Human T-Cell Leukemia Virus Type II Infection Frequently Goes Undetected in Contemporary US Blood Donors

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Serologic screening for human T-cell leukemia virus type I (HTLV-I) infection was begun in US blood banks with the licensure of enzyme-linked immunosorbent assays (ELISA) in December 1988. We examined the donation histories of the first 60 Western blot (WB)-confirmed HTLV-I/II positive donors to one blood center and found 8 had made 16 previous donations that scored negative on the screening ELISA. All 16 donations had ELISA absorbance below the cutoff for a positive assay, but still well above that of the average donation (17.6% ± 5.7% of the cutoff). In a more extensive study, 17 donations from a total of 61,752 at six blood centers were both ELISA-positive and WB-positive for HTLV-I (4) or HTLV-II (13), and 218 samples had ELISA absorbance greater than 50% of the ELISA cutoff. One hundred seventy-eight of the 218 were tested further by WB and 11 were found positive. All 11 positives were confirmed by polymerase chain reaction; 10 had HTLV-II and 1 had HTLV-I. Thus, the HTLV-I–based screening ELISA missed at least 10 of 23, or 43% (95% confidence interval, 23% to 66%), of HTLV-II infections, compared with 1 of 5, or 20%, of HTLV-I infections.
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HUMAN T-CELL leukemia/lymphoma virus type I (HTLV-I) is a blood-borne, pathogenic RNA tumor virus that is endemic in the southern islands of Japan, as well as in sub-Saharan Africa, Brazil, and the Caribbean.[1] HTLV-I causes adult T-cell leukemia/lymphoma as well as a chronic paralytic disease in a fraction of infected persons. HTLV-II, a related virus with antigenic cross-reactivity, is endemic in new world Indian tribes, but has not been unequivocally proven to be a pathogen. A high fraction of users of injectable drugs (IDU) are infected with HTLV-II.[1]

US blood centers screen for HTLV-I/II antibodies because HTLV-I is known to be an etiologic agent for at least two serious diseases, is present among US blood donors, and because diagnostic tests for antibodies against HTLV-I are judged to be sensitive and specific indicators of infection.[1,2] It has recently been appreciated that infection with HTLV-II is responsible for about 50% of HTLV seropositivity in the US donors.[3,4] Existing antibody screening enzyme-linked immunosorbent assays (ELISA) and Western blots (WB), having been designed for detection of antibodies to HTLV-I, use lysates of HTLV-I as substrate. There has been doubt about the sensitivity of such reagents for detecting HTLV-II antibodies. The measured sensitivity of licensed anti-HTLV-I ELISAs for HTLV-II have ranged from 55% to 91%.[5,6] The importance of reliable detection of HTLV-II has recently been highlighted as examples of apparent HTLV-II–related neurologic disease have been uncovered.[7-9]

Routine testing for HTLV-I/II antibodies consists of an ELISA screen, followed by WB of ELISA-positive sera.[2] Recently, the lysate-based WB has been refined by addition of recombinant gp21^{e} *envelope* antigens, as well as sensitive, type-specific peptide antigens that allow the differentiation of HTLV-I from HTLV-II antibodies.[10,11] A serum is judged positive for HTLV-I/II antibodies if reactivity to both the p24^{gag} and rgp21^{e} antigens is present. Differentiation of HTLV-I from -II can also be accomplished with type-specific peptide antigens in ELISA format. Polymerase chain reaction (PCR), performed on peripheral blood mononuclear cells (PBMC) DNA, remains the standard for diagnosis of infection and for distinction of HTLV-I from -II.[1] Antibody tests for HTLV-I/II are used for routine diagnostic purposes, however, because expense, labor-intensiveness, and difficulty in automation militate against the use of PCR for that purpose.

