Immunodetection of Human T-Cell Lymphotropic Virus Type I Core Protein in Biological Samples by Using a Monoclonal Antibody Immunoassay

LAWRENCE PAPSIDERO,^{1*} FRANKLIN SWARTZWELDER,¹ MICHAEL SHEU,¹ RICHARD MONTAGNA,¹ GARTH EHRLICH,² SATYAKAM BHAGAVATI,³ HARVEY DOSIK,⁴ JOHN SNINSKY,⁵ AND BERNARD POIESZ²

Cellular Products, Inc., 688 Main Street, Buffalo, New York 14202¹; Department of Medicine, Health Science Center, State University of New York, Syracuse, New York 13210²; Department of Neurology, Health Science Center, State University of New York, Brooklyn, New York 11203³; Department of Medicine, Interfaith Medical Center, Brooklyn, New York 11238⁴; and Cetus Corp., Emeryville, California 94608⁵

Received 25 October 1989/Accepted 5 February 1990

A monoclonal antibody-based enzyme immunoassay (EIA) has been developed for the detection of human T-cell lymphotropic virus type I (HTLV-I) core protein. The monoclonal antibody (clone 6.11) specifically recognizes the p19 gag gene-encoded protein of the virus. The EIA was over 100 times more sensitive than reverse transcriptase measurement and was capable of responding to less than 500 pg of whole-virus lysate. The assay exhibited type specificity in that HTLV-II antigens failed to produce a positive signal. In addition, a panel of other viruses demonstrated no antigenic cross-reactivity. These included herpesviruses, measles virus, human immunodeficiency viruses, and others. Viral p19 was followed during the course of density gradient ultracentrifugation in the presence of detergent, where it was noted to associate with viral membrane proteins. In comparison, reverse transcriptase activity localized in fractions of higher density containing envelope-free cores. Of clinical interest, the EIA was used to detect HTLV-I antigen in the viral cultures of patients with HTLV-I-associated myelopathies and from symptom-free individuals with proviral integration.

Human T-cell lymphotropic virus type I (HTLV-I) is associated with various forms of lymphoproliferation and leukemogenesis, including polyclonal lymphocytosis of T cells (9, 32), adult T-cell leukemia and lymphoma (14, 31, 41), and B-cell chronic lymphocytic leukemia (25). Seroepidemiologic and molecular biologic studies have also associated the viral infection with chronic myelopathies (3, 18, 21) and polymyositis (40). HTLV-I infections have been reported as the leading cause of hematopoietic neoplasms in Japan and the Caribbean, where the rate of infection among asymptomatic individuals reaches as high as 37% (4, 15, 17).

Recent information suggests that HTLV-I infection and associated leukemia may be more common in the United States than was previously recognized (24, 27, 30, 33). Inasmuch as the clinical presentations are highly variable, efforts to improve upon the laboratory diagnosis of HTLV-I infection are needed.

The present work describes a sensitive enzyme immunoassay (EIA) for the measurement of HTLV-I core protein, encoded by the viral *gag* gene (36). Analogous tests have proven to be valuable for the diagnostic evaluation of human immunodeficiency virus type 1 (HIV-1) antigenemia and for the monitoring of viral cultures (2, 26). The present assay provides significant advantages over the use of conventional methodologies to detect HTLV-I (e.g., reverse transcriptase [RT]), including high sensitivity and assay throughput. In addition, the EIA is type specific because of the employment of a monoclonal antibody (MAb) to p19. Initial data indicate that the assay is of use for monitoring viral antigen during the course of disease pathogenesis in samples including viral cultures and infected cell lysates and also for following viral protein during biochemical fractionations.

MATERIALS AND METHODS

Cells. The following retrovirus-producing cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and antibiotics: HUT-102 (HTLV-I [31]), MT-2 (HTLV-I [41]), SLB-I (HTLV-I [20]), MoT (HTLV-II [5, 6]), LAV-2 (HIV-2 [7]), and HIV-1_{AAV}/HUT-78 (HIV-1 [22]). Cell-free culture supernatants were obtained at the log phase of growth (6×10^5 to 8×10^5 cells per ml) by sedimentation of the cells at $5,000 \times g$.

Viral antigen preparations. Purified virus samples were obtained from the supernatants of producer cell lines as described previously (29). Purified virions were disrupted in phosphate-buffered saline (PBS; 50 mM phosphate-0.15 M NaCl; pH 7.2) containing 0.5% Triton X-100 detergent. After 30 min at 37°C, the lysates were centrifuged at $100,000 \times g$ for 1 h at 4°C, and the detergent was removed by two cycles of ether extraction. Viral lysates were adjusted to 1 mg of protein per ml and stored in aliquots at -70°C.

Commercial preparations of several viruses were also obtained (Lee Biomolecular, San Diego, Calif.). These included herpes simplex virus types 1 and 2, Epstein-Barr virus, measles virus, and influenza A virus.

