Absence of Human T-Cell Lymphotropic Virus Type I Coinfection in Human Immunodeficiency Virus-Infected Hemophilic Men

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Concern for transmission of human T-cell lymphotropic virus, type 1 (HTLV-1) infection to recipients of infected cellular blood products has prompted development of tests to eliminate blood units with HTLV-I antibodies. Most hemophilic men from the United States became infected with human immunodeficiency virus (HIV) before HIV donor screening and before blood products were processed to inactivate the virus. To assess whether these men might also be infected with HTLV-I, we examined the HTLV-I antibody status of 127 factor VIII (hemophilia A) recipients and 71 factor IX (hemophilia B) recipients. One HIV-seronegative and four HIV-seropositive persons were HTLV-I reactive by enzyme-linked immunosorbent assay (ELISA). Four of five ELISA-reactive serum samples were negative by HTLV-I immunoblot assay (IB); 1 reactive and 1 borderline reactive serum were indeterminate on IB (p19 reactivity), but negative by radioimmunoprecipitation assay (RIPA). Peripheral blood mononuclear cells from one patient with indeterminate HTLV-I IB were negative for HTLV-I genomic sequences by polymerase chain reaction. The other indeterminate patient's serum antibody pattern was stable over a 2-year period, suggesting this was not an instance of early HTLV-I seroconversion. These results reaffirm the safety of factor components in the United States with regard to HTLV-I but emphasize the importance and need for further testing of reactive HTLV-I ELISA results with a second more specific technique.

HUMAN T-CELL lymphotropic virus I (HTLV-I) is considered an etiologic agent for development of adult T-cell lymphoma/leukemia (ATLL)[1] and more recently was associated with a spastic paraparesis syndrome [HTLV-I–associated myelopathy (HAM) or tropical spastic paraparesis (TSP)].[2] The virus infection is endemic in southwestern Japan,[3] the Caribbean,[4] South America,[5] Africa,[6] and among certain risk groups [eg intravenous (IV) drug users] in the United States.[7] The virus is transmitted through transfusion of cellular blood products containing HTLV-I–infected cells,[8] by sharing of virus-contaminated needles among drug users,[7] through sexual contact,[9] and from mother to offspring, predominantly by breast feeding.[10] Concern about transmission of HTLV-I infection from contaminated blood products has been intensified by recent reports of HTLV-I infection in multiple transfused patients[11] and by serologic evidence of HTLV-I in volunteer blood donor populations in the United States.[12]

Coinfection with HTLV-I and human immunodeficiency virus (HIV) occurs in certain patients with acquired immunodeficiency syndrome (AIDS)[13,14] and in some groups at risk for AIDS,[7] in part because of similar modes of transmission of the viruses. Hemophilic patients who received HIV-contaminated blood components before blood screening and inactivation procedures for HIV were implemented have elevated rates of HIV-specific antibodies and represent a significant risk group for development of AIDS.[15] Most US hemophilic men became infected with HIV before HIV donor-screened, virus-inactivated blood products were used.[16] In preliminary studies, hemophilia A patients receiving noncellular blood products did not have HTLV-I antibodies.[17] This report details the HTLV-I status of 127 factor VIII (hemophilia A) recipients and 71 factor IX (hemophilia B) recipients. Failure to find evidence of HTLV-I infection in this cohort reaffirms the safety of US factor components in regard to HTLV-I.

MATERIALS AND METHODS

Patient selection. The details of patient selection, immunological evaluation of patients, blood products received within groups of patients, and HIV antibody status of this cohort have been reported.[15,16] Hemophilia patients from 32 US hemophilia treatment centers were voluntarily enrolled in 1984 in a longitudinal cohort study of withdrawn lots of factors VIII and IX. The participants receiving withdrawn factor lots were matched to recipients of other factor lots according to the following criteria: type of factor received (VIII or IX), age (±5 years), sex, race, type of hemophilia (A or B) and, for factor VIII recipients, approximate amount of factor received each year. Patient's sera were evaluated for immunocomplexes by a staphylococcal binding assay (SBA) and for Clq complement by an ^{125}I-labeled Clq binding assay as previously described.[15] All participants received factor products from 1981 to 1988. Serum samples were obtained approximately yearly from 1984 through 1988, and the latest serum sample for each person was tested for HTLV-I antibody. Informed consent was obtained from all participants.

