EDTA. Approximately 0.4-ml fractions were collected and 2000 cpm from each fraction were analyzed by agarose gel and autoradiography. Fractions containing molecules between 500 and 5000 bp were pooled, the NaCl was adjusted to 200 m*M*, and the DNA was concentrated to 0.4 ml by passage through an Elutip-D cartridge (S&S) as described by the manufacturer. One microgram of dephosphorylated λgt11 (Promega Biotech) was added and the DNA precipitated at −20°C after the addition of 0.1 volume of 8*M* LiCl and 2.5 volumes of ethanol. The DNA pellets were redissolved in 20 μl of water and ligated.
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32. We thank R. Doolittle for helpful discussions, E. D. Korn and J. A. Hammer III for first suggesting that Sm-97 might be paramyosin, J. E. Coligan for use of the MicroGenie software, and C. Cohen for the *Mytilus* paramyosin. This work was supported in part by grants from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and The Edna McConnell Clark Foundation.

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Isolation of a New Virus, HBLV, in Patients with Lymphoproliferative Disorders

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A novel human B-lymphotropic virus (HBLV) was isolated from the peripheral blood leukocytes of six individuals: two HTLV-III seropositive patients from the United States (one with AIDS-related lymphoma and one with dermatopathic lymphadenopathy), three HTLV-III seronegative patients from the United States (one with angioimmunoblastic lymphadenopathy, one with cutaneous T-cell lymphoma, and one with immunoblastic lymphoma), and one HTLV-III seronegative patient with acute lymphocytic leukemia from Jamaica. All six isolates were closely related by antigenic analysis, and sera from all six virus-positive patients reacted immunologically with each virus isolate. In contrast, only four sera from 220 randomly selected healthy donors and none from 12 AIDS patients without associated lymphoma were seropositive. The virus selectively infected freshly isolated human B cells and converted them into large, refractile mono- or binucleated cells with nuclear and cytoplasmic inclusion bodies. HBLV is morphologically similar to viruses of the herpesvirus family but is readily distinguishable from the known human and nonhuman primate herpesviruses by host range, in vitro biological effects, and antigenic features.

THE IDENTIFICATION OF THE CAUSative agents (HTLV-I and HTLV-III) of two human hematopoietic cell diseases, adult T-cell leukemia (ATL) and acquired immune deficiency syndrome (AIDS), respectively, was possible because of the propagation of specific cell types in culture (*1–5*). Related retroviruses have been isolated from healthy Africans (HTLV-IV) and from some Africans with AIDS (LAV-2) (*6*). We have developed and utilized methods for activation and long-term cultivation of different types of fresh human lymphocytes, monocyte-macrophages, granulopoietic cells, and other cell types in liquid suspension culture for the purpose, in part, of finding new viruses.

In this study, cells primarily derived from fresh peripheral blood mononuclear cells from patients with associated lymphoproliferative disorders (Table 1) were grown in cell culture in RPMI 1640 supplemented with 20% fetal bovine serum and hydrocortisone at 5 μg/ml (*7*). In three instances (Table 1, patients 1–3), a small number of short-lived, large, refractile cells were observed in the primary cell cultures, which subsequently proved to be of value in predicting the presence of the new virus in other patients. The large cells were generally mono- or binucleated (Fig. 1a) and these cells frequently contained intranuclear and/or intracytoplasmic inclusion bodies. Electron microscopic examination (Fig. 2a) revealed a virus that we will call human B-lymphotropic virus (HBLV) (*8*). These large cells were also the only ones in culture that expressed viral antigens (described below) as measured by fixed-cell indirect immunofluorescence assays (IFA) (*9*). We also found a low number of these cells in fresh and/or cultured cells from three additional patients (Table 1, patients 4–6).

HBLV from the six patients was transmitted to freshly isolated phytohemagglutinin (PHA)-stimulated human leukocytes from umbilical-cord blood, adult peripheral blood, bone marrow, and spleen. After infection in vitro large refractile cells, similar to those noted in primary cultures, appeared within 2–4 days (Fig. 1b). These cells may initially have a growth advantage because they eventually became the predominant cells in the culture, surviving for an additional 8 to 12 days while other cells in the culture rapidly died. As in primary cell cultures, these large cells expressed viral nucleic acids as shown by in situ hybridization (Fig. 3) (*10*), and viral antigens as detected by IFA (Fig. 4, a and b). Virus production was confirmed by electron microscopy (Fig. 2b) (*8*).

The main features of this virus are: (i) uniform shape and size of virion, (ii) abundant extracellular virus production by infected cells, (iii) absence of perichromatin-like granules in the nucleus, (iv) absence of any cytoplasmic aggregates (the inclusion bodies of HBLV-infected cells are not virus clusters although dispersed single virus particles are present in the cytoplasm), (v) icosahedral

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Table 1. Presence of HBLV in peripheral blood lymphocytes of patients with lymphoproliferative disorders.

