLV strains in other areas of the world. Alternatively, people in southern Japan may have greater resistance to the T-cell suppressive effect of the virus. There are, of course, many precedents for variations in the susceptibility of different populations to a microorganism.

Whether or not the etiological agent of AIDS is an HTLV, it is interesting to consider the possible routes of transmission of such an agent. Most findings suggest transmission by blood transfusion and by intravenous drug administration. However, the high incidence of AIDS in homosexuals who apparently have not received blood transfusions or used intravenously administered drugs suggests that the agent is also transmitted during sexual contact by sperm or saliva. It may therefore be possible to detect HTLV antigen in these secretions and to transmit the virus to cord blood by the coculturing method. Since HTLV can also infect marmoset and other primate T cells (*32*), should this virus be established as the causative agent of AIDS it may also be possible to develop an animal model of the disease.

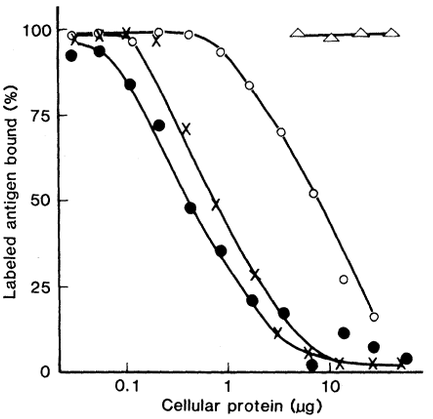

Fig. 2. Homologous competitive radioimmunoprecipitation assay of the major core protein p24 with cell extracts of established T-cell lines. Symbols: ○, cells from AIDS patient EP; ●, C183/EP cells (cord blood T cells infected with $HTLV_{EP}$); △, normal cord blood T cells; and X, positive control cells (from a patient with a mature T-cell malignancy known to release HTLV).

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