Serology. Patients' serum samples were initially tested for HTLV-I antibodies in a research approved enzyme-linked immunosorbent assay (ELISA, Dupont, Wilmington, DE) according to the manufacturer's recommendations. The specificity (99.8%) and sensitivity (94%) of the ELISA test (using the manufacturer's cutoff) was previously determined in an HTLV-I risk group.[18] Sera repeatedly reactive (above manufacturer's cutoff, 60% mean of positive control samples) and sera whose absorbances were within 20% below the manufacturer's cutoff (to ensure maximum sensitivity) were

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tested further by immunoblotting (IB) and if necessary (reactive on IB) by radioimmunoprecipitation assay.

Samples were tested by HTLV-I immunoblotting as described[19] with modifications. HTLV-I antigen from an HTLV-I–infected cell line (MT-2)[20] was obtained from a commercial source (Hillcrest Biologicals, Cypress, CA). HTLV-I antigen was suspended in sample buffer (0.1 mol/L Tris buffer, pH 6.8, containing 0.5% sodium dodecyl sulfate (SDS), 0.10 μg/mL bromophenol blue, 20% vol/vol glycerol, and 10% vol/vol 2-mercaptoethanol), heated at 95°C for 4 minutes and electrophoresed in a single well of a 10% polyacrylamide gel. Resolved proteins were electrophoretically transferred to nitrocellulose sheets which were then blocked for 5 hours in phosphate-buffered saline (PBS, pH 7.4) containing 0.5% Tween 20 (Sigma Chemical, St Louis, MO) and 5 g/100 mL nonfat dry milk, and cut into 3.0-mm strips. Individual strips were incubated with dilutions of 1:100 of serum overnight at 4°C, washed, incubated for 60 minutes at room temperature with 5 μg/mL biotinylated goat anti-human IgG (H & L Vector Laboratory, Burlingame, CA). After a repeated wash step, strips were then incubated with avidin-biotin-peroxidase complex according to the manufacturer's instructions (Vector). Color reactions were developed with 3,3′ diaminobenzidine, nickel chloride and hydrogen peroxide (Sigma). For each IB trial, banding patterns were compared with those of a known positive serum (adult T-cell leukemia patient). Specificity of banding patterns was further compared with strips tested for reactivity to monoclonal antibodies (MoAbs) against HTLV-I–specific proteins (p19, p24, and gp68).[21,22] Serum samples were tested for antibody to HIV by IB as previously described,[16] at a dilution of 1:100. Serologic reactions to HIV antigen with 18-Kd, 25-Kd, and 41-Kd proteins of HIV were scored as positive.

To perform HTLV-I radioimmunoprecipitation assay (RIPA), HTLV-I–infected MT-2 cells were metabolically labeled with ^{35}S cysteine and ^{35}S methionine (New England Nuclear, Boston, MA), disrupted with RIPA lysing buffer [0.02 mol/L NaH_2PO_4, 0.01 mol/L Na_2HPO_4-$7H_2O$, 0.02 mL Triton-X 100 (Sigma), 1.0 g/L SDS, 1.0 g/L sodium azide, 0.05 mol/L NaCl), and centrifuged. The lysate supernatants (200 μL were reacted with each serum (20 μL) for 16 hours at 4°C. Immunocomplexes were precipitated with Protein A-Sepharose CL-4B (Sigma) for 1.5 hours at 4°C. Protein-A-Sepharose/immunocomplexes were washed with RIPA lysing buffer, and immunocomplexes were eluted in sample buffer by boiling. Samples were electrophoretically analyzed in 10% polyacrylamide gels followed by autoradiography of the dried gel. Patients sera banding patterns were compared with positive (adult T-cell leukemia patient) and negative (normal donor) sera and to carbon 14 molecular weight (mol wt) standards with each RIPA gel.

A serum sample was determined to be HTLV-I positive if antibody reactivity was detected to *gag* gene product p24 and to an *env* gene product (gp46 and/or gp68).[23] Serum specimens not satisfying these criteria but having immunoreactivities to at least one suspected HTLV-I gene product were designated indeterminate. Serum specimens with no immunoreactivity to any HTLV-I gene products were considered negative. Because of extensive crossreactivity among viral proteins, our serologic methods did not distinguish antibody reactivity to HTLV-I from reactivity to HTLV-II. Our immunoblot strips used to detect HTLV-I antibodies failed to react to panels of sera confirmed to have HIV-1 and HIV-2 antibodies.