Patient*	Description†	Diagnosis–risk factor	IgG titer§ to		
			HBLV	EBV	CMV
1	WM,29	AIDS-KS, B-cell lymphoma–homosexual‡	1:80	1:320	≥1:640
2	BM,66	Mycosis fungoides($T4^+$), cutaneous T-cell lymphoma–unknown	1:80	1:160	≥1:160
3	BF,35	Immunoblastic lymphoma–unknown	1:80	1:80	1:320
4	WM,40	Dermatopathic lymphadenopathy $T8^+$ skin infiltrate–intravenous drug abuse	1:80	1:160	1:80
5	WM,57	Angioimmunoblastic lymphadenopathy–open-heart surgery	1:40	1:160	1:160
6	BM,17	Acute lymphocytic leukemia, T-cell type–unknown	1:160	1:160	1:40

* Peripheral blood leukocytes from patients were cultured as the primary source of virus. Virus particles were transmitted to fresh human umbilical-cord blood lymphocytes. Positive cell cultures were identified by morphology, immunofluorescence assay, and electron microscopy. †Race (W-white, B-black); sex (M-male, F-female); age (years). ‡AIDS-KS, acquired immune deficiency syndrome with Kaposi's sarcoma. §Serology was done by indirect immunofluorescence using as standard a reference HBLV isolated from patient 6. This virus showed the highest titer and infectivity for cell cultures. Patients 1 and 4 were also positive for HTLV-III; patients 4 and 6 were positive for HTLV-II. For EBV analysis, P3HR-1 cells were used. For CMV, Flow 7000 (fibroblast) cells infected with the AD-169 strain of CMV were used. For all three viruses, IgG titers to the viral capsid antigens were measured.

symmetry with 162 capsomers enveloped in a lipid membrane, and (vi) a diameter of the enveloped particle of about 200 nm. These virus particles mature in the nucleus and are initially released from the cell by exocytosis and subsequently as a result of cell death. A particle count has shown approximately 10^6 particles per ml in HBLV-infected culture supernatants after infection of 10^6 core-blood leukocytes (5 ml) with one particle per cell (infected as described in Fig. 2b).

HBLV-infected cells were typed for surface markers defined by specific monoclonal antibodies and were found to express antigens recognized by B cell–specific Leu-12, Leu-16, B1, and B4 monoclonal antibodies. These cells lacked T-cell markers as measured by OKT-3, OKT-4, and OKT-8 monoclonal antibodies.

Despite morphological similarities to human herpesviruses, the host range of HBLV is different from that of known members of the human herpesvirus group, as HBLV only infects freshly isolated B cells. Attempts to transmit the virus to a number of T and B lymphoblastoid cell lines, and to a variety of other commonly used cell types, were unsuccessful (Table 2). In contrast, Epstein-Barr virus (EBV) infects most B cells and some epithelial cells (*11*). Unlike EBV-infected cells, B cells infected with HBLV were not immortalized. The infected cells developed into characteristic large, refractile cells and exhibited a slight growth advantage over other cells in culture. However,