Polyclonal antibodies. New Zealand rabbits were primed at intramuscular and intradermal sites with 100 µg of HTLV-I lysate emulsified in complete Freund adjuvant. After 2 months, the animals received a similar dose of antigen intradermally in incomplete Freund adjuvant. Thereafter, the rabbits received booster doses at bimonthly intervals for 18 months.

The immunoglobulin G (IgG) fractions of preimmune and immune sera were obtained by protein A-Sepharose (Pharmacia LKB, Piscataway, N.J.) affinity chromatography. Sera were diluted 1/4 in PBS and passed over the affinity gel at 20 ml/h. Nonbound proteins were eluted with 10 bed volumes of PBS, and specifically bound IgG was desorbed at

* Corresponding author.

a low pH (0.1 M glycine-HCl, pH 2.8). IgG was immediately neutralized and extensively dialyzed into PBS at 4°C. The purity of the IgG was determined by polyacrylamide gel electrophoresis (23) under reducing and nonreducing conditions.

MAb to HTLV-I p19 protein. A BALB/c mouse was primed intraperitoneally with 50 µg of HTLV-I in complete Freund adjuvant. At 3 and 6 months, the animal received a booster intraperitoneally with 25 µg of antigen in incomplete Freund adjuvant. Three days following the second boost, splenocytes were fused with murine myeloma cells (P3x63Ag.8.653) in the presence of 50% polyethylene glycol, according to standard protocols (16). Hybridomas were selected in the presence of medium containing hypoxanthine-aminopterin-thymidine, and cultures of interest were cloned by limiting dilution. Reactivity of hybridoma antibodies to HTLV-I p19 protein was determined by Western immunoblotting, and viral specificity was confirmed by immunofluorescence assay with infected and noninfected cells.

MAbs were isotypized by double immunodiffusion with subclass-specific antisera, and antibodies were isolated from culture fluids by ammonium sulfate precipitation followed by protein A chromatography (13).

Western immunoblotting. MT-2 cells or HTLV-I virions purified from the supernatants of HUT-102 cell cultures were extracted in the presence of 1% Triton X-100-PBS, and soluble proteins were overlaid onto 12.5% polyacrylamide slab gels (23). Following electrophoresis, the fractionated proteins were electrotransferred onto sheets of nitrocellulose paper as described elsewhere (37). The sheets were cut into 3-mm strips, which were allowed to react with rabbit antisera (1:100 dilution) or MAbs (10 µg/ml) for 18 h at 23°C. After being washed in PBS containing 0.05% Tween 20, the strips were incubated in pretitrated dilutions of biotin-labeled antiglobulins for 1 h at 37°C. Thereafter, strips were incubated in solutions containing streptavidin conjugated with peroxidase for 30 min at 37°C. Washed strips were finally immersed in substrate solution (1 mg of 4-chloro-1-naphthol–0.03% hydrogen peroxide–citrate buffer per ml, the last ingredient of which was pH 5.5) for 5 to 10 min, and the reactions were stopped in deionized water.

Immunofluorescence assay. Virus-infected and control T cells were washed in PBS and fixed in 2% formaldehyde–PBS for 30 min at 23°C. Fixed cells (10 µl) were dropped onto glass microscope slides at 10⁶ cells per ml of PBS and air dried. Cells were postfixed in acetone at 4°C for 15 min and allowed to dry. Serum samples (1:20 dilution) or MAbs (10 µg/ml) were added to a smear for 1 h at 37°C in a moist chamber and then washed in PBS at 23°C with stirring. Fluorescein isothiocyanate-labeled goat antibodies to murine or rabbit IgG were next applied for 30 min at 37°C. After a further wash, the slides were mounted in buffered glycerol and read by fluorescence microscopy.

EIA for detection of HTLV-I p19 protein. A sandwich-type immunoassay was constructed with rabbit anti-HTLV-I antibodies as the capture phase. Microdilution plates were incubated overnight with 0.06 µg of immune IgG in carbonate-bicarbonate buffer (pH 9.6) per well. Plates were blocked with PBS containing 0.5% Triton X-100 for 1 h at 23°C, washed six times with PBS, and air dried. To perform the EIA, specimens were brought to 0.5% Triton X-100 by using a 5% stock solution and 200 µl was added to each microwell for 2 h at 37°C. After six washes in PBS–0.05% Tween 20, MAb against HTLV-I p19 (clone 6.11) was added (200 µl at 1 µg/ml) for 1 h at 37°C. Thereafter, washed wells received

200 µl of pretitrated goat antibodies against murine IgG which were conjugated with peroxidase. After an incubation at 37°C for 1 h, the wells were washed and the substrate was applied. The substrate contained 0.42 mM tetramethylbenzidine, 1.3 mM hydrogen peroxide, and 0.1 M citrate-acetate buffer (pH 4.5). After 30 min at 23°C, the reactions were stopped by the addition of 50 µl of 2 N sulfuric acid and optical density was measured at 450 nm. Standard curves were constructed with increasing dilutions of crude p19 protein consisting of whole-HTLV-I lysate. Results were expressed as units of p19 protein, in which 1 ng of virions contains 100 U of specific antigen.