MATERIALS AND METHODS

Identification of donors with anti-HTLV antibodies with negative ELISA. Two ways of determining the rate of HTLV infection in ELISA-negative donors were used. In the first method, the donation histories of ELISA-positive, WB-positive donors were retrospectively examined for previous donations that had passed the ELISA screen. In the second method, sera were collected prospectively from consecutive blood donors with high-negative screening ELISA (>50% of ELISA cutoff for positivity) over a 2.5-month period (February 13 through April 30, 1992), and subjected to WB. Because this interval is greater than 8 weeks (long enough that some donors could donate twice during the study), we looked for February donations among the 41 donors with high-negative samples who gave blood in the last 2 weeks of April. Seven of the 225 high-negative donations were found to be duplicate donations, and were subtracted from the total. A comparable fraction was subtracted from the total donations (63,735), based on the assumption that the average donor donates with a frequency comparable to that of the high-negative donors. Institutional Review Board approval was obtained in accordance with an assurance filed with, and approved by, the Department of Health and Human Services.

Serologic studies and PCR. Screening ELISA (Abbott Laboratories, North Chicago, IL) was performed as recommended by the manufacturer. All ELISA high-negative or positive samples in this study were subjected to rgp21^{e}-supplemented WB (Cambridge Biotech, Rockville, MD).[10] WB-positive samples were subjected to peptide epitope ELISA (Coulter Select ELISA, Coulter Corp, Hialeah, FL). ELISA-negative, WB-positive sera were also tested with peptide-enhanced WB (Diagnostic Biotechnology Ltd, Singapore),[11] as were some ELISA-positive samples that were negative with the Coulter

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Select peptide epitope ELISA. PCR was performed on donor PBMC DNA as described,[4] using primers in *tax* and *pol*.

Statistical methods. The confidence intervals for proportions were determined using a two-tailed Fisher's exact test on the binomial. The *P* value for comparison of the gender distribution of ELISA-positive, WB-positive donors and ELISA-negative, WB-positive donors was determined using a two-tailed Fisher's exact test.

RESULTS

We examined the donation histories of the first 60 ELISA-positive, WB-confirmed donors to a single blood center with high HTLV-II prevalence (United Blood Services, Albuquerque, NM).[4] Seropositive donations by these 60 donors were collected between December 1988 and June 1991. The 60 donors were determined to have HTLV-I (7) or -II (53) by PCR and/or peptide epitope ELISA in 57 cases; 3 were diagnosed with HTLV-II solely on the basis of WB pattern.[10,12] Of the 60, 8 had made 16 previous donations that had passed the screening ELISA. Thus, the total donations made by the 60 HTLV-positive donors was 76; 16 of those were negative by screening ELISA, for a calculated rate of undetected anti-HTLV antibodies of 21% (16/76). Six of the 8 donors were tested by PCR and were positive; for the remaining 2, viral type was determined by peptide epitope ELISA. One of the 8 (a man of African ancestry) had HTLV-I, and the remainder had HTLV-II. Of the 7 with HTLV-II, 3 had American Indian ancestry,[4] 1 each had distant IDU history or transfusion exposure, and 2 women had long-standing relationships with HTLV-II-seropositive IDU men. ELISA absorbance/cutoff values from the 16 ELISA-negative donations ranged from 41% to 95%, which was far above the average of 17.6% ± 5.7% (mean ± SD) for all ELISA-negative donations to the blood center in question. However, there were many other donations with ELISA absorption values comparable with the absorption of ELISA-negative donations from the infected cohort; there was no clear hiatus between the 16 donations by the infected cohort and donations at large. The high ELISA absorbances observed in the past donations, coupled with the risk histories suggesting distant exposure to the virus, led us to believe that these donors were infected at the time of their previous ELISA-negative donations, and were not undergoing recent seroconversion (Fig 1).