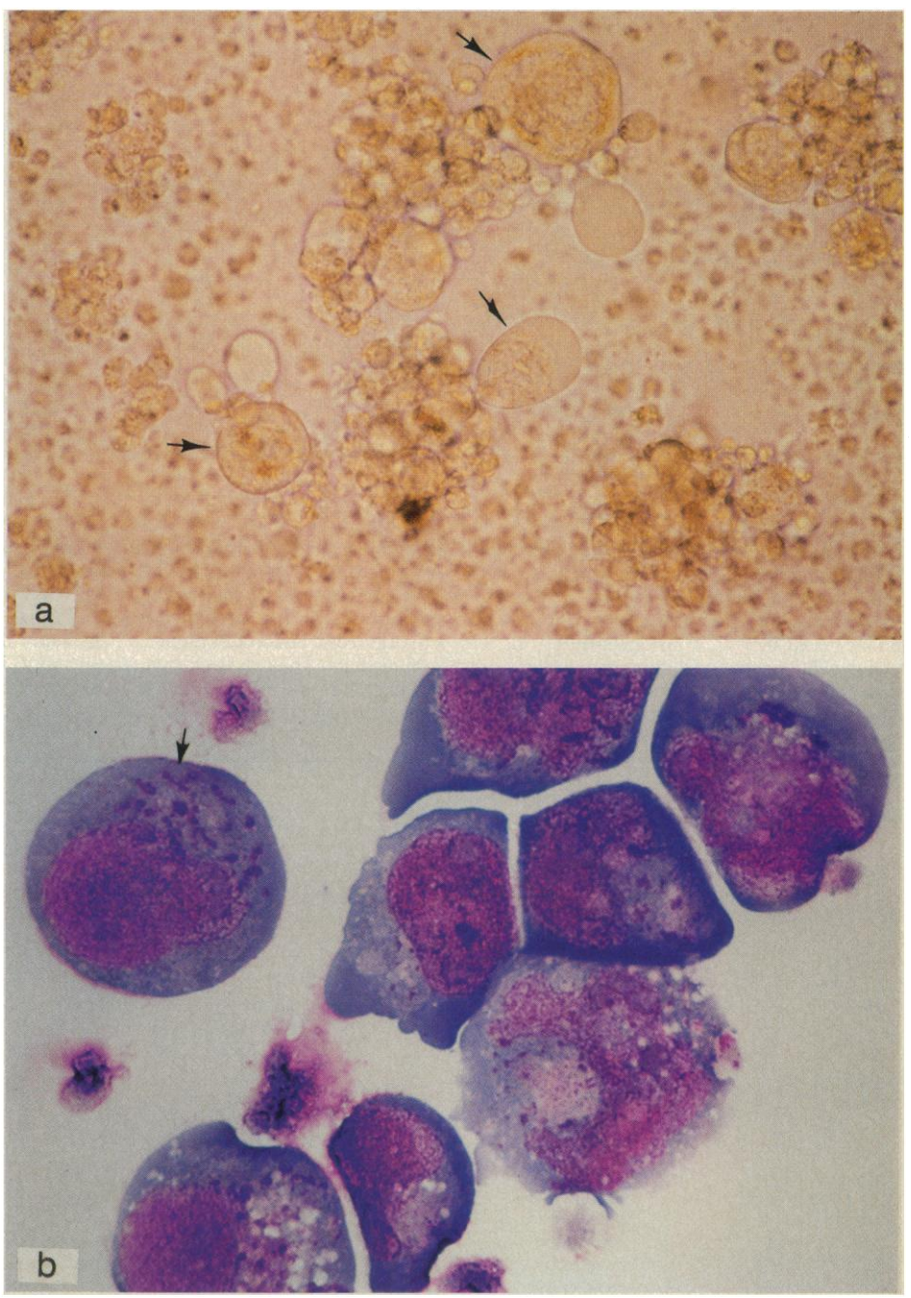

Fig. 1. Peripheral blood leukocytes from an HBLV-infected patient with AIDS-associated lymphoma and HBLV-infected umbilical-cord blood leukocytes in cell culture. (a) Heparanized peripheral blood leukocytes collected from a patient with AIDS-associated lymphoma were banded in Ficoll-Hypaque and established in cell culture at 36°C after PHA-P (5 μg/ml) stimulation for 48 hours. Cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; heat inactivated, 56°C, 30 minutes) and hydrocortisone (5 μg/ml) (*7*). Unstained cells are shown after 5 days in culture. Arrows indicate the large, refractile, HBLV-infected cells. (b) Human umbilical-cord blood mononuclear cells, established in cell culture as described in (a), were treated for 24 hours with polybrene (2 μg/ml), and infected with cell-free virus collected from cultured primary cells. Arrow indicates an infected cell with characteristic cytoplasmic inclusion bodies.

these blast-like cells also died within 10–12 days. Furthermore, other herpesviruses, such as cytomegalovirus (CMV), herpes simplex virus (HSV)–1 and –2, and varicella-zoster virus (VZV), infect a variety of the cell types shown in Table 2, often inducing cytopathic effects that are the bases for quantitation and biological cloning of these viruses (*12*). In addition, no EBV nuclear antigens (*11*) were detected in HBLV-infected umbilical-cord blood mononuclear cells.

Serological comparisons further demonstrated lack of immunological cross-reactivity with known herpesviruses. An IFA assay, developed according to techniques originally described for other herpesviruses (*9, 11*), was used to analyze sera from patients and healthy controls and to monitor infected cells. As shown in Table 1, sera from the six HBLV-positive patients had an immunoglobulin G (IgG) antibody titer to viral capsid antigens >1:40. In contrast, only 4 of 220 sera from randomly selected healthy donors were even weakly positive. Twelve sera from AIDS patients with no known lymphomas were also tested, and they were negative for antibody to HBLV. The pattern of immunofluorescent staining in fixed, infected cells varied from punctate nuclear staining to diffuse staining of the entire cell (Fig. 4a). In live cells the staining was confined to the cell surface as small patches (Fig. 4b). Uninfected umbilical-cord blood mononuclear cells were negative when tested with sera from the HBLV-positive pa-