A neutralization test was used to confirm the immunoreactivity demonstrated by the EIA procedure. Prior to the performance of the EIA procedure, the test specimen was treated with 10 µl of hyperimmune serum (HTLV-I seropositive, titer >500); the same volume of nonimmune serum was used as a control in a replicate sample. Treated samples were then assayed for residual antigen content by the EIA. Specific reduction in the test signal (optical density) by more than 50% was considered to represent the occurrence of HTLV-I antigen.

Detection of HTLV-I provirus. DNA was isolated from peripheral blood mononuclear cells (PBMC) as described previously (1). One microgram of DNA (equivalent to ca. 150,000 cells) was amplified in 100-µl reaction volumes of 50 mM KCl, 10 mM Tris (pH 8.3), 2.5 mM MgCl₂, 225 µM deoxynucleoside triphosphate, 0.02% gelatin, each primer at 100 nM, and 2.5 U of *Thermus aquaticus* polymerase (*Taq*) (Perkin-Elmer Cetus Corp., Norwalk, Conn.) (35). Amplifications were carried out for 30 cycles in a Perkin-Elmer Cetus thermal cycler. The HTLV-I-specific and generic oligonucleotide primer pairs and probes have been previously described (10–12). The HTLV-I-specific primers and probes utilized were SK54/55 and SK56 (*pol* gene), SG293/220 and SG225 (*env* gene), and SG221/227 and SG229 (*env* gene). The HTLV-II type-specific primers and probes were SG638/639 and SG640 (*env* gene). The generic primers and probes for HTLV-I and HTLV-II were SK43/44 and SK45 (*tax* gene) and SK110/111 and SK115 (*pol* gene).

Amplified DNA products were detected by liquid hybridization analysis as described elsewhere (1). Briefly, a 30-µl sample of each amplification mixture was hybridized for 1 h with 250,000 cpm of the appropriate ³²P-end-labeled detector oligonucleotide at 55°C in 250 mM NaCl–1 mM EDTA. The reaction products were then separated in a gel retardation assay followed by autoradiography.

Viral cultures. Viral cultures were performed essentially as described before (31). PBMC from HTLV-I-seropositive patients or normal donors were cultured in RPMI 1640 medium containing 10% fetal bovine serum, antibiotics, and 20% (vol/vol) T-cell growth factor (Cellular Products, Inc.). The cultures were given fresh culture medium three times per week. Virus growth was monitored weekly by p19 EIA on culture fluids.

Recovery of virus-infected cells. HTLV-I-infected cells (MT-2) and noninfected T cells (HUT-78) were separately washed in PBS, and each was adjusted to 2 × 10⁶ cells per ml of PBS. Increasing numbers of infected cells were added to a constant number (10⁶) of noninfected cells in individual tubes. Thereafter, the cell mixtures were subjected to centrifugation at 3,000 × *g* for 15 min, and the cell pellets were dissolved in 1 ml of PBS containing 0.5% Triton X-100. After 15 min at 4°C, the samples were clarified by centrifugation at 10,000 × *g* and 200 µl of the clear supernatant was examined

for p19 antigen content by EIA. Results were expressed as viral antigen per 10^4 noninfected cells.

Reverse transcriptase (RT) measurements. RT was measured with poly(rC)-oligo(dG) as the template primer as described before (34). Reaction mixtures (100 μ l) contained 50 mM Tris hydrochloride (pH 7.9), 50 mM KCl, 2 mM dithiothreitol, 0.01% Triton X-100, 1 mg of bovine serum albumin per ml, 20 mM $MgCl_2$, 10 μ g of the template primer per ml, 10 μ Ci of [3H]dGTP, and 20% glycerol. Incorporation was allowed to proceed for 4 h at 37°C, and acid-precipitable counts were determined by beta spectrometry.

Isolation of retroviral cores. Transcriptionally active HTLV-I ribonucleoprotein cores were isolated from whole virions as described previously (8). Virions from 30 liters of HUT-102 cultures were purified as above and divided into two aliquots, one of which was subjected to detergent exposure in order to release ribonucleoprotein cores. In brief, virions were pelleted at $40,000 \times g$ for 1 h at 4°C and suspended in 5 ml of TNE buffer (10 mM Tris hydrochloride [pH 8.0], 0.15 M NaCl, 2 mM EDTA). An equal volume of detergent buffer (2% Nonidet P-40, 10% sucrose, 0.2 M dithiothreitol) was added, and the mixture was incubated on ice for 5 min. The mixture was then loaded onto a linear sucrose gradient (20 to 70%, prepared in TNE-0.02 M dithiothreitol) and centrifuged at $189,000 \times g$ for 2 h at 4°C. Fractions of 0.5 ml were collected and assayed for optical density at 280 nm, RT activity, and HTLV-I p19 antigen. Control, buffer-treated preparations of HTLV-I virions were also run.