The overall rate of undetected HTLV antibodies in our study of 60 seropositive donors was 21%, including 1 of 8 (13%) donations from HTLV-I-positive donors and 15 of 68 donations (22%) from HTLV-II-positive donors. The 21% rate of undetected HTLV-positive donations derived from study of the 60 HTLV-I/II-seropositive donors may be an underestimate, because we selected those donors who ultimately tested ELISA-positive. To estimate more precisely the actual rate, we prospectively collected 178 of the 218 sera from 61,752 (estimated) donations that scored "high-negative" (>50% of the cutoff for positivity) in the ELISA. Sera were collected from donations made over a 2.5-month interval in early 1992 from six US blood centers (Albuquerque, NM; El Paso, TX; Phoenix, AZ; Lafayette, LA; Reno, NV; and Las Vegas, NV). Collectively, about 80% of WB-positive donors to these six centers have had HTLV-II in past years.

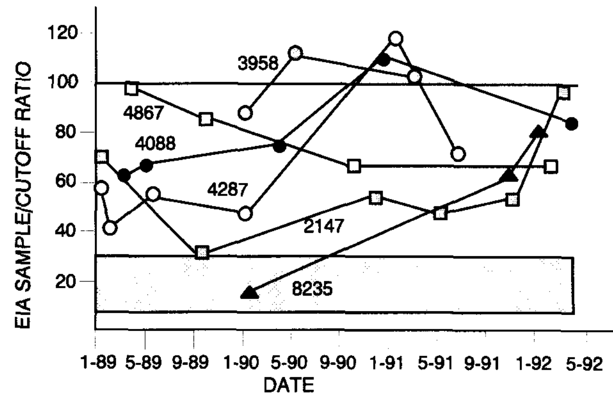

Fig 1. Serial ELISA absorbances are plotted against donation date for three WB-positive donors (open, closed, or shaded circles) who ultimately tested positive in the ELISA. Two had HTLV-II (4088 and 4287) and one had HTLV-I (3958). Absorbance of sera obtained after their ELISA-positive, WB-positive donation, which excluded them from further donation, are also plotted. Absorbance values from two WB-positive donors with persistently negative ELISAs, detected in a prospective study of ELISA-negative donors, are also plotted (open or shaded boxes). Both of these donors had HTLV-II DNA by PCR. The ELISA absorbances of donations from a donor with possible seroconversion (8235) to HTLV-II is depicted with closed triangles. The rectangular area represents 95% confidence interval (17.6% ± 11.3%) for average ELISA absorbance of donors.

During the 2.5 months we were collecting sera from donations with high-negative ELISA, 49 ELISA-positive donations were made. Seventeen of the 49 were found to be positive in the WB. Peptide epitope ELISA and "enhanced" WB[11] studies indicated that 4 of the 17 had HTLV-I, 9 had HTLV-II, and for 4 donations the viral antibodies could not be subtyped by those methods. Of the 4 sera that could not be subtyped, all had an HTLV-II-pattern WB,[12] and 2 of those donors were studied by PCR and found to have HTLV-II DNA; these results indicate that at least 11, and probably 13, of the 17 $ELISA^+/WB^+$ donors were infected with HTLV-II.

Sera with high-negative ELISA absorbance were stratified into two groups according to absorbance value relative to the cutoff for positivity. Of 178 high-negative ELISA donations tested, 119 had absorbance less than 70% of the cutoff, and 5 of those (4%) were positive in the WB. Of the 59 donations that scored higher than 70% of the cutoff, 6 (10%) were positive. WB patterns from 2 of the 11 WB-positive donors, both in the greater than 70% ELISA absorbance group, were of the pattern usually seen with HTLV-I infection (p19 reactivity ≥ p24). The remaining nine positive samples had patterns typical for HTLV-II.[12] Four of the 9 with HTLV-II-pattern WB also had antibodies reactive to the $p40^{tax}$ trans-activator gene product of HTLV-I/II.