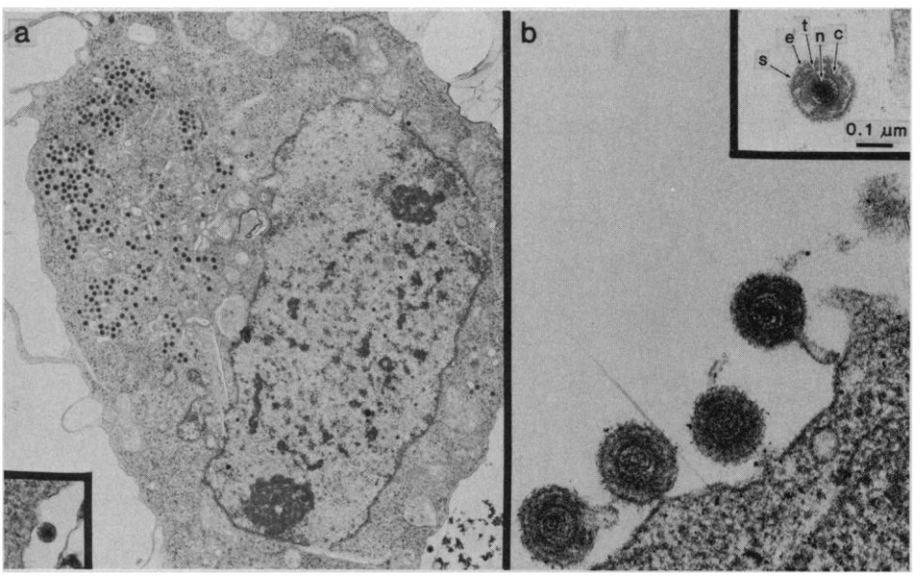

Fig. 2. Electron micrographs of a cultured cell producing HBLV. (a) Peripheral blood mononuclear cells from a patient with AIDS-associated B-cell lymphoma were grown in cell culture as described (Fig. 1). Intracytoplasmic and extracellular HBLV particles are shown. Inset shows higher magnification of a virus particle. (b) An infected cell from a culture of PHA-P–stimulated umbilical-cord blood mononuclear cells, 5 days postinfection. Cells were cultured and infected by cell-free HBLV as described (Fig. 1). Extracellular particles initially appear prior to cell lysis. Inset shows a mature HBLV particle measuring about 200 nm in diameter; s, surface spikes; e, envelope; t, tegument; n, nucleocapsid; c, core.

Table 2. Cell lines that could not be infected with HBLV. The hematopoietic cells were grown at a density of 1×10^6 cells per milliliter in RPMI 1640 supplemented with 20% FBS. These cells were treated with polybrene (2 μg/ml, Aldrich Chemical), for 12–14 hours. Cells were centrifuged gently at 300*g* for 10 minutes at room temperature and treated with either herpesvirus (10 plaque-forming units per cell) or HBLV (10 or 1 particles per cell). (The virus particles were concentrated by negative-pressure ultracentrifugation). The cells were incubated with the respective viruses for 90 minutes and medium was added and incubated at 37°C. The nonhematopoietic cells were cultured in Dulbecco's modified Eagle's medium with 10% FBS. Cultures were treated with DEAE-dextran (25 μg/ml) for 30 minutes and rinsed twice with medium. The cells were then treated with 0.2 ml of virus suspension and allowed to adsorb for 90 minutes. Fresh medium was added to all cultures and incubation continued at 37°C. The hematopoietic as well as nonhematopoietic cells were monitored for at least 1 month by morphology, transmission of cell-free virus to fresh umbilical-cord blood cells, dot immunoblot, and (in case of suspected positivity) by electron microscopy. HVA, *Herpesvirus atteles*.

Human nonhematopoietic cells	Nonhuman nonhematopoietic cells	Human hematopoietic cells			Nonhuman hematopoietic cells
		B cells	T cells	Other cells	
Fibroblast	*Nonhuman primate*	*EBV(+)*	Hut-78	K562	Two marmoset T-cell lines (transformed with HVS)
HT29*	Owl monkey kidney (OMK)	Raji	MOLT-3	THPN-1	
PC-109 (foreskin)†	Rhesus monkey kidney (RMK)	NC-37	MOLT-4	HL60	
Flow 7000	Vero	LDV-7	H-9	PL-1	Two owl monkey T-cell lines (transformed with HVS)
WI-38	Marmoset kidney (MK)	Ly38	CEM	PL-2	
K-82 (skin)	Marmoset fibroblast (MF)	NAB-1	JM	H-69‡	
Epithelial	*Other animals*	PA (non-producer)	81-66-45	NMC-1	Inbred rabbit T-cell lines (transformed with HVS and HVA)
FRN	Dog kidney (MDCK)	Splenic tumor (AG-F)	Alex	NMC-5	
HeLa	Rabbit kidney (RK)	Infectious mononucleosis B-cell (IMB)		VDSO	
KHOS	Sheep kidney (SK)	Craig		Bovine spleen (primary)	
HOS	Rabbit cornea (SARC)	Stanley		BM1009 (megakaryocyte)	
D-98	Bat lung (CCL88)	SB-2		Nalm-1 (pre-b, pre-T)	
D98/Raji-hybrid	Mink lung (CCL64)	*EBV(−)*		U937 (monocyte)	
HEK	Horse epidermal (CCL57)	Ramos		CCL127	
A204 (human rhabdomyosarcoma	Dog thymus (A7573)	Bjab			
	Murine cells (Micl S^+L^-)	Louks			

*Malignant cells from human colon carcinoma. †This cell line is extremely sensitive to CMV(AD-169) infection. ‡This cell line was established from oat cell carcinoma of lung.