Anion-exchange chromatography. HTLV-I viral lysate (20 mg) was dialyzed into running buffer (25 mM Tris hydrochloride, pH 8.0) and applied to a column (2.5 by 20 cm) packed with DEAE-Sephacel (Pharmacia) equilibrated in running buffer. Nonreactive components were eluted with 2 bed volumes of running buffer, and a linear gradient of NaCl was then applied. Fractions of 1 ml were collected and assayed for optical density at 280 nm, conductivity, and p19 activity.

RESULTS AND DISCUSSION

Immunization of rabbits with HTLV-I lysate yielded antisera which predominantly reacted with viral *gag* gene-encoded proteins (Fig. 1) as determined by Western immunoblotting. Antibody activity was also observed against a protein of between 50 and 60 kilodaltons. This protein may represent a *gag* polypeptide precursor or may be a comigrating infected-cell protein. The unabsorbed rabbit antibodies produced strong immunofluorescent staining of HTLV-I-infected cells (MT-2 and HUT-102) but also yielded a weak but unequivocal reactivity with control T cells (data not shown). Inasmuch as retroviral preparations express cell surface antigens of the carrier cell, these data were not unexpected. The IgG fraction of antiserum was used as the capture phase of an HTLV-I EIA. To contribute viral specificity to the assay, a murine MAb to p19 protein was chosen as the detector reagent. This MAb (clone 6.11) demonstrated strong reactivity with HTLV-I p19 and p32 on Western immunoblotting with HUT-102 virus (Fig. 1). Binding to p28 was also observed with the MT-2 strain of virus. Control preimmune sera and subclass-matched MAbs failed to react with *gag*-associated structural proteins of the virus.

Utilizing the above immune reagents, an EIA was constructed for immune detection of HTLV-I core antigen. Antigen which adsorbed to the solid-phase antibody was revealed by sequential incubations with MAb to p19 followed by peroxidase conjugates of goat antibodies to mouse

FIG. 1. Western immunoblot reactivity of polyclonal antibodies and MAbs to HTLV-I. The target was detergent lysate of sucrose gradient-purified HTLV-I virions, approximately 20 μ g per strip. Strip 1, Rabbit antiserum to HTLV-I (EIA capture antibody); strip 2, preimmune rabbit serum; strip 3, monoclonal antibody to HTLV-I p19 (clone 6.11; IgG1 subclass); strip 4, MAb to HIV-1 transmembrane protein, gp41 (IgG1 subclass [27]).

IgG. Since homogeneous preparations of p19 protein were not currently available to serve as a calibrator for the assay, whole-HTLV-I lysate was used as a reference standard. HTLV-I lysate generated a strong dose-response effect in the p19-specific immunoassay (Fig. 2). The sensitivity of the assay with whole-HTLV-I protein was 500 pg/ml. Inasmuch as p19 core protein most likely contributes less than 5 to 10% of the total mass of the HTLV-I virion, as it does for other retroviruses (19), the absolute sensitivity of the EIA may lie in the range of 25 to 50 pg/ml. At present, it was chosen to assign arbitrary units to reflect changes in the target antigen concentration. For this purpose, a solution of HTLV-I lysate at a protein concentration of 1 ng/ml was assigned a value of

FIG. 2. EIA for detection of HTLV-I p19 protein. Detergent lysates of HTLV-I were serially diluted and subjected to EIA analysis in the absence (●) or presence (○) of human serum which was HTLV-I antibody seropositive.

FIG. 3. Dilutional sensitivity of RT versus HTLV-I EIA. Culture fluids (approximately 500 ml) obtained from HUT-102 cells were centrifuged at $5,000 \times g$ to remove cells, and then viral particles in the supernatant were sedimented at $50,000 \times g$. Pelleted virus was solubilized in 1 ml of lysis buffer and used to prepare a serial dilution series. Each dilution was assayed by EIA.

100 arbitrary units per ml. The dose response generated with HTLV-I antigen was completely quenched in the presence of HTLV-I-seropositive human serum (Fig. 2). Antibody-negative serum failed to perturb the dose-response effect, further indicating a virus-specific interaction.

The relative sensitivities of the EIA and conventional RT measurements were compared. The dilutional sensitivity of each test (Fig. 3) shows that the EIA procedure provides a 100- to 200-fold increase in sensitivity over RT measurements when HTLV-I virions are the target sample under study.