HTLV-I polymerase chain reaction. Polymerase chain reaction (PCR) was performed with total genomic DNA isolated from patients' peripheral blood leukocytes using reaction conditions as described.[24] Oligonucleotide primer pairs from the *pol* and *gag* genes of HTLV-I were used to amplify 1 μg total genomic DNA for each PCR amplification.[25] The amplified products were analyzed on a 5.0% polyacrylamide gel and confirmed further by Southern blot hybridization using specific *pol* and *gag* nucleotide ^{32}P-labeled probes.[26] Genomic DNA from MT-2 cells (HTLV-I–positive cell line) and HuT 78 (uninfected T-cell line) were used as positive and negative controls, respectively. Sensitivity of our PCR procedure for HTLV-I (detection of one copy of HTLV-I DNA in 10^6 cells) was estimated by serial dilution of MT-2 cells with negative control cells (HuT78).

RESULTS

Of 198 hemophilic patients whose serum was assayed for antibodies reactive against HTLV-I, 153 (77.3%) were seropositive for HIV-1, as previously reported.[15] Among the 127 factor VIII recipients, 116 (91.3%) of these patients had HIV antibodies and only 11 (8.7%) lacked antibodies to HIV. Of the 71 factor IX recipients, 37 (52.1%) were seropositive for HIV antibodies, and 34 (47.9%) did not have antibodies directed against HIV.

When these 198 sera were tested by HTLV-I ELISA, 5 (2.5%) were reactive for HTLV-I. Of these ELISA-reactive samples, four of five failed to react to HTLV-I antigen in immunoblotting assays (Table 1); sample E reacted to HTLV-I *gag* proteins p19 and p28 but not to other HTLV-I–specific proteins. Another sample (ELISA negative but near cutoff) reacted in a pattern similar to that with the IB assay (sample F, Table 1). These two IB-reactive samples (E and F) failed to react to HTLV-I antigens in RIPA. The remaining 193 (97.5%) serum samples failed to react to HTLV-I antigen by ELISA. The HTLV-I reactivity of samples E and F did not correlate with the patient's HIV antibody status. Sample F (reactive to HTLV-I p19 and p28) was negative for HIV, whereas sample E (also reactive to HTLV-I p19 and p28) was from a patient seropositive for HIV (Table 1).

To define the HTLV-I infection status of these patients with indeterminate p19 reactive immunoblots further, cells from patient E and an additional earlier serum sample from patient F were tested further. HTLV-I genetic sequences were not detected by PCR on DNA extracted from patient E (Fig 1). Patient F's p19 reactive status on immunoblot did not change when an earlier sample was tested; this stable pattern suggested that the reactivity to HTLV-I p19 did not represent early seroconversion to HTLV-I.

Patient serum samples reactive to HTLV-I by ELISA were examined for Clq complement (Clq binding assay) and immune complex levels (SBA). Values for percentage binding for Clq complement and staphylococcal binding assay were within expected ranges (data not shown) for this cohort of patients.[16]

DISCUSSION

The absence of confirmed HTLV-I antibodies in this cohort of hemophilic men indicates that this retrovirus, in contrast to HIV, has not been transmitted through factor products VIII or IX. The importance of cell-associated transmission of HTLV-I was recently confirmed by evidence for infection with HTLV-I in 6 of 211 patients receiving multiple blood transfusions in the United States.[11] A recent study of approximately 40,000 blood donors found 10 HTLV-I–positive patients,[12] suggesting that the opportunity

Table 1. HTLV-I Testing of Hemophilia Cohort: ELISA-Reactive Samples

Patient/Age (yr)	Factor Type	Amount of Factor Units	HIV Antibody	HTLV-I			
				EIA	IB	RIPA	PCR
A/15	VIII	166,452	+	+	–	–	ND
B/13	IX	24,160	+	+	–	–	ND
C/10	IX	8,850	–	+	–	–	ND
D/35	IX	23,010	+	+	–	–	ND
E/19	VIII	43,000	+	+	p19/28	–	–
F/29	IX	79,960	–	±	p19/28*	–	ND

HTLV-I EIA reactive samples derived from total sera tested from 127 factor VIII and 71 factor IX recipients.

Abbreviation: ND, not determined.

*Samples tested from 1984 and 1987 both p19-reactive Western IB.

exists for HTLV-I–positive units to be used in production of blood factor products. Our results affirm the relative safety of factor components in regard to HTLV-I transmission. These data suggest that factor components do not transmit HTLV-I and/or that procedures used for HIV inactivation are effective in preventing transmission of the virus.