Table 3. Reactivity of HBLV-infected cells with human and nonhuman primate herpesviruses. Monoclonal antibodies and hyperimmune sera prepared against human and nonhuman primate herpesviruses (*16*) were tested for reactivity with HBLV-infected cells by indirect immunofluorescence procedures as described (Fig. 4). Monoclonal antibodies to EBV were used at 1:40 dilution. Two were specific for EBV early antigens R and D; two for EBV viral capsid antigen, and two for EBV membrane glycoproteins. Monoclonal antibodies to HCMV were also used at a 1:40 dilution. One was specific for early antigen, and two were for late viral capsid proteins. Monoclonal antibodies to HSV-1 and -2, VZV, and HVS were used at a 1:10 dilution; normal mouse ascites fluid was used at 1:5 and 1:10 dilutions as control. Monoclonal antibodies to HSV-1 and -2 were specific for early and late antigen, while others were specific for VZV or HVS late antigens. The monoclonal antibodies recognize all the known serotypes of respective herpesviruses (*11, 13, 14*). Hyperimmune sera to African Green and rhesus monkey CMV and HVS were heat-inactivated (56°C, 30 minutes), clarified by centrifugation at 10,000 rev/min, and were used at 1:10 dilutions. African Green monkey and rhesus sera containing antibody to CMV were also negative when tested with HBLV (*16*).

Antibody*	Number tested	Viruses used to infect target cells							
		HBLV	EBV	HCMV	HSV-1 and -2	VZV	HVS	AGM-CMV	Rhesus-CMV
EBV monoclonal antibodies	6	–	+	–	–	–	–	–	–
Human sera(VCA, EA, MA, EBNA)	5	–	+	ND	ND	ND	ND	ND	ND
HCMV monoclonal antibodies	3	–	–	+	–	–	–	–	–
Human sera (VCA, EA, MA)	4	–	ND	+	ND	ND	ND	ND	ND
HSV-1 and -2: monoclonal antibodies (early and late)	3	–	–	–	+	–	–	–	–
Human sera (early and late, neutralizing) and MA	4	–	ND	ND	+	ND	–	–	–
VZV: monoclonal antibody	1	–	–	–	–	+	–	–	–
Human sera (early and late, MA, neutralizing)	4	–	–	–	ND	+	ND	ND	ND
HVS: monoclonal antibody	5	–	–	–	–	–	+	–	–
Hyperimmune serum	1	–	–	–	–	ND	+	–	–
AGM	1	–	–	–	–	–	–	+	–
CMV (hyperimmune serum)	1								
Rhesus monkey	1	–	–	–	–	–	–	–	+
CMV (hyperimmune serum)	1								

*Abbreviations used: AGM, African Green monkey; HBLV, human B lymphotropic virus; EBV, Epstein-Barr virus; HCMV, human cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus; HVS, *Herpesvirus saimiri*; VCA, viral capsid antigen; EA, early antigen; MA, membrane antigen; ND, not done.

tients, and HBLV-infected cells showed no reactivity with sera lacking antibody to the virus (Fig. 4c). Thus, the sera were specific for infected cells.

Cells infected by HBLV were also used to directly compare immunological cross-reactivities with other human herpesvirus–specific monoclonal antibodies and hyperimmune sera, or well-characterized human sera containing antibodies to early and late viral antigens from EBV, CMV, HSV, or VZV, or sera from control donors. The monoclonal antibodies used in these experiments recognize all known serotypes of the herpesvirus, including EBV, CMV, HSV-1, and HSV-2; lack of reactivity to these antibodies is strong evidence for the absence of cross-reacting epitopes on HBLV-infected cells. Monoclonal and polyclonal antibodies to EBV, CMV, HSV, VZV, and *Herpesvirus saimiri* (HVS) (*11, 13–16*) and hyperimmune sera to rhesus CMV, African Green CMV, and HVS did not react with HBLV-infected cells (Table 3). Furthermore, the reactivity to other human herpesviruses (EBV, CMV, HSV, or VZV) found in human sera was completely removed by adsorption with cells infected by these viruses without significantly affecting the antibody titer to HBLV. Also, conversely, adsorption with HBLV-infected cells removed antibody to HBLV without significantly affecting the titer to the other human herpesviruses. Comparison of the titers of antibody to EBV, CMV, and HBLV in the original six patients yielded a distinct titer for HBLV as compared to that for EBV and CMV (Table 1).