The specificity of the assay was further probed with a panel of viral antigens, including other retroviruses. Results indicated that the EIA failed to react with samples containing HIV-1, HIV-2, Epstein-Barr virus, herpes simplex virus, measles virus, and influenza virus (Tables 1 and 2). Interestingly, fluids containing genetically related HTLV-II also did not elicit a positive response in the assay, strongly indicating type specificity of the EIA.

To determine if the EIA was capable of detecting small percentages of infected cells, recovery experiments were performed. For this purpose, increasing numbers of HTLV-I-infected cells were mixed with noninfected T cells. These cell mixtures were collected by centrifugation, and the detergent-lysed cells were assayed for viral antigen content. It was possible to detect as few as 1 infected cell per 10,000

TABLE 1. HTLV-I EIA reactivity of viral specimens

Specimen (virus)	EIA reactivity ^a in culture fluid that was:	
	Undiluted	Diluted 1/10
HTLV-III _B /H9 (HIV-1)	0.04	NR ^b
AAV/HUT-78 (HIV-1)	0.05	NR
LAV-2/CEM (HIV-2)	0.05	NR
MoT (HTLV-II)	0.04	NR
HUT-102 (HTLV-I)	>4.0	1.04
SLB-1 (HTLV-I)	>4.0	3.88
MT-2 (HTLV-I)	>4.0	>4.0

^a Optical density at 450 nm.

^b NR, Not reactive.

TABLE 2. HTLV-I EIA reactivity of viral specimens

Specimen	EIA reactivity ^a in viral lysates at:	
	10 µg/ml	0.1 µg/ml
HIV-1	0.04	NR ^b
Measles virus	0.03	NR
Epstein-Barr virus	0.04	NR
Herpes simplex virus type 1	0.04	NR
Herpes simplex virus type 2	0.04	NR
Influenza A virus	0.04	NR
HTLV-I (HUT-102)	>4.0	>4.0

^a Optical density at 450 nm.

^b NR, Not reactive.

noninfected T cells by the p19 EIA (Fig. 4). At a specimen volume of 0.2 ml containing 10^6 noninfected cells per ml, this represents approximately 20 infected cells. This level of sensitivity approaches that associated with the use of polymerase chain reaction (PCR) DNA amplification (1, 21, 28). In contrast to gene amplification techniques, however, the EIA relies upon the occurrence of specific gene expression and will not yield a positive signal during periods of viral latency.

The biochemical and physical behavior of p19 was investigated by ultracentrifugation and liquid chromatography, as monitored by the EIA. When HTLV-I was subjected to density gradient ultracentrifugation in linear gradients of sucrose, p19 activity was associated with the peak of RT activity. This activity occurred in fractions containing intact virions at densities of around 1.14 g/cm^3 (Fig. 5A). After removal of the viral envelope by detergent treatment, RT activity was localized to denser regions of the gradient ($>1.2 \text{ g/cm}^3$; Fig. 5B), which putatively contained viral cores (8). In contrast, p19 immunoreactivity remained quantitatively associated with lower-density fractions. These data point to a stable interaction of p19 with the viral envelope, probably imparted by the occurrence of myristic acid on the protein. This type of cotranslational modification (39) has been described for the 5' *gag* proteins of many retroviruses (38).

At the biochemical level, the immunoassay was of value to study the chromatographic behavior of the p19 protein.

FIG. 4. Recovery of HTLV-I-infected cells from infected- and noninfected-cell mixtures. Increasing numbers of infected cells were added to 10^4 noninfected T cells (HUT-78). Cells were pelleted and lysed in detergent buffer, and each sample was subjected in triplicate to EIA analysis.

FIG. 5. Characterization of transcriptionally active cores isolated from HTLV-I virions. HTLV-I virions were buffer treated (A) or detergent treated (B) and subjected to density gradient ultracentrifugation in a linear gradient of sucrose. Fractions thus obtained were examined for p19 protein and RT activity. RT activity was associated with viral cores, whereas p19 immunoreactivity was associated with less-dense (membrane) regions of the gradient.

Under conditions of low ionic strength, the protein was retained on DEAE gels under conditions in which the majority of infected-cell proteins demonstrated no interaction (Fig. 6). Antigenic activity was observed to desorb from the anion-exchange resin after the introduction of a salt gradient. The major peak of antigen eluted at around 0.1 M NaCl (Fig. 6). In addition, a minor peak of immunoreactivity

FIG. 6. Interaction of HTLV-I p19 protein with anion-exchange medium. Samples (20 mg) of gradient-purified HTLV-I virions were lysed in detergent buffer, dialyzed into running buffer (25 mM Tris hydrochloride, pH 8.0), and applied to a column packed with DEAE-Sephacel. Following the recovery of protein in the flowthrough, bound proteins were desorbed under a gradient of NaCl. Collected fractions were examined for protein at 280 nm and for p19 content. The bar represents fractions which were pooled and further characterized.