Further serologic testing, donor interviews, and PCR were conducted to exclude the possibility of false-positive WB reactivity (Table 1). Seven of the 9 ELISA-negative sera with HTLV-II-pattern WB were positive for HTLV-II using peptide epitope ELISA, as was 1 of the 2 with HTLV-I-pattern WB (donation 2147). The remaining HTLV-I-pattern serum (5077) reacted only with the HTLV-I-specific peptide ELISA. Similar results were obtained using WB strips enhanced with

Table 1. Laboratory Results, Risk-Factor Histories, and Ethnic Backgrounds of 11 Donors With Negative ELISA and Positive WB

			Antibody to Peptide[11]:						
Donor	ELISA (%)	WB Pattern[10]	HTLV1 MTA	HTLV2 K55	Select ELISA	PCR Tax	PCR Pol	Ethnic	Risk
1965	84	HTLV2	Neg	Neg	HTLV2	HTLV2	HTLV2	NHW	None
5077	81	HTLV1	1+	Neg	HTLV1	HTLV1	Neg	NHW	TxR
8235	84	HTLV2	Neg	1+	HTLV2	HTLV2	Neg	His	IDU-S
6872	69	HTLV2	Neg	1+	HTLV2	HTLV2	HTLV2	NHW	TxR
9872	96	HTLV2	Neg	1+	HTLV2	HTLV2	Neg	Ind	Tattoo
4867	68	HTLV2	Neg	1+	HTLV2	HTLV2	HTLV2	Ind	TxR
0097	84	HTLV2	Neg	1+	Neg	HTLV2	HTLV2	His	None
3785	51	HTLV2	Neg	1+	HTLV2	HTLV2	HTLV2	NHW	None
2147	IR	HTLV1	Neg	2+	HTLV2	HTLV2	HTLV2	His	None
8737	69	HTLV2	Neg	Neg	HTLV2	HTLV2	HTLV2	His	IDU-S
5211	61	HTLV2	Neg	1+	Neg	HTLV2	Neg	His	IDU-S

Abbreviations: IDU-S, IDU spouse; TxR, transfusion recipient; NHW, non-Hispanic white; Ind, American Indian; His, Hispanic; IR, initially reactive, but repeated negative twice.

type-specific peptide antigens, in that 7 of the 9 sera with HTLV-II WB pattern and serum 2147 were reactive with the HTLV-II–specific K55 antigen and sample 5077 was reactive with the HTLV-I–specific MTA-1 antigen. *Tax* gene PCR verified the presence of HTLV-I proviral DNA in PBMC obtained from donor 5077, and HTLV-II DNA from donor 2147, as well as in all nine of the donors with HTLV-II–pattern WB. As reported previously, PCR using HTLV-I– or -II–specific primers in *pol* was less sensitive than *tax* gene PCR,[4] producing amplification product in 7 of the 11 DNAs.

The 11 ELISA-negative, WB-positive donors were interviewed (Table 1); 10 were women, and 7 were of Hispanic or Indian ancestry. For one donor, American Indian ancestry and receipt of a tattoo were the sole risk factors.[4] Three of the 11 had a history of blood transfusion (including the HTLV-I–infected donor 5077) and 3 had sexual exposure to an IDU partner.[3] By comparison, similar direct or indirect exposure to blood or needles was uncovered in 9 of 14 of the 17 ELISA-positive, WB-positive donors available for interview, and 3 others in that group were born in HTLV-I or -II–endemic areas and were breast-fed as infants. An additional donor from the ELISA$^+$/WB$^+$ group had received multiple tatoos and had an HTLV-II–seropositive wife.

Repeat testing with the Abbott lysate ELISA was performed in two laboratories. Three of the 11 sera now scored positive in one lab, and 4 of the 11 in the other. Two HTLV-II PCR-positive donors (9872 and 0097) were among the positives in both laboratories. Repeat ELISA absorbances on the sera from those two donors ranged from 101% to 134% of the cutoff. When the 11 sera were tested with a second licensed screening ELISA (DuPont, Wilmington, DE), only 2 samples were positive, including that from the HTLV-I–infected donor 5077.