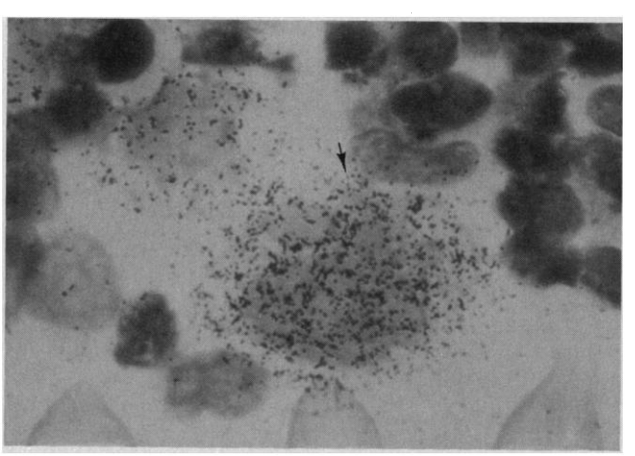

Fig. 3. In situ hybridization of HBLV-infected human umbilical-cord blood cells. Experiments were performed utilizing ^{35}S-labeled RNA probes derived from clone pZVH14 of the HBLV genome (*17*). This clone was used as a template for synthesis of radiolabeled RNA by means of T7 RNA polymerase, ^{35}S-labeled dGTP, and unlabeled ribotriphosphates. Less than one grain per cell was observed in uninfected negative control cultures examined. Arrow shows large refractile cells characteristic of HBLV-infected cultures.

Since HBLV, like EBV, appears to be B-lymphotropic, and since several species of Old World primates harbor B-tropic viruses that are partially related to EBV (possess approximately 40% homology and cross-react immunologically), sera from several Old World and New World primates were

Table 4. Reactivity of nonhuman primate sera with HBLV-, EBV-, and HVS-infected cells.

Serum sources	Positive reactivity/ total tested		
	HBLV	EBV	HVS
*Old World primates**			
Chimpanzee	0/5	5/5	0/4
Gorilla	0/3	2/3	0/3
Orangutan	0/2	1/2	0/2
Baboons	0/3	3/3	0/3
Stumptail	0/2	1/2	0/2
Rhesus	0/9	6/9	0/7
African Green	0/10	6/10	0/10
New World primates†			
Squirrel monkey	0/10	0/10	8/10
Owl monkey	0/6	0/6	3/6
Marmoset (common)	0/6	0/6	0/6
Marmoset (cottontop)	0/3	0/3	1/3

*Sera from Old World (*16*) and New World primates were tested for antibody to HBLV by indirect immunofluorescence as described in Fig. 4. All sera were heat-inactivated at 56°C for 30 minutes and clarified by centrifugation before use. HBLV-infected umbilical-cord blood leukocytes, P3HR-1 (an established cell line expressing EBV viral capsid antigen), and owl monkey kidney cells infected by HVS-11 were used. When infected cells showed cytopathic effects, the cells were fixed in acetone and used for the IFA. †Three owl monkeys and one cottontop marmoset were previously inoculated with HVS. Sera from these animals possessed antibody to HVS late antigen that cross-reacted with *Herpesvirus ateles* (*15*).