TABLE 3. Relationship among proviral status, antibody seropositivity, and expression of HTLV-I in viral cultures^a

Patient	Provirus ^b	Serology ^c	Viral culture ^d
Normal donor	—	—	—
20844 ^e	+	+	—
20845 ^e	+	+	—
20846 ^e	+	+	+
23374 ^e	+	+	+
23472 ^e	+	+	+
24165 ^f	+	+	+

^a +, Test results were positive; —, test results were negative.

^b As determined by PCR with HTLV-I-specific primer pairs and oligonucleotide probes.

^c The presence of circulating antibody was determined using a Food and Drug Administration-licensed enzyme-linked immunosorbent assay (Cellular Products, Inc.).

^d Monitored for viral expression by HTLV-I p19 EIA.

^e Asymptomatic, virus-positive individuals (southern black or Caribbean descent).

^f Individual with HTLV-I-associated myelopathy.

was more strongly bound and required 0.4 M NaCl to effect dissociation from the matrix. Examination of the major peak of activity indicated a 300-fold enrichment, associated with over 70% recovery. Electrophoresis revealed that this fraction exhibited several minor protein contaminants, some of which may represent nonacetylated forms of the protein, and efforts to achieve homogeneous preparations are in progress.

In order to determine the potential clinical utility of antigen measurements, the relationship among proviral status, antibody seropositivity, and expression of HTLV-I in viral cultures was examined. Six individuals with proviral DNA were studied (one with myelopathy and five symptom-free carriers) (Table 3). Each of these individuals was antibody positive. Of the cultures established from the PBMC of the individuals, four demonstrated the expression of viral antigen. Results from the two culture-negative patients suggest that PCR amplification of proviral DNA provides higher sensitivity than a viral culture does. However, since PCR amplifies relatively short sequences of DNA (100 to 500 base pairs), it remains possible that noninfectious, defective genomes will elicit a PCR-reactive result. Thus, complementary data are expected from biologic techniques such as the detection of antigen expression by viral cultures.

Figure 7 illustrates the profile of antigen activity in four series of viral cultures established from individuals with HTLV-I proviral integration. PBMC cultures were maintained in the presence of interleukin-2 and were monitored for viral proliferation by the EIA. Three of the four donors demonstrated significant viral antigen expression which was noted to increase over time in culture. The culture-positive specimens were from two symptom-free carriers in addition to one patient with HTLV-I-associated myelopathy. The culture-negative sample was obtained from a disease-free carrier, presumably at low viral load. Overall, it appears that PCR, EIA, and serology may complement one another and offer strategies to identify individuals in distinct periods of viral latency and activity, with varying viral loads, or infected with cross-reactive but distinct viral pathogens.

ACKNOWLEDGMENTS

We appreciate the excellent technical support of Ray Dittmer, Stephen Lenhart, John Mikula, Diane Waterman, Michelle Wysocki, Christine Stephens, Christopher Stephens, Mary Rubert, Barbara Jones, and Virginia Bryz-Gornia. We also thank Steven Greenberg for graciously providing some of the oligonucleotides used for the PCR analyses.

FIG. 7. Detection of HTLV-I antigen in cultures of viruses obtained from individuals with proviral integration. PBMC were cultured in the presence of cytokine as described in Materials and Methods. The occurrence of HTLV-I proviral integration was confirmed by a polymerase chain reaction with virus-specific primer pairs and probes. Cultures were maintained for 28 days, and supernatants were assayed for viral antigen at 7-day intervals. Patients: 23374, 23472, and 20845, asymptomatic carriers; 24165, HTLV-I-associated myelopathy. All patients were HTLV-II provirus negative as determined by using PCR. AU, Arbitrary units.

This work was supported in part by Public Health Service contract no. NO1HB67021 and grant no. RO1HL43602-01 from the National Institutes of Health.