Review of the past donation histories of the 11 ELISA-negative, WB-positive donors showed that 7 had made a total of 17 previous donations that had scored negative in the screening ELISA. The previous absorbance values for three of these donors are plotted in Fig 1. Two of the 17 previous donations were below 50% of the cutoff for positivity. In one case (donor 8235), the absorbance data suggested a possible seroconversion to HTLV-II infection. It should be noted that a very limited review of the past donations of ELISA-"high negative," WB-indeterminate controls suggested that a history of previous ELISA-"high negative" donations does not necessarily indicate that a donor is infected with an HTLV.

DISCUSSION

We estimate that at least 11 of 28 donations (39%) made by donors with HTLV infection were missed by the licensed Abbott screening ELISA. In addition, serum aliquots from 40 donations with high-negative ELISA absorbance were not sampled, suggesting that another two to three WB-positive donations may have been missed in our survey. Furthermore, we did not attempt to determine the prevalence of HTLV infection in those donors with ELISA absorbance less than 50%. Our review of previous donation history of infected donors identified in this study uncovered donations with absorbance less than 50% made by this group (see Fig 1 for examples).

Our donors with high-negative ELISA absorbance and positive WB are considered to be genuinely infected according to a variety of criteria. Ten of 11 WB-positive donors with negative ELISA were infected with HTLV-II, compared with 11 to 13 of 17 WB-positive donors with positive ELISA. It appears that by selecting for donors with negative ELISA, we may have enriched for HTLV-II infection at the expense of HTLV-I infection. These results suggest that HTLV-I–based screening ELISAs are detecting HTLV-II infections inefficiently among contemporary donors. This study may underestimate the sensitivity of the screening ELISA at detecting all HTLV infections because of the predominance of HTLV-II in the population studied, and because some donors with strongly positive screening ELISA have already been removed from the donor pool after more than 3 years of anti-HTLV antibody screening.

Of the 8 donors in our cohort of 60 ELISA-positives who had made previous donations with negative ELISA, 3 should have been deferred from donation because of past use of injectable drugs or sexual relations with IDU. There were also 3 donors with sexual exposure to IDU in the cohort of

11 WB-positive donors identified in the prospective study (Table 1). Our experience has been that donors who continue to donate despite such risk history usually do so because (1) drug usage ended before the onset of the acquired immunodeficiency syndrome (AIDS) epidemic around 1980; and/or (2) the IDU partner has been tested and found to be seronegative for the human immunodeficiency virus (HIV). Few donors are aware that IDU and their partners have increased risk of acquiring viruses other than HIV.

The sensitivity of the Abbott screening ELISA is less than that determined for the same test in an HTLV-I–endemic population (93.2%),[13] or in a population of HTLV-positive IDU in California in which the viral subtype had not been determined (91%).[6] This may reflect the effect of long-term screening of blood donors in our study, the exclusion of "WB-indeterminate" sera that come from infected patients by use of WB strips lacking enhanced sensitivity to *envelope* product antibodies,[13] or insensitivity of immunofluorescence assay confirmatory testing.[6] However, the 57% (maximum) sensitivity to HTLV-II we obtained is very similar to that obtained with a licensed ELISA by Wiktor et al[5] (58%) in a WB-positive IDU population with a high fraction of HTLV-II.

We do not know what factors predispose some infected persons to produce low levels of antibody in response to HTLV-II or -I infection. In our ELISA-negative group, there was an enrichment for female donors (10 of 11) compared with the ELISA-positive group (9 of 17; $P < .05$ by two-tailed Fisher's exact test), and slight enrichment for donors with no known risk factors. One possible explanation is that poor antibody response may be more frequent in those who acquire infection later in life, especially via sexual exposure. In a single case (donor 8235), serial absorbance data from past donations suggested that a seroconversion in response to recent exposure to HTLV-II was occurring (Fig 1). Finally, HTLV-I–based ELISAs may be insufficiently sensitive to HTLV-II antibodies because of incomplete cross-reactivity. ELISA screening tests designed for greater sensitivity to HTLV-II antibodies are in development (G. Robertson, Abbott Laboratories, personal communication, August 1992).

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