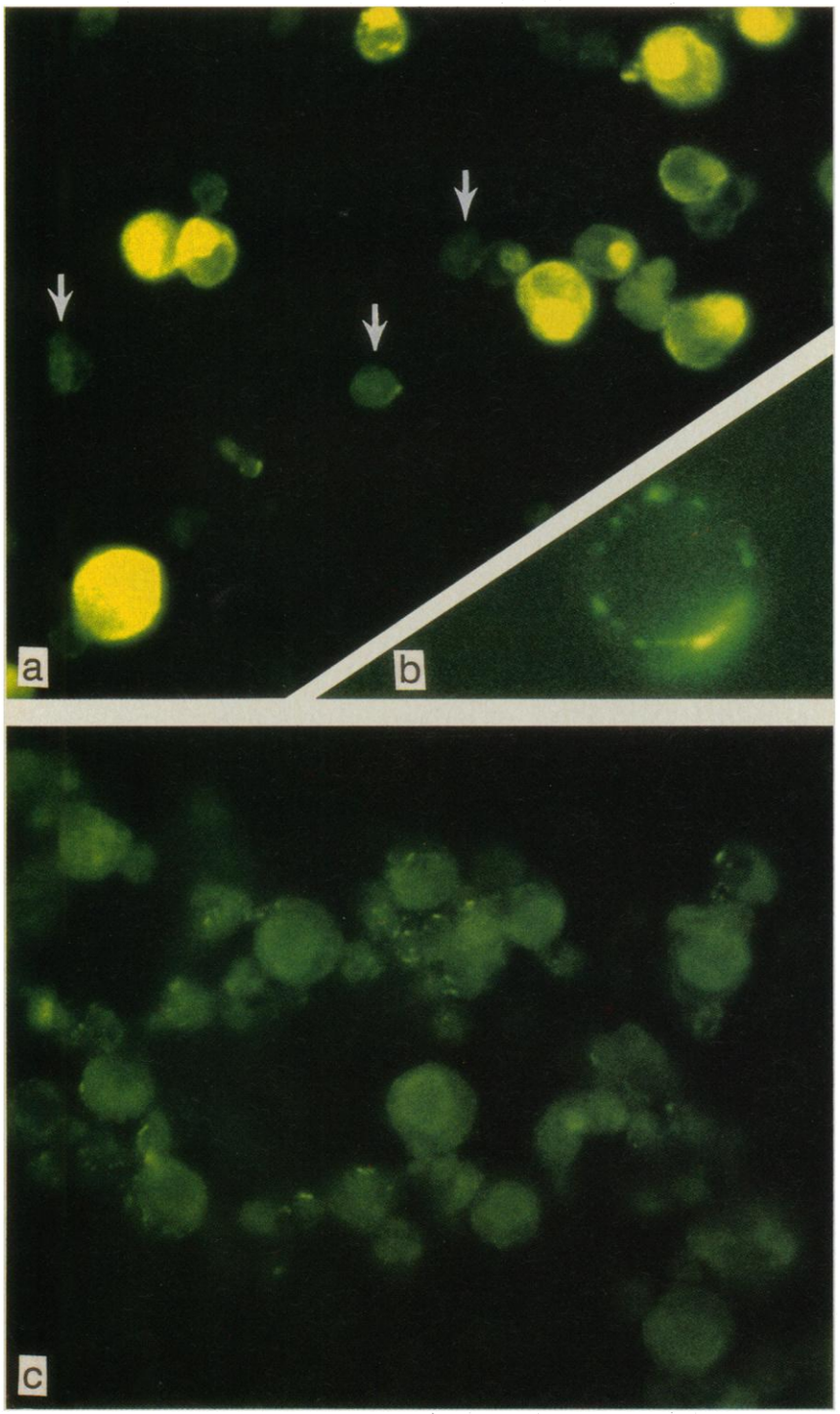

Fig. 4. Immunofluorescent analysis of HBLV-infected cells. (a) Detection of antibody to HBLV capsid antigens. A modification of the indirect immunofluorescence (IF) assay developed by Henle *et al.* was used (*9*). For this assay, HBLV-infected or uninfected human umbilical-cord blood mononuclear cells were washed three times for 10 minutes with phosphate-buffered saline (PBS), resuspended in PBS, deposited on teflon-coated slides, air-dried, and fixed in cold acetone for 10 minutes. Patients' sera (heat-inactivated at 56°C for 30 minutes and clarified by centrifugation) were added to the fixed cells, incubated in a humidity chamber at 37°C for 40 minutes. They were then washed with PBS, air-dried, and stained with affinity-purified fluoroscein isothiocyanate (FITC)–conjugated anti-human IgG for 40 minutes. The cells were again washed as above, air-dried, counter-stained with Evans Blue for 5 minutes and mounted with IF mounting solution. HBLV-infected, enlarged cells (5 days after infection) exhibited granular, nuclear, and cytoplasmic immunofluorescent staining. Small cells in the background (arrows) did not react with patient's serum. (b) Detection of viral membrane antigen. HBLV-infected as well as uninfected live cells (nonfixed) were washed three times in serum-free medium and treated with patient's serum for 30 minutes at 4°C. The cells were then washed, treated with affinity-purified FITC–anti-human IgG for another 30 minutes, washed in medium again, and examined for membrane fluorescence. An HBLV-infected cell with patchy surface fluorescence is shown. (c) HBLV-infected umbilical-cord blood mononuclear cells were stained with an HBLV-negative serum as described in (a).

also tested (*15, 16*). These sera did not react with HBLV-infected cells (Table 4). This does not appear to be a recent infection of man by one of the known simian herpesviruses. We conclude from these serological studies that all six HBLV isolates are the same, and have immunological features that distinguish them from the known human herpesviruses. Thus, the immunological results support the in vitro biological studies indicating that HBLV is a novel human herpes-like virus. These conclusions are supported by genomic analysis (*17*).

Even though HBLV was associated in two instances with HTLV-III/LAV seropositive donors, other evidence demonstrates that HBLV is not exclusively an AIDS-associated agent. Not only did both HTLV-III seropositive patients have complicating lymphoproliferative disorders, but HBLV was also isolated from four HTLV-III seronegative patients. Furthermore, additional seroepidemiological analysis (*18*) has recently shown HBLV antibodies in a patient population clearly dissociated from HTLV-III infection. These results focus our attention on the possible role of HBLV in some lymphoproliferative and immune abnormalities of man.

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Genomic Analysis of the Human B-Lymphotropic Virus (HBLV)

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The human B-lymphotropic virus (HBLV) has a double-stranded DNA genome of greater than 110 kilobase pairs, which is consistent with its morphological classification as a herpesvirus. A 9000–base pair cloned probe of HBLV detected specific sequences in DNA and RNA of infected cells but did not hybridize to the genomic DNA of other human herpesviruses including the Epstein-Barr virus, human cytomegalovirus, herpes simplex type I, and varicella-zoster virus. Conversely, while probes obtained from each of the known human herpesvirus readily detected the homologous viral DNA, they did not hybridize to genomic HBLV DNA. This evidence, in addition to serological and morphological distinctions and the biological effects of this virus demonstrate that HBLV is a novel human herpesvirus.