LITERATURE CITED

- Abbott, M. A., B. J. Poiesz, B. C. Byrne, S. Kwok, J. J. Sninsky, and G. D. Ehrlich. 1988. Enzymatic gene amplification: qualitative and quantitative methods for detecting proviral DNA amplified in vitro. *J. Infect. Dis.* **158**:1158-1169.
- Allain, J.-P., Y. Laurian, D. A. Paul, F. Verroust, M. Leuther, C. Gazengel, D. Senn, M.-J. Larrieu, and C. Bosser. 1987. Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. Potential clinical importance. *N. Engl. J. Med.* **317**:1114-1121.
- Bhagavati, S., G. Ehrlich, R. Kula, S. Kwok, J. Sninsky, V. Udani, and B. J. Poiesz. 1988. Detection of human T-cell lymphoma/leukemia virus type I (HTLV-I) in the spinal fluid and blood of cases of chronic progressive myelopathy and a clinical, radiological and electrophysiological profile of HTLV-I associated myelopathy. *N. Engl. J. Med.* **318**:1141-1147.
- Blattner, W. A., W. N. Gibbs, C. Saxinger, M. Robert-Guroff, J. Clark, W. Lofters, B. Hanchard, M. Campbell, and R. C. Gallo. 1983. Human T-cell leukemia/lymphoma virus-associated lymphoreticular neoplasia in Jamaica. *Lancet* **ii**:61-64.
- Chen, I. S. Y., J. McLaughlin, J. C. Gasson, S. C. Clark, and D. W. Golde. 1983. Molecular characterization of genome of a unique human T-cell leukaemia virus. *Nature (London)* **305**: 502-505.
- Chen, I. S. Y., S. G. Quan, and D. W. Golde. 1983. Human T-cell leukemia virus type II transforms normal human lymphocytes. *Proc. Natl. Acad. Sci. USA* **80**:7006-7009.
- Clavel, F., D. Guetard, F. Brun-Vesinet, S. Chamaret, M. Rey, M. Santos-Ferreira, A. G. Laurent, C. Dauge, C. Katlama, C. Rouzioux, D. Klatzman, J. L. Champalimaud, and L. Montagnier. 1986. Isolation of a new human retrovirus from West African patients with AIDS. *Science* **233**:343-346.
- David, N. L., and R. R. Rueckert. 1972. Properties of a ribonucleoprotein particle isolated from Nonidet P-40-treated Rous sarcoma virus. *J. Virol.* **10**:1010-1020.
- Ehrlich, G. D., F. R. Davey, J. J. Kirshner, J. J. Sninsky, K. S. Kwok, D. J. Slamon, R. Kalish, and B. J. Poiesz. 1989. A polyclonal CD4+ and CD8+ lymphocytosis in a patient doubly infected with HTLV-I and HIV-1: a clinical and molecular analysis. *Am. J. Hematol.* **30**:128-139.
- Ehrlich, G. D., J. B. Glaser, M. A. Abbott, D. J. Slamon, D. Keith, M. Sliwowski, J. Brandis, E. Keitelman, Y. Teramoto, L. Papsidero, H. Simpkins, J. J. Sninsky, and B. Poiesz. 1989. Detection of anti-HTLV-I tax antibodies in HTLV-I enzyme-linked immunosorbent assay-negative individuals. *Blood* **74**: 1066-1072.
- Ehrlich, G. D., J. B. Glaser, K. LaVigne, D. Quan, D. Mildvan, J. J. Sninsky, S. Kwok, L. Papsidero, and B. J. Poiesz. 1989. Prevalence of human T-cell leukemia/lymphoma virus (HTLV) type II infection among high-risk individuals: type-specific identification of HTLVs by polymerase chain reaction. *Blood* **74**: 1658-1664.
- Ehrlich, G. D., S. J. Greenberg, and M. A. Abbott. 1989. Detection of human T-cell lymphoma/leukemia viruses (HTLV), p. 325-336. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), *PCR protocols: a guide to methods and applications*. Academic Press, Inc., San Diego, Calif.
- Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG1, IgG2a, and IgG3 immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry* **15**:429-438.
- Gallo, R. C. 1984. Human T-cell leukemia-lymphoma virus and T-cell malignancies in adults. *Cancer Surv.* **3**:113-159.
- Gessain, A., A. Jouanelle, P. Escarmant, A. Celander, L. Schaffer-Deshayes, and G. de-The. 1984. HTLV antibodies in patients with non-Hodgkin lymphoma in Martinique. *Lancet* **i**:1183-1184.
- Goding, J. W. 1980. Antibody production by hybridomas. *J. Immunol. Methods* **29**:285-308.
- Hinuma, Y., H. Komoda, T. Chosa, T. Kondo, M. Kohakura, T. Takenaka, M. Kikuchi, M. Ichimura, K. Yunoki, I. Sato, R. Matsuo, Y. Takiuchi, H. Uchino, and M. Hanoaka. 1982. Antibodies to adult T-cell leukemia virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nationwide sero-epidemiological study. *Int. J. Cancer* **29**:631-635.
- Jacobson, S., C. S. Raine, E. S. Mingioli, and D. E. McFarlin. 1988. Isolation of an HTLV-I-like retrovirus from patients with tropical spastic paraparesis. *Nature (London)* **331**:540-543.
- Joklik, W. K. 1988. Tumor viruses, p. 106-133. In W. K. Joklik (ed.), *Virology*, 3rd edition. Appleton and Lange, Norwalk, Conn.
- Koeffler, H. P., I. S. Y. Chen, and D. W. Golde. 1984. Characterization of a novel HTLV-infected cell line. *Blood* **64**:482-491.
- Kwok, S., D. Kellogg, G. Ehrlich, B. Poiesz, S. Bhagavati, and J. J. Sninsky. 1988. Characterization of a sequence of human T cell leukemia virus type I from a patient with chronic progressive myelopathy. *J. Infect. Dis.* **158**:1193-1197.
- Kwok, S., D. H. Mack, K. B. Mullis, B. Poiesz, G. Ehrlich, D. Blair, A. Friedman-Kien, and J. J. Sninsky. 1987. Identification of human immunodeficiency virus sequences by using in vitro enzymatic amplification and oligomer cleavage detection. *J. Virol.* **61**:1690-1694.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.
- Levine, P. H., W. A. Blattner, J. Clark, R. Tarone, E. M. Maloney, E. M. Murphy, R. C. Gallo, M. Robert-Guroff, and W. C. Saxinger. 1988. Geographic distribution of HTLV-I and identification of a new high-risk population. *Int. J. Cancer* **42**:7-12.
- Mann, D. L., P. DeSantis, G. Mark, A. Pfeifer, M. Newman, N. Gibbs, M. Popovic, M. G. Sarngadharan, R. C. Gallo, J. Clark, and W. Blattner. 1987. HTLV-I-associated B-cell CLL: indirect role for retrovirus in leukemogenesis. *Science* **236**:1103-1106.
- McDougal, J. S., S. P. Cort, M. S. Kennedy, C. D. Cabridilla, P. M. Feorino, D. P. Francis, D. Hicks, V. S. Kalyanaraman, and L. S. Martin. 1985. Immunoassay for the detection and quantitation of infectious human retrovirus, lymphadopathy-associated virus (LAV). *J. Immunol. Methods* **76**:171-183.
- Minamoto, G. Y., J. W. M. Gold, D. A. Scheinberg, W. D. Hardy, N. Chein, E. Zuckerman, L. Reich, K. Dietz, T. Gee, J. Hoffer, K. Mayer, L. Gabrilove, B. Clarkson, and D. Armstrong. 1988. Infection with human T-cell leukemia virus type I in