We found that 5 of 198 samples were reactive in an ELISA test, but we could not confirm these to be positive by immunoblot and RIPA assays. These results verify the importance of confirming positive ELISA results with a second, more specific technique.[23] The occurrence of false-positive samples did not correlate with levels of Clq complement component or with immune complex levels of patients reactive to HTLV-I by ELISA, because patients in the cohort with equally elevated levels of Clq and immune complexes did not react when EIA was used.

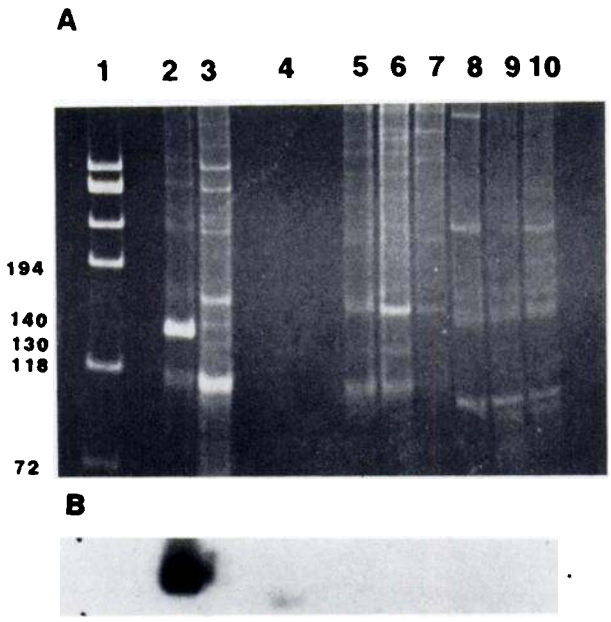

Fig 1. HTLV-I *pol* and *gag* gene amplification analysis of DNAs isolated from positive and negative control cell lines and patients' PBMNCs. (A) 5.0% polyacrylamide gel stained with ethidium bromide; (B) hybridization blot with labeled oligonucleotide ^{32}P probe of *pol* (lanes 2, 5 through 7) and *gag* (lanes 4, 8 through 10) primer amplified sequences]. Lane 1, λ Phage 174 mol wt marker; lane 2, MT-2 HTLV-I–positive cell line DNA for *pol* primer pair; lane 3, HuT 78-negative cell line control; lane 4, MT-2 HTLV-I–positive cell line for *gag* primer pair; lanes 5 and 8, patient E DNA (HTLV-I p19 IB indeterminate); lanes 6, 7, 9, and 10, control hemophilic patients (HTLV-I seronegative).

Two samples from the cohort were considered indeterminate for antibodies to HTLV-I (reactive to *gag* p19, p28 only). Both of these samples were negative when RIPA was used to measure HTLV-I antibody. These two samples may be nonspecifically binding these viral antigens in IB. HTLV-I p19 antigen may contain similar epitopes to certain host cell components, as MoAbs against HTLV-I p19 can recognize host cellular antigens.[21] Multiply transfused patients, such as those in our cohort, may have developed antibodies to antigens that also react with HTLV-I p19 or cellular antigens, causing an elevated false-positive rate for the ELISA test (2.5%). This would also explain similar reactions to HTLV-I p28 in the immunoblot test because this antigen is an incompletely cleaved gene product which contains the p19 protein.[27]

The failure to demonstrate HTLV-I genetic sequence from extracted cellular DNA from one patient (patient E) with p19 immunoblot reactive sera indicates that the reactivity in IB was not specific for HTLV-I. We were unable to demonstrate seroconversion to a positive pattern in a sequential serum sample from patient F in our present study and from two other persons with this pattern of reactivity.[28] Together these data suggest that the p19 reactivity on IB from these two hemophilic patients was not a result of HTLV-I infection. We have not eliminated the possibility that reactivity to p19 may represent a crossreaction to a closely related retrovirus. This reactivity to p19 on HTLV-I IB was probably not owing to crossreactivity to HIV antibodies, because our IB strips failed to react panels of HIV-1 and HIV-2 sera.

In summary, we have not confirmed HTLV-I antibodies in any of our hemophilic cohort patients. These data are consistent with the known transmission routes for HTLV-I; cell-associated transmission of the virus is necessary for infection. Our findings provide evidence that blood factor components do not transmit HTLV-I and indicate the importance of confirming reactive screening test results with a second technique.

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