THE HUMAN B-LYMPHOTROPIC VIRUS (HBLV) is a herpes-like virus on the basis of its icosahedral core structure of 162 capsomers. However, it has several unusual features in terms of host-range, morphology, and serology (*1*) that distinguish it from other known members of the human herpesvirus family [the Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), herpes simplex type 1 and type 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV)] (*2*). We report here that HBLV contains a large molecular weight, double-stranded DNA genome that is distinct from the DNA of known human herpesviruses by Southern blot analyses.

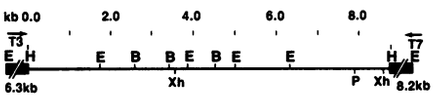

Fig. 1. A restriction map of the HBLV clone pZVH14 obtained as described (*3*). The insert size is 9.0 kb. Shown are the restriction enzyme sites for Bam HI (B), Eco RI (E), Xho I (Xh), Pst I (P), and Hind III (H). The dark boxes indicate the position and size of flanking viral Eco RI fragments detected in genomic viral and infected cellular DNA. The positions of the T3 and T7 promoters contained in the vector are shown by the labeled arrows.

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The isolation of HBLV was performed by cocultivation of peripheral blood lymphocytes (from patients) with phytohemagglutinin-stimulated human umbilical-cord blood lymphocytes and by cell-free transmission of virus from HBLV-positive patients. Propagation was continued by reinfection of fresh umbilical-cord blood cells with filtered (0.45-μm filters) culture supernatants as described (*1*). The supernatants were then layered onto 20% glycerol cushions and centrifuged at 100,000*g* for 3 hours and the DNA ($HBLV_{dp}$ DNA) was extracted from the pelleted virions (*3*).

A molecular clone designated pZVH14 (Fig. 1) was then obtained from the $HBLV_{dp}$ DNA (*3*). When used as a probe, this clone specifically hybridized to DNA of HBLV-infected human umbilical-cord blood cells harvested after several rounds of cell-free virus transmission (Fig. 2) and to the cytoplasmic messenger RNA (mRNA) of infected cells but not to DNA or RNA of uninfected cells (*1*).

The pZVH14 probe was also useful for detection of virus in sucrose gradients by quantitative DNA dot-blot analyses (*4*). Two major peaks were evident (Fig. 3). The first was in fractions 3–5 (density 1.13 g/ml), which was probably enveloped virus; and the second was the pellet, which probably contained the unenveloped capsids. Fractions 3–5, which contained the majority of the virus, were pooled and used to obtain DNA from gradient-banded virions ($HBLV_{bv}$ DNA) (*5*). Bam-HI digests of end-labeled (*6*) $HBLV_{bv}$ DNA indicate a minimum molecular weight estimate for HBLV of >110,000 base pairs (data not shown).

To determine whether there is any significant homology of HBLV to other human herpesviruses we performed Southern blot hybridizations (*7*) with two sets of filters (Fig. 4, A and B) that contained the restriction enzyme–treated genomic DNA's (*8, 9*) of $HBLV_{bv}$, $HBLV_{dp}$, CMV, HSV-1, EBV, VZV, and *Herpesvirus saimiri* (HVS) of squirrel monkeys. Each set of filters was hybridized to a ^{32}P-labeled viral DNA probe (*8, 9*), then washed and analyzed by autoradiography. The probe was then removed by boiling the filters before each successive hybridization. In some of the rehybridized filters, weak bands could be seen as a result of incomplete removal of the previously hybridized probe. These proved useful in aligning the filters and as convincing reference points for the new bands that appeared after each new hybridization. Set A was first hybridized to the HBLV probe pZVH14

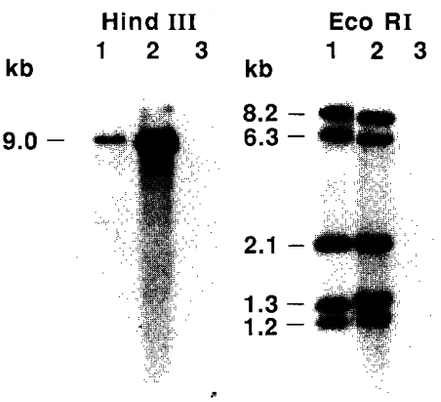

Fig. 2. Hybridization of HBLV clone pZVH14 to DNA's digested with Hind III and Eco RI (*5*) (Fig. 5). Clone pZVH14 hybridized specifically to extracellular virus DNA ($HBLV_{dp}$ DNA) (lanes 1) or infected umbilical-cord blood cell DNA (lanes 2), but not to the uninfected control DNA isolated from the skin of an AIDS patient (lanes 3). The infected cell DNA shown in lane 2 was isolated after several rounds of cell-free virus transmission in human umbilical-cord blood cells.