- patients with leukemia. *N. Engl. J. Med.* **318**:219–222.
28. Ou, C.-Y., S. Kwok, S. W. Mitchell, D. H. Mack, J. J. Sninsky, J. W. Krebs, P. Feorino, D. Warfield, and G. Schochetman. 1988. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. *Science* **239**:295–297.
 29. Papsidero, L. D., B. J. Poiesz, and R. A. Montagna. 1988. Monoclonal antibody identifies a highly conserved and immunodominant epitope of the human immunodeficiency virus transmembrane protein. *Hybridoma* **7**:117–128.
 30. Phillips, P. E., S. L. Johnston, L. A. Runge, J. L. Moore, and B. J. Poiesz. 1986. High IgM antibody to human T-lymphotropic virus type I in systemic lupus erythematosus. *J. Clin. Immunol.* **6**:234–241.
 31. Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA* **77**:7415–7419.
 32. Posnett, D. N., H. A. McGrath, R. A. Scott, A. S. McNutt, R. Folkl, T. D. Hickey, M. J. Macera, and P. Szabo. 1988. Polyclonal lymphocytosis of T-cells associated with human T-cell leukemia virus I. *Cancer Res.* **48**:2585–2589.
 33. Ratner, L., and B. J. Poiesz. 1989. Leukemias associated with human T-cell lymphotropic virus type I in a non-endemic region. *Medicine (Baltimore)* **67**:401–422.
 34. Rho, H. M., B. J. Poiesz, F. W. Ruscetti, and R. C. Gallo. 1981. Characterization of the reverse transcriptase from a new retrovirus (HTLV) produced by a human cutaneous T-cell lymphoma cell line. *Virology* **112**:355–360.
 35. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**:487–491.
 36. Seiki, M., S. Hattori, Y. Hirayama, and M. Yoshida. 1983. Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc. Natl. Acad. Sci. USA* **80**:3618–3622.
 37. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350–4356.
 38. Veronese, F. D., T. D. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sarngadharan. 1988. Biochemical and immunological analysis of human immunodeficiency virus *gag* gene products p17 and p24. *J. Virol.* **62**:795–801.
 39. Wilcox, C., L.-S. Hu, and N. E. Olson. 1987. Acylation of proteins with myristic acid occurs cotranslationally. *Science* **238**:1275–1278.
 40. Wiley, C. A., M. Nerenberg, D. Cros, and M. C. Soto-Aguilar. 1989. HTLV-I polymyositis in a patient also infected with the human immunodeficiency virus. *N. Engl. J. Med.* **320**:992–995.
 41. Yoshida, M., I. Miyoshi, and Y. Hinuma. 1982. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication for the disease. *Proc. Natl. Acad. Sci. USA* **79**:2031